TITLE OF THE INVENTION

"IMMUNOGENIC COMPOSITIONS AND USES THEREFOR"

FIELD OF THE INVENTION

[0001] This application claims priority to Australian Provisional Application No. 2017904540 entitled "Immunogenic compositions and uses therefor" filed 8 November 2017, the contents of which are incorporated herein by reference in their entirety.

[0002] This invention relates generally to immunogenic compositions. More particularly, the present invention relates to the use of protein kinase C (PKC-θ) inhibitors for enhancing the immune effector function of functionally repressed T-cells that have undergone epithelial to mesenchymal transition (EMT). In specific embodiments, PKC-θ inhibitors are used to enhance susceptibility of exhausted T-cells to reinvigoration by PD-1 binding antagonists. The compositions of the present invention find utility in treating a range of disorders including T-cell dysfunctional disorders such as pathogenic infections and hyperproliferative disorders.

BACKGROUND OF THE INVENTION

[0003] Programmed death receptor 1 (PD-1) is an immunecheckpoint regulator that is expressed in various immune cells including T-cells, B-cells, natural killer (NK) cells, NK T (NKT) cells, monocytes, macrophages, and dendritic cells (DCs) following their activation. PD-1 binds to its two ligands: programmed cell death 1 ligand-1 (PD-L1; B7-H1; CD274) and PD-L2 (B7-DC; CD273), both of which are B7 family members. PD-L1 is constitutively expressed in a wide range of cells including hematopoietic and non-hematopoietic cells. In contrast, PD-L2 expression is restricted to professional antigen presenting cells (APCs; monocytes, macrophages, and DCs) and a certain subset of B cells. Inflammatory cytokines such as interferons (IFNs; α, β, and γ) are potent regulators of both PD-L1 and PD-L2 expression.

[0004] PD-1 is induced by T-cell receptor (TCR) signaling, and when PD-1 binds to PD-L1 or PD-L2, it inhibits TCR/CD28 signaling and T-cell activation. These immunoregulatory roles of PD-1 are responsible for limiting excessive T-cell activation to prevent immune-mediated tissue damage. However, prolonged TCR stimulation and PD-1 expression lead to T-cell exhaustion, which is a state of T-cell dysfunction defined by poor T-cell effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T-cells, and which is commonly associated with inefficient control of tumors and persistent viral infections (Wherry, EJ., 2011. *Nature Immunology* 12: 492-499). As such, the PD-1 pathway is an important determinant of the outcome of the T-cell response, regulating the balance between effective host defense and immunopathology, implicating the potential for manipulating the PD-1 pathway against various human diseases.

[0005] Blockade of the PD-1 pathway has been used to reinvigorate exhausted T-cells and restore anti-tumor or anti-pathogen immune responses. Indeed, antibodies that block the PD-1 pathway have shown promising clinical results in a significant number of advanced-stage cancer patients. However, clinical trial data to date show a high variety of response rates among different types of cancers to PD-1 immunecheckpoint inhibition therapy, with a range of 18% to 87%. These trials have also found that patients can present with primary, adaptive, or even acquired resistance to PD-1 immune-checkpoint inhibition therapy. Furthermore, emerging data demonstrate that certain patients experience hyperprogressive disease status after receiving anti-PD-1 antibodies.

[0006] Recently, Huang *et al.* (2017, *Nature* 545: 60-65) used immune profiling of peripheral blood from patients with stage IV melanoma before and after treatment with the anti-PD-1 antibody, pembrolizumab, to identify pharmacodynamic changes in circulating exhausted-phenotype CD8 T-cells (T_{ex} cells). Most of the patients demonstrated an immunological response to pembrolizumab but this was short lived. Clinical failure in many patients was not solely due to an inability to induce immune reinvigoration, but rather resulted from an imbalance between T-cell reinvigoration and tumor burden. The magnitude of reinvigoration of circulating T_{ex} cells determined in relation to pretreatment tumor burden correlated with clinical response, raising the possibility that even robust reinvigoration by anti-PD-1 therapy may be clinically ineffective if the tumor burden is high.

SUMMARY OF THE INVENTION

[0007] The present invention arises from the unexpected finding that increased translocation of protein kinase C theta (PKC-θ) in the nucleus of a T-cells (*e.g.*, $CD8^+$ T-cells) induces epithelial to mesenchymal transition (EMT) of the cells with repression of their immune effector function, including decreased expression of biomarkers of T-cell activation and effector capacity (*e.g.*, interleukin-2 (IL-2), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)), and increased expression of Zinc Finger E-Box Binding Homeobox 1 (ZEB1), which is a negative regulator of T-cell responses linked with cancer progression and T-cell effector inhibition, including repressing the expression of IL-2 and E-cadherin, and inducing EMT. Unexpectedly, PKC-θ and ZEB1 were found to co-localize in the nucleus and that this co-localization contributes at least in part to the repression of T-cell function. Notably, the inventors have determined that PKC-θ and ZEB1 are in close proximity in the nucleus and form a complex that is predicted to be a repressor of T-cell function.

[0008] The present inventors have also found that exposure of these mesenchymal, functionally repressed T-cells to PKC-θ inhibitors results in epigenetic reprogramming of the T-cells with remarkable de-repression of their immune effector function, including elevated expression of biomarkers of T-cell activation and effector capacity (*e.g.*, IL-2, IFN-γ and TNF-α), decreased expression of biomarkers of T-cell effector inhibition and cancer progression (*e.g.*, ZEB1), as well as decreased expression of biomarkers of T-cell exhaustion (*e.g.*, PD-1 and Eomesodermin (EOMES)) and elevated expression of the transcription factor TBET, which increases production of IFN-γ in cells of the adaptive and innate immune systems. Surprisingly, it has also been found that PKC-θ inhibitor-mediated epigenetic reprogramming confers enhanced susceptibility of exhausted T-cells to reinvigoration by PD-1 binding antagonists. These findings have been reduced to practice in methods and compositions for enhancing the immune effector function of T-cells and for treating diseases or conditions associated with T-cell dysfunction, as described hereafter.

[0009] Accordingly, in one embodiment, the present invention provides compositions for enhancing T-cell (*e.g.*, $CD8^+$ T-cell) function, or for treating a T-cell dysfunctional disorder. These compositions generally comprise, consist or consist essentially of a PKC-θ inhibitor and a PD-1 binding antagonist. The PKC-θ inhibitor is suitably selected from inhibitors of PKC-θ enzymatic activity and inhibitors of PKC-θ nuclear translocation. In specific embodiments, the PKC-θ inhibitor is an inhibitor of PKC-θ nuclear translocation, non-limiting examples of which include peptides corresponding to the nuclear localization site of PKC-θ, such as those disclosed for example in International Publication WO 2017/132728 A1 (*e.g.*, importinib4759). The PD-1 binding antagonist suitably inhibits the binding of PD-1 to PD-L1 and/or PD-L2. In preferred embodiments, the PD-1

binding antagonist is an anti-PD-1 antagonist antibody, illustrative examples of which include nivolumab, pembrolizumab, lambrolizumab and pidilizumab. In other embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, AMP-224). In some embodiments, the compositions further comprise an ancillary agent (*e.g.*, a chemotherapeutic agent) for treating, or for aiding in the treatment of, a T-cell dysfunctional disorder. The compositions are typically pharmaceutical compositions or formulations, which optionally comprise a pharmaceutically acceptable carrier.

[0010] Another embodiment of the present invention provides methods of enhancing T-cell function. These methods generally comprise, consist or consist essentially of contacting a T-cell with a PKC-θ inhibitor and a PD-1 binding antagonist, to thereby enhance T-cell function. Suitably, the enhanced T-cell function includes any one or more of increased production of cytokines such as such as IL-2, IFN-γ, TNF-α, increased activation of $CD8^+$ T-cells, increased recognition of an antigen or an antigen peptide derived from an antigen in the context of MHC class I molecules by T-cell receptors, increased elimination of cells presented in the context of MHC class I molecules and increased cytolytic killing of antigen expressing target cells. In some embodiments, the T-cell has a mesenchymal phenotype. Suitably, the T-cell has aberrant expression of nuclear PKC-θ. In representative examples of this type, the T-cell expresses nuclear PKC-θ at a higher level than the level of expression of TBET in the same T-cell, and/or at a higher level than in an activated T-cell. In some of the same and other embodiments, the T-cell is one exhibiting T-cell exhaustion or anergy. In non-limiting examples of this type, the T-cell expresses a higher level of EOMES than TBET and/or has elevated expression of PD-1. Preferably, the T-cell is a $CD8^+$ T-cell.

[0011] The present inventors propose that since PKC-θ-mediated EMT occurs both in tumor cells and in T-cells, which are unrelated cell types, PKC-θ-mediated epigenetic reprogramming is also likely to occur more broadly, including in other immune effector cells that express PD-1 (*e.g.*, T-cells, B-cells, NK cells, NKT cells, monocytes, macrophages and DCs), to thereby repress their immune effector function. Accordingly, in another embodiment, the present invention provides methods of enhancing immune effector function of an immune effector cell that expresses PD-1. These methods generally comprise, consist or consist essentially of contacting the immune effector cell with a PKC-θ inhibitor and a PD-1 binding antagonist, to thereby enhance the immune effector function of the immune effector cell. Suitably, the enhanced immune effector function includes any one or more of increased recognition of an antigen or an antigen peptide derived from an antigen in the context of MHC class II molecules by T-cell receptors, increased release of cytokines and/or the activation of $CD8^+$ lymphocytes (CTLs) and/or B-cells, increased recognition of an antigen or an antigen peptide derived from an antigen in the context of MHC class I molecules by T-cell receptors, increased elimination of cells presented in the context of MHC class I molecules, *i.e.*, cells characterized by presentation of an antigen with class I MHC, for example, via apoptosis or perforin-mediated cell lysis, increased production of cytokines such as Il-2, IFN-γ and TNF-α, and increased specific cytolytic killing of antigen expressing target cells. Suitably, the immune effector cell has aberrant expression of nuclear PKC-θ. In representative examples of this type, the immune effector expresses nuclear PKC-θ at a higher level than the level than in a control immune effector cell (*e.g.*, an immune effector cells with normal or non-repressed immune effector function).

[0012] In yet another embodiment, the present invention provides methods of treating a T-cell dysfunctional disorder in a subject. These methods generally comprise, consist or consist essentially of administering concurrently to the subject a PKC-θ inhibitor and a PD-1 binding

antagonist in effective amounts to treat the T-cell dysfunctional disorder. Suitably, the PKC-θ inhibitor and PD-1 binding antagonist are administered in synergistically effective amounts. In some embodiments, the T-cell dysfunctional disorder is a disorder or condition of T-cells characterized by decreased responsiveness to antigenic stimulation and/or increased inhibitory signal transduction through PD-1. In some of the same and other embodiments, the T-cell dysfunctional disorder is one in which the T-cells have decreased ability to secrete cytokines, proliferate, or execute cytolytic activity. In illustrative examples of this type, the decreased responsiveness to antigenic stimulation results in ineffective control of a pathogen or tumor. In some embodiments, the T-cell dysfunctional disorder is one in which T-cells are anergic. Representative examples of T-cell dysfunctional disorders include unresolved acute infection, chronic infection and tumor immunity. In preferred embodiments, the T-cell dysfunctional disorder is a cancer or infection that comprises a T-cell (*e.g.*, a $CD8^+$ T-cell) with a mesenchymal phenotype. In representative examples of this type, the T-cell expresses nuclear PKC-θ at a higher level than the level of expression of TBET in the same T-cell, and/or at a higher level than in an activated T-cell. In some of the same and other embodiments, the T-cell is one exhibiting T-cell exhaustion or anergy. In non-limiting examples of this type, the T-cell expresses a higher level of EOMES than TBET and/or has elevated expression of PD-1. In some embodiments, the T-cell is a tumor-infiltrating lymphocyte. In other embodiments, the T-cell is a circulating lymphocyte. In some embodiments, the cancer is skin cancer (*e.g.*, melanoma), lung cancer, breast cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer, prostate cancer, colorectal cancer, glioblastoma, neuroblastoma, or hepatocellular carcinoma. In preferred embodiments, the cancer is a metastatic cancer. Preferably, the metastatic cancer is metastatic melanoma or metastatic lung cancer. In some embodiments, the methods further comprise further administering concurrently to the subject, with the PKC-θ inhibitor and the PD-1 binding antagonist, an ancillary agent (*e.g.*, a chemotherapeutic agent) or ancillary therapy (*e.g.*, ablation or cytotoxic therapy) for treating, or for aiding in the treatment of, a T-cell dysfunctional disorder.

[0013] In related embodiments, the present invention provides methods of treating or delaying the progression of cancer in a subject. These methods generally comprise, consist or consist essentially of administering concurrently to the subject a PKC-θ inhibitor and a PD-1 binding antagonist in effective amounts to treat or delay the progression of the cancer. In some embodiments, the subject has been diagnosed with cancer, wherein a T-cell in a tumor sample of the cancer from the subject expresses nuclear PKC-θ at a higher level than the level of expression of TBET in the same T-cell, and/or at a higher level than in an activated T-cell.

[0014] In other related embodiment, the present invention provides methods of enhancing immune function (*e.g.*, immune effector function) in an individual having cancer. These methods generally comprise, consist or consist essentially of administering concurrently to the individual a PKC-θ inhibitor and a PD-1 binding antagonist in effective amounts to enhance the immune function. In some embodiments, the individual has been diagnosed with cancer, wherein a T-cell in a tumor sample of the cancer taken from the individual expresses nuclear PKC-θ at a higher level than the level of expression of TBET in the same T-cell, and/or at a higher level than in an activated T-cell.

[0015] In further embodiments, provided herein are methods of treating infection (*e.g.*, with a bacteria or virus or other pathogen). These methods generally comprise, consist or consist

essentially of administering concurrently to the individual a PKC-θ inhibitor and a PD-1 binding antagonist in effective amounts to treat the infection. In some embodiments, the infection is with virus and/or bacteria. In some embodiments, the infection is with a pathogen. In some embodiments, the infection is an acute infection. In some embodiments, the infection is a chronic infection.

[0016] In other related embodiments, the present invention provides methods of enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having an infection. These methods generally comprise, consist or consist essentially of administering concurrently to the individual a PKC-θ inhibitor and a PD-1 binding antagonist in effective amounts to enhance the immune function. In some embodiments, the individual has been diagnosed with the infection, wherein a T-cell in a sample taken from the individual expresses nuclear PKC-θ at a higher level than the level of expression of TBET in the same T-cell, and/or at a higher level than in an activated T-cell.

[0017] Another embodiment of the present invention provides use of a PKC-θ inhibitor and a PD-1 binding antagonist for treating a T-cell dysfunctional disorder, or for enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, for treating or delaying the progression of cancer, or for treating infection. The PKC-θ inhibitor and PD-1 binding antagonist are generally used in the manufacture of medicaments for this purpose. Suitably, the PKC-θ inhibitor and PD-1 binding antagonist are formulated for concurrent administration.

[0017a] Disclosed herein is the use of a PKC-θ inhibitor and a PD-1 binding antagonist in the manufacture of a medicament for treating a T-cell dysfunctional disorder in a subject, wherein the T-cell dysfunctional disorder is a cancer that is resistant to one or more PD-1 binding antagonists, and wherein the PKC-θ inhibitor is an inhibitor of PKC-θ nuclear translocation.

[0018] In a related embodiment, the present invention provides use of a PKC-θ inhibitor, a PD-1 binding antagonist and an ancillary agent (*e.g.*, a chemotherapeutic agent) for treating, or for aiding in the treatment of, a T-cell dysfunctional disorder, or for enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, for treating or delaying the progression of cancer, or for treating infection. The PKC-θ inhibitor, PD-1 binding antagonist and ancillary agent (*e.g.*, a chemotherapeutic agent) are typically used in the manufacture of medicaments for this purpose. Suitably, the PKC-θ inhibitor, PD-1 binding antagonist and ancillary agent (*e.g.*, a chemotherapeutic agent) are formulated for concurrent administration.

[0019] In some embodiments, the methods for treating a T-cell dysfunctional disorder, or for enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, for treating or delaying the progression of cancer, or for treating infection comprise detecting an elevated level of nuclear PKC-θ (*i.e.*, PKC-θ localized in the nucleus) in a T cell (*e.g.*, relative to the level of TBET in the same T-cell or the level of nuclear PKC-θ in an activated T-cell) in a sample obtained from the subject, prior to the concurrent administration.

[0020] In some embodiments, the methods for treating a T-cell dysfunctional disorder comprise detecting an elevated level of nuclear PKC-θ (*i.e.*, PKC-θ localized in the nucleus) in a T cell (*e.g.*, relative to the level of TBET in the same T-cell or the level of nuclear PKC-θ in an activated T-cell) and an elevated level of ZEB1 in the nucleus of the T cell (*e.g.*, relative to the

level of TBET in the same T-cell or the level of ZEB1 in the nucleus of an activated T-cell) in a sample obtained from the subject, prior to the concurrent administration. In representative examples of this type, these methods comprise detecting an elevated level of a complex comprising PKC-θ and ZEB1, suitably in the nucleus of the T-cell.

[0021] In related embodiments, the present invention provides kits comprising a medicament comprising a PKC-θ inhibitor and an optional pharmaceutically acceptable carrier, and a package insert comprising instructional material for concurrent administration of the medicament with another medicament comprising a PD-1 binding antagonist and an optional pharmaceutically acceptable carrier for treating a T-cell dysfunctional disorder, or for enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, for treating or delaying the progression of cancer, or for treating infection in an individual.

[0022] In other related embodiments, the present invention provides kits comprising a medicament comprising a PD-1 binding antagonist and an optional pharmaceutically acceptable carrier, and a package insert comprising instructional material for concurrent administration of the medicament with another medicament comprising a PKC-θ inhibitor and an optional pharmaceutically acceptable carrier for treating a T-cell dysfunctional disorder, or for enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, for treating or delaying the progression of cancer, or for treating infection in an individual.

[0023] In still other related embodiments, the present invention provides kits comprising a first medicament comprising a PKC-θ inhibitor and an optional pharmaceutically acceptable carrier, and a second medicament comprising a PD-1 binding antagonist and an optional pharmaceutically acceptable carrier for treating a T-cell dysfunctional disorder, or for enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, for treating or delaying the progression of cancer, or for treating infection in an individual. In some embodiments, the kits further comprise a package insert comprising instructional material for administering concurrently the first medicament and the second medicament for treating a T-cell dysfunctional disorder, or for enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, for treating or delaying the progression of cancer, or for treating infection in an individual.

[0024] In some embodiments of the methods, uses, compositions, formulations and kits described above and elsewhere herein, $CD8^+$ T cells in the individual have enhanced priming, activation, proliferation and/or cytolytic activity as compared to before the administration of the combination of PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, the number of $CD8^+$ T cells is elevated as compared to before administration of the combination. In some embodiments, the $CD8^+$ T cell is an antigen-specific $CD8^+$ T cell. In some embodiments, Treg function is suppressed as compared to before administration of the combination of the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, T cell exhaustion is decreased as compared to before administration of the combination of the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, number of Treg cells is decreased as compared to before administration of the combination of the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, plasma IFN-γ is increased as compared to before administration of the combination of the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, plasma TNF-α is increased as compared to before administration of the combination of the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, plasma IL-2 is increased as compared to before

administration of the combination of the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, the number of memory T effector cells is increased as compared to before administration of the combination of the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, memory T effector cell activation and/or proliferation is increased as compared to before administration of the combination of the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, memory T effector cells are detected in peripheral blood. In some embodiments, detection of memory T effector cells is by detection of CXCR3.

[0025] In some embodiments of the methods, uses, formulations, and kits described above and elsewhere herein, the PKC-θ inhibitor and/or PD-1 binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments of the methods, uses, compositions, and kits described above and herein, the treatment further comprises administering an ancillary agent (*e.g.*, a chemotherapeutic agent) for treating or delaying progression of cancer in an individual. In some embodiments, the individual has been treated with a chemotherapeutic agent before the combination treatment with the PKC-θ inhibitor and PD-1 binding antagonist. In some embodiments, the individual treated is refractory to a chemotherapeutic agent treatment. Some embodiments of the methods, uses, compositions, and kits described throughout the application, further comprise administering a chemotherapeutic agent for treating or delaying progression of cancer.

[0026] A further embodiment of the present invention provides methods of diagnosing the presence of a T-cell dysfunctional disorder in a subject. These methods generally comprise, consist or consist essentially of:

(i) obtaining a sample from the subject, wherein the sample comprises a T-cell (*e.g.*, $CD8^+$ T-cell);

(ii) contacting the sample with a first binding agent that binds to PKC-θ in the sample and a second binding agent that binds to ZEB1 in the sample; and

(iii) detecting localization of the first and second binding agents in the nucleus of the T-cell;

wherein localization of the first and second binding agents in the nucleus of the T-cell is indicative of the presence of the T-cell dysfunctional disorder in the subject.

[0027] In yet another embodiment, the present invention provides methods of diagnosing the presence of a T-cell dysfunctional disorder in a subject. These methods generally comprise, consist or consist essentially of:

(i) obtaining a sample from the subject, wherein the sample comprises a T-cell (*e.g.*, $CD8^+$ T-cell);

(ii) contacting the sample with a first binding agent that binds to PKC-θ in the sample and a second binding agent that binds to ZEB1 in the sample; and

(iii) detecting the first and second binding agents when bound to a PKC-θ—ZEB1 complex in the sample;

wherein an elevated level of PKC-θ—ZEB1 complex detected in the sample relative to a level of PKC-θ—ZEB1 complex detected in a control sample (*e.g.*, one comprising an activated T-cell) is indicative of the presence of the T-cell dysfunctional disorder in the subject.

[0028] A further embodiment of the present invention provides methods of monitoring the treatment of a subject with a T-cell dysfunctional disorder. These methods generally comprise, consist or consist essentially of:

(i) obtaining a sample from the subject following treatment of the subject with a therapy for the T-cell dysfunctional disorder, wherein the sample comprises a T-cell (*e.g.*, $CD8^+$ T-cell);

(ii) contacting the sample with a first binding agent that binds to PKC-θ in the sample and a second binding agent that binds to ZEB1 in the sample; and

(iii) detecting the first and second binding agents when bound to a PKC-θ—ZEB1 complex in the sample;

wherein a lower level of PKC-θ—ZEB1 complex detected in the sample relative to a level of PKC-θ—ZEB1 complex detected in a control sample taken from the subject prior to the treatment is indicative of an increased clinical benefit (*e.g.*, enhanced immune effector function such as enhanced T-cell function) to the subject, and

wherein a higher level of PKC-θ—ZEB1 complex detected in the sample relative to a level of PKC-θ—ZEB1 complex detected in a control sample taken from the subject prior to the treatment is indicative of no or negligible clinical benefit (*e.g.*, enhanced immune effector function such as enhanced T-cell function) to the subject.

[0029] In still another embodiment, a kit is provided for diagnosing the presence of a T-cell dysfunctional disorder in a subject. These kits generally comprise, consist or consist essentially of: (i) a first binding agent that binds to PKC-θ, (ii) a second binding agent that binds to ZEB1; and (iii) a third agent comprising a label, which is detectable when each of the first and second binding agents is bound to a PKC-θ—ZEB1 complex. In specific embodiments, the third agent is a binding agent that binds to the first and second binding agent.

[0030] In a related embodiment, the present invention provides a complex comprising PKC-θ and ZEB1, a first binding agent that is bound to PKC-θ of the complex, a second binding agent bound to ZEB1 of the complex; and (iii) a third agent comprising a label, which is detectable when each of the first and second binding agents is bound to the PKC-θ—ZEB1 complex. In specific embodiments, the PKC-θ—ZEB1 complex is located in a T-cell. In specific embodiments, the third agent is a binding agent that binds to the first and second binding agent.

[0031] In still another embodiment, the present invention provides a T-cell that comprises a complex comprising PKC-θ and ZEB1, a first binding agent that is bound to PKC-θ of the complex, a second binding agent bound to ZEB1 of the complex; and (iii) a third agent comprising a label, which is detectable when each of the first and second binding agents is bound to the PKC-θ—ZEB1 complex. In specific embodiments, the third agent is a binding agent that binds to the first and second binding agent.

[0032] In any of the above embodiment, respective binding agents are preferably antibodies.

[0033] The above diagnostic methods and kits are useful as companion diagnostics for the treatment methods of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] Figure 1 is a graphical, schematic and photographic representation showing that PKC-θ can be targeted for therapeutic intervention. A) Depicts the strength of binding of

recombinant purified His_6-PKC-θ to increasing concentrations of different subunits and subunit combinations (α2, α2β1, and β1) of the importin α/β heterodimer nuclear transport receptor using an AlphaScreen® binding assay as described in Wagstaff *et al.* (2011. *Journal of Biomolecular Screening* 16(2):192-200). B-C) Depicts protein structure of PKC-θ indicating binding locations for the PKC-θ peptide inhibitor, PKCθi (RKEIDPPFRPKVK), including the nuclear localization sequence (NLS) of PKC-θ. D) The specificity of PKCθi was examined on cells treated with this peptide inhibitor. Cells were screened with primary antibodies to PKC-θ (T538p), PKC-β2, PKC-β1, PKC-α, PKC-ε and PKC-γ. The nuclear to cytoplasmic fluorescence ratio (Fn/c) using the equation: Fn/c = (Fn − Fb)/(Fc − Fb), where Fn is nuclear fluorescence, Fc is cytoplasmic fluorescence, and Fb is background fluorescence was used to determine the impact on nuclear translocation/localization. E) EC_{50} of PKCθi peptide inhibitor, PKCθi, on MDA-MB-231 cells using WST-1 assay (Sigma). EC^{50} of inhibitors calculated using GraphPad PRISM software. F) The PKC-θ kinase activity was measured using the PKC kinase activity kit from ENZO life sciences and recombinant PKC-θ; C27 is an inhibitor of PKC-θ kinase activity, which is disclosed in Jimenez *et al.* (2013. *J Med Chem* 56(5):1799–810).

[0035] Figure 2 is a photographic and graphical representation depicting a PKC-θ resistance signature in $CD8^+$ T-cells from BRAF negative melanoma patients. A) Melanoma patient formalin-fixed paraffin-embedded (FFPE) tissue from primary tumor baseline biopsy for either a complete response (CR), stable disease (SD) or progressive disease (PD) was processed by 3D high resolution microscopy using a Leica Bond RX Stainer. FFPE tissues were fixed and immunofluorescence microscopy was performed probing with primary antibodies to anti-PD1, anti-PKC-θ (T538p) and anti-CD8 with DAPI. Representative images for each dataset are shown. Graph plots represent the TCFI values for CD8, TNFI for PKC-θ and TCFI for PD1 measured using ImageJ minus background (N=40 cells per a patient sample). B) Peripheral blood mononuclear cells (PBMCs) isolated from melanoma patient liquid biopsies were pre-clinically screened with either control or PKCθi nuclear peptide inhibitor. PBMC Samples were screened in triplicate for expression of IL-2, IFN-γ and TNF-α. C) Melanoma patient FFPE tissue from primary tumor baseline biopsy for CR, SD and two PD cohorts defined by responder or resistant to Dual Immunotherapy was processed by 3D high resolution microscopy using a Leica Bond RX Stainer. FFPE tissues were fixed and immunofluorescence microscopy was performed probing with primary antibodies to anti-PD1, anti-PKC-θ (T538p), anti-CD8 and anti-ZEB1 with DAPI. Representative images for each dataset are shown. Graph plots represent the TCFI values for CD8, TNFI for PKC-θ and TNFI for ZEB1 measured using ImageJ minus background (N=40 cells per a patient sample).

[0036] Figure 3 is a photographic representation depicting a PKC-θ resistance signature in $CD8^+$ T-cells. A-B) CD8s were isolated from melanoma patient liquid biopsies (CR = complete response, SD = Stable Disease, PD = progression of disease) and were stimulated with PMA/CI and pre-clinically screened with either vehicle control or PKCθi nuclear peptide inhibitor. Samples were fixed and immunofluorescence microscopy was performed on these cells with primary antibodies for ZEB1, PKC-θ, and CD8. Representative images for each dataset are shown. Graph represents the TNFI for PKC-Theta and TNFI for ZEB1 measured using ImageJ to select the nucleus minus background (n= >20 cells/sample). Plot profiles for each cohort for ZEB1 and PKC-θ are also depicted (RED = ZEB1, Green = PKC-θ) with the PCC indicated. Graphs depicting the % inhibition or induction based on protein expression where also plotted for each protein target relative to untreated sample. C-D) CD8s were isolated from melanoma patient liquid biopsies (CR = complete

response, PR = partial response, PD = progression of disease) and were stimulated with PMA/CI and pre-clinically screened with either vehicle control or our PKCθi nuclear peptide inhibitor. Samples were fixed and immunofluorescence microscopy was performed on these cells with primary antibodies for TNF-α and IFN-γ, and CD8. Representative images for each dataset are shown. Graph represents the TNFI for TNF-α and TNFI for IFN-γ measured using ImageJ to select the nucleus minus background (n= >20 cells/sample). Graphs depicting the % inhibition or induction based on protein expression where also plotted for each protein target relative to untreated sample.

[0037] Figure 4 is graphical representation showing expression of EOMES, TBET and PD-1 in $CD8^+$ T-cells of melanoma patient liquid biopsies in the presence and absence of PKCθi peptide inhibitor. A) $CD8^+$ T-cells were isolated from melanoma patient liquid biopsies (CR = complete response, SD = Stable Disease, PD = progression of disease) and were stimulated with PMA/CI and pre-clinically screened with either vehicle control or PKCθi peptide inhibitor. Samples were then fixed and immunofluorescence microscopy was performed on these cells with primary antibodies for EOMES, TBET, and PD-1. Representative images for each dataset are shown. Graph represents the TCFI for PD1, TNFI for EOMES and TNFI for TBET measured using ImageJ to select the nucleus minus background (n= >20 cells/sample). Graphs depicting the % inhibition or induction based on protein expression where also plotted for each protein target relative to untreated sample.

[0038] Some figures and text contain color representations or entities. Color illustrations are available from the Applicant upon request or from an appropriate Patent Office. A fee may be imposed if obtained from a Patent Office.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0039] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0040] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.* to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0041] The term "about" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*.

[0042] "Activation", as used herein, refers to the state of a cell following sufficient cell surface moiety ligation to induce a noticeable biochemical or morphological change. Within the context of T cells, such activation refers to the state of a T cell that has been sufficiently stimulated to induce cellular proliferation. Activation of a T cell may also induce cytokine production and detectable effector functions, including performance of regulatory or cytolytic effector functions. Within the context of other cells, this term infers either up or down regulation of a particular

physico-chemical process. Activation can also be associated with induced cytokine production, and detectable effector functions.

[0043] The term "activated T-cell" means a T-cell that is currently undergoing cell division, detectable effector functions, including cytokine production, performance of regulatory or cytolytic effector functions, and/or has recently undergone the process of "activation".

[0044] The terms "administration concurrently" or "administering concurrently" or "co-administering" and the like refer to the administration of a single composition containing two or more actives, or the administration of each active as separate compositions and/or delivered by separate routes either contemporaneously or simultaneously or sequentially within a short enough period of time that the effective result is equivalent to that obtained when all such actives are administered as a single composition. By "simultaneously" is meant that the active agents are administered at substantially the same time, and desirably together in the same formulation. By "contemporaneously" it is meant that the active agents are administered closely in time, *e.g.*, one agent is administered within from about one minute to within about one day before or after another. Any contemporaneous time is useful. However, it will often be the case that when not administered simultaneously, the agents will be administered within about one minute to within about eight hours and suitably within less than about one to about four hours. When administered contemporaneously, the agents are suitably administered at the same site on the subject. The term "same site" includes the exact location, but can be within about 0.5 to about 15 centimeters, preferably from within about 0.5 to about 5 centimeters. The term "separately" as used herein means that the agents are administered at an interval, for example at an interval of about a day to several weeks or months. The active agents may be administered in either order. The term "sequentially" as used herein means that the agents are administered in sequence, for example at an interval or intervals of minutes, hours, days or weeks. If appropriate the active agents may be administered in a regular repeating cycle.

[0045] The term "agent" includes a compound that induces a desired pharmacological and/or physiological effect. The term also encompass pharmaceutically acceptable and pharmacologically active ingredients of those compounds specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the above term is used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc. The term "agent" is not to be construed narrowly but extends to small molecules, proteinaceous molecules such as peptides, polypeptides and proteins as well as compositions comprising them and genetic molecules such as RNA, DNA and mimetics and chemical analogs thereof as well as cellular agents. The term "agent" includes a cell that is capable of producing and secreting a polypeptide referred to herein as well as a polynucleotide comprising a nucleotide sequence that encodes that polypeptide. Thus, the term "agent" extends to nucleic acid constructs including vectors such as viral or non-viral vectors, expression vectors and plasmids for expression in and secretion in a range of cells.

[0046] "Amplification," as used herein generally refers to the process of producing multiple copies of a desired sequence. "Multiple copies" mean at least two copies. A "copy" does not necessarily mean perfect sequence complementarity or identity to the template sequence. For example, copies can include nucleotide analogs such as deoxyinosine, intentional sequence alterations (such as sequence alterations introduced through a primer comprising a sequence that

is hybridizable, but not complementary, to the template), and/or sequence errors that occur during amplification.

[0047] The "amount" or "level" of a biomarker is a detectable level in a sample. These can be measured by methods known to one skilled in the art and also disclosed herein. The expression level or amount of biomarker assessed can be used to determine the response to treatment.

[0048] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

[0049] The term "anergy" refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T-cell receptor (*e.g.* increase in intracellular Ca^{2+} in the absence of ras-activation). T-cell anergy can also result upon stimulation with antigen in the absence of co-stimulation, resulting in the cell becoming refractory to subsequent activation by the antigen even in the context of co-stimulation. The unresponsive state can often be overridden by the presence of IL-2. Anergic T-cells do not undergo clonal expansion and/or acquire effector functions.

[0050] The term "antagonist" or "inhibitor" refers to a substance that prevents, blocks, inhibits, neutralizes, or reduces a biological activity or effect of another molecule, such as a receptor.

[0051] The term "antagonist antibody" refers to an antibody that binds to a target and prevents or reduces the biological effect of that target. In some embodiments, the term can denote an antibody that prevents the target, *e.g.*, PD-1, to which it is bound from performing a biological function.

[0052] As used herein, an "anti-PD-1 antagonist antibody" refers to an antibody that is able to inhibit PD-1 biological activity and/or downstream events(s) mediated by PD-1. Anti-PD-1 antagonist antibodies encompass antibodies that block, antagonize, suppress or reduce (to any degree including significantly) PD-1 biological activity, including inhibitory signal transduction through PD-1 and downstream events mediated by PD-1, such as PD-L1 binding and downstream signaling, PD-L2 binding and downstream signaling, inhibition of T cell proliferation, inhibition of T cell activation, inhibition of IFN secretion, inhibition of IL-2 secretion, inhibition of TNF secretion, induction of IL-10, and inhibition of anti-tumor immune responses. For purposes of the present invention, it will be explicitly understood that the term "anti-PD-1 antagonist antibody" (interchangeably termed "antagonist PD-1 antibody", "antagonist anti-PD-1 antibody" or "PD-1 antagonist antibody") encompasses all the previously identified terms, titles, and functional states and characteristics whereby PD-1 itself, a PD-1 biological activity, or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree. In some embodiments, an anti-PD-1 antagonist antibody binds PD-1 and upregulates an anti-tumor or anti-pathogen immune response. Examples of anti-PD-1 antagonist antibodies are provided herein.

[0053] The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0054] An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an antibody is purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0055] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0056] The term "constant domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the C_{H1}, C_{H2} and C_{H3} domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

[0057] The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as "V_H." The variable domain of the light chain may be referred to as "V_L." These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

[0058] The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The

constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0059] The "light chains" of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains.

[0060] The term IgG "isotype" or "subclass" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions.

[0061] Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, γ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas *et al.* Cellular and Mol. Immunology, 4th ed. (W.B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

[0062] The terms "full length antibody," "intact antibody" and "whole antibody" are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region.

[0063] The term "naïve T-cells" refers to immune cells that comprise antigen-inexperienced cells, *e.g.*, immune cells that are precursors of memory T effector cells. In some embodiments, naïve T cells may be differentiated, but have not yet encountered their cognate antigen, and therefore are activated T cells or memory effector T cells. In some embodiments, naïve T cells may be characterized by expression of CD62L, CD27, CCR7, CD45RA, CD28, and CD127, and the absence of CD95, or CD45RO isoform.

[0064] A "naked antibody" for the purposes herein is an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0065] "Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. In some embodiments, the antibody fragment described herein is an antigen-binding fragment. Examples of antibody fragments include Fab, Fab', F(ab'$_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0066] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0067] "Fv" is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one

light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a "dimeric" structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0068] The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0069] "Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see, *e.g.*, Pluckthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0070] The term "diabodies" refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson *et al.*, Nat. Med. 9:129-134 (2003); and Hollinger *et al.*, Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson *et al.*, Nat. Med. 9:129-134 (2003).

[0071] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are identical except for possible mutations, *e.g.*, naturally occurring mutations, that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific

antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[0072] The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein, Nature, 256:495-97 (1975); Hongo *et al.*, Hybridoma, 14 (3): 253-260 (1995), Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: Monoclonal Antibodies and T-cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, Nature, 352: 624-628 (1991); Marks *et al.*, J. Mol. Biol. 222: 581-597 (1992); Sidhu *et al.*, J. Mol. Biol. 338(2): 299-310 (2004); Lee *et al.*, J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee *et al.*, J. Immunol. Methods 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, Proc. Natl. Acad. Sci. USA 90: 2551 (1993); Jakobovits *et al.*, Nature 362: 255-258 (1993); Bruggemann *et al.*, Year in Immunol. 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and U.S. Pat. No. 5,661,016; Marks *et al.*, Bio/Technology 10: 779-783 (1992); Lonberg *et al.*, Nature 368: 856-859 (1994); Morrison, Nature 368: 812-813 (1994); Fishwild *et al.*, Nature Biotechnol. 14: 845-851 (1996); Neuberger, Nature Biotechnol. 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13: 65-93 (1995).

[0073] The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, *e.g.*, U.S. Pat. No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)). Chimeric antibodies include PRIMATTZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with the antigen of interest.

[0074] "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a HVR of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise

residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, Nature 321:522-525 (1986); Riechmann *et al.*, Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also, *e.g.*, Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0075] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks *et al.*, J. Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner *et al.*, J. Immunol., 147(1):86-95 (1991). See also van Dijk and van de Winkel, Curr. Opin. Pharmacol., 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0076] A "species-dependent antibody" is one which has a stronger binding affinity for an antigen from a first mammalian species than it has for a homologue of that antigen from a second mammalian species. Normally, the species-dependent antibody "binds specifically" to a human antigen (*e.g.*, has a binding affinity (Kd) value of no more than about $1x10^{-7}$ M, preferably no more than about $1x10^{-8}$ M and preferably no more than about $1x10^{-9}$ M) but has a binding affinity for a homologue of the antigen from a second nonhuman mammalian species which is at least about 50-fold, or at least about 500-fold, or at least about 1000-fold, weaker than its binding affinity for the human antigen. The species-dependent antibody can be any of the various types of antibodies as defined above, but preferably is a humanized or human antibody.

[0077] The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Xu *et al.*, Immunity 13:37-45 (2000); Johnson and Wu, in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, N.J., 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the

absence of light chain. See, *e.g.*, Hamers-Casterman *et al.*, Nature 363:446-448 (1993); Sheriff *et al.*, Nature Struct. Biol. 3:733-736 (1996).

[0078] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0079] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al.*, supra, for each of these definitions.

[0080] "Framework" or "FR" residues are those variable domain residues other than the HVR residues as herein defined. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3 (L3)-FR4.

[0081] The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain variable domains or light chain variable domains of the compilation of antibodies in Kabat *et al.*, supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0082] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (*e.g.*, Kabat *et al.*, Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant

region (*e.g.*, the EU index reported in Kabat *et al.*, supra). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[0083] The expression "linear antibodies" refers to the antibodies described in Zapata *et al.* (1995 Protein Eng, 8(10):1057-1062). Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0084] As used herein, the term "antigen" and its grammatically equivalents expressions (*e.g.*, "antigenic") refer to a compound, composition, or substance that may be specifically bound by the products of specific humoral or cellular immunity, such as an antibody molecule or T-cell receptor. Antigens can be any type of molecule including, for example, haptens, simple intermediary metabolites, sugars (*e.g.*, oligosaccharides), lipids, and hormones as well as macromolecules such as complex carbohydrates (*e.g.*, polysaccharides), phospholipids, and proteins. Common categories of antigens include, but are not limited to, viral antigens, bacterial antigens, fungal antigens, protozoa and other parasitic antigens, tumor antigens, antigens involved in autoimmune disease, allergy and graft rejection, toxins, and other miscellaneous antigens.

[0085] As use herein, the term "binds", "specifically binds to" or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that binds to or specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (Kd) of ≤1 μM, ≤100 nM, ≤10 nM, ≤1 nM, or ≤0.1 nM. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[0086] As used herein, the term "binding agent" refers to an agent that binds to a target antigen and does not significantly bind to unrelated compounds. Examples of binding agents that can be effectively employed in the disclosed methods include, but are not limited to, lectins, proteins, and antibodies, such as monoclonal antibodies, chimeric antibodies, or polyclonal antibodies, or antigen-binding fragments thereof, as well as aptamers, Fc domain fusion proteins, and aptamers having or fused to hydrophobic protein domain, e.g, Fc domain, etc. In an embodiment the binding agent is an exogenous antibody. An exogenous antibody is an antibody not naturally produced in a mammal, e.g. in a human, by the mammalian immune system.

[0087] The term "biomarker" as used herein refers to an indicator, *e.g.*, predictive, diagnostic, and/or prognostic, which can be detected in a sample. The biomarker may serve as an indicator of a particular subtype of a disease or disorder (*e.g.*, T-cell dysfunctional disorder) characterized by certain, molecular, pathological, histological, and/or clinical features. In some embodiments, a biomarker is a gene. Biomarkers include, but are not limited to, polynucleotides (*e.g.*, DNA, and/or RNA), polynucleotide copy number alterations (*e.g.*, DNA copy numbers),

polypeptides, polypeptide and polynucleotide modifications (*e.g.*, posttranslational modifications), carbohydrates, and/or glycolipid-based molecular markers.

[0088] The terms "biomarker signature," "signature," "biomarker expression signature," or "expression signature" are used interchangeably herein and refer to one or a combination of biomarkers whose expression is an indicator, *e.g.*, predictive, diagnostic, and/or prognostic. The biomarker signature may serve as an indicator of a particular subtype of a disease or disorder (*e.g.*, T-cell dysfunctional disorder) characterized by certain molecular, pathological, histological, and/or clinical features. In some embodiments, the biomarker signature is a "gene signature." The term "gene signature" is used interchangeably with "gene expression signature" and refers to one or a combination of polynucleotides whose expression is an indicator, *e.g.*, predictive, diagnostic, and/or prognostic. In some embodiments, the biomarker signature is a "protein signature." The term "protein signature" is used interchangeably with "protein expression signature" and refers to one or a combination of polypeptides whose expression is an indicator, *e.g.*, predictive, diagnostic, and/or prognostic.

[0089] The terms "cancer" and "cancerous" refer to or describe the physiological condition in subjects that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but not limited to, squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by the antibodies of the invention include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, glioblastoma, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the cancer is selected from: small cell lung cancer, glioblastoma, neuroblastomas, melanoma, breast carcinoma, gastric cancer, colorectal cancer (CRC), and hepatocellular carcinoma. Yet, in some embodiments, the cancer is selected from: non-small cell lung cancer, colorectal cancer, glioblastoma and breast carcinoma, including metastatic forms of those cancers.

In specific embodiments, the cancer is melanoma or lung cancer, suitably metastatic melanoma or metastatic lung cancer.

[0090] The terms "cell proliferative disorder", "proliferative disorder" and "hyperproliferative disorder" are used interchangeably herein to refer to disorders that are associated with some degree of abnormal cell proliferation. In some embodiments, the cell proliferative disorder is cancer. In some embodiments, the cell proliferative disorder is a tumor, including a solid tumor.

[0091] "Chemotherapeutic agent" includes compounds useful in the treatment of cancer. Examples of chemotherapeutic agents include erlotinib (TARCEVA®, Genentech/OSI Pharm.), bortezomib (VELCADE®, Millennium Pharm.), disulfiram, epigallocatechin gallate, salinosporamide A, carfilzomib, 17-AAG (geldanamycin), radicicol, lactate dehydrogenase A (LDH-A), fulvestrant (FASLODEX®, AstraZeneca), sunitib (SUTENT®, Pfizer/Sugen), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), finasunate (VATALANIB®, Novartis), oxaliplatin (ELOXATIN®, Sanofi), 5-FU (5-fluorouracil), leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), Lonafamib (SCH 66336), sorafenib (NEXAVAR®, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), AG1478, alkylating agents such as thiotepa and CYTOXAN® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including topotecan and irinotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); adrenocorticosteroids (including prednisone and prednisolone); cyproterone acetate; 5α-reductases including finasteride and dutasteride); vorinostat, romidepsin, panobinostat, valproic acid, mocetinostat dolastatin; aldesleukin, talc duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin γ1I and calicheamicin ω1I (Angew Chem. Intl. Ed. Engl. 1994 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-

azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamnol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, TAXOL (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumberg, Ill.), and TAXOTERE® (docetaxel, doxetaxel; Sanofi-Aventis); chloranmbucil; GEMZAR® (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0092] Chemotherapeutic agent also includes (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, iodoxyfene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifine citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestanie, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide and goserelin; buserelin, tripterelin, medroxyprogesterone acetate, diethylstilbestrol, premarin, fluoxymesterone, all transretionic acid, fenretinide, as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-α, Ralf and H-Ras; (vii) ribozymes such as VEGF expression inhibitors (*e.g.*, ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN®, rIL-2; a topoisomerase 1 inhibitor such as LURTOTECAN®; ABARELIX® rmRH; and (ix) pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0093] Chemotherapeutic agent also includes antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG®, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar,

Corixia), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth). Additional humanized monoclonal antibodies with therapeutic potential as agents in combination with the compounds of the invention include: apolizumab, aselizumab, atlizumab, bapineuzumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti-interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) which is a recombinant exclusively human-sequence, full-length IgG.sub.1 .lamda. antibody genetically modified to recognize interleukin-12 p40 protein.

[0094] Chemotherapeutic agent also includes "EGFR inhibitors," which refers to compounds that bind to or otherwise interact directly with EGFR and prevent or reduce its signaling activity, and is alternatively referred to as an "EGFR antagonist." Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, U.S. Pat. No. 4,943,533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (U.S. Pat. No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in U.S. Pat. No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF or Panitumumab (see WO98/50433, Abgenix/Amgen); EMD 55900 (Stragliotto *et al.* Eur. J. Cancer 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF-α for EGFR binding (EMD/Merck); human EGFR antibody, HuMax-EGFR (GenMab); fully human antibodies known as E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6. 3 and E7.6. 3 and described in U.S. Pat. No. 6,235,883; MDX-447 (Medarex Inc); and mAb 806 or humanized mAb 806 (Johns *et al.*, J. Biol. Chem. 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, *e.g.*, EP659439A2, Merck Patent GmbH). EGFR antagonists include small molecules such as compounds described in U.S. Pat. Nos. 5,616,582, 5,457,105, 5,475,001, 5,654,307, 5,679,683, 6,084,095, 6,265,410, 6,455,534, 6,521,620, 6,596,726, 6,713,484, 5,770,599, 6,140,332, 5,866,572, 6,399,602, 6,344,459, 6,602,863, 6,391,874, 6,344,455, 5,760,041, 6,002,008, and 5,747,498, as well as the following PCT publications: WO98/14451, WO98/50038, WO99/09016, and WO99/24037. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, TARCEVA® Genentech/OSI Pharmaceuticals); PD 183805 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quin-azolinyl]-, dihydrochloride, Pfizer Inc.); ZD1839, gefitinib (IRESSA®) 4-(3'-Chloro-4'-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoli- ne, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenyl-amino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methyl-piperidin-4-yl)-pyrimido[5,4-- d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[4-[(1-phenylethyl)amino]-1H-pyrrolo[2,3-

d]pyrimidin-6-yl]-phenol)- ; (R)-6-(4-hydroxyphenyl)-4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d]pyrimi- dine); CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide); EKB-569 (N-[4-[(3-chloro-4-fluorophenyl)amino]-3-cyano-7-ethoxy-6-quinolinyl]-4-(- dimethylamino)-2-butenamide) (Wyeth); AG1478 (Pfizer); AG1571 (SU 5271; Pfizer); dual EGFR/HER2 tyrosine kinase inhibitors such as lapatinib (TYKERB®, GSK572016 or N-[3-chloro-4-[(3 fluorophenyl)methoxy]phenyl]-6[5[[[2methylsulfonyl)ethyl]amino]methyl]-2-- furanyl]-4-quinazolinamine).

[0095] Chemotherapeutic agents also include "tyrosine kinase inhibitors" including the EGFR-targeted drugs noted in the preceding paragraph; small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda; CP-724,714, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase (Pfizer and OSI); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; lapatinib (GSK572016; available from Glaxo-SmithKline), an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibit Raf-1 signaling; non-HER targeted TK inhibitors such as imatinib mesylate (GLEEVEC®, available from Glaxo SmithKline); multi-targeted tyrosine kinase inhibitors such as sunitinib (SUTENT®, available from Pfizer); VEGF receptor tyrosine kinase inhibitors such as vatalanib (PTK787/ZK222584, available from Novartis/Schering AG); MAPK extracellular regulated kinase I inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lamber); antisense molecules (*e.g.* those that bind to HER-encoding nucleic acid); quinoxalines (U.S. Pat. No. 5,804,396); tryphostins (U.S. Pat. No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); imatinib mesylate (GLEEVEC®); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Pfizer); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone), rapamycin (sirolimus, RAPAMUNE®); or as described in any of the following patent publications: U.S. Pat. No. 5,804,396; WO 1999/09016 (American Cyanamid); WO 1998/43960 (American Cyanamid); WO 1997/38983 (Warner Lambert); WO 1999/06378 (Warner Lambert); WO 1999/06396 (Warner Lambert); WO 1996/30347 (Pfizer, Inc); WO 1996/33978 (Zeneca); WO 1996/3397 (Zeneca) and WO 1996/33980 (Zeneca).

[0096] Chemotherapeutic agents also include dexamethasone, interferons, colchicine, metoprine, cyclosporine, amphotericin, metronidazole, alemtuzumab, alitretinoin, allopurinol, amifostine, arsenic trioxide, asparaginase, BCG live, bevacuzimab, bexarotene, cladribine, clofarabine, darbepoetin alfa, denileukin, dexrazoxane, epoetin alfa, elotinib, filgrastim, histrelin acetate, ibritumomab, interferon alfa-2a, interferon alfa-2b, lenalidomide, levamisole, mesna, methoxsalen, nandrolone, nelarabine, nofetumomab, oprelvekin, palifermin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed disodium, plicamycin, porfimer sodium, quinacrine, rasburicase, sargramostim, temozolomide, VM-26, 6-TG, toremifene, tretinoin, ATRA, valrubicin, zoledronate, and zoledronic acid, and pharmaceutically acceptable salts thereof.

[0097] Chemotherapeutic agents also include hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate and fluprednidene acetate; immune selective anti-inflammatory peptides (ImSAIDs) such as phenylalanine-glutamine-glycine (FEG) and its D-isomeric form (feG) (IMULAN BioTherapeutics, LLC); anti-rheumatic drugs such as azathioprine, ciclosporin (cyclosporine A), D-penicillamine, gold salts, hydroxychloroquine, leflunomideminocycline, sulfasalazine, tumor necrosis factor α (TNF-α) blockers such as etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi), Interleukin 1 (IL-1) blockers such as anakinra (Kineret), T-cell costimulation blockers such as abatacept (Orencia), Interleukin 6 (IL-6) blockers such as tocilizumab (ACTEMERA®); Interleukin 13 (IL-13) blockers such as lebrikizumab; Interferon α (IFN) blockers such as Rontalizumab; Beta 7 integrin blockers such as rhuMAb Beta7; IgE pathway blockers such as Anti-M1 prime; Secreted homotrimeric LTa3 and membrane bound heterotrimer LTa1/β2 blockers such as Anti-lymphotoxin α (LTa); radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); miscellaneous investigational agents such as thioplatin, PS-341, phenylbutyrate, ET-18-OCH_3, or farnesyl transferase inhibitors (L-739749, L-744832); polyphenols such as quercetin, resveratrol, piceatannol, epigallocatechine gallate, theaflavins, flavanols, procyanidins, betulinic acid and derivatives thereof; autophagy inhibitors such as chloroquine; delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; acetylcamptothecin, scopolectin, and 9-aminocamptothecin); podophyllotoxin; tegafur (UFTORAL®); bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine; perifosine, COX-2 inhibitor (*e.g.* celecoxib or etoricoxib), proteosome inhibitor (*e.g.* PS341); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

[0098] Chemotherapeutic agents also include non-steroidal anti-inflammatory drugs with analgesic, antipyretic and anti-inflammatory effects. NSAIDs include non-selective inhibitors of the enzyme cyclooxygenase. Specific examples of NSAIDs include aspirin, propionic acid derivatives such as ibuprofen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin and naproxen, acetic acid derivatives such as indomethacin, sulindac, etodolac, diclofenac, enolic acid derivatives such as piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam and isoxicam, fenamic acid derivatives such as mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, and COX-2 inhibitors such as celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, rofecoxib, and valdecoxib. NSAIDs can be indicated for the symptomatic relief of conditions such as rheumatoid arthritis,

osteoarthritis, inflammatory arthropathies, ankylosing spondylitis, psoriatic arthritis, Reiter's syndrome, acute gout, dysmenorrhoea, metastatic bone pain, headache and migraine, postoperative pain, mild-to-moderate pain due to inflammation and tissue injury, pyrexia, ileus, and renal colic.

[0099] As used herein, a "companion diagnostic" refers to a diagnostic method and or reagent that is used to identify subjects susceptible to treatment with a particular treatment or to monitor treatment and/or to identify an effective dosage for a subject or sub-group or other group of subjects. For purposes herein, a companion diagnostic refers to reagents, such as a reagent for detecting, measuring or localizing a T-cell function biomarker (*e.g.*, as described herein) in a sample. The companion diagnostic refers to the reagents and also to the test(s) that is/are performed with the reagent.

[0100] As used herein, the term "complex" refers to an assemblage or aggregate of molecules (*e.g.*, peptides, polypeptides, *etc.*) in direct and/or indirect contact with one another. In specific embodiments, "contact", or more particularly, "direct contact" means two or more molecules are close enough so that attractive noncovalent interactions, such as Van der Waal forces, hydrogen bonding, ionic and hydrophobic interactions, and the like, dominate the interaction of the molecules. In such embodiments, a complex of molecules (*e.g.*, a peptide and polypeptide) is formed under conditions such that the complex is thermodynamically favored (*e.g.*, compared to a non-aggregated, or non-complexed, state of its component molecules). The term "polypeptide complex" or "protein complex," as used herein, refers to a trimer, tetramer, pentamer, hexamer, heptamer, octamer, nonamer, decamer, undecamer, dodecamer, or higher order oligomer. In specific embodiments, the polypeptide complexes are formed by self-assembly of PKC-θ and ZEB1.

[0101] Throughout this specification, unless the context requires otherwise, the words "comprise," "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. Thus, use of the term "comprising" and the like indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0102] The terms "correlate" and "correlating" generally refers to determining a relationship between one type of data with another or with a state. In various embodiments, TBET and/or CXCR3 expression or a TBET:EOMES ratio, is correlated with the presence, absence or degree of an inflammatory or activation state of T cells.

[0103] By "corresponds to" or "corresponding to" is meant an amino acid sequence that displays substantial sequence similarity or identity to a reference amino acid sequence. In general the amino acid sequence will display at least about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81,

82, 83, 84, 85, 86, 97, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or even up to 100% sequence similarity or identity to at least a portion of the reference amino acid sequence.

[0104] As used herein, the term "cytolytic activity" refers to ability of a cell, *e.g.*, a $CD8^+$ cell or an NK cell, to lyse target cells. Such cytolytic activity can be measured using standard techniques, *e.g.*, by radioactively labeling the target cells.

[0105] The term "cytotoxic agent" as used herein refers to any agent that is detrimental to cells (*e.g.*, causes cell death, inhibits proliferation, or otherwise hinders a cellular function). Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Exemplary cytotoxic agents can be selected from anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors, immunotherapeutic agents, proapoptotic agents, inhibitors of LDH-A, inhibitors of fatty acid biosynthesis, cell cycle signalling inhibitors, HDAC inhibitors, proteasome inhibitors, and inhibitors of cancer metabolism. In some embodiments, the cytotoxic agent is a taxane. In representative examples of this type, the taxane is paclitaxel or docetaxel. In some embodiments, the cytotoxic agent is a platinum agent. In some embodiments, the cytotoxic agent is an antagonist of EGFR. In representative examples of this type, the antagonist of EGFR is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (*e.g.*, erlotinib). In some embodiments, the cytotoxic agent is a RAF inhibitor. In non-limiting examples of this type, the RAF inhibitor is a BRAF and/or CRAF inhibitor. In other non-limiting examples, the RAF inhibitor is vemurafenib. In one embodiment the cytotoxic agent is a PI3K inhibitor.

[0106] As used herein, the term "cytotoxic therapy" refers to therapies that induce cellular damage including but not limited to radiation, chemotherapy, photodynamic therapy, radiofrequency ablation, anti-angiogenic therapy, and combinations thereof. A cytotoxic therapeutic may induce DNA damage when applied to a cell.

[0107] As used herein, "delaying progression of a disease" or "decreasing the rate of progression of a disease" means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as a T-cell dysfunctional disorder). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0108] The term "detection" includes any means of detecting, including direct and indirect detection.

[0109] The term "diagnosis" is used herein to refer to the identification or classification of a molecular or pathological state, disease or condition (*e.g.*, T-cell dysfunctional disorder). For example, "diagnosis" may refer to identification of a particular type of T-cell dysfunctional disorder. "Diagnosis" may also refer to the classification of a particular subtype of T-cell dysfunctional disorder, e.g., by histopathological criteria, or by molecular features (*e.g.*, a subtype characterized

by expression of one or a combination of biomarkers (*e.g.*, particular genes or proteins encoded by said genes)).

[0110] The term "aiding diagnosis" is used herein to refer to methods that assist in making a clinical determination regarding the presence, or nature, of a particular type of symptom or condition of a disease or disorder (*e.g.*, T-cell dysfunctional disorder). For example, a method of aiding diagnosis of a disease or condition (*e.g.*, T-cell dysfunctional disorder) can comprise measuring certain biomarkers in a biological sample from an individual.

[0111] A "disorder" is any condition that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose a subject to the disorder in question.

[0112] The term "dysfunction" in the context of immune dysfunction, refers to a state of reduced immune responsiveness to antigenic stimulation. The term includes the common elements of both exhaustion and/or anergy in which antigen recognition may occur, but the ensuing immune response is ineffective to control infection or tumor growth.

[0113] The term "dysfunctional", as used herein, also includes refractory or unresponsive to antigen recognition, specifically, impaired capacity to translate antigen recognition into down-stream T-cell effector functions, such as proliferation, cytokine production (*e.g.*, IL-2, IFN-γ, TNF-α, *etc.*) and/or target cell killing.

[0114] An "effective amount" is at least the minimum amount required to effect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (*i.e.*, slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the cancer or tumor. In the case of an infection, an effective amount of the drug may have the effect in reducing pathogen (bacterium, virus, *etc.*) titers in the circulation or tissue; reducing the number of pathogen infected cells; inhibiting (*i.e.*, slow to some extent or desirably stop) pathogen infection of organs; inhibit (*i.e.*, slow to some extent and desirably stop) pathogen growth; and/or relieving to some extent one or more of the symptoms associated with the infection. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic

or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0115] An "effective response" of a patient or a patient's "responsiveness" to treatment with a medicament and similar wording refers to the clinical or therapeutic benefit imparted to a patient at risk for, or suffering from, a disease or disorder, such as cancer. In one embodiment, such benefit includes any one or more of: extending survival (including overall survival and progression free survival); resulting in an objective response (including a complete response or a partial response); or improving signs or symptoms of cancer. A patient who "does not have an effective response" to treatment refers to a patient who does not have any one of extending survival (including overall survival and progression free survival); resulting in an objective response (including a complete response or a partial response); or improving signs or symptoms of cancer.

[0116] "Enhancing T-cell function" means to induce, cause or stimulate a T-cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T-cells. Examples of enhancing T-cell function include any one or more of: increased secretion of IFN-γ, increased secretion of TNF-α, increased secretion of IL-2 from $CD8^+$ T-cells, increased proliferation, increased antigen responsiveness (*e.g.*, viral, pathogen, or tumor clearance) relative to such levels before the intervention. In some embodiments, the level of enhancement is as least 50%, alternatively 60%, 70%, 80%, 90%, 100%, 120%, 150%, 200%. The manner of measuring this enhancement is known to one of ordinary skill in the art.

[0117] The term "epithelial phenotype" is understood in the art, and can be identified by morphological, molecular and/or functional characteristics. For example, epithelial cells generally have a rounded or cobblestone appearance, express the epithelial marker E-cadherin, are rapidly dividing and/or have relatively low levels of motility, invasiveness and/or anchorage-independent growth as compared with mesenchymal cells.

[0118] As used herein, the term "epithelial-to-mesenchymal transition" (EMT) refers to the conversion from an epithelial to a mesenchymal phenotype, which is a normal process of embryonic development. EMT is also the process whereby injured epithelial cells that function as ion and fluid transporters become matrix remodeling mesenchymal cells. In carcinomas, this transformation typically results in altered cell morphology, the expression of mesenchymal proteins and increased invasiveness. The criteria for defining EMT *in vitro* involve the loss of epithelial cell polarity, the separation into individual cells and subsequent dispersion after the acquisition of cell motility (see, Vincent-Salomon *et al.*, Breast Cancer Res. 2003; 5(2): 101-106). Classes of molecules that change in expression, distribution, and/or function during EMT, and that are causally involved, include growth factors (*e.g.*, transforming growth factor-β (TGF-β), wnts), transcription factors (*e.g.*, Snail, SMAD, LEF, and nuclear β-catenin), molecules of the cell-to-cell adhesion axis (cadherins, catenins), cytoskeletal modulators (Rho family), and extracellular proteases (matrix metalloproteinases, plasminogen activators) (see, Thompson *et al.*, Cancer Research 65, 5991-5995, Jul. 15, 2005). In specific embodiments, EMT refers to a process whereby epithelial cancer cells take on a mesenchymal phenotype, which may be associated with

metastasis. These mesenchymal cells may display reduced adhesiveness, increased motility and invasiveness and are relatively resistant to immunotherapeutic agents, chemotherapeutic agents and/or radiation (*e.g.*, treatments that target rapidly dividing cells).

[0119] The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen-binding regions. Epitopes often consist of a surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. In some embodiments, the epitope can be a protein epitope. Protein epitopes can be linear or conformational. In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. A "non-linear epitope" or "conformational epitope" comprises non-contiguous polypeptides (or amino acids) within the antigenic protein to which an antibody specific to the epitope binds. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, *e.g.*, using the techniques described in the present specification. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition and cross-competition studies to find antibodies that compete or cross-compete with one another for binding to a target antigen (*e.g.*, PD-1), *e.g.*, the antibodies compete for binding to the antigen.

[0120] The term "exhaustion" refers to T-cell exhaustion as a state of T-cell dysfunction that arises from sustained TCR signaling that occurs during many chronic infections and cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T-cells. Exhaustion prevents optimal control of infection and tumors. Exhaustion can result from both extrinsic negative regulatory pathways (*e.g.*, immunoregulatory cytokines) as well as cell intrinsic negative regulatory (costimulatory) pathways (PD-1, B7-H3, B7-H4, *etc.*).

[0121] The term "expression" with respect to a gene sequence refers to transcription of the gene to produce a RNA transcript (*e.g.*, mRNA, antisense RNA, siRNA, shRNA, miRNA, *etc.*) and, as appropriate, translation of a resulting mRNA transcript to a protein. Thus, as will be clear from the context, expression of a coding sequence results from transcription and translation of the coding sequence. Conversely, expression of a non-coding sequence results from the transcription of the non-coding sequence.

[0122] The terms "level of expression" or "expression level" in general are used interchangeably and generally refer to the amount of a biomarker in a sample. "Expression" generally refers to the process by which information (*e.g.*, gene-encoded and/or epigenetic) is converted into the structures present and operating in the cell. Therefore, as used herein, "expression" may refer to transcription into a polynucleotide, translation into a polypeptide, or even polynucleotide and/or polypeptide modifications (*e.g.*, posttranslational modification of a polypeptide). Fragments of the transcribed polynucleotide, the translated polypeptide, or polynucleotide and/or polypeptide modifications (*e.g.*, post-translational modification of a polypeptide) shall also be regarded as expressed whether they originate from a transcript generated by alternative splicing or a degraded transcript, or from a post-translational processing

of the polypeptide, *e.g.*, by proteolysis. "Expressed genes" include those that are transcribed into a polynucleotide as mRNA and then translated into a polypeptide, and also those that are transcribed into RNA but not translated into a polypeptide (*e.g.*, transfer and ribosomal RNAs).

[0123] "Elevated expression," "elevated expression levels," or "elevated levels" refers to an increased expression or increased levels of a biomarker in an individual or part of an individual (*e.g.*, a cell, tissue or organ) relative to a control, such as an individual or individuals who are not suffering from the disease or disorder (*e.g.*, T-cell dysfunctional disorder)or parts thereof (*e.g.*, a cell, tissue or organ) or an internal control (*e.g.*, housekeeping biomarker).

[0124] "Reduced expression", "reduced expression levels", or "reduced levels" refers to a decreased expression or decreased levels of a biomarker in an individual or part of an individual (*e.g.*, a cell, tissue or organ) relative to a control, such as an individual or individuals who are not suffering from the disease or disorder (*e.g.*, T-cell dysfunctional disorder) or parts thereof (*e.g.*, a cell, tissue or organ) or an internal control (*e.g.*, housekeeping biomarker). In some embodiments, reduced expression is little or no expression.

[0125] The term "housekeeping biomarker" refers to a biomarker or group of biomarkers (*e.g.*, polynucleotides and/or polypeptides) which are typically similarly present in all cell types. In some embodiments, the housekeeping biomarker is a "housekeeping gene." A "housekeeping gene" refers herein to a gene or group of genes which encode proteins whose activities are essential for the maintenance of cell function and which are typically similarly present in all cell types.

[0126] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell either *in vitro* or *in vivo*. In one embodiment, growth inhibitory agent is growth inhibitory antibody that prevents or reduces proliferation of a cell expressing an antigen to which the antibody binds. In another embodiment, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., The Molecular Basis of Cancer, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (W.B. Saunders, Philadelphia, 1995), *e.g.*, p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0127] The term "immune effector cells" in the context of the present invention relates to cells which exert effector functions during an immune reaction. For example, such cells secrete cytokines and/or chemokines, kill microbes, secrete antibodies, recognize infected or cancerous cells, and optionally eliminate such cells. For example, immune effector cells comprise T-cells

(cytotoxic T-cells, helper T-cells, tumor infiltrating T-cells), B-cells, natural killer (NK) cells, lymphokine-activated killer (LAK) cells, neutrophils, macrophages, and dendritic cells.

[0128] The term "immune effector functions" in the context of the present invention includes any functions mediated by components of the immune system that result, for example, in the killing of virally infected cells or tumor cells, or in the inhibition of tumor growth and/or inhibition of tumor development, including inhibition of tumor dissemination and metastasis. Preferably, the immune effector functions in the context of the present invention are T-cell mediated effector functions. Such functions comprise in the case of a helper T-cell ($CD4^+$ T-cell) the recognition of an antigen or an antigen peptide derived from an antigen in the context of MHC class II molecules by T-cell receptors, the release of cytokines and/or the activation of $CD8^+$ lymphocytes (CTLs) and/or B-cells, and in the case of CTL the recognition of an antigen or an antigen peptide derived from an antigen in the context of MHC class I molecules by T-cell receptors, the elimination of cells presented in the context of MHC class I molecules, *i.e.*, cells characterized by presentation of an antigen with class I MHC, for example, via apoptosis or perforin-mediated cell lysis, production of cytokines such as IFN-γ and TNF-α, and specific cytolytic killing of antigen expressing target cells.

[0129] The term "immune response" refers to any detectable response to a particular substance (such as an antigen or immunogen) by the immune system of a host mammal, such as innate immune responses (e.g., activation of Toll receptor signaling cascade), cell-mediated immune responses (e.g., responses mediated by T cells, such as antigen-specific T cells, and non-specific cells of the immune system), and humoral immune responses (e.g., responses mediated by B cells, such as generation and secretion of antibodies into the plasma, lymph, and/or tissue fluids).

[0130] The term "immunogenic" refers to the ability of a substance to cause, elicit, stimulate, or induce an immune response including an enhanced T-cell (*e.g.*, $CD8^+$ T-cell) immune response, or to improve, enhance, increase or prolong a pre-existing immune response, against a particular antigen, whether alone or when linked to a carrier, in the presence or absence of an adjuvant.

[0131] "Immunogenicity" refers to the ability of a particular substance to provoke an immune response. Tumors are immunogenic and enhancing tumor immunogenicity aids in the clearance of the tumor cells by the immune response. Examples of enhancing tumor immunogenicity include treatment with a PKC-θ inhibitor and a PD-1 binding antagonist.

[0132] The term "infection" refers to invasion of body tissues by disease-causing microorganisms, their multiplication and the reaction of body tissues to these microorganisms and the toxins they produce. "Infection" includes but are not limited to infections by viruses, prions, bacteria, viroids, parasites, protozoans and fungi. Non-limiting examples of viruses include *Retroviridae* human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III); and other isolates, such as HIV-LP); *Picornaviridae* (*e.g.*, polio viruses, hepatitis A virus; enteroviruses, human *Coxsackie* viruses, rhinoviruses, echoviruses); *Calciviridae* (*e.g.*, strains that cause gastroenteritis, including Norwalk and related viruses); *Togaviridae* (*e.g.*, equine encephalitis viruses, rubella viruses); *Flaviridae* (*e.g.*, dengue viruses, encephalitis viruses, yellow fever viruses); *Coronaviridae* (*e.g.*, coronaviruses); *Rhabdoviridae* (*e.g.*, vesicular stomatitis viruses, rabies viruses); *Filoviridae* (*e.g.*, ebola viruses); *Paramyxoviridae* (*e.g.*, parainfluenza

viruses, mumps virus, measles virus, respiratory syncytial virus, Metapneumovirus); *Orthomyxoviridae* (*e.g.*, influenza viruses); *Bunyaviridae* (*e.g.*, Hantaan viruses, bunya viruses, phleboviruses and Nairo viruses); *Arenaviridae* (hemorrhagic fever viruses); *Reoviridae* (*e.g.*, reoviruses, orbiviruses and rotaviruses); *Bimaviridae*; *Hepadnaviridae* (Hepatitis B virus); *Parvoviridae* (parvoviruses); *Papovaviridae* (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); *Herpesviridae* (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus); *Poxviridae* (variola viruses, VACV, pox viruses); and *Iridoviridae* (*e.g.*, African swine fever virus); and unclassified viruses (*e.g.*, the etiological agents of *Spongiform* encephalopathies, the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1 = internally transmitted; class 2 = parenterally transmitted (i.e., Hepatitis C); and astroviruses. Representative bacteria that are known to be pathogenic include pathogenic *Pasteurella* species (*e.g.*, *Pasteurella multocida*), *Staphylococcus* species (*e.g.*, *Staphylococcus aureus*), *Streptococcus* species (*e.g.*, *Streptococcus pyogenes* (Group A *Streptococcus*), *Streptococcus agalactiae* (Group B *Streptococcus*), *Streptococcus* (viridans group), *Streptococcus faecalis, Streptococcus bovis, Streptococcus* (anaerobic sps.), *Streptococcus pneumoniae*), *Neisseria* species (*e.g.*, *Neisseria gonorrhoeae, Neisseria meningitidis*), *Escherichia* species (*e.g.*, enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC)), *Bordetella* species, *Campylobacter* species, *Legionella* species (*e.g.*, *Legionella pneumophila*), *Pseudomonas* species, *Shigella* species, *Vibrio* species, *Yersinia* species, *Salmonella* species, *Haemophilus* species (*e.g.*, *Haemophilus influenzae*), *Brucella* species, *Francisella* species, *Bacteroides* species, *Clostridiium* species (*e.g.*, *Clostridium difficile, Clostridium perfringens, Clostridium tetani*), *Mycobacteria* species (*e.g.*, *M. tuberculosis, M. avium, M. intracellulare, M. kansaii, M. gordonae*), *Helicobacter pyloris, Borelia burgdorferi, Listeria monocytogenes, Chlamydia trachomatis, Enterococcus* species, *Bacillus anthracis, Corynebacterium diphtheriae, Erysipelothrix rhusiopathiae, Enterobacter aerogenes, Klebsiella pneumoniae, Fusobacterium nucleatum, Streptobacillus moniliformis, Treponema pallidium, Treponema pertenue, Leptospira, Rickettsia*, and *Actinomyces israeli.* Non-limiting pathogenic fungi include *Cryptococcus neoformans, Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis, Candida albicans, Candida glabrata, Aspergillus fumigata, Aspergillus flavus*, and *Sporothrix schenckii*. Illustrative pathogenic protozoa, helminths, *Plasmodium*, such as *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale*, and *Plasmodium vivax; Toxoplasma gondii; Trypanosoma brucei, Trypanosoma cruzi; Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum; Leishmania donovani; Giardia intestinalis; Cryptosporidium parvum*; and the like.

[0133] As used herein, "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the compositions and methods of the invention. The instructional material of the kit of the invention may, for example, be affixed to a container which contains the therapeutic or diagnostic agents of the invention or be shipped together with a container which contains the therapeutic or diagnostic agents of the invention.

[0134] The term "label" when used herein refers to a detectable compound or composition. The label is typically conjugated or fused directly or indirectly to a reagent, such as a polynucleotide probe or an antibody, and facilitates detection of the reagent to which it is conjugated or fused. The label may itself be detectable (*e.g.*, radioisotope labels or fluorescent

labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which results in a detectable product.

[0135] The term "leukocytes" or "white blood cell" as used herein refers to any immune cell, including monocytes, neutrophils, eosinophils, basophils, and lymphocytes.

[0136] The term "lymphocytes" as used herein refers to cells of the immune system which are a type of white blood cell. Lymphocytes include, but are not limited to, T-cells (cytotoxic and helper T-cells), B-cells and natural killer cells (NK cells). The term "tumor infiltrating lymphocyte" as used herein refers to lymphocytes that are present in a solid tumor. The term "circulating lymphocyte" as used herein refers to lymphocytes that are present in the circulation (*e.g.*, present in blood).

[0137] By "memory T effector cells" is meant a subset of T-cells including CTL and helper T-cells that have previously encountered and responded to their cognate antigen; thus, the term antigen-experienced T-cell is often applied. Such T-cells can recognize foreign microbes, such as bacteria or viruses, as well as cancer cells. Memory T effector cells have become "experienced" by having encountered antigen during a prior infection, encounter with cancer, or previous vaccination. At a second encounter with the microbe, memory T effector cells can reproduce to mount a faster and stronger immune response than the first time the immune system responded to the microbe. This behaviour is utilized in T lymphocyte proliferation assays, which can reveal exposure to specific antigens.

[0138] The term "mesenchymal phenotype" is understood in the art, and can be identified by morphological, molecular and/or functional characteristics. For example, mesenchymal cells generally have an elongated or spindle-shaped appearance, express the mesenchymal markers vimentin, fibronectin and N-cadherin, divide slowly or are non-dividing and/or have relatively high levels of motility, invasiveness and/or anchorage-independent growth as compared with epithelial cells.

[0139] As used herein, the term "mesenchymal-to-epithelial transition" (MET) is a reversible biological process that involves the transition from motile, multipolar or spindle-shaped mesenchymal cells to planar arrays of polarized cells called epithelia. MET is the reverse process of EMT. METs occur in normal development, cancer metastasis, and induced pluripotent stem cell reprogramming. In specific embodiments, MET refers to the reprogramming of cells that have undergone EMT to regain one or more epithelial characteristics (*e.g.*, as described above). For example, such cells typically exhibit reduced motility and/or invasiveness and/or are rapidly dividing, and may thereby regain sensitivity to immunotherapeutics and/or cytotoxic agents.

[0140] The term "multiplex-PCR" refers to a single PCR reaction carried out on nucleic acid obtained from a single source (*e.g.*, an individual) using more than one primer set for the purpose of amplifying two or more DNA sequences in a single reaction.

[0141] The terms "patient", "subject", "host" or "individual" used interchangeably herein, refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, any member of the subphylum Chordata including primates (*e.g.*, humans, monkeys and apes, and includes species of monkeys such from the genus *Macaca* (*e.g.*, cynomologus monkeys such as *Macaca fascicularis*, and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*), as well as marmosets

(species from the genus *Callithrix*), squirrel monkeys (species from the genus *Saimiri*) and tamarins (species from the genus *Saguinus*), as well as species of apes such as chimpanzees (*Pan troglodytes*)), rodents (*e.g.*, mice rats, guinea pigs), lagomorphs (*e.g.*, rabbits, hares), bovines (*e.g.*, cattle), ovines (*e.g.*, sheep), caprines (*e.g.*, goats), porcines (*e.g.*, pigs), equines (*e.g.*, horses), canines (*e.g.*, dogs), felines (*e.g.*, cats), avians (*e.g.*, chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars *etc.*), marine mammals (*e.g.*, dolphins, whales), reptiles (snakes, frogs, lizards *etc.*), and fish. A preferred subject is a human in need of eliciting an immune response, including an immune response with enhanced T-cell activation. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

[0142] The term "pharmaceutical composition" or "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient(s) to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the composition or formulation would be administered. Such formulations are sterile. "Pharmaceutically acceptable" excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0143] As used herein, the term "PD-1" refers to any form of PD-1 and variants thereof that retain at least part of the activity of PD-1. Unless indicated differently, such as by specific reference to human PD-1, PD-1 includes all mammalian species of native sequence PD-1, *e.g.*, human, canine, feline, equine, and bovine. One exemplary human PD-1 is found as UniProt Accession Number Q15116.

[0144] The term "PD-1 binding antagonist" refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to one or more of its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In some embodiments, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T-cells mediated through PD-1 so as to render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is MDX-1106 (nivolumab). In another specific aspect, a PD-1 binding antagonist is MK-3475 (pembrolizumab). In another specific aspect, a PD-1 binding antagonist is CT-011 (pidilizumab). In still another specific aspect, a PD-1 binding antagonist is AMP-224.

[0145] In the context of the present invention the term "priming" refers to the induction of a first contact of the T-cell (typically a naïve T-cell) with its specific antigen (*e.g.*, by antigen-presenting cells presenting the antigen to T-cells), which causes the differentiation of the T-cell into an effector-T cell (*e.g.*, a cytotoxic T cell or a T helper cell).

[0146] By "radiation therapy" is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell

altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0147] The term "sample" as used herein includes any biological specimen that may be extracted, untreated, treated, diluted or concentrated from a subject. Samples may include, without limitation, biological fluids such as whole blood, serum, red blood cells, white blood cells, plasma, saliva, urine, stool (*i.e.*, feces), tears, sweat, sebum, nipple aspirate, ductal lavage, tumor exudates, synovial fluid, ascitic fluid, peritoneal fluid, amniotic fluid, cerebrospinal fluid, lymph, fine needle aspirate, amniotic fluid, any other bodily fluid, cell lysates, cellular secretion products, inflammation fluid, semen and vaginal secretions. Samples may include tissue samples and biopsies, tissue homogenates and the like. Advantageous samples may include ones comprising any one or more biomarkers as taught herein in detectable quantities. Suitably, the sample is readily obtainable by minimally invasive methods, allowing the removal or isolation of the sample from the subject. In certain embodiments, the sample contains blood, especially peripheral blood, or a fraction or extract thereof. Typically, the sample comprises blood cells such as mature, immature or developing leukocytes, including lymphocytes, polymorphonuclear leukocytes, neutrophils, monocytes, reticulocytes, basophils, coelomocytes, hemocytes, eosinophils, megakaryocytes, macrophages, dendritic cells natural killer cells, or fraction of such cells (*e.g.*, a nucleic acid or protein fraction). In specific embodiments, the sample comprises leukocytes including peripheral blood mononuclear cells (PBMC).

[0148] A "reference sample", "reference cell", "reference tissue", "control sample", "control cell", or "control tissue", as used herein, refers to a sample, cell, tissue, standard, or level that is used for comparison purposes. In one embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (*e.g.*, tissue or cells) of the same subject or individual. For example, healthy and/or non-diseased cells or tissue adjacent to the diseased cells or tissue (*e.g.*, cells or tissue adjacent to a tumor). In another embodiment, a reference sample is obtained from an untreated tissue and/or cell of the body of the same subject or individual. In yet another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (*e.g.*, tissues or cells) of an individual who is not the subject or individual. In even another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from an untreated tissue and/or cell of the body of an individual who is not the subject or individual.

[0149] By "tissue sample" or "cell sample" is meant a collection of similar cells obtained from a tissue of a subject or individual. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ, tissue sample, biopsy, and/or aspirate; blood or any blood constituents such as plasma; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may also be primary or cultured cells or cell lines. Optionally, the tissue or cell sample is obtained from a disease tissue/organ. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

[0150] The term "sequence identity" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by an appropriate method. For example, sequence identity analysis may be carried out using the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software.

[0151] As used herein a "small molecule" refers to a compound that has a molecular weight of less than 3 kiloDalton (kDa), and typically less than 1.5 kiloDalton, and more preferably less than about 1 kiloDalton. Small molecules may be nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic (carbon-containing) or inorganic molecules. As those skilled in the art will appreciate, based on the present description, extensive libraries of chemical and/or biological mixtures, often fungal, bacterial, or algal extracts, may be screened with any of the assays of the invention to identify compounds that modulate a bioactivity. A "small organic molecule" is an organic compound (or organic compound complexed with an inorganic compound (e.g., metal)) that has a molecular weight of less than 3 kiloDalton, less than 1.5 kiloDalton, or even less than about 1 kDa.

[0152] "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

[0153] "Stringent conditions" or "high stringency conditions", as defined herein, can be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50.degree. C.; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42º C; or (3) overnight hybridization in a solution that employs 50% formamide, 5xSSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5xDenhardt's solution, sonicated salmon sperm DNA (50 µg/mL),

0.1% SDS, and 10% dextran sulfate at 42º C, with a 10 minute wash at 42º C in 0.2xSSC (sodium chloride/sodium citrate) followed by a 10 minute high-stringency wash consisting of 0.1xSSC containing EDTA at 55º C.

[0154] "Sustained response" refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the beginning of the administration phase. In some embodiments, the sustained response has a duration at least the same as the treatment duration, at least 1.5x, 2.0x, 2.5x, or 3.0x length of the treatment duration.

[0155] As used herein, the term "synergistic" means that the therapeutic effect of a PKC-θ inhibitor when administered in combination with a PD-1 binding antagonist (or *vice-versa*) is greater than the predicted additive therapeutic effects of the PKC-θ inhibitor and the PD-1 binding antagonist when administered alone. The term "synergistically effective amount" as applied to a PKC-θ inhibitor and a PD-1 binding antagonist refers to the amount of each component in a composition (generally a pharmaceutical formulation), which is effective for enhancing immune effector function including any one or more of increased recognition of an antigen or an antigen peptide derived from an antigen in the context of MHC class II molecules by T-cell receptors, increased release of cytokines and/or the activation of $CD8^+$ lymphocytes (CTLs) and/or B-cells, increased recognition of an antigen or an antigen peptide derived from an antigen in the context of MHC class I molecules by T-cell receptors, increased elimination of cells presented in the context of MHC class I molecules, *i.e.*, cells characterized by presentation of an antigen with class I MHC, for example, via apoptosis or perforin-mediated cell lysis, increased production of cytokines such as Il-2, IFN-γ and TNF-α, and increased specific cytolytic killing of antigen expressing target cells, and which produces an effect which does not intersect, in a dose-response plot of the dose of PKC-θ inhibitor *versus* a dose of PD-1 binding antagonist *versus* enhancing immune effector function as illustrated for example above, either the dose PKC-θ inhibitor axis or PD-1 binding antagonist axis. The dose response curve used to determine synergy in the art is described for example by Sande *et al.* (*see*, p. 1080-1105 in A. Goodman *et al.*, ed., the Pharmacological Basis of Therapeutics, MacMillan Publishing Co., Inc., New York (1980)). The optimum synergistic amounts can be determined, using a 95% confidence limit, by varying factors such as dose level, schedule and response, and using a computer-generated model that generates isobolograms from the dose response curves for various combinations of the PKC-θ inhibitor and the PD-1 binding antagonist. The highest enhancement of immune effector function on the dose response curve correlates with the optimum dosage levels.

[0156] A "T-cell dysfunctional disorder" is a disorder or condition of T-cells characterized by decreased responsiveness to antigenic stimulation. In a particular embodiment, a T-cell dysfunctional disorder is a disorder that is specifically associated with inappropriate increased signaling through PD-1. In another embodiment, a T-cell dysfunctional disorder is one in which T-cells are anergic or have decreased ability to secrete cytokines, proliferate, or execute cytolytic activity. In a specific aspect, the decreased responsiveness results in ineffective control of a pathogen or tumor expressing an immunogen. Examples of T-cell dysfunctional disorders characterized by T-cell dysfunction include unresolved acute infection, chronic infection and tumor immunity.

[0157] As used herein, the term "treatment" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical

pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with a T-cell dysfunctional disorder are mitigated or eliminated, including, but are not limited to, reducing the proliferation of (or destroying) cancerous cells, reducing pathogen infection, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[0158] As used herein, the expressions "Treg" and "regulatory T-cells", formerly known as suppressor T-cells, refer to T lymphocytes that maintain immunological tolerance. During an immune response, Tregs inhibit T cell-mediated immunity and suppress auto-reactive T cells that have escaped negative selection within the thymus. Adaptive Treg cells (called Th3 or Tr 1 cells) are thought to be generated during an immune response. Naturally occurring Treg cells ($CD4^+CD25^+FoxP3^+$ Treg cells) are generated in the thymus and have been linked to interactions between developing T-cells with both myeloid ($CD11c^+$) and plasmacytoid ($CD123^+$) dendritic cells that have been activated with the cytokine thymic stromal lymphopoietin (TSLP). The presence of FoxP3 in naturally occurring Treg cells distinguishes them from other T-cells.

[0159] "Tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer", "cancerous", "cell proliferative disorder", "proliferative disorder" "hyperproliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

[0160] "Tumor immunity" refers to the process in which tumors evade immune recognition and clearance. Thus, as a therapeutic concept, tumor immunity is "treated" when such evasion is attenuated, and the tumors are recognized and attacked by the immune system. Examples of tumor recognition include tumor binding, tumor shrinkage and tumor clearance.

[0161] As used herein, underscoring or italicizing the name of a gene shall indicate the gene, in contrast to its protein product, which is indicated by the name of the gene in the absence of any underscoring or italicizing. For example, "*PKC-θ*" shall mean the *PKC-θ* gene, whereas "PKC-θ" shall indicate the protein product or products generated from transcription and translation and/or alternative splicing of the *PKC-θ* gene.

[0162] Each embodiment described herein is to be applied *mutatis mutandis* to each and every embodiment unless specifically stated otherwise.

2. Agents for enhancing T cell function

[0163] The present invention is based in part of the determination that exposure of functionally repressed T-cells of a mesenchymal phenotype to PKC-θ inhibitors results in epigenetic reprogramming of the T-cells with de-repression of their immune effector function, including elevated expression of biomarkers of T-cell activation and effector capacity (*e.g.*, IL-2, IFN-γ and TNF-α), decreased expression of biomarkers of T-cell effector inhibition and cancer progression (*e.g.*, ZEB1), as well as decreased expression of biomarkers of T-cell exhaustion (*e.g.*, PD-1 and EOMES) and elevated expression of the transcription factor TBET, which increases production of IFN-γ in cells of the adaptive and innate immune systems. The present inventors have also found that PKC-θ inhibitor-mediated epigenetic reprogramming confers enhanced susceptibility of exhausted T-cells to reinvigoration by PD-1 binding antagonists.

[0164] Thus, in accordance with the present invention, compositions and methods are provided that take advantage of a PKC-θ inhibitor (*e.g.*, an inhibitor of PKC-θ kinase activity or an inhibitor of PKC-θ nuclear translocation/localization) and a PD-1 binding antagonist to enhance immune effector function, and/or to enhance T-cell (*e.g.*, $CD8^+$ T-cell) function, including increasing T-cell activation and enhancing susceptibility of exhausted T-cells to reinvigoration by PD-1 binding antagonists . The methods and compositions of the present invention are thus particularly useful in the treatment of T-cell dysfunctional disorders including cancers and infections.

2.1 PKC-θ inhibitors

[0165] The PKC-θ inhibitor includes and encompasses any active agent that reduces the accumulation, function (*e.g.*, enzymatic activity, nuclear translocation/localization *etc.*) or stability of PKC-θ; or decrease expression of *PKC-θ*, and such inhibitors include without limitation, small molecules and macromolecules such as nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, polysaccharides, lipopolysaccharides, lipids or other organic (carbon containing) or inorganic molecules.

[0166] The PKC-θ inhibitor includes and encompasses any active agent that reduces the accumulation, function or stability of a PKC-θ; or decreases expression of a *PKC-θ* gene, and such inhibitors include without limitation, small molecules and macromolecules such as nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, polysaccharides, lipopolysaccharides, lipids or other organic (carbon containing) or inorganic molecules.

[0167] In some embodiments, the PKC-θ inhibitor is an antagonistic nucleic acid molecule that functions to inhibit the transcription or translation of *PKC-θ* transcripts. Representative transcripts of this type include nucleotide sequences corresponding to any one the following sequences: (1) human *PKC-θ* nucleotide sequences as set forth for example in GenBank Accession Nos. XM_005252496, XM_005252497, XM_005252498, and XM_005252499, (2) nucleotide sequences that share at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence identity with any one of the sequences referred to in (1); (3) nucleotide sequences that hybridize under at least low, medium or high stringency conditions to the sequences referred to in (1); (4) nucleotide sequences that encode any one of the following amino acid sequences: human PKC-θ amino acid sequences as set forth for example in GenPept Accession Nos. XP_005252553, XP_005252554, XP_005252555 and XP_005252556; (5) nucleotide sequences that encode an amino acid sequence that shares at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence similarity with any one of the sequences referred to in (4); and nucleotide sequences that encode an amino acid sequence that shares at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% sequence identity with any one of the sequences referred to in (4).

[0168] Illustrative antagonist nucleic acid molecules include antisense molecules, aptamers, ribozymes and triplex forming molecules, RNAi and external guide sequences. The nucleic acid molecules can act as effectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules can possess a *de novo* activity independent of any other molecules.

[0169] Antagonist nucleic acid molecules can interact with any macromolecule, such as DNA, RNA, polypeptides, or carbohydrate chains. Thus, antagonist nucleic acid molecules can interact with *PKC-θ* mRNA or the genomic DNA of *PKC-θ* or they can interact with a PKC-θ polypeptide. Often antagonist nucleic acid molecules are designed to interact with other nucleic acids based on sequence homology between the target molecule and the antagonist nucleic acid molecule. In other situations, the specific recognition between the antagonist nucleic acid molecule and the target molecule is not based on sequence homology between the antagonist nucleic acid molecule and the target molecule, but rather is based on the formation of tertiary structure that allows specific recognition to take place.

[0170] In some embodiments, anti-sense RNA or DNA molecules are used to directly block the translation of *PKC-θ* by binding to targeted mRNA and preventing protein translation. Antisense molecules are designed to interact with a target nucleic acid molecule through either canonical or non-canonical base pairing. The interaction of the antisense molecule and the target molecule may be designed to promote the destruction of the target molecule through, for example, RNAseH mediated RNA-DNA hybrid degradation. Alternatively the antisense molecule may be designed to interrupt a processing function that normally would take place on the target molecule, such as transcription or replication. Antisense molecules can be designed based on the sequence of the target molecule. Numerous methods for optimization of antisense efficiency by finding the most accessible regions of the target molecule exist. Non-limiting methods include *in vitro* selection experiments and DNA modification studies using DMS and DEPC. In specific examples, the antisense molecules bind the target molecule with a dissociation constant (K_d) less than or equal to 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. In specific embodiments, antisense oligodeoxyribonucleotides derived from the translation initiation site, *e.g.*, between –10 and +10 regions are employed.

[0171] Aptamers are molecules that interact with a target molecule, suitably in a specific way. Aptamers are generally small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stem-loops or G-quartets. Aptamers can bind small molecules, such as ATP and theophylline, as well as large molecules, such as reverse transcriptase and thrombin. Aptamers can bind very tightly with Kds from the target molecule of less than 10^{-12} M. Suitably, the aptamers bind the target molecule with a K_d less than 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}. Aptamers can bind the target molecule with a very high degree of specificity. For example, aptamers have been isolated that have greater than a 10,000 fold difference in binding affinities between the target molecule and another molecule that differ at only a single position on the molecule. It is desirable that an aptamer have a K_d with the target molecule at least 10-, 100-, 1000-, 10,000-, or 100,000-fold lower than the K_d with a background-binding molecule. A suitable method for generating an aptamer to a target of interest (*e.g.*, PKC-θ) is the "Systematic Evolution of Ligands by EXponential Enrichment" (SELEX™). The SELEX™ method is described in U.S. Pat. No. 5,475,096 and U.S. Pat. No. 5,270,163 (see also WO 91/19813). Briefly, a mixture of nucleic acids is contacted with the target molecule under conditions favorable for binding. The unbound nucleic acids are partitioned from the bound nucleic acids, and the nucleic acid-target complexes are dissociated. Then the dissociated nucleic acids are amplified to yield a ligand-enriched mixture of nucleic acids, which is subjected to repeated cycles of binding, partitioning, dissociating and amplifying as desired to yield highly specific high affinity nucleic acid ligands to the target molecule.

[0172] In other embodiments, anti-*PKC-θ* ribozymes are used for catalyzing the specific cleavage of *PKC-θ* RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by a endonucleolytic cleavage. There are several different types of ribozymes that catalyze nuclease or nucleic acid polymerase type reactions, which are based on ribozymes found in natural systems, such as hammerhead ribozymes, hairpin ribozymes, and tetrahymena ribozymes. There are also a number of ribozymes that are not found in natural systems, but which have been engineered to catalyze specific reactions *de novo*. Representative ribozymes cleave RNA or DNA substrates. In some embodiments, ribozymes that cleave RNA substrates are employed. Specific ribozyme cleavage sites within potential RNA targets are initially identified by scanning the target molecule for ribozyme cleavage sites, which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

[0173] Triplex forming functional nucleic acid molecules are molecules that can interact with either double-stranded or single-stranded nucleic acid. When triplex molecules interact with a target region, a structure called a triplex is formed, in which there are three strands of DNA forming a complex dependent on both Watson-Crick and Hoogsteen base pairing. Triplex molecules are preferred because they can bind target regions with high affinity and specificity. It is generally desirable that the triplex forming molecules bind the target molecule with a K_d less than 10^{-6}, 10^{-8}, 10^{-10}, or 10^{-12}.

[0174] External guide sequences (EGSs) are molecules that bind a target nucleic acid molecule forming a complex, and this complex is recognized by RNAse P, which cleaves the target molecule. EGSs can be designed to specifically target a RNA molecule of choice. RNAse P aids in processing transfer RNA (tRNA) within a cell. Bacterial RNAse P can be recruited to cleave virtually any RNA sequence by using an EGS that causes the target RNA:EGS complex to mimic the natural tRNA substrate. Similarly, eukaryotic EGS/RNAse P-directed cleavage of RNA can be utilized to cleave desired targets within eukaryotic cells.

[0175] In other embodiments, RNA molecules that mediate RNA interference (RNAi) of a *PKC-θ* gene or *PKC-θ* transcript can be used to reduce or abrogate gene expression. RNAi refers to interference with or destruction of the product of a target gene by introducing a single-stranded or usually a double-stranded RNA (dsRNA) that is homologous to the transcript of a target gene. RNAi methods, including double-stranded RNA interference (dsRNAi) or small interfering RNA (siRNA), have been extensively documented in a number of organisms, including mammalian cells and the nematode *C. elegans* (Fire *et al.*, 1998. *Nature* 391, 806-811). In mammalian cells, RNAi can be triggered by 21- to 23-nucleotide (nt) duplexes of small interfering RNA (siRNA) (Chiu *et al.*, 2002 *Mol. Cell.* 10:549-561; Elbashir *et al.*, 2001. *Nature* 411:494-498), or by micro-RNAs (miRNA), functional small-hairpin RNA (shRNA), or other dsRNAs which are expressed *in vivo* using DNA templates with RNA polymerase III promoters (Zeng *et al.*, 2002. *Mol. Cell* 9:1327-1333 ; Paddison *et al.*, 2002. *Genes Dev.* 16:948-958; Lee *et al.*, 2002. *Nature Biotechnol.* 20:500-505; Paul *et al.*, 2002. *Nature Biotechnol.* 20:505-508; Tuschl, T., 2002. *Nature Biotechnol.* 20:440-

448; Yu *et al.*, 2002. *Proc. Natl. Acad. Sci. USA* 99(9):6047-6052; McManus *et al.*, 2002. *RNA* 8:842-850; Sui *et al.*, 2002. *Proc. Natl. Acad. Sci. USA* 99(6):5515-5520).

[0176] In specific embodiments, dsRNA *per se* and especially dsRNA-producing constructs corresponding to at least a portion of a *PKC-θ* gene are used to reduce or abrogate its expression. RNAi-mediated inhibition of gene expression may be accomplished using any of the techniques reported in the art, for instance by transfecting a nucleic acid construct encoding a stem-loop or hairpin RNA structure into the genome of the target cell, or by expressing a transfected nucleic acid construct having homology for a *PKC-θ* gene from between convergent promoters, or as a head to head or tail to tail duplication from behind a single promoter. Any similar construct may be used so long as it produces a single RNA having the ability to fold back on itself and produce a dsRNA, or so long as it produces two separate RNA transcripts, which then anneal to form a dsRNA having homology to a target gene.

[0177] Absolute homology is not required for RNAi, with a lower threshold being described at about 85% homology for a dsRNA of about 200 base pairs (Plasterk and Ketting, 2000, *Current Opinion in Genetics and Dev.*10: 562-67). Therefore, depending on the length of the dsRNA, the RNAi-encoding nucleic acids can vary in the level of homology they contain toward the target gene transcript, *i.e.*, with dsRNAs of 100 to 200 base pairs having at least about 85% homology with the target gene, and longer dsRNAs, *i.e.*, 300 to 100 base pairs, having at least about 75% homology to the target gene. RNA-encoding constructs that express a single RNA transcript designed to anneal to a separately expressed RNA, or single constructs expressing separate transcripts from convergent promoters, are suitably at least about 100 nucleotides in length. RNA-encoding constructs that express a single RNA designed to form a dsRNA *via* internal folding are usually at least about 200 nucleotides in length.

[0178] The promoter used to express the dsRNA-forming construct may be any type of promoter if the resulting dsRNA is specific for a gene product in the cell lineage targeted for destruction. Alternatively, the promoter may be lineage specific in that it is only expressed in cells of a particular development lineage. This might be advantageous where some overlap in homology is observed with a gene that is expressed in a non-targeted cell lineage. The promoter may also be inducible by externally controlled factors, or by intracellular environmental factors.

[0179] In some embodiments, RNA molecules of about 21 to about 23 nucleotides, which direct cleavage of specific mRNA to which they correspond, as for example described by Tuschl *et al.* in U.S. 2002/0086356, can be utilized for mediating RNAi. Such 21- to 23-nt RNA molecules can comprise a 3' hydroxyl group, can be single-stranded or double stranded (as two 21- to 23-nt RNAs) wherein the dsRNA molecules can be blunt ended or comprise overhanging ends (*e.g.*, 5', 3').

[0180] In some embodiments, the antagonist nucleic acid molecule is a siRNA. siRNAs can be prepared by any suitable method. For example, reference may be made to International Publication WO 02/44321, which discloses siRNAs capable of sequence-specific degradation of target mRNAs when base-paired with 3' overhanging ends, which is incorporated by reference herein. Sequence specific gene silencing can be achieved in mammalian cells using synthetic, short double-stranded RNAs that mimic the siRNAs produced by the enzyme dicer. siRNA can be chemically or *in vitro*-synthesized or can be the result of short double-stranded hairpin-like RNAs (shRNAs) that are processed into siRNAs inside the cell. Synthetic siRNAs are generally designed

using algorithms and a conventional DNA/RNA synthesizer. Suppliers include Ambion (Austin, Tex.), ChemGenes (Ashland, Mass.), Dharmacon (Lafayette, Colo.), Glen Research (Sterling, Va.), MWB Biotech (Esbersberg, Germany), Proligo (Boulder, Colo.), and Qiagen (Vento, The Netherlands). siRNA can also be synthesized in vitro using kits such as Ambion's SILENCER™ siRNA Construction Kit.

[0181] The production of siRNA from a vector is more commonly done through the transcription of a short hairpin RNAs (shRNAs). Kits for the production of vectors comprising shRNA are available, such as, for example, Imgenex's GENESUPPRESSOR™ Construction Kits and Invitrogen's BLOCK-IT™ inducible RNAi plasmid and lentivirus vectors. In addition, methods for formulation and delivery of siRNAs to a subject are also well known in the art. See, e.g., US 2005/0282188; US 2005/0239731; US 2005/0234232; US 2005/0176018; US 2005/0059817; US 2005/0020525; US 2004/0192626; US 2003/0073640; US 2002/0150936; US 2002/0142980; and US 2002/0120129, each of which is incorporated herein by reference.

[0182] Illustrative RNAi molecules (*e.g.*, *PKC-θ* siRNA and shRNA) are described in the art (*e.g.*, Ma *et al.*, 2013. BMC Biochem. 14: 20; and Kim *et al.*, 2013. Immune Netw. 13(2):55-62) or available commercially from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), OriGene Technologies, Inc. (Rockville, MD, USA), Sigma-Aldrich Pty Ltd (Castle Hill, NSW, Australia).

[0183] The present invention further contemplates peptide or polypeptide based inhibitor compounds. For example, various PKC-θ isozyme- and variable region-specific peptides are known, illustrative examples of which include:

[0184] (a) θV1 derived peptides θV1-1 and θV1-2, having the amino acid sequence GLSNFDCG (PKC-θ residues 8-15) or YVESENGQMYI [SEQ ID NO:1] (PKC-θ residues 36-46), respectively, as disclosed for example in US Patent No. 5,783,405, which is hereby incorporated by reference herein in its entirety;

[0185] (b) θV5 derived peptides having the amino acid sequence VKSPFDCS (PKC-θ residues 655-662) or DRALINS, or modified peptide VrSPFDCS, as disclosed for example in US 2004/0009922, which is hereby incorporated by reference herein in its entirety; and

[0186] (c) Ψθ RACK derived peptides having the amino acid sequence KGDNVDLI, KGENVDLI, KGKEVDLI, KGKNVDLI, RGKNVELA, RGENVELA, KGKQVNLI, KGKQVNLI, KGDQVNLI, or KGEQVNLI as disclosed for example in US 2010/0311644, which is hereby incorporated by reference herein in its entirety.

[0187] PKC-θ inhibitory peptides, as described for example above may be modified by being part of a fusion protein. The fusion protein may include a transport protein or peptide that functions to increase the cellular uptake of the peptide inhibitors, has another desired biological effect, such as a therapeutic effect, or may have both of these functions. The fusion protein may be produced by methods known to the skilled artisan. The inhibitor peptide may be bound, or otherwise conjugated, to another peptide in a variety of ways known to the art. For example, the inhibitor peptide may be bound to a carrier peptide or other peptide described herein *via* cross-linking wherein both peptides of the fusion protein retain their activity. As a further example, the peptides may be linked or otherwise conjugated to each other by an amide bond from the C-terminal of one peptide to the N-terminal of the other peptide. The linkage between the inhibitor peptide and the other member of the fusion protein may be non-cleavable, with a peptide bond, or cleavable with, for example, an ester or other cleavable bond known to the art.

[0188] In some embodiments, the transport protein or peptide may be, for example, a *Drosophila* Antennapedia homeodomain-derived sequence comprising the amino acid sequence CRQIKIWFQNRRMKWKK [SEQ ID NO:2], and may be attached to the inhibitor by cross-linking *via* an N-terminal Cys-Cys bond (as discussed, for example, in Theodore *et al.*, 1995. J. Neurosci. 15:7158-7167; Johnson *et al.*, 1996. Circ. Res 79:1086). Alternatively, the inhibitor may be modified by a transactivating regulatory protein (Tat)-derived transport polypeptide (such as from amino acids 47-57 of Tat shown in SEQ ID NO:3; YGRKKRRQRRR) from the human immunodeficiency virus, Type 1, as described in Vives *et al.*, 1997. J. Biol. Chem, 272:16010-16017, U.S. Pat. No. 5,804,604 and GenBank Accession No. AAT48070; or with polyarginine as described in Mitchell *et al.*, 2000. J. Peptide Res. 56:318-325 and Rolhbard *et al.*, 2000. Nature Med. 6:1253-1257). The inhibitors may be modified by other methods known to the skilled artisan in order to increase the cellular uptake of the inhibitors.

[0189] A PKC-θ inhibitory peptide can also be introduced into a cell by introducing into the cell a nucleic acid comprising a nucleotide sequence that encodes a PKC-θ inhibitory peptide. The nucleic acid can be in the form of a recombinant expression vector. The PKC-θ inhibitory peptide-encoding sequence can be operably linked to a transcriptional control element(s), *e.g.*, a promoter, in the expression vector. Suitable vectors include, *e.g.*, recombinant retroviruses, lentiviruses, and adenoviruses; retroviral expression vectors, lentiviral expression vectors, nucleic acid expression vectors, and plasmid expression vectors. In some cases, the expression vector is integrated into the genome of a cell. In other cases, the expression vector persists in an episomal state in a cell.

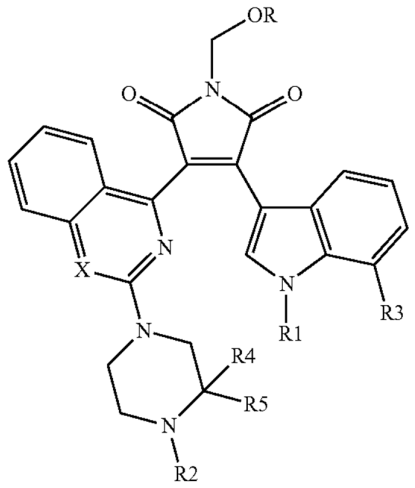
[0190] Suitable expression vectors include, but are not limited to, viral vectors (*e.g.*, viral vectors based on vaccinia virus; poliovirus; adenovirus (see, *e.g.*, Li *et al.*, Invest Opthalmol Vis Sci 35:2543 2549, 1994; Borras *et al.*, Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto *et al.*, H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, *e.g.*, Ali *et al.*, Hum Gene Ther 9:8186, 1998, Flannery *et al.*, PNAS 94:6916 6921, 1997; Bennett *et al.*, Invest Opthalmol Vis Sci 38:2857 2863, 1997; Jomary *et al.*, Gene Ther 4:683 690, 1997, Rolling *et al.*, Hum Gene Ther 10:641 648, 1999; Ali *et al.*, Hum Mol Genet. 5:591 594, 1996; Srivastava in WO 93/09239, Samulski *et al.*, J. Vir. 63:3822-3828, 1989; Mendelson *et al.*, Virol. 166:154-165, 1988; and Flotte *et al.*, PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, *e.g.*, Miyoshi *et al.*, PNAS 94:10319 23, 1997; Takahashi *et al.*, J Virol 73:7812 7816, 1999); a retroviral vector (*e.g.*, murine leukemia virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous sarcoma virus, Harvey sarcoma virus, avian leucosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

[0191] The present invention also contemplates small molecule agents that reduce the functional activity of PKC-θ (*e.g.*, reduce PKC-θ-mediated phosphorylation, inhibit binding of PKC-θ to the promoter of *CD44* or *uPAR*, reduce binding of PKC-θ (*e.g.*, active PKC-θ) to chromatin; reduce PKC-θ-mediated inhibition of guanine exchange factor, GIV/Girdin, reduce PKC-θ-mediated inhibition of regulatory T cell function, reduce PKC-θ-mediated EMT *etc.*).

[0192] Small molecule agents that reduce functional activity of PKC-θ that are suitable for use in the present invention include pyridine derivatives that inhibit PKC-θ functional activity; purine compounds that inhibit PKC-θ functional activity, pyrimidine derivatives that inhibit PKC-θ

functional activity; aniline compounds that inhibit PKC-θ functional activity, indole derivatives that inhibit PKC-θ functional activity, and the like.

[0193] In some embodiments, small molecule PKC-θ inhibitors are selected from substituted indole derivatives as described for example by Cooke *et al.* in US Publication No. 2013/0157980, which is incorporated herein by reference in its entirety. Illustrative derivatives of this type include compounds according to formula (I):



(I)

[0194] or a pharmaceutically acceptable salt, or hydrate thereof.

[0195] In some embodiments of the compounds according to formula (I):

[0196] X is CH or N;

[0197] R is H or PO_3H_2;

[0198] R1 is H; or C_{1-4}alkyl; R2 is H; or C_{1-4}alkyl; R3 is H; C_{1-4}alkyl; CN; Hal; or OH; and R4 and R5 are independently from each other H, or C_{1-4}alkyl; or R4 and R5 form together with the carbon atom to which they are attached a 3-6 membered cycloalkyl group.

[0199] In other embodiments of the compounds according to formula (I):

[0200] X is CH;

[0201] R is PO_3H_2;

[0202] R1 is H;

[0203] R2 is H; or C_{1-4}alkyl; R3 is H; or C_{1-4}alkyl; and R4 and R5 are independently from each other H; or R4 and R5 form together with the carbon atom to which they are attached a 3-6 membered cycloalkyl group.

[0204] In still other embodiments of the compounds according to formula (I):

[0205] X is CH;

[0206] R is H;

[0207] R1 is H;

[0208] R2 is H; or C_{1-4}alkyl; R3 is H; or C_{1-4}alkyl; and R4 and R5 are independently from each other H; or R4 and R5 form together with the carbon atom to which they are attached a 3-6 membered cycloalkyl group.

[0209] In still other embodiments of the compounds according to formula (I):

[0210] X is N;

[0211] R is PO_3H_2;

[0212] R1 is H;

[0213] R2 is H; or C_{1-4}alkyl;

[0214] R3 is H; and

[0215] R4 and R5 are independently from each other H; or R4 and R5 form together with the carbon atom to which they are attached a 3-6 membered cycloalkyl group.

[0216] In still other embodiments of the compounds according to formula (I):

[0217] X is N;

[0218] R is PO_3H_2;

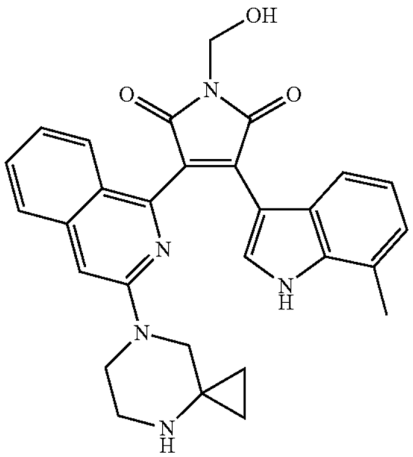
[0219] R1 is H;

[0220] R2 is H; or C_{1-4}alkyl;

[0221] R3 is H; and

[0222] R4 and R5 are independently from each other H; or C_{1-4}alkyl.

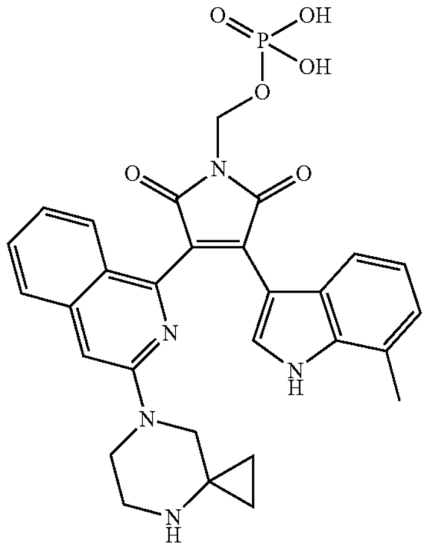
[0223] In some embodiments, the substituted indole derivatives that inhibit PKC-θ functional activity include compounds according to formula (II):



(II)

[0224] or a pharmaceutically acceptable salt thereof.

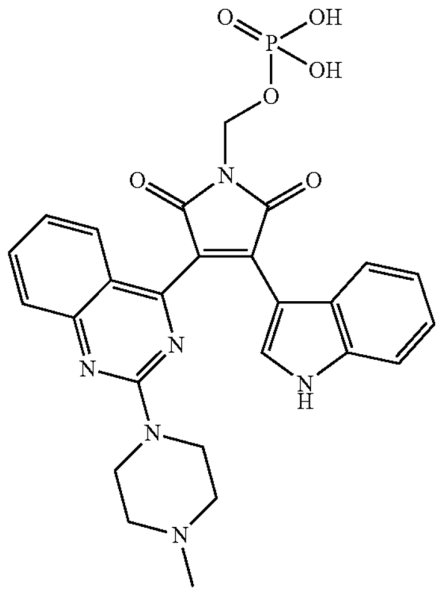
[0225] In other embodiments, the substituted indole derivatives that inhibit PKC-θ functional activity include compounds according to formula (III):



(III)

[0226] or a pharmaceutically acceptable salt or hydrate thereof.

[0227] In still other embodiments, the substituted indole derivatives that inhibit PKC-θ functional activity include compounds according to formula (IV):



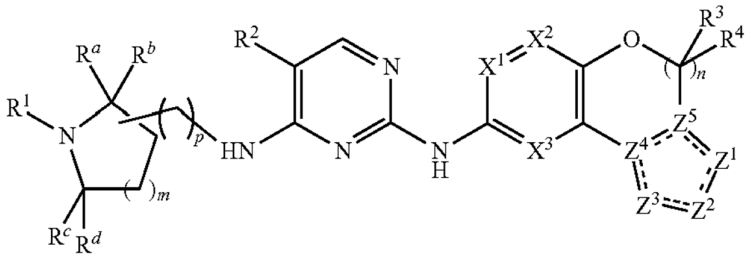
(IV)

[0228] or a pharmaceutically acceptable salt thereof.

[0229] Representative examples of compounds according to formula (I) include: phosphoric acid mono-[3-[3-(4,7-diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-4-(7-methyl-1- H-indol-3-yl)-2,5-dioxo-2,5-dihydro-pyrrol-1-ylmethyl]ester, mono-hydrate; 3-[3-(4,7-diaza-spiro[2.5]oct-7-yl)-isoquinolin-1-yl]-1-hydroxymethyl-4-(- 7-methyl-1H-indol-3-yl)-pyrrole-2,5-dione or a pharmaceutically acceptable salt thereof; and phosphoric acid mono-{3-(1H-indol-3-yl)-4-[2-(4-methyl-piperazin-1-yl)-quinazolin-4-yl]-2,5-dioxo-2,5-dihydro-pyrrol-1-ylmethyl}ester or a pharmaceutically acceptable salt thereof.

[0230] In other embodiments, small molecule PKC-θ inhibitors are selected from pyrimidine diamine derivatives as described for example by Zhao *et al.* in US Publication No.

2013/0143875, which is incorporated herein by reference in its entirety. Representative derivatives of this type include compounds according to formula (V):



(V)

[0231] wherein:

[0232] R^1 is selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, $-C(O)OR^{1a}$, $-S(O)R^{1b}$, and $-S(O)_2R^{1c}$; wherein each of R^{1a}, R^{1b}, and R^{1c} is independently hydrogen, alkyl or phenyl-alkyl;

[0233] R^a, R^b, R^c and R^d independently are selected from hydrogen and alkyl;

[0234] m is an integer from one to five;

[0235] p is an integer from zero to six;

[0236] R^2 is selected from acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, substituted alkyl, substituted alkoxy, amino, substituted amino, aminoacyl, acylamino, azido, carboxyl, carboxylalkyl, cyano, halogen, nitro, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, –SO-alkyl, –SO-substituted alkyl, –SO-aryl, –SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl, and trihalomethyl;

[0237] X^1, X^2, and X^3 are CR^5 or one of X^1, X^2, and X^3 is N and rest are CR^5;

[0238] R^5 is selected from hydrogen, halogen, alkyl and substituted alkyl;

[0239] R^3 and R^4 are, for each occurrence, independently selected from hydrogen, alkyl, substituted alkyl, alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, hydroxyamino, alkoxyamino, nitro, –SO-alkyl, –SO-substituted alkyl, –SO-aryl, –SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl; or R^3 and R^4 together with the carbon atom to which they are attached form a carbocyclic or heterocyclic 4 to 8-membered ring;

[0240] n is an integer from one to three;

[0241] Z^1, Z^2, and Z^3 are selected from CR^6R^{6a}, N, O, and S;

[0242] Z^4 and Z^5 are selected from N, C, and CR^6;

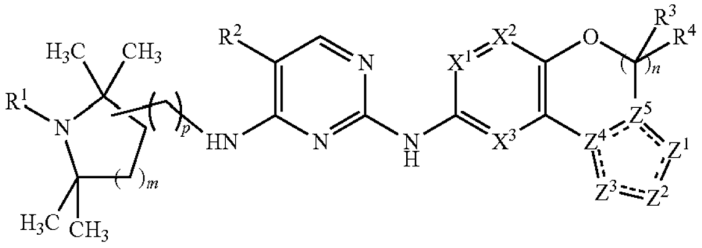
[0243] R^6 is selected from hydrogen, halogen, alkyl and substituted alkyl;

[0244] R^{6a} is selected from hydrogen, halogen, alkyl and substituted alkyl or is absent to satisfy valence requirements; and

[0245] the dashed lines represent a single bond or double bond;

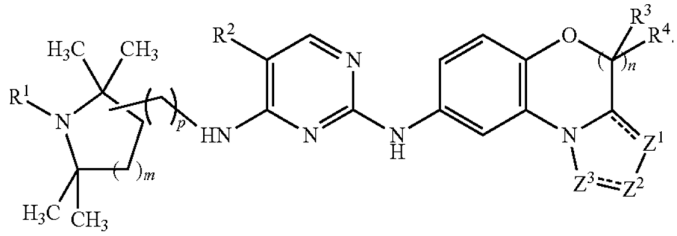
[0246] or a salt or solvate or stereoisomer thereof.

[0247] In some embodiments of compounds according formula (V), R^a, R^b, R^c and R^d represent lower alkyl groups. Illustrative examples of such compounds include those wherein R^a, R^b, R^c and R^d are methyl groups and have formula (VI):



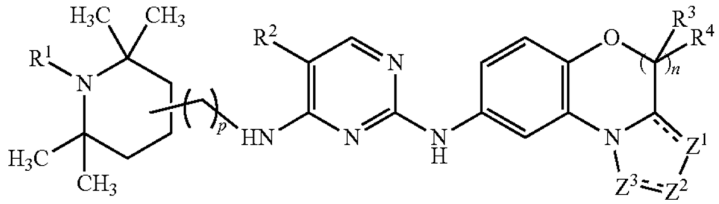
(VI)

[0248] In other embodiments of compounds according formula (V), X^1, X^2, and X^3 are each CH. These compounds have the following formula (VII):



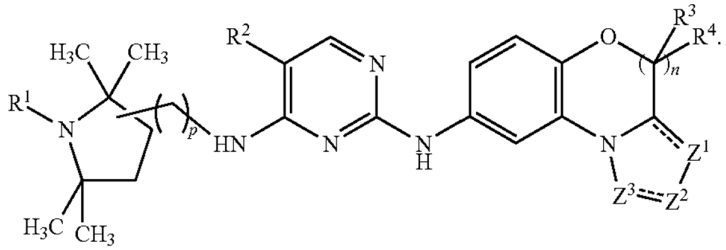
(VII)

[0249] In other embodiments of compounds according formula (V), X^1, X^2, and X^3 are each CH; and m is 2. These compounds have the following formula (VIII):



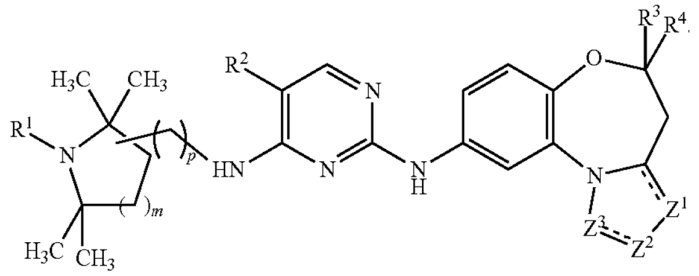
(VIII)

[0250] In still other embodiments of compounds according formula (V), X^1, X^2, and X^3 are each CH; and m is one. These compounds have the following formula (IX):



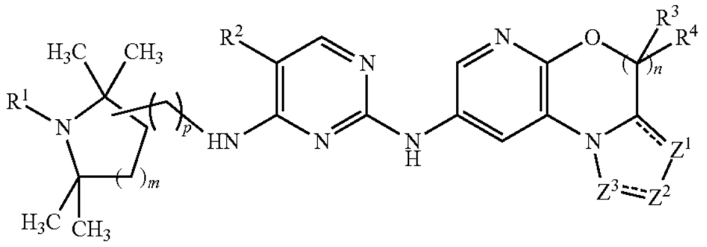
(IX)

[0251] In still other embodiments of compounds according formula (V), X^1, X^2, and X^3 are each CH; n is 2; and one set of R^3 and R^4 is hydrogen. These compounds have the following formula (X):



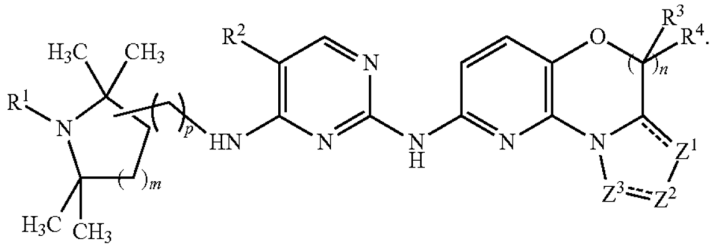
(X)

[0252] In still other embodiments of compounds according formula (V), X^2 is N and X^1 and X^3 are each CH. These compounds have the following formula (XI):



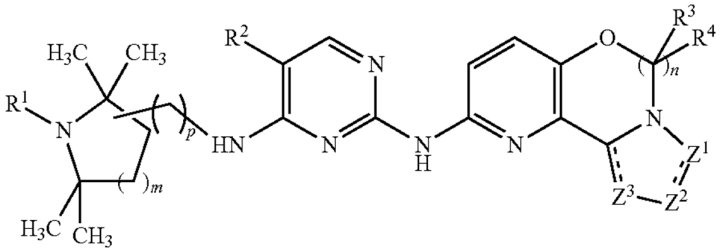
(XI)

[0253] In still other embodiments of compounds according formula (V), X^3 is N and X^1 and X^2 are each CH. These compounds have the following formula (XII):



(XII)

[0254] In other embodiments of compounds according formula (V), Z^4 is C and Z^5 is N. Such compounds have the following formula (XIII):



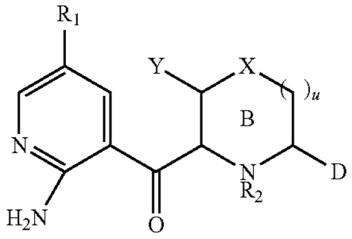
(XIII)

[0255] Exemplary compounds of formula V include: N2-(4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4-(2,2,6,6-tet- ramethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4-(1,2,2,6,6-p- entamethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(4H-benzo[b]pyrrolo[1,2-d][1,4]oxazin-8-yl)-5-fluoro-N4-(2,2,6,6-tetra- methylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(4H-benzo[b]pyrrolo[1,2-d][1,4]oxazin-8-yl)-5-fluoro-N4-(1,2,2,6,6-pentamethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(4,4-difluoro-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4- -(2,2,6,6-tetramethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(4,4-difluoro-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4- -(1,2,2,6,6-pentamethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(4,4-dimethyl-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4- -(1,2,2,6,6-pentamethylpiperidin-4-

yl)pyrimidine-2,4-diamine; N2-(4,4-dimethyl-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4- -(2,2,6,6-tetramethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(5,5-dimethyl-5H-benzo[e]tetrazolo[1,5-c][1,3]oxazin-9-yl)-5-fluoro-N4- -(2,2,6,6-tetramethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(5,5-dimethyl-5H-benzo[e]tetrazolo[1,5-c][1,3]oxazin-9-yl)-5-fluoro-N4- -(1,2,2,6,6-pentamethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(8,9-dihydrospiro[benzo[b]tetrazolo[1,5-d][1,4]oxazine-4,1'-cyclobutan- e]-8-yl)-5-fluoro-N4-(2,2,6,6-tetramethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(8,9-dihydrospiro[benzo[b]tetrazolo[1,5-d][1,4]oxazine-4,1'-cyclobutane]-8-yl)-5-fluoro-N4-(1,2,2,6,6-pentamethylpiperidin-4-yl)pyrimidine-2,4-diamine; 5-fluoro-N2-(4-methyl-8,9-dihydro-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin– 8-yl)-N4-(2,2,6,6-tetramethylpiperidin-4-yl)pyrimidine-2,4-diamine; 5-fluoro-N2-(4-methyl-8,9-dihydro-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin– 8-yl)-N4-(1,2,2,6,6-pentamethylpiperidin-4-yl)pyrimidine-2,4-diamine; N2-(4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4-((1,2- ,2,5,5-pentamethylpyrrolidin-3-yl)methyl)pyrimidine-2,4-diamine; N2-(4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4-((2,2,5,5-te- tramethylpyrrolidin-3-yl)methyl)pyrimidine-2,4-diamine; N2-(4,4-dimethyl-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4- -((1,2,2,5,5-pentamethylpyrrolidin-3-yl)methyl)pyrimidine-2,4-diamine; N2-(4,4-dimethyl-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fl- uoro-N4-((2,2,5,5-tetramethylpyrrolidin-3-yl)methyl)pyrimidine-2,4-diamine- ; N2-(4,4-dimethyl-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-f- luoro-N4-(((3S)-2,2,5-trimethylpyrrolidin-3-yl)methyl)pyrimidine-2,4-diami- ne; and N2-(4,4-dimethyl-4H-benzo[b]tetrazolo[1,5-d][1,4]oxazin-8-yl)-5-fluoro-N4- -(((3R)-2,2,5-trimethylpyrrolidin-3-yl)methyl)pyrimidine-2,4-diamine,

[0256] or salts or solvates or stereoisomers thereof.

[0257] Alternative small molecule PKC-θ inhibitors compounds may be selected from aminopyridine compounds as described for example by Maltais *et al.* in US Publication No. 2013/0137703, which is incorporated herein by reference in its entirety. Non-limiting compounds of this type have the formula (XIV):



(XIV)

[0258] or a pharmaceutically acceptable salt thereof

[0259] wherein:

[0260] R_1 is –H, C1-C3 aliphatic, F, or Cl. Ring B is a 5- or 6-membered monocyclic heteroaromatic ring. X is –CH–, –S–, or –NR_2–. R_2 is absent or –H. Y is –Y1 or -Q1. Y1 a C1-10 aliphatic group optionally and independently substituted with one or more F.

[0261] Q1 is phenyl or a 5-6 membered monocyclic heteroaryl ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and Q1 is optionally and independently substituted with one or more J_a.

[0262] D is ring C or -Q-R_3.

[0263] Ring C is a 6-8-membered non-aromatic monocyclic ring having 1-2 nitrogen atoms, or an 8-12 membered non-aromatic bridged bicyclic ring system having 1-3 heteroatoms selected from nitrogen and oxygen; and ring C is optionally and independently substituted with one or more J_b.

[0264] Q is –NH–, or –O–.

[0265] R_3 is a C1-10 alkyl substituted with –OH, or $–NH_2$; wherein three to six methylene units in R_3 may optionally form a C3-C6 membered cycloalkyl ring; and R_3 is further independently optionally and independently substituted with one or more J_e.

[0266] Each J_a is independently F or C1-C6 alkyl.

[0267] J_b is C1-C10 alkyl wherein up to three methylene units are optionally replaced –O–; and wherein the C1-C10 alkyl is optionally and independently substituted with or more J_c; or J_b is C3-C6 cycloalkyl, or C5-C6 heteroaryl; or J_b is phenyl optionally and independently substituted with J_d; or two J_b on the same carbon atom form =O or spiro C3-C6 cycloalkyl.

[0268] Each J_c is independently F, –OH, or C3-C6 cycloalkyl.

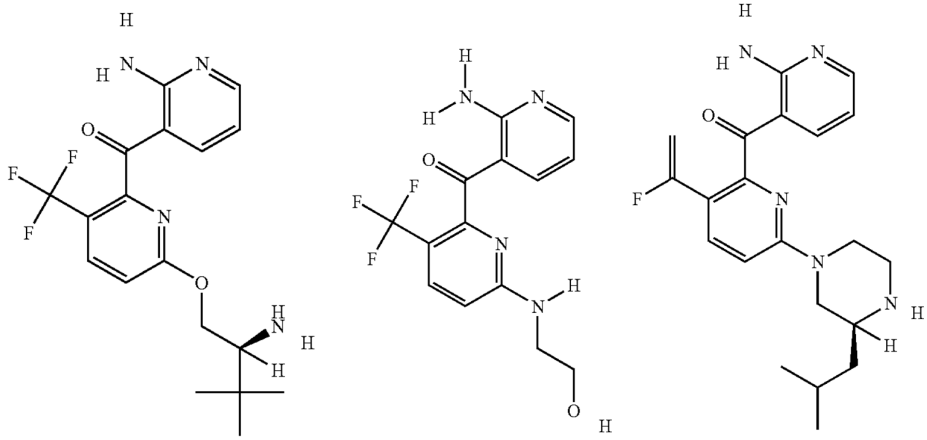
[0269] Each J_d is independently F or Cl.

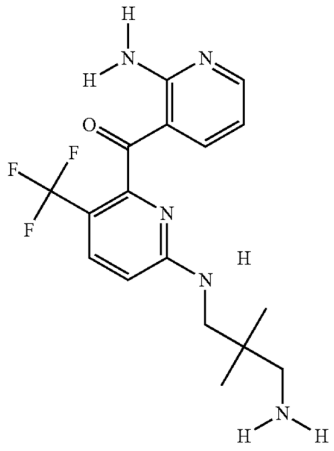
[0270] Each J_e is independently phenyl, a 5-6-membered monocyclic aromatic or non-aromatic ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or two J_e on the same carbon atom form a spiro C3-C6 cycloalkyl.

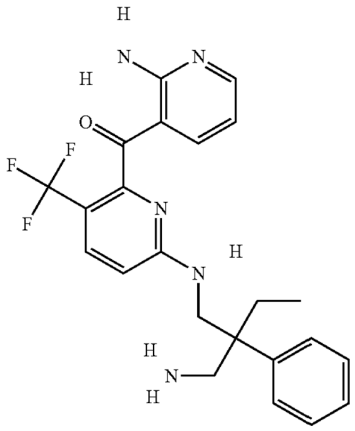
[0271] u is 0 or 1.

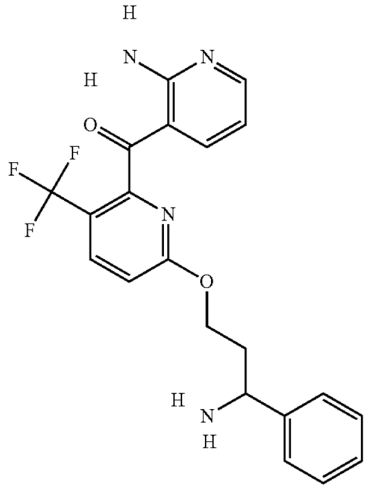
[0272] In some embodiments, ring B is pyridyl; ring C is selected from the group consisting of piperidinyl, piperizinyl, diazepanyl, triazepanyl, azocanyl, diazocanyl, triazocanyl, indolyl, indazolyl, or diazabicyclooctyl; and ring C is optionally and independently substituted with one or more J_b and the remainder of the variables are as described above.

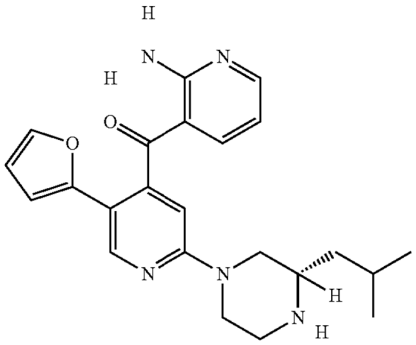
[0273] Representative compounds according to formula (XIV) include:

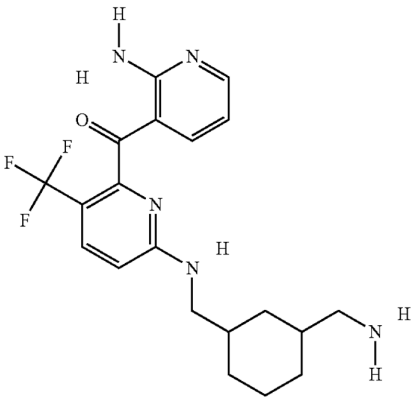


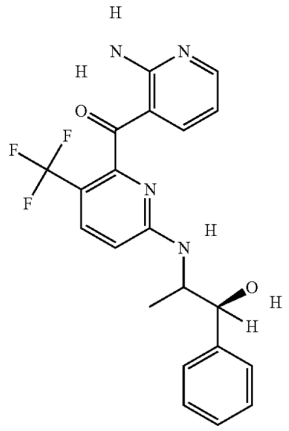


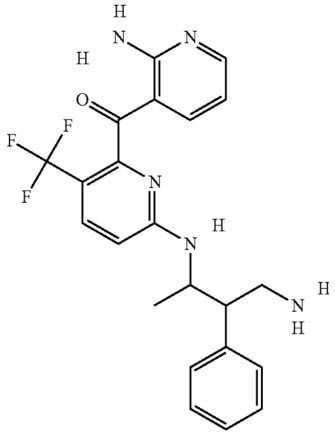


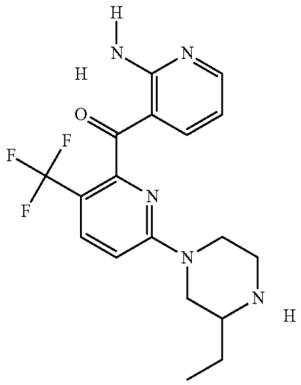


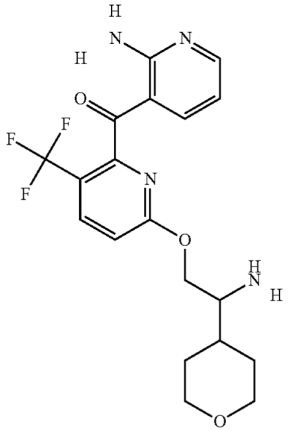


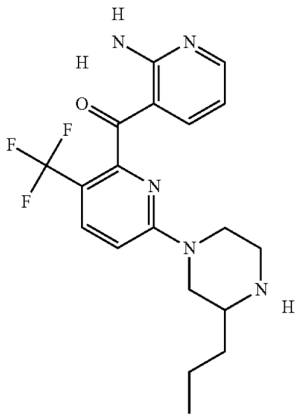
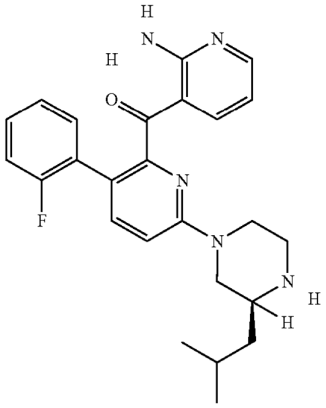
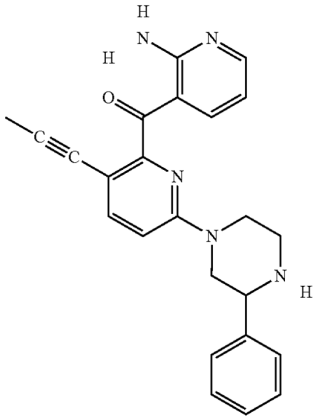
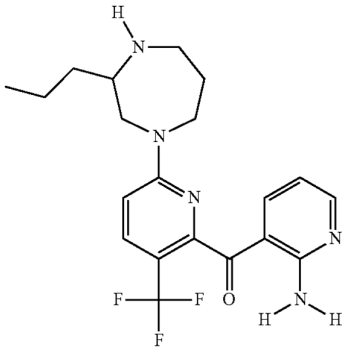
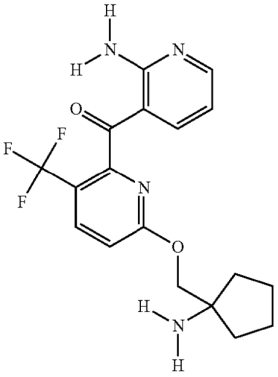
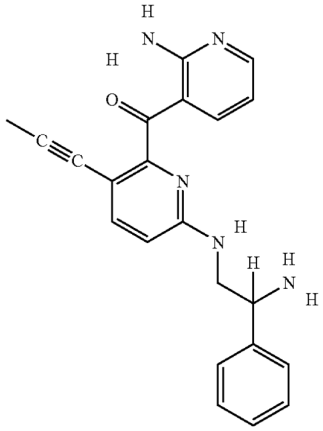
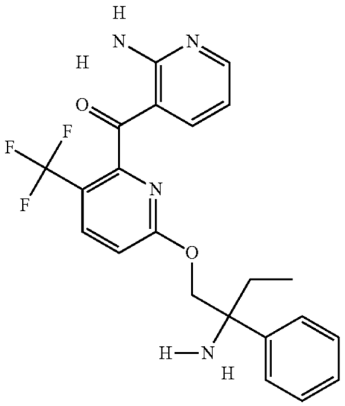
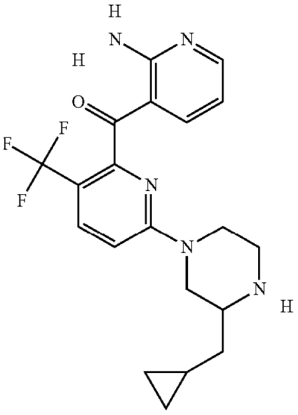
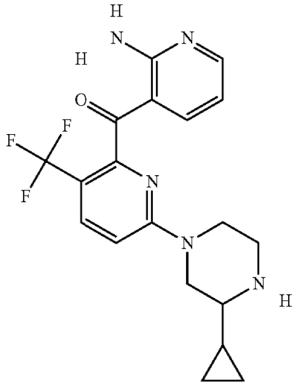
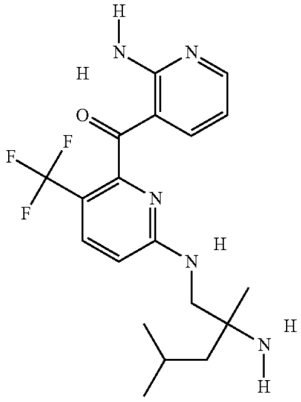
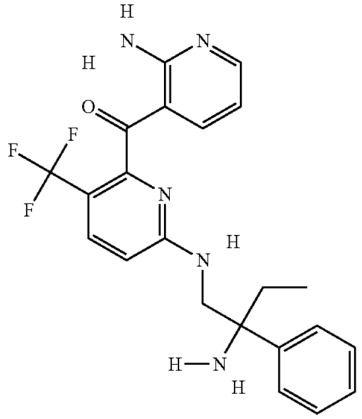




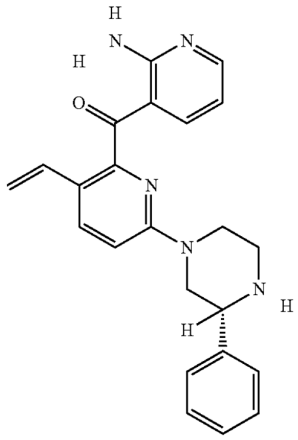


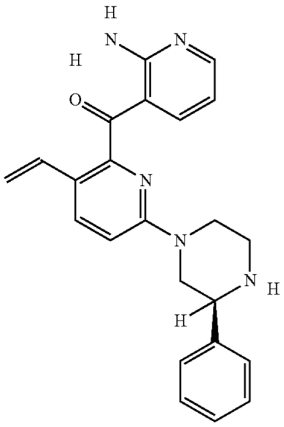


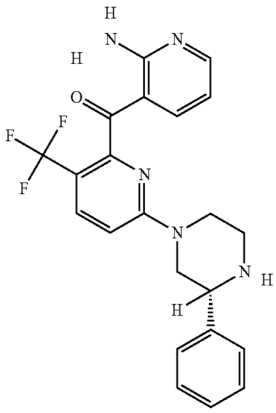


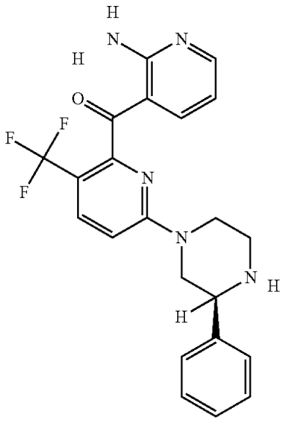












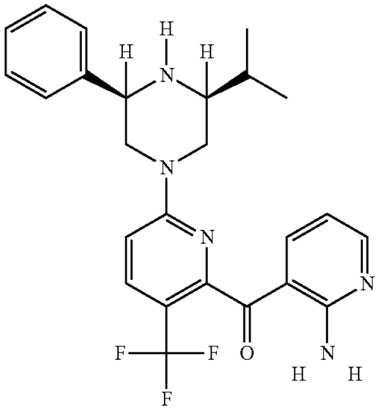
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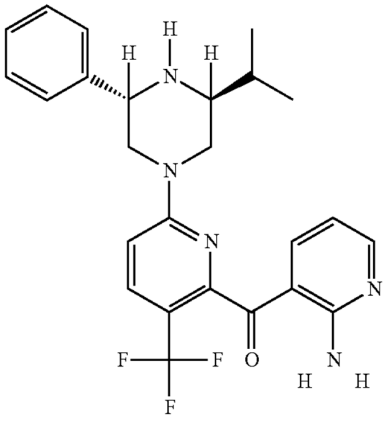


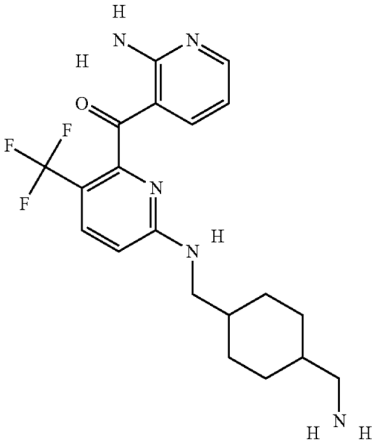


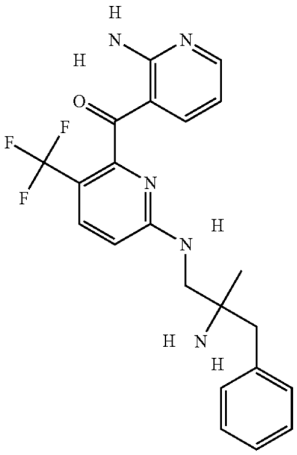


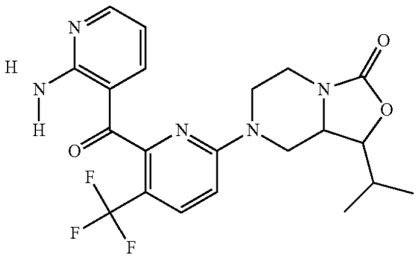


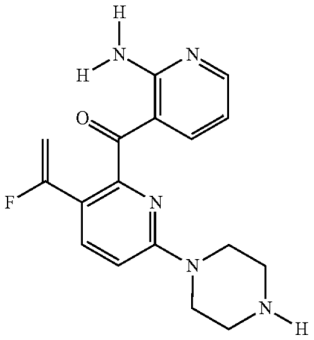


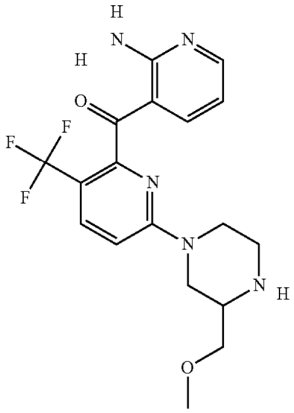


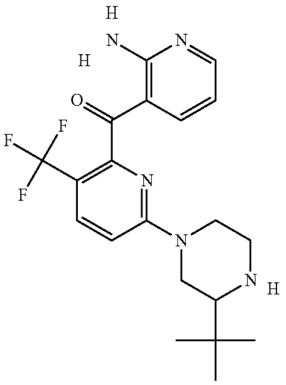


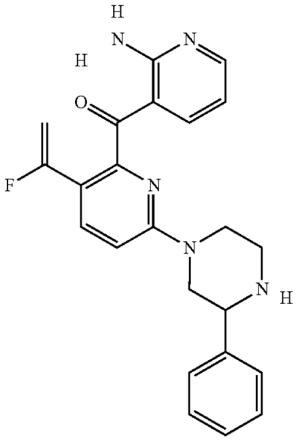


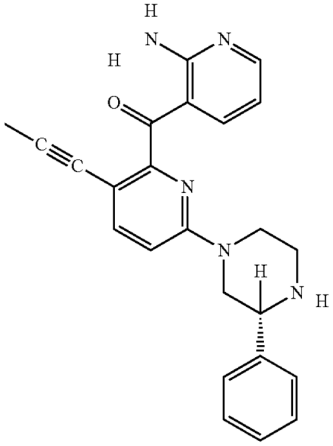


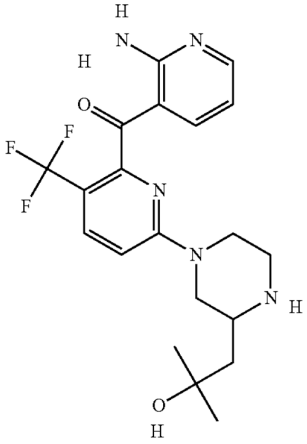


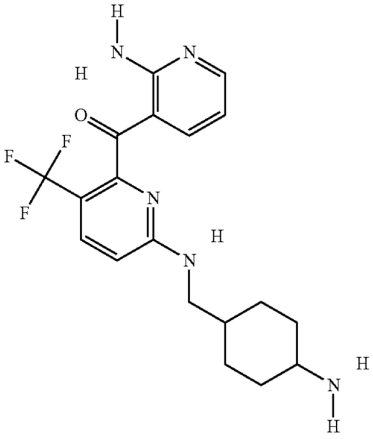


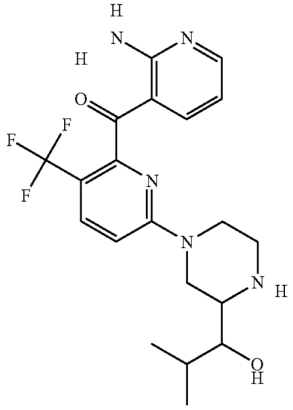


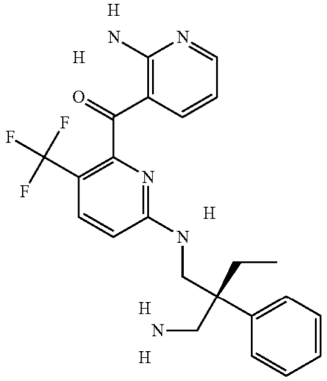


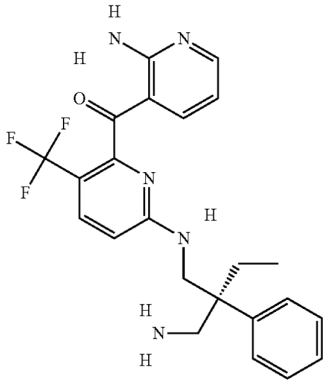


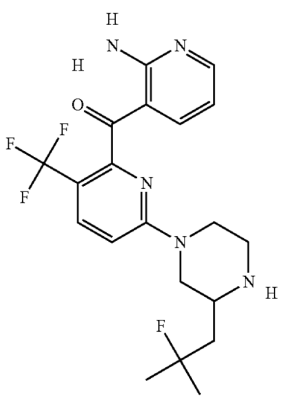


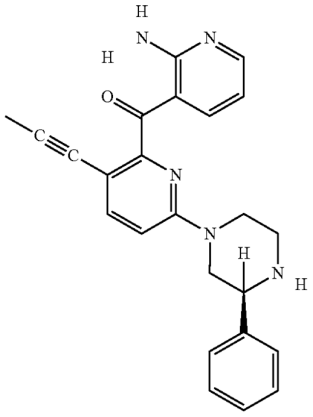


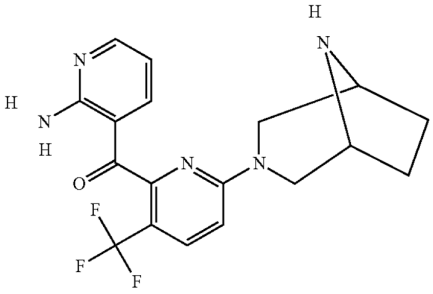


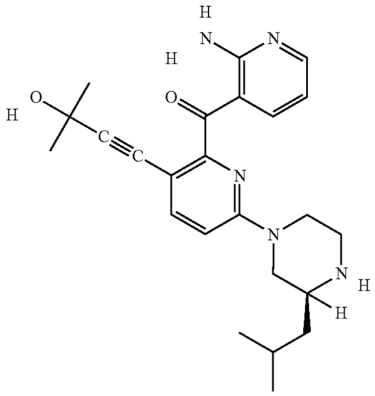


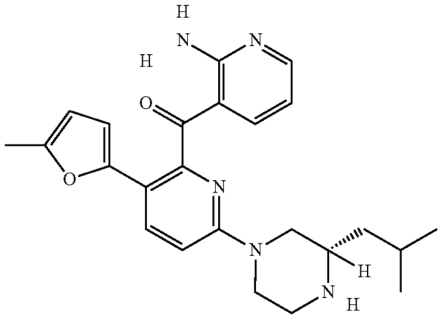


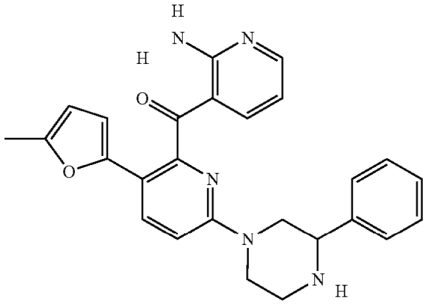


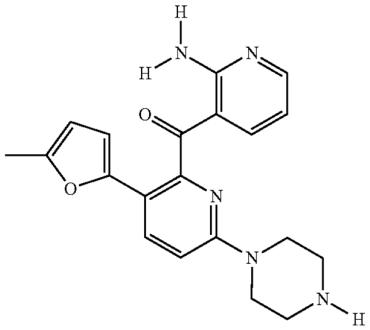


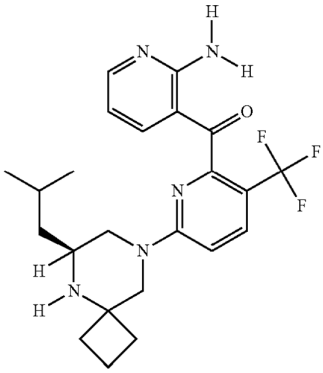


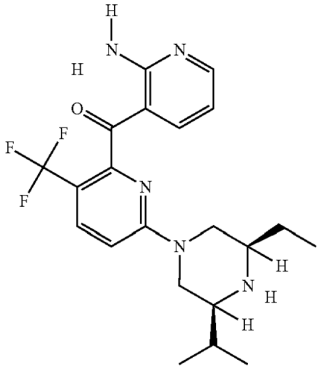


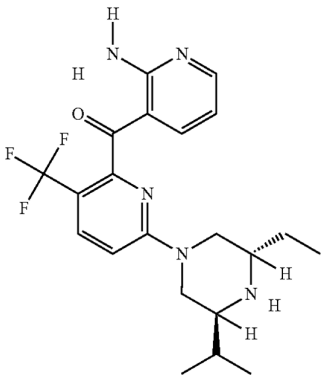


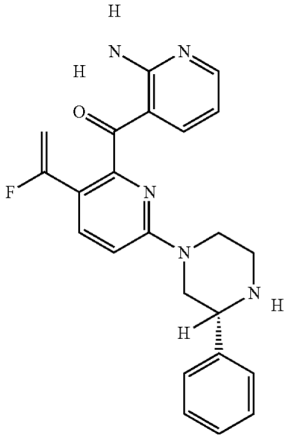


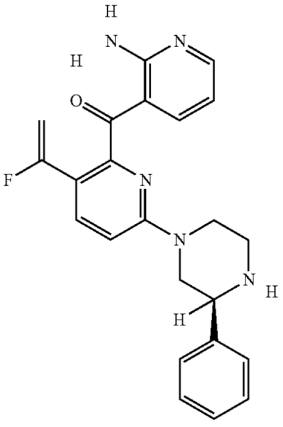




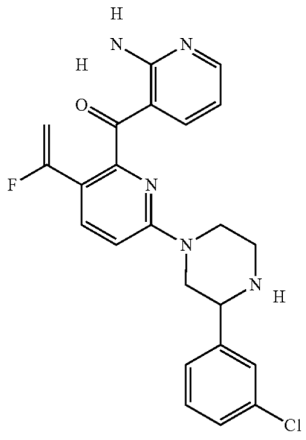


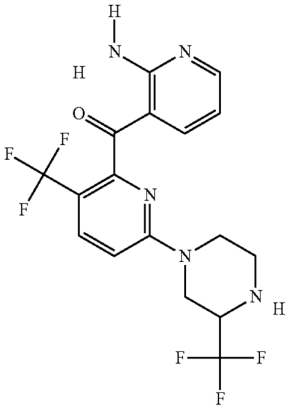


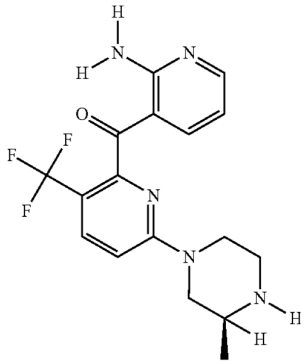


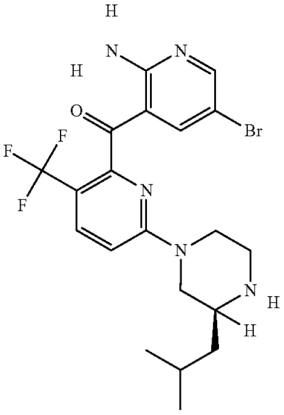


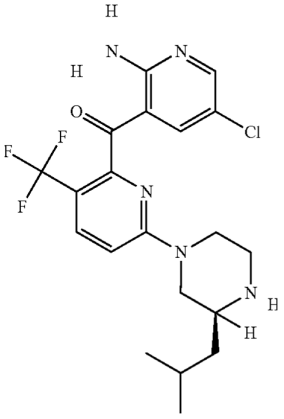
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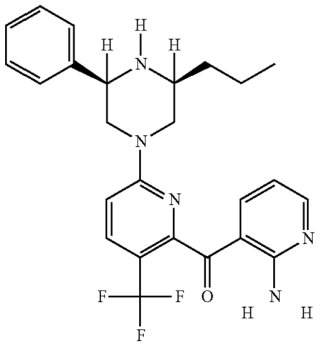


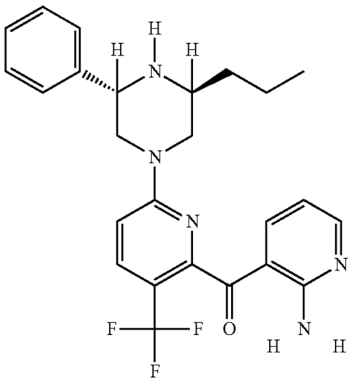


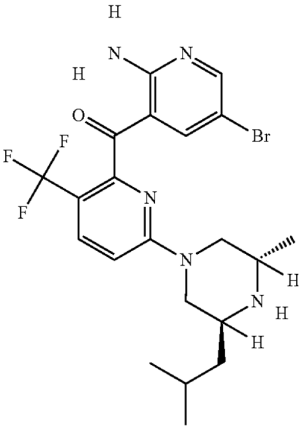


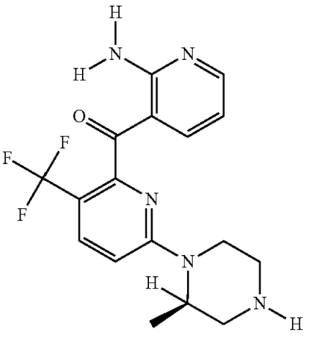


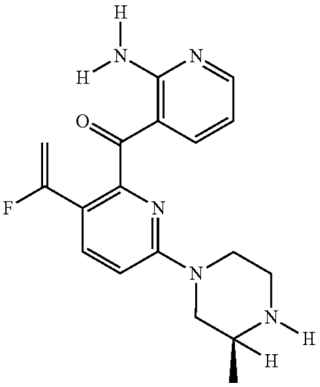




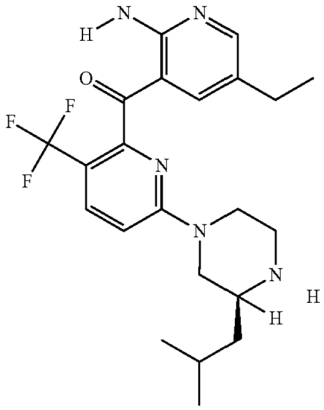


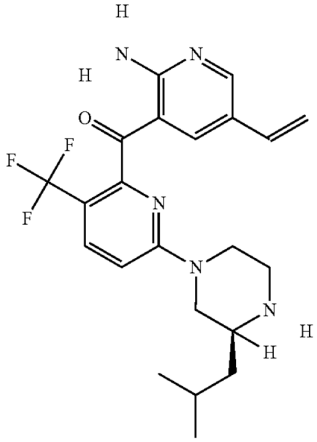


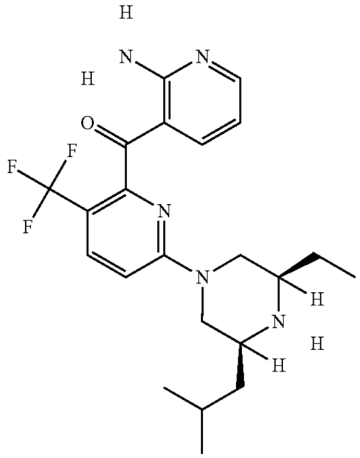


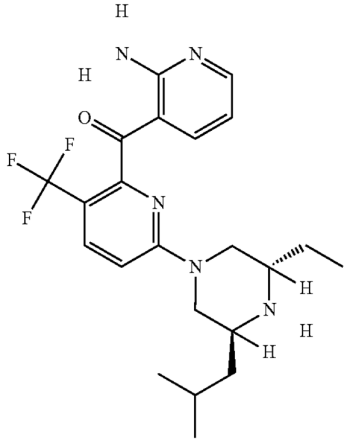


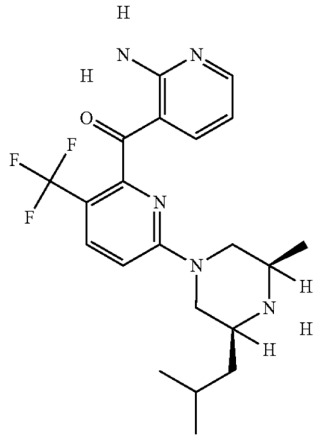
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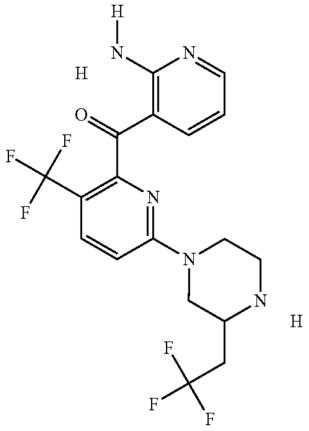


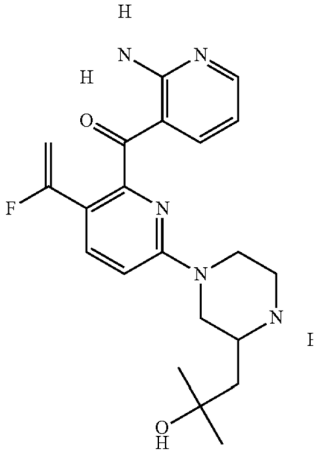


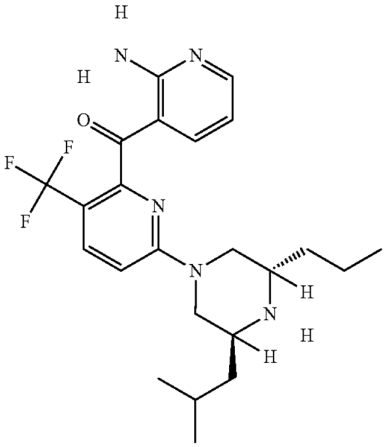


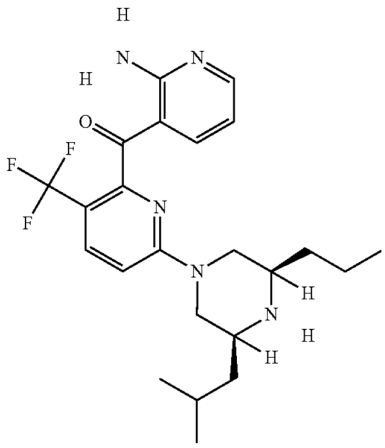


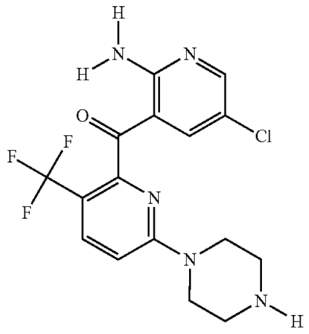


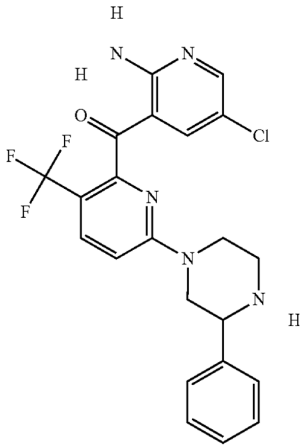


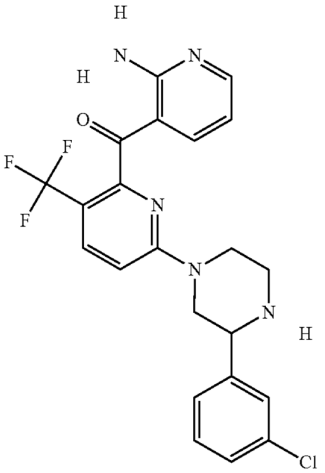


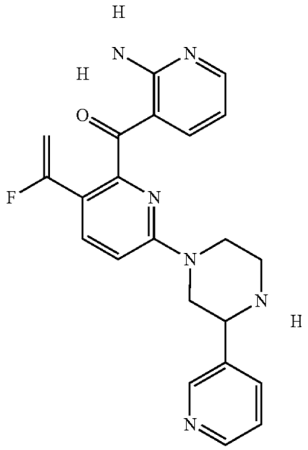


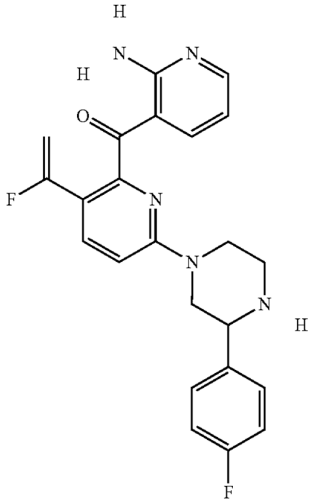


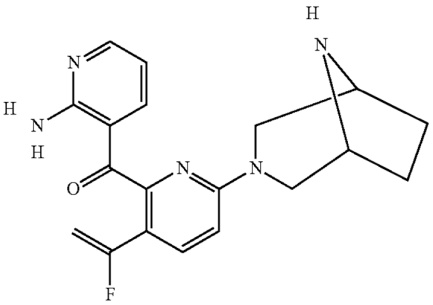


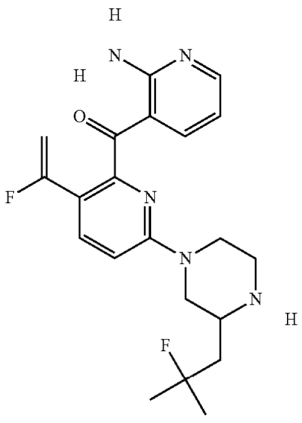


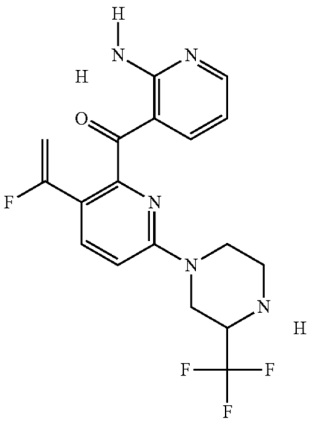


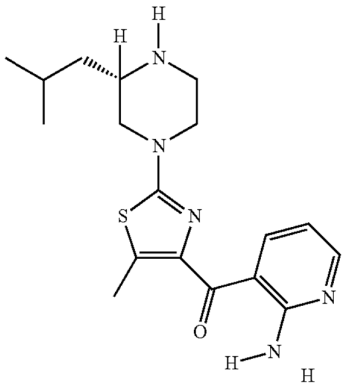


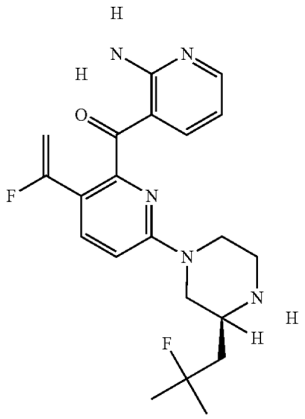


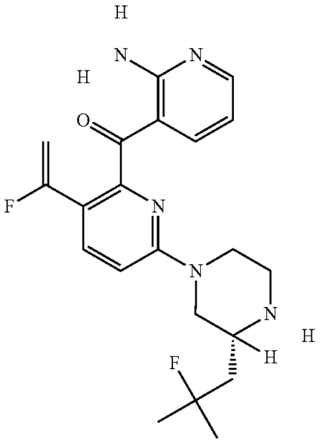


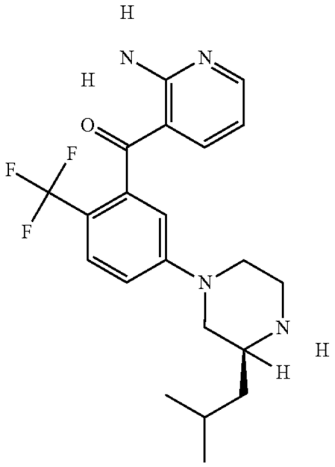


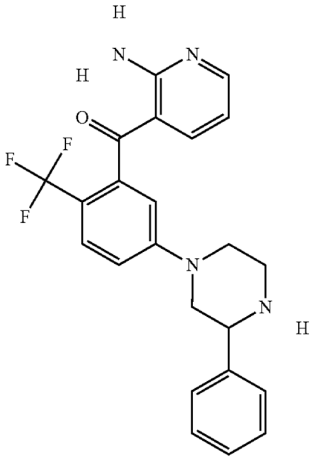


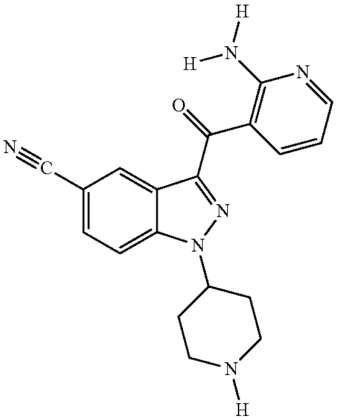


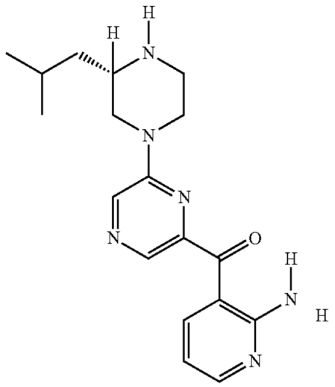


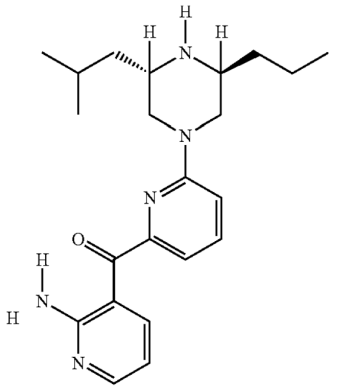


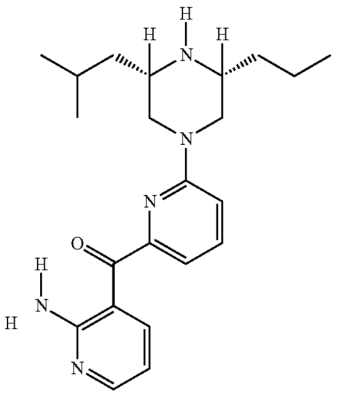


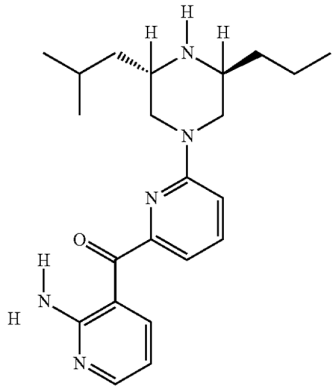


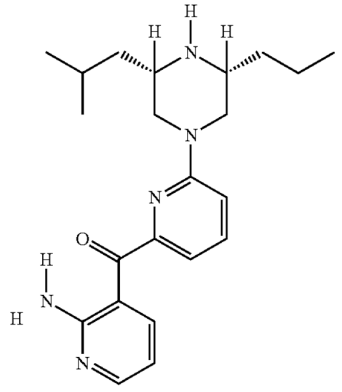




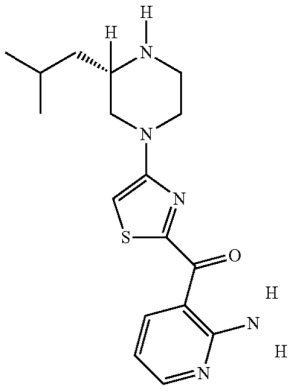


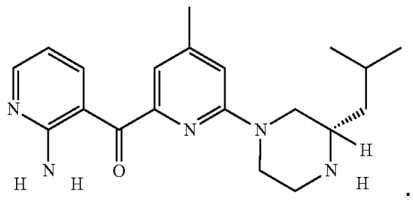




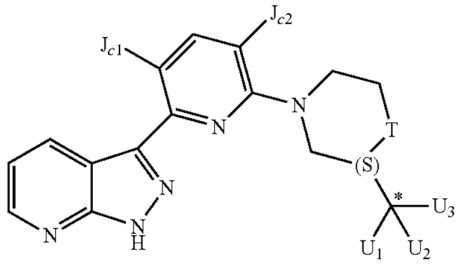


5



and .

[0274] The present invention also contemplates pyrazolopyridine compounds as described for example by Jimenez *et al.* in International Publication WO2011/094273 and US Publication No. 2013/0053395, each of which is incorporated herein by reference in its entirety. Illustrative derivatives of this type include compounds according to formula (XV):



(XV)

[0275] or a pharmaceutically acceptable salt thereof,

[0276] wherein:

[0277] T is –NH– or absent;

[0278] each J_{c1} and J_{c2} is independently –CN, –F, –Cl, –OR, $–CH_2OR$, or $–CF_3$;

[0279] each U_1, U_2, and U_3 is independently –H, Z, or J_b wherein no more than one of U_1, U_2, and U_3 is –H; or two of U_1, U_2, and U_3 join together to form a C_{1-6} cycloalkyl ring having 0-1 heteroatoms optionally and independently substituted with one or more J_e;

[0280] Z is Y2-Q2;

[0281] Y2 is absent or C_{1-6} alkyl optionally and independently substituted with one or more J_d.

[0282] Q2 is absent or C_{3-8} cycloalkyl having 0-1 heteroatoms optionally and independently substituted with one or more J_e, wherein Y2 and Q2 are not both absent;

[0283] each J_b is independently –F, –OR, –CN, $–CF_3$, $–N(R)_2$, $–C(O)N(R)_2$, C_{1-6} alkyl optionally and independently substituted with one or more J_a;

[0284] each J_a is independently –F, –OR, $–N(R)_2$, or $–C(O)N(R)_2$;

[0285] each J_d is independently –OR, –CN, $–C(O)N(R)_2$, $–N(R)_2$ or F;

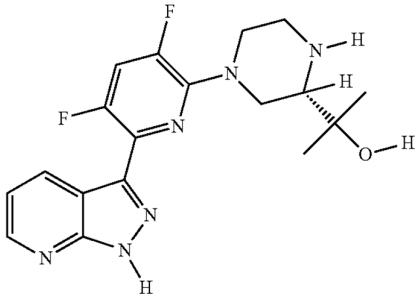
[0286] each J_e is independently C_{1-6} alkyl, –OR, $–N(R)_2$, $–CF_3$, or F; and

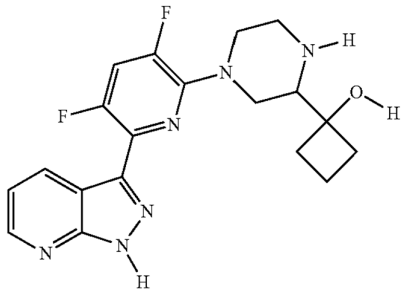
[0287] each R is –H or C_{1-6} alkyl.

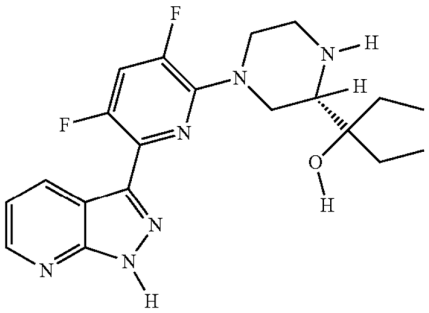
[0288] In some embodiments there is an achiral center at the carbon indicated by *

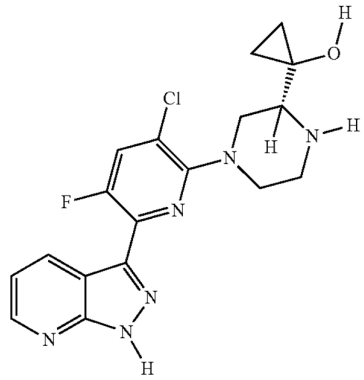
[0289] In representative compounds according to formula (XV): U_1 is Z and U_3 is J_b; and/or U_1 and U_2 are Z and U_3 is J_b; and/or Y_2 is C_1-C_3 alkyl optionally and independently substituted with one or more J_d, Q2 is absent or C_3-C_6 alkyl optionally and independently substituted with one or more J_e, and each J_d is independently -OR, or F; and/or J_b is -OH or $-NH_2$; and/or each J_{c1} and J_{C2} is independently -CF3, -CN, -F, or –Cl, or J_{c1} is F and J_{c2} is CI; or J_{c1} is Cl and J_{c2} is F.

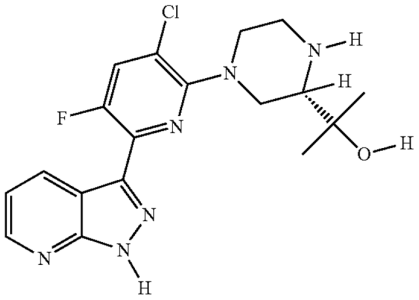
[0290] Non-limiting examples of compounds according to formula (XV) include compounds represented by the following structures:

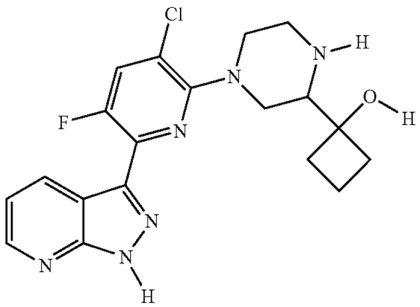


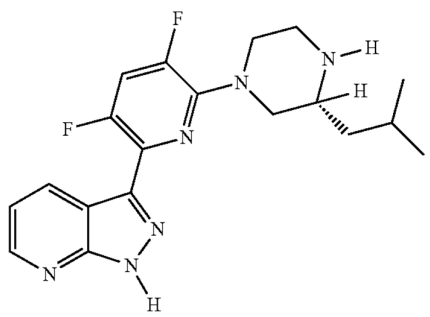


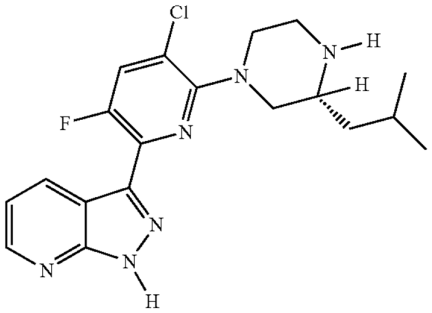


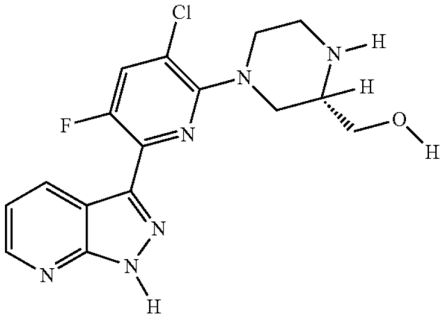


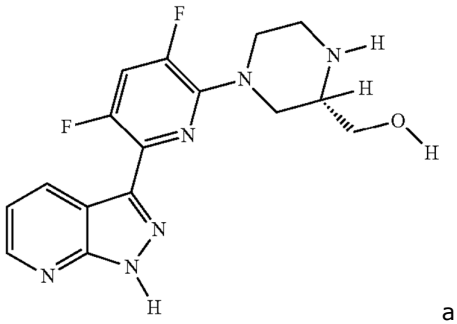




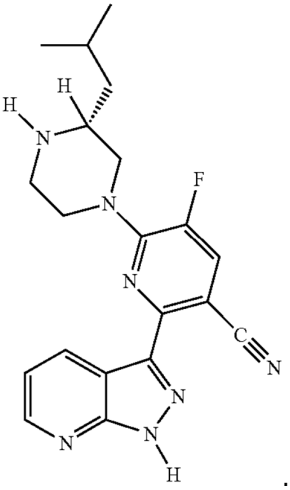






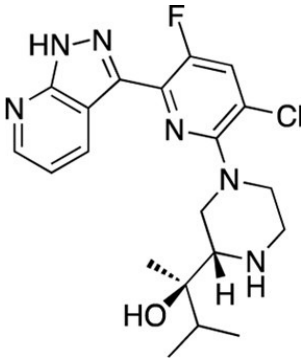


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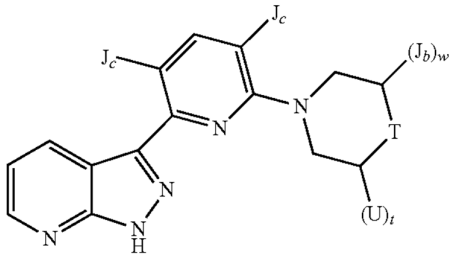
[0291] In specific embodiments, the pyrazolopyridine compound of formula (XV) is represented by the formula (XV1):



(XV1)

[0292] This compound is designated in Jimenez *et al.* (2013, *J. Med. Chem.* 56 1799-180) as (*R*)-2-((*S*)-4-(3-chloro-5-fluoro-6-(1H-pyrazolo[3,4-*b*]pyridin-3-yl)pyridin-2-yl)piperazin-2-yl)-3-methylbutan-2-ol or Compound 27 (also referred to herein as "C27").

[0293] In still other embodiments, small molecule PKC-θ inhibitors are selected from pyrazolopyridine compounds as described for example by Boyall *et al.* in US Publication No. 2012/0071494, which is incorporated herein by reference in its entirety. Non-limiting compounds of this type are represented by formula (XVa):



(XVa)

[0294] or a pharmaceutically acceptable salt thereof,

[0295] wherein:

[0296] t is 0, 1, or 2;

[0297] w is 0 or 1;

[0298] each J_c is independently –CN, –F, –Cl, –OR, $–CH_2OR$, or $–CF_3$;

[0299] U is Z or J_b;

[0300] Z is Y2-Q2;

[0301] Y2 is absent or C_{1-6} alkyl optionally and independently substituted with one or more J_d;

[0302] Q2 is absent or C_{3-8} cycloalkyl having 0-1 heteroatoms optionally and independently substituted with one or more J_e, wherein Y2 and Q2 are not both absent;

[0303] each J_b is independently –F, –OR, –CN, $–CF_3$, $–N(R)_2$, $–C(O)N(R)_2$, C_{1-6} alkyl optionally and independently substituted with one or more J_a;

[0304] each J_a is independently –F, –OR, $–N(R)_2$, or $–C(O)N(R)_2$;

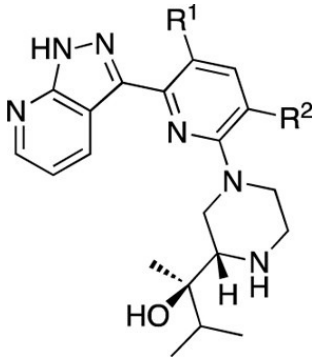
[0305] each J_d is independently –OR, –CN, $–C(O)N(R)_2$, $–N(R)_2$ or F;

[0306] each J_e is independently –OR, $–CF_3$, $–N(R)_2$, or F;

[0307] T is $–CH_2–$, $-CH(J_b)–$, $–C(J_b)_2-$, –NH– or $–N(J_b)–$; and

[0308] each R is –H or C_{1-6} alkyl.

[0309] In specific embodiments, compounds according to formula XVa are represented by formula XVa1, as disclosed for example by Jimenez *et al.* (2013, *J. Med. Chem.* 56 1799-180), which is incorporated herein by reference in its entirety:



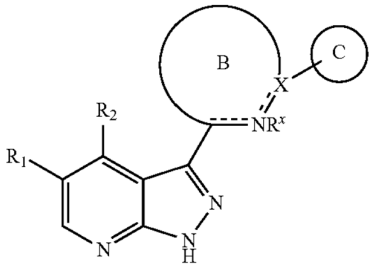
(XVa1)

[0310] wherein:

[0311] R^1 is independently F, Cl or CF_3; and

[0312] R^2 is independently H, F, Cl, OH, CN or CH_2OH.

[0313] In still other embodiments, small molecule PKC-θ inhibitors are selected from tri-cyclic pyrazolopyridine compounds as described for example by Brenchley *et al.* in US Publication No. 2012/0184534, which is incorporated herein by reference in its entirety. Non-limiting compounds of this type are represented by formula (XVI):



(XVI)

[0314] or a pharmaceutically acceptable salt thereof,

[0315] wherein:

[0316] R_1 is –H, halogen, –OR', $–N(R')_2$, –C(O)OR', $–C(O)N(R')_2$, –NR'C(O)R', NR'C(O)OR', –CN, $–NO_2$, C_{1-10} aliphatic optionally and independently substituted with one or more J_a, or C_{3-8} cycloaliphatic optionally and independently substituted with one or more J_b.

[0317] R_2 is –H, halogen, –CN, $–NO_2$, –OR', $–N(R')_2$, –C(O)OR', $–C(O)N(R')_2$, –NR'C(O)R', –NR'C(O)OR', C_{1-10} aliphatic optionally and independently substituted with one or more J_a, or C_{3-8} cycloaliphatic optionally and independently substituted with one or more J_b.

[0318] X is –C– or –N–.

[0319] R^x is absent or –H.

[0320] Ring B is a 5-membered monocyclic heteroaromatic ring optionally fused to an aromatic or non-aromatic ring; and ring B is optionally substituted with one Y and independently further optionally and independently substituted with one or more J_c.

[0321] Y is –Y1-Q1.

[0322] Y1 is absent, or C_{1-10} aliphatic, wherein up to three methylene units of Y1 are optionally and independently replaced with G' wherein G' is –O–, –C(O)–, –N(R')–, or $–S(O)_p–$; and Y1 is optionally and independently substituted with one or more J_d.

[0323] Q1 is absent, or a C_{3-8} membered saturated, partially unsaturated, or fully unsaturated monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and Q1 is optionally and independently substituted with one or more J_b; wherein Y1 and Q1 are not both absent.

[0324] Ring C is a 3-8-membered saturated, partially unsaturated, or fully unsaturated monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an 8-12 membered saturated, partially unsaturated, or fully unsaturated bicyclic ring system having 0-5 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and ring C is optionally substituted with one Z and independently further optionally and independently substituted with one or more J_b.

[0325] Z is –Y2-Q2.

[0326] Y2 is absent, or C_{1-10} aliphatic, wherein up to three methylene units of Y2 are optionally and independently replaced with G' wherein G' is –O–, –C(O)–, –N(R')–, or $–S(O)_p–$; and Y2 is optionally and independently substituted with one or more J_d.

[0327] Q2 is absent, C_{3-8} membered saturated, partially unsaturated, or fully unsaturated monocyclic ring having 0-3 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or an 8-12 membered saturated, partially unsaturated, or fully unsaturated bicyclic ring system having 0-5 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and Q2 is optionally and independently substituted with one or more J_e; wherein Y2 and Q2 are not both absent.

[0328] Each R' is independently –H, or C_{1-6} alkyl optionally and independently substituted with one or more J_a.

[0329] Each J_a is independently halogen, –OR, $–N(R)_2$, –C(O)OR, $–C(O)N(R)_2$, –NRC(O)R, –NRC(O)OR, –CN, $–NO_2$, or oxo.

[0330] Each J_b is independently halogen, –OR, $–N(R)_2$, –C(O)OR, $–C(O)N(R)_2$, –NRC(O)R, –NRC(O)OR, –CN, $–NO_2$, oxo, or C1-C6 alkyl optionally and independently substituted with J_a.

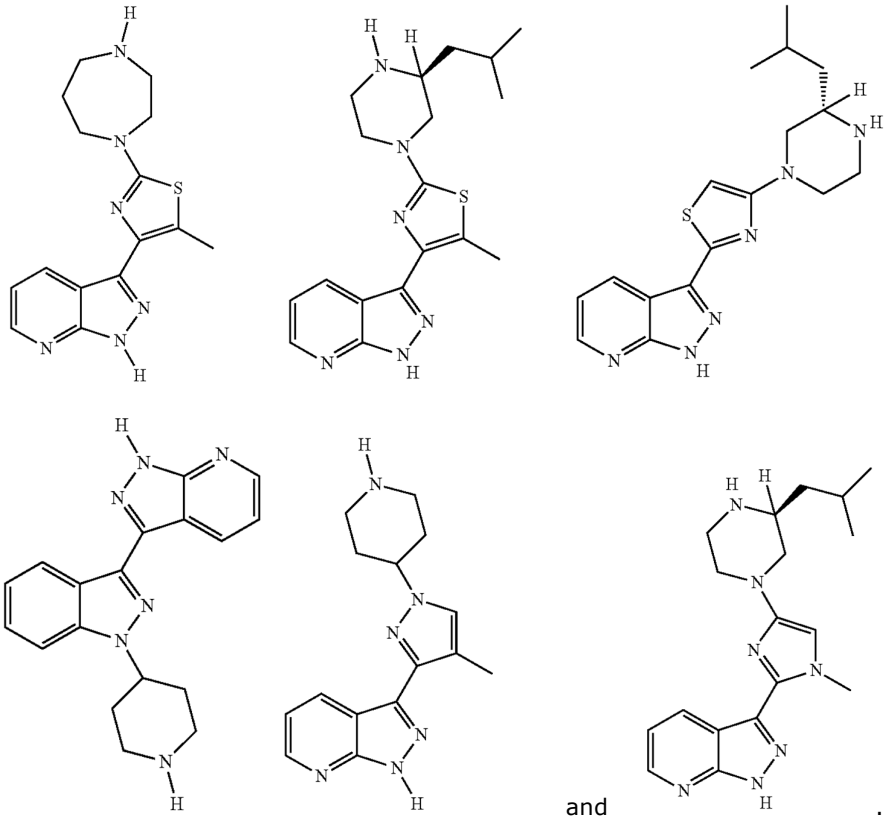
[0331] Each J_c is independently halogen, –OR', $–N(R')_2$, –C(O)OR', $–C(O)N(R')_2$, –NR'C(O)R', –NR'C(O)OR', –CN, $–NO_2$, or C1-C10 aliphatic optionally and independently substituted with one or more J_a, or C3-C8 cycloaliphatic optionally and independently substituted with one or more J_b.

[0332] Each J_d is independently halogen, –CN, or $–NO_2$. Each J_e is independently halogen, –CN, $–NO_2$, oxo, C1-10 aliphatic, wherein up to three methylene units are optionally and independently replaced with G' wherein G' is –O–, –C(O)–, –N(R')–, or $–S(O)_p–$ and the aliphatic group is optionally and independently substituted with one or more J_d, or J_e is C_{3-8} cycloaliphatic optionally and independently substituted with one or more J_b.

[0333] Each R is independently –H or C_{1-6} alkyl.

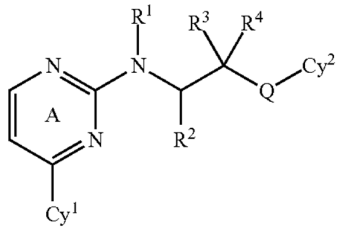
[0334] Each p is independently 0, 1, or 2.

[0335] Representative examples of compounds according to formula (XVI) include:



and .

[0336] Still other embodiments of small molecule PKC-θ inhibitors include 2-(amino-substituted)-4-aryl pyrimidine compounds as described for example by Fleming *et al.* in US Publication No. 2011/0071134, which is incorporated herein by reference in its entirety. Representative compounds of this type are represented by formula (XVII):



(XVII)

[0337] or a pharmaceutically acceptable salt thereof,

[0338] wherein:

[0339] R^1 and R^2 are each independently H, C_{1-3} alkyl or C_3-5cycloalkyl;

[0340] R^3 is H or F;

[0341] R^4 is H, F, $-OR^a$, $-C(O)R^a$, $-C(O)OR^a$ or $-N(R^a)_2$; or R^3 and R^4 together with the carbon atom to which they are attached form a carbonyl group; wherein each occurrence of R^a is independently H, C_{1-3}alkyl or C_3-5cycloalkyl;

[0342] Ring A is optionally substituted with 1 or 2 independent occurrences of R^5, wherein each R^5 is independently selected from halo, C_{1-4} aliphatic, $-CN$, $-OR^b$, $-SR^C$, $-N(R^b)_2$, $-NR^bC(O)R^b$, $-NR^bC(O)N(R^b)_2$, $-NR^bCO_2R^C$, $-CO_2R^b$, $-C(O)R^b$, $-C(O)N(R^b)_2$, $-OC(O)N(R^b)_2$, $-S(O)_2R^C$, $-SO_2N(R^b)_2$, $-S(O)R^C$, $-NR^bSO_2N(R^b)_2$, $-NR^bSO_2R^C$, or C_{1-4}aliphatic optionally substituted with halo,

–CN, –OR^b, –SR^C, –$N(R^b)_2$, $NR^bC(O)R^b$, –$NR^bC(O)N(R^b)_2$, –$NR^bCO_2R^C$, –CO_2R^b, –$C(O)R^b$, –$C(O)N(R^b)_2$, –$OC(O)N(R^b)_2$, –$S(O)_2R^C$, –$SO_2N(R^b)_2$, –$S(O)R^C$, –$NR^bSO_2N(R^b)_2$, or –$NR^bSO_2R^C$, wherein each occurrence of R^b is independently H or C_{1-4}aliphatic; or two R^b on the same nitrogen atom taken together with the nitrogen atom form a 5-8 membered aromatic or non-aromatic ring having in addition to the nitrogen atom 0-2 ring heteroatoms selected from N, O or S; and each occurrence of R^C is independently C_{1-4} aliphatic;

[0343] Cy^1 is selected from: a) a 6-membered aryl or heteroaryl ring substituted by one occurrence of W at the meta or para position of the ring; or b) a 5-membered heteroaryl ring substituted by one occurrence of W;

[0344] wherein Cy^1 is optionally further substituted by one to three independent occurrences of R^6, wherein each occurrence of R^6 is independently selected from -halo, C_{1-8} aliphatic, –CN, –OR^b, –SR^D, –$N(R^E)_2$, –$NR^EC(O)R^b$, –$NR^EC(O)N(R^E)_2$, –$NR^ECO_2R^D$, –CO_2R^b, –$C(O)R^b$, –$C(O)N(R^E)_2$, –$OC(O)N(R^E)_2$, –$S(O)_2R^D$, –$SO_2N(R^E)_2$, –$S(O)R^D$, –$NR^ESO_2N(R^E)_2$, –$NR^ESO_2R^D$, –$C(=NH)–N(R^E)_2$, or C_{1-8} aliphatic optionally substituted with halo, –CN, –OR^b, –SR^D, –$N(R^E)_2$, –$NR^EC(O)R^b$, –$NR^EC(O)N(R^E)_2$, –$NR^ECO_2R^D$, –CO_2R^b, –$C(O)R^b$, –$C(O)N(R^E)_2$, –$OC(O)N(R^E)_2$, –$S(O)_2R^D$, –$SO_2N(R^E)_2$, –$S(O)R^D$, –$NR^ESO_2N(R^E)_2$, –$NR^ESO_2R^D$, or –$C(=NH)–N(R^E)_2$, wherein each occurrence of R^D is C_{1-6} aliphatic and each occurrence of R^E is independently H, C_{1-6} aliphatic, –$C(=O)R^b$, –$C(O)OR^b$ or –SO_2R^b; or two R^E on the same nitrogen atom taken together with the nitrogen atom form a 5-8 membered aromatic or non-aromatic ring having in addition to the nitrogen atom 0-2 ring heteroatoms selected from N, O or S;

[0345] W is –R^8, V–R^8, L_1-R^7, V-L_1-R^7, L_1-V–R^8, or L_1-V-L_2-R^7; wherein: L_1 and L_2 are each independently an optionally substituted C_{1-6} alkylene chain; V is –CH_2–, –O–, –S–, –S(O)–, –$S(O)_2$–, –C(O)–, –CO_2–, –NR^E–$NR^EC(O)$–, –NR^ECO_2–, –NR^ESO_2–, –$C(O)N(R^b)$–, –$SO_2N(R^b)$–, –$NR^EC(O)N(R^b)$– or –OC(O)–; R^7 is H, halo, –OH, –$N(R^F)_2$, –CN, –OR^G, –$C(O)R^G$, –CO_2H, –CO_2R^G, –SR^G, –$S(O)R^G$, –$S(O)_2R^G$, –$N(R^E)C(O)R^G$, –$N(R^E)CO_2R^G$, –$N(R^E)SO_2R^G$, –$C(O)N(R^F)_2$, –$SO_2N(R^F)_2$, –$N(R^E)C(O)N(R^F)_2$, –$OC(O)R^F$ or an optionally substituted group selected from C_{1-10} aliphatic, C_{6-10}aryl, 3-14 membered heterocyclyl or 5-14 membered heteroaryl, wherein each occurrence of R^F is independently H, C_{1-6} aliphatic, C_{6-10}aryl, 3-14 membered heterocyclyl, 5-14 membered heteroaryl, –$C(=O)R^b$, –$C(O)OR^b$ or –SO_2R^b; or two R^F on the same nitrogen atom taken together with the nitrogen atom form an optionally substituted 5-8 membered aromatic or non-aromatic ring having in addition to the nitrogen atom 0-2 ring heteroatoms selected from N, O or S; and each occurrence of R^G is C_{1-6} aliphatic, C_{6-10}aryl, 3-14 membered heterocyclyl, or 5-14 membered heteroaryl; R^8 is an optionally substituted group selected from C_{1-10} aliphatic, C_{6-10} aryl, 3-14 membered heterocyclyl or 5-14 membered heteroaryl;

[0346] Q is a bond, CH_2 or C(=O);

[0347] Cy^2 is a C_{6-10} aryl, a 5-10 membered heteroaryl, or a 5-10 membered heterocyclyl ring, wherein each ring is optionally substituted by one to three independent occurrences of R^9 and one occurrence of R^{10},

[0348] wherein each occurrence of R^9 is independently selected from C_{1-4}aliphatic, –$N(R^b)_2$, halo, NO_2, –CN, –OR^b, –$C(O)R^a$, –CO_2R^a, –SR^C, –$S(O)R^C$, –$S(O)_2R^C$, –$OS(O)_2R^C$–, $N(R^b)C(O)R^a$, –$N(R^b)CO_2R^a$, –$N(R^b)SO_2R^a$, –$C(O)N(R^b)_2$, –$SO_2N(R^b)_2$, –$N(R^b)C(O)N(R^b)_2$, –$OC(O)R^a$, or C_{1-4} aliphatic optionally substituted by –$N(R^b)_2$, halo, NO_2, –CN, –OR^b, –$C(O)R^a$, –CO_2R^a, –SR^C, –

$S(O)R^C$, $-OS(O)_2R^C$, $-S(O)_2R^C$, $-N(R^b)C(O)R^a$, $-N(R^b)CO_2R^a$, $-N(R^b)SO_2R^a$, $-C(O)N(R^b)_2$, $-SO_2N(R^b)_2$, $-N(R^b)C(O)N(R^b)_2$, or $-OC(O)R^a$, and

[0349] R^{10} is selected from phenyl, or a 5-6 membered heterocyclyl or heteroaryl ring.

[0350] In certain embodiments, compounds of formula XVII are subject to one or more, or all of, the following limitations:

[0351] 1) when Cy^1 is phenyl substituted in the meta position with W then:

[0352] a) when W is –OMe, R^1, R^2, R^3, and R^4 are each hydrogen, and Q is a bond, then when ring A is further substituted with R^5, R^5 is a group other than $-CF_3$ or $-C(O)N(R^b)_2$; and

[0353] b) when W is –OMe, R^1, R^2, R^3, and R^4 are each hydrogen, and Q is $-CH_2-$, then Cy^2 is other than 1H-benzimidazol-1-yl;

[0354] 2) when Cy^1 is phenyl substituted in the para position with W, and R^1, R^2, R^3, and R^4 are each hydrogen then:

[0355] a) when Q is a bond, W is other than: i) $-CONH_2$; ii) $-CONHR^8$, where R^8 is an optionally substituted group selected from phenyl, -alkylphenyl, alkyl, or -alkylheterocycle; iii) $-CF_3$; iv) $-SO_2Me$; v) $-NH_2$; vi) -tBu; vii) $-CO_2H$ when Cy^2 is morpholine; viii) –O(phenyl) when Cy^2 is indole; and ix) –OMe;

[0356] b) when Q is $-CH_2-$, W is other than: i) $-CONH_2$, when Cy^2 is optionally substituted imidazole or benzimidazole; ii) $-CONHR^8$, where R^8 is an optionally substituted group selected from phenyl, -alkylphenyl, or -alkylheterocycle; iii) $-CF_3$; iv) $-SO_2Me$; v) –OH, where Cy^2 is a 5-10 membered heterocyclyl ring; vi) tBu, when Cy^2 is a 5-10 membered heterocyclyl ring; and vii) –OMe; and 3) when Cy^1 is a 5-membered heteroaryl ring then:

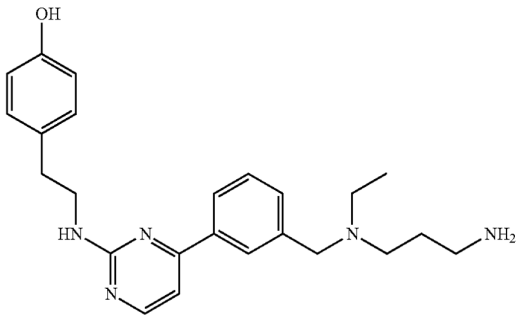
[0357] a) when Cy^1 is isoxazole, R^1, R^2, R^3, and R^4 are each hydrogen, Q is a bond, and W is p-fluoro-phenyl, then Cy^2 is a group other than pyridyl or N-pyrrolidinyl;

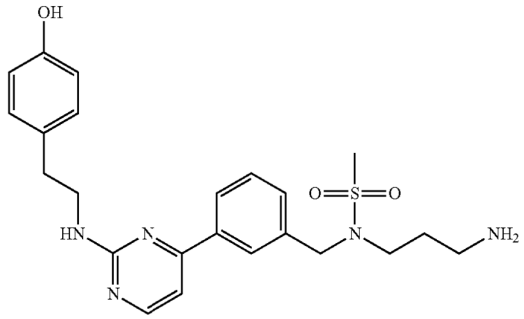
[0358] b) when Cy^1 is triazolyl, R^1, R^2, R^3, and R^4 are each hydrogen, Q is a bond, and W is $-(CH_2)_2N(cyclopentyl)C(O)CH_2(naphthyl)$, then Cy^2 is a group other than N-piperidinyl;

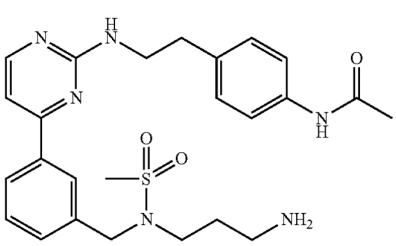
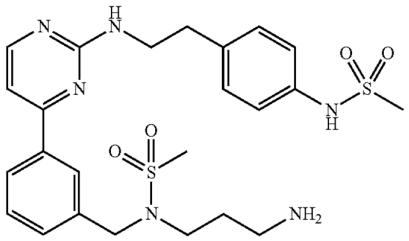
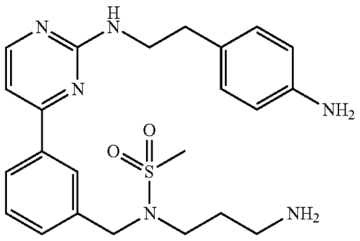
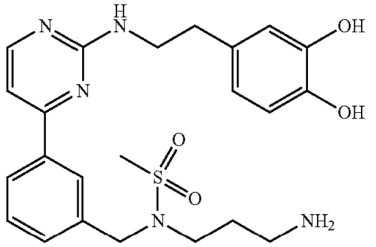
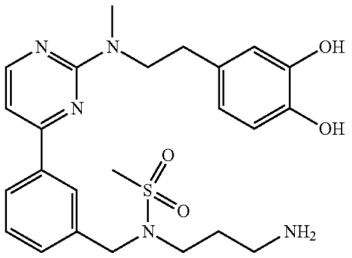
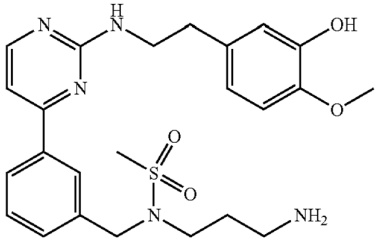
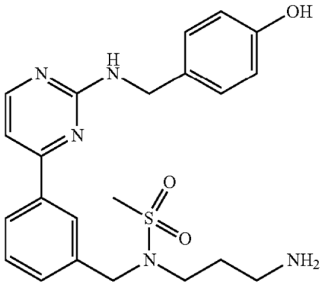
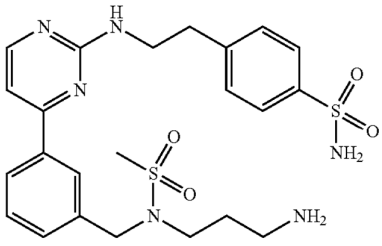
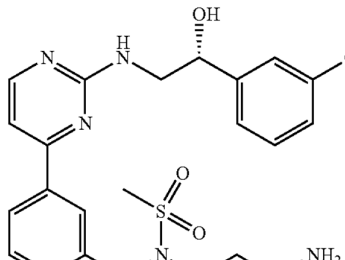
[0359] c) when Cy^1 is imidazolyl, R^1, R^2, R^3, and R^4 are each hydrogen, Q is a bond, and W is meta-CF_3-phenyl, then R^6 is a group other than $C(O)OCH_2CH_3$; and

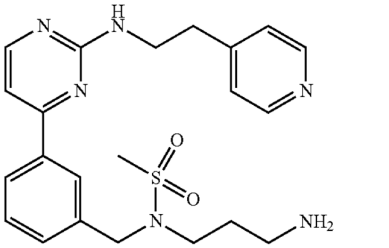
[0360] d) when Cy^1 is imidazol-5-yl and W is para-fluoro-phenyl, then R^6 is a group other than cyclohexyl.

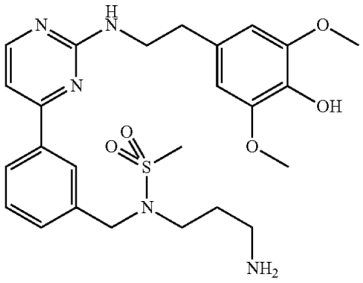
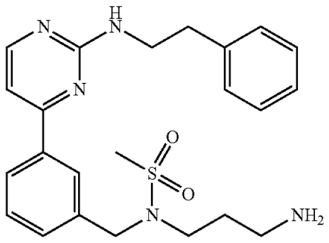
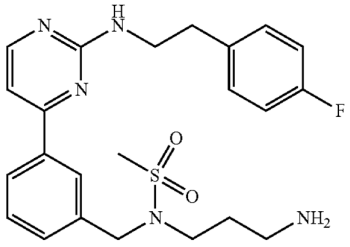
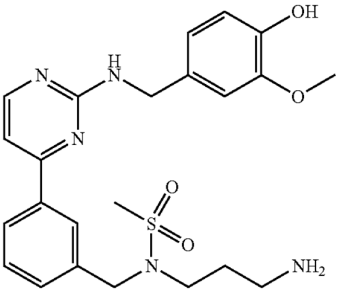
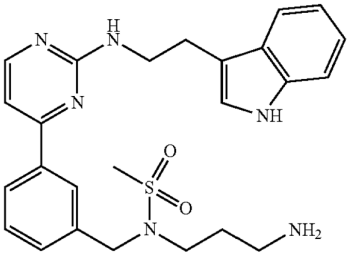
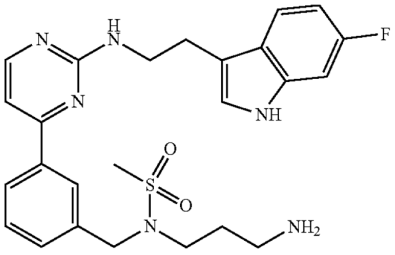
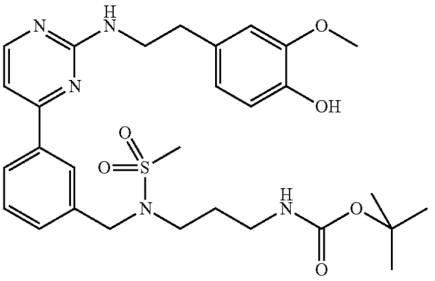
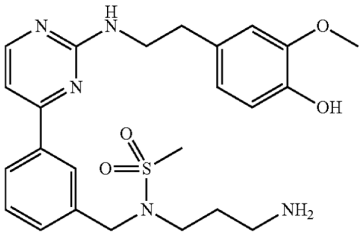
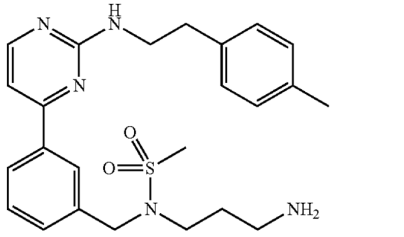
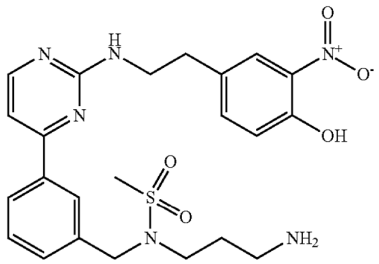
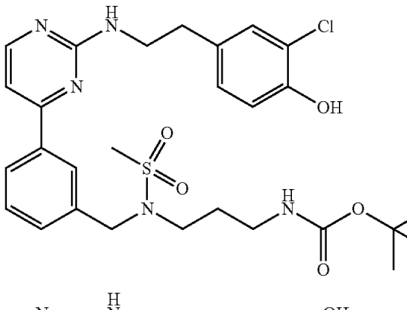
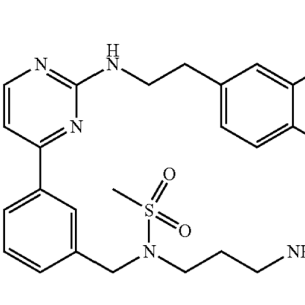
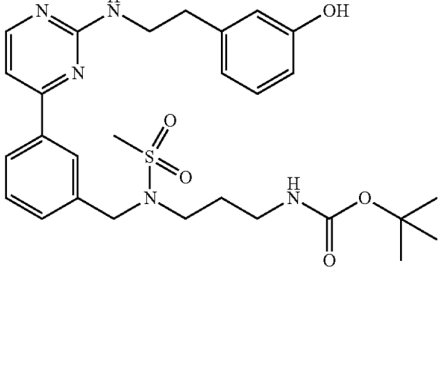
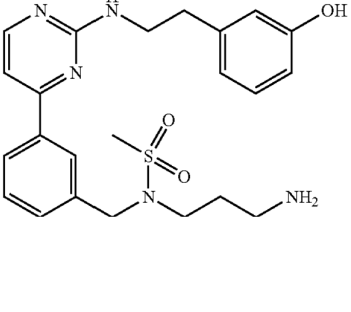
[0361] Non-limiting compounds of this type are represented by the following structures:



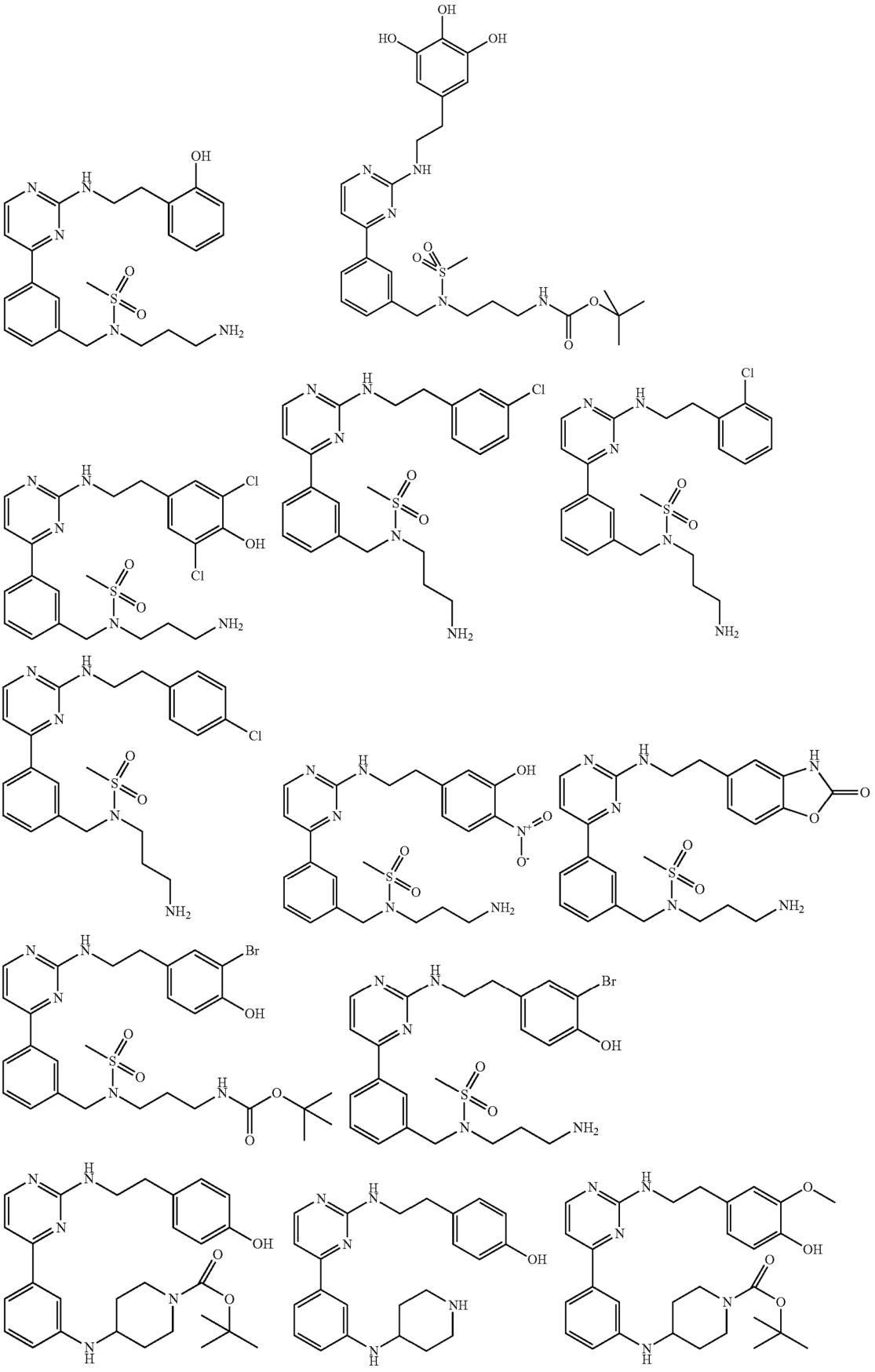


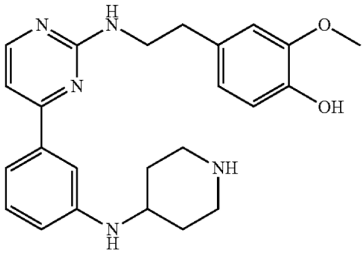










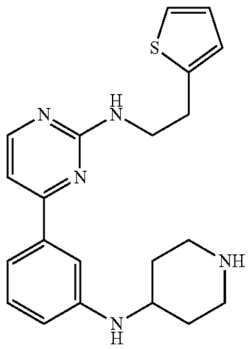

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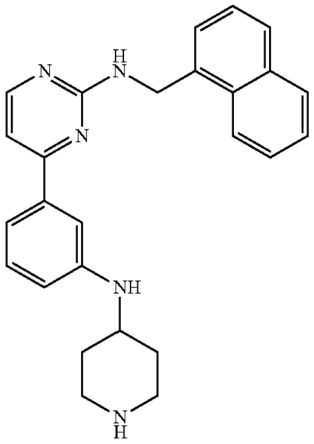















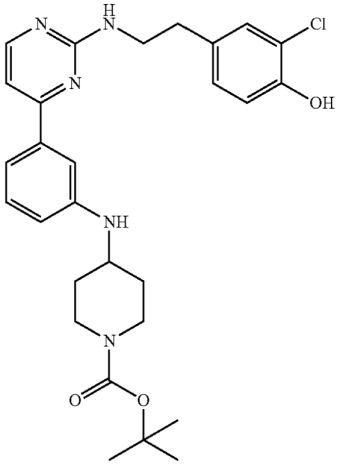
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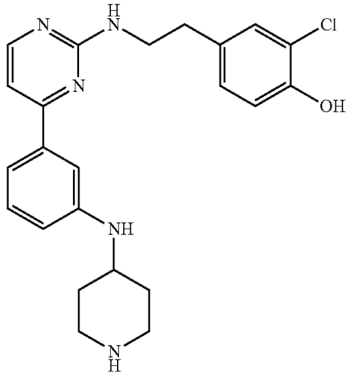


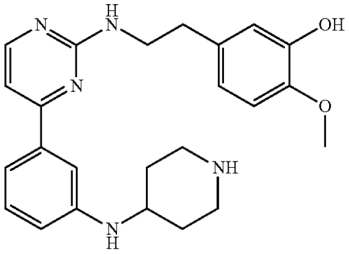


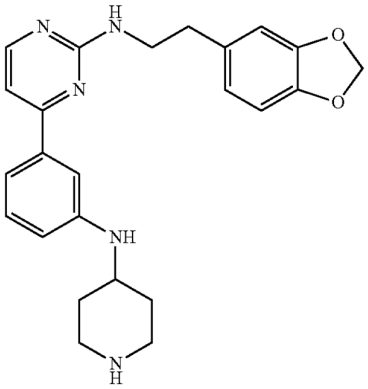


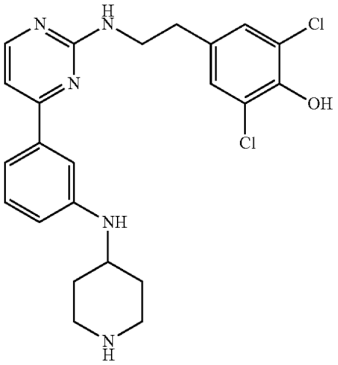


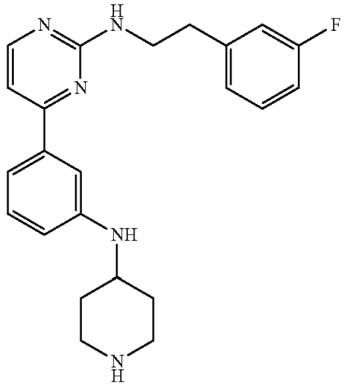




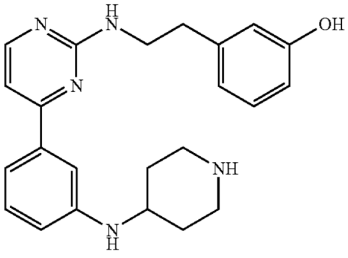


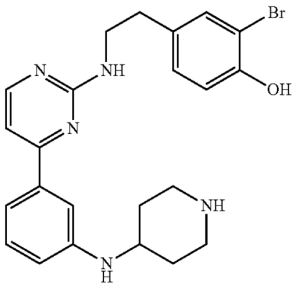


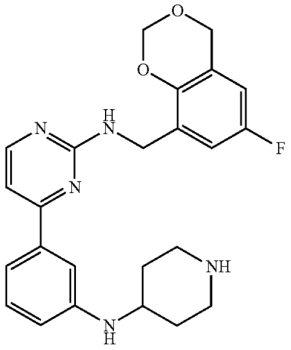


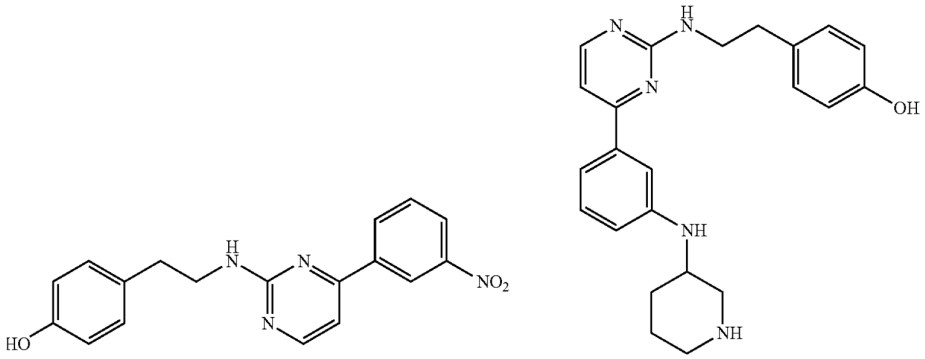


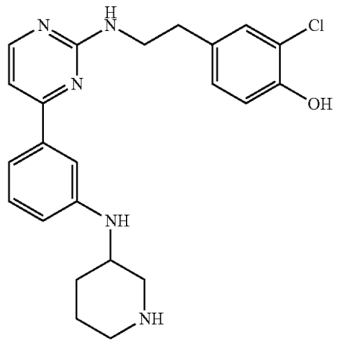
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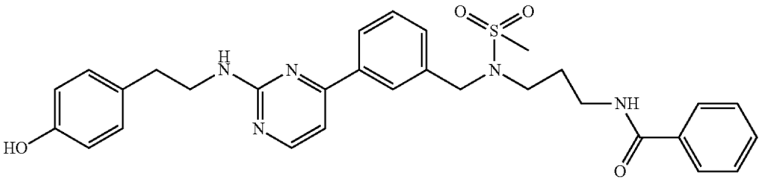


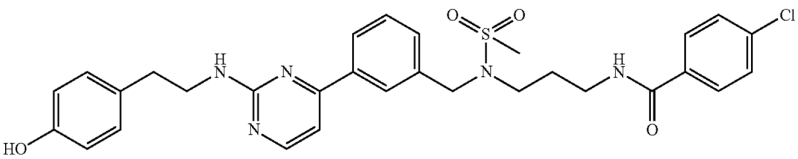


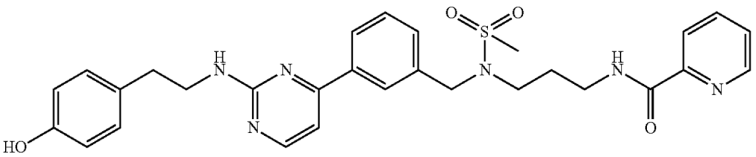


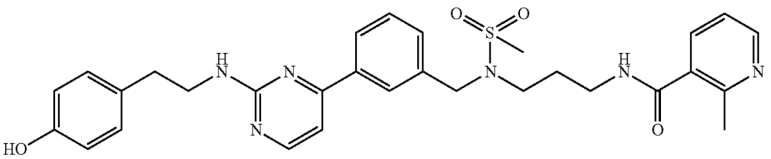


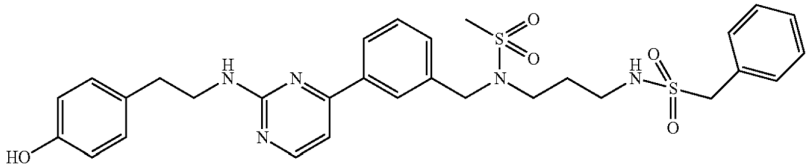


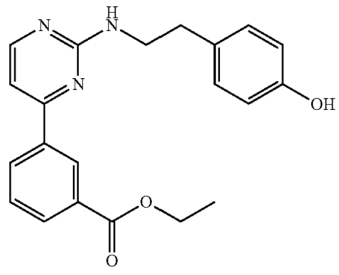


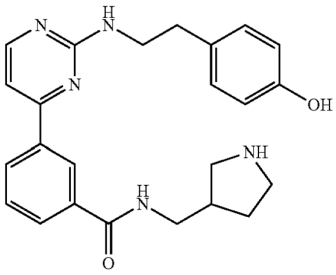


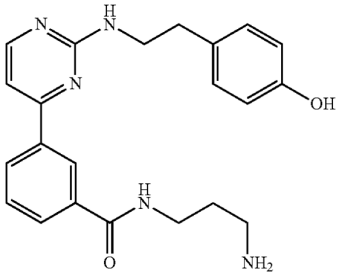




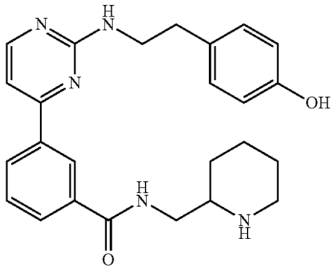


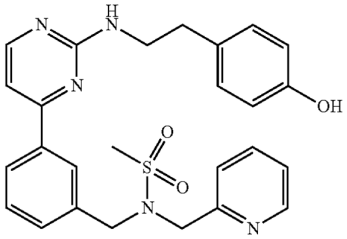


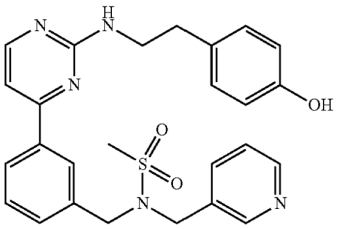


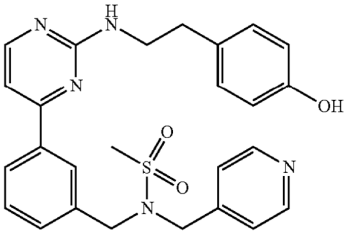


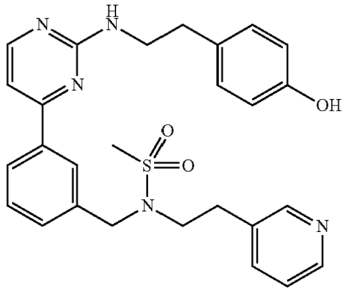
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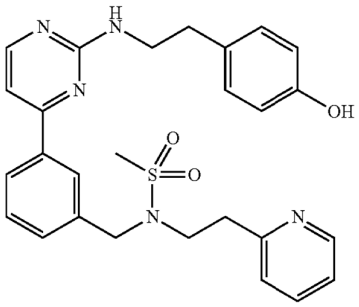


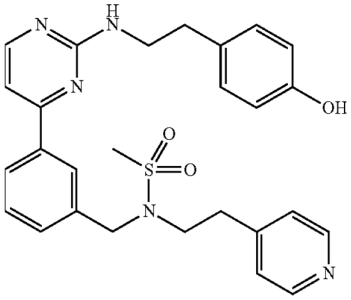


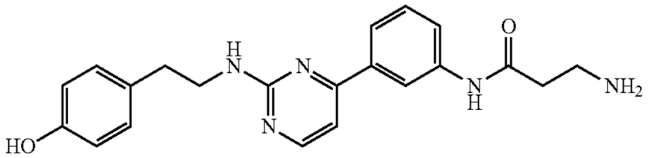




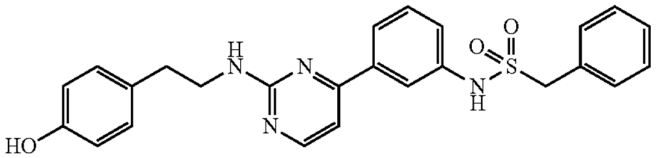


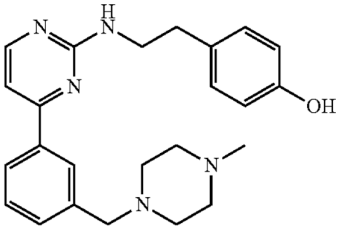


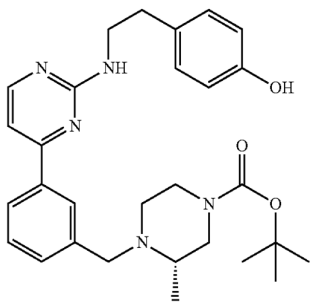


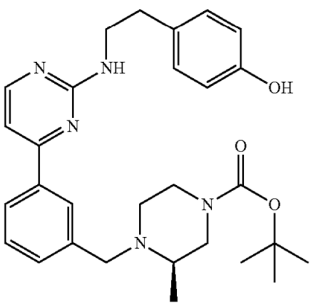


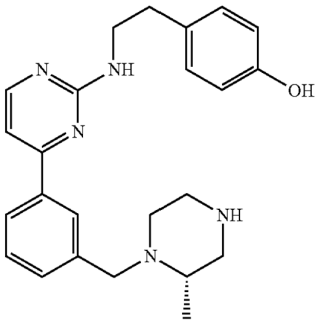
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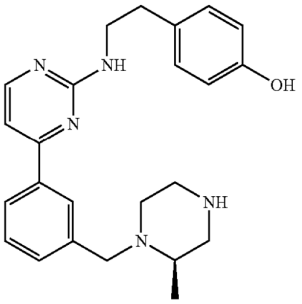


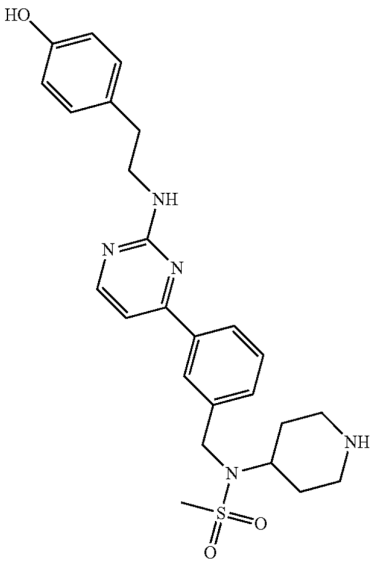


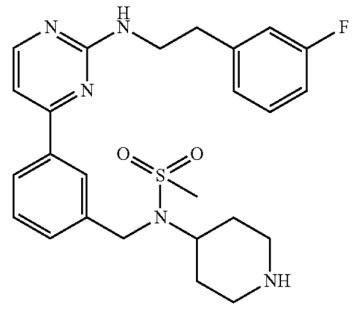


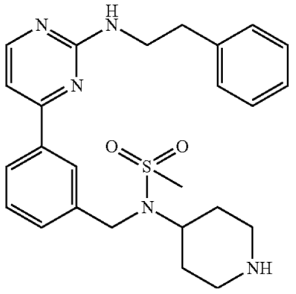


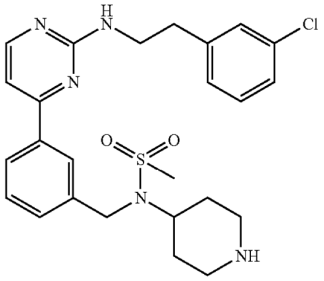


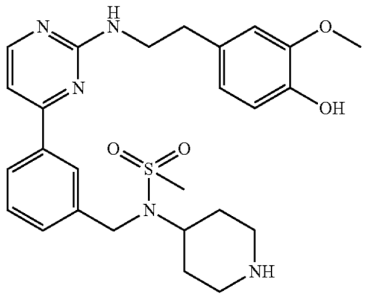


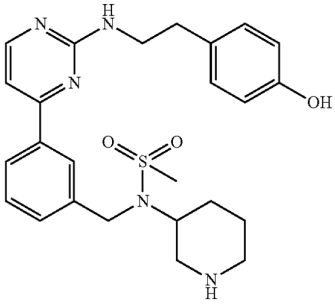


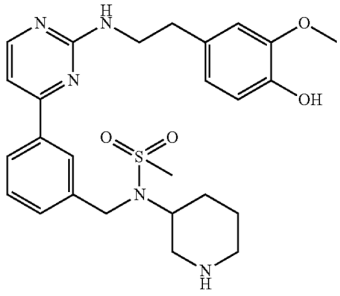


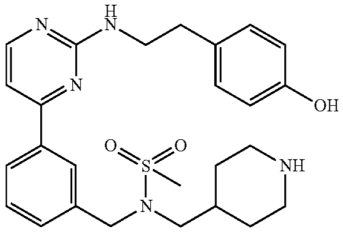


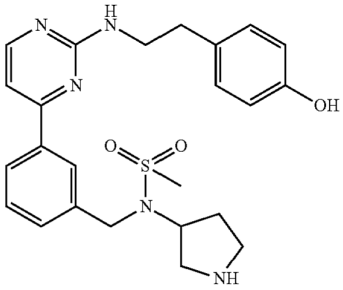


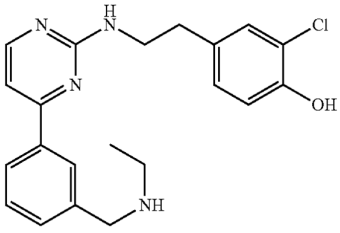


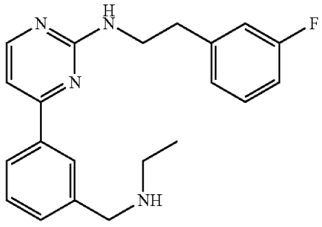


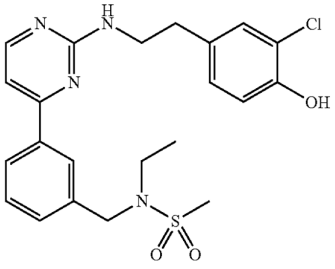


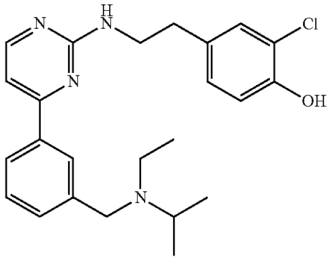


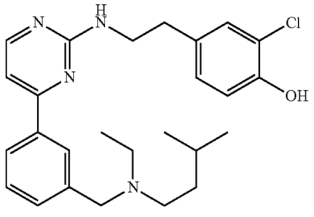




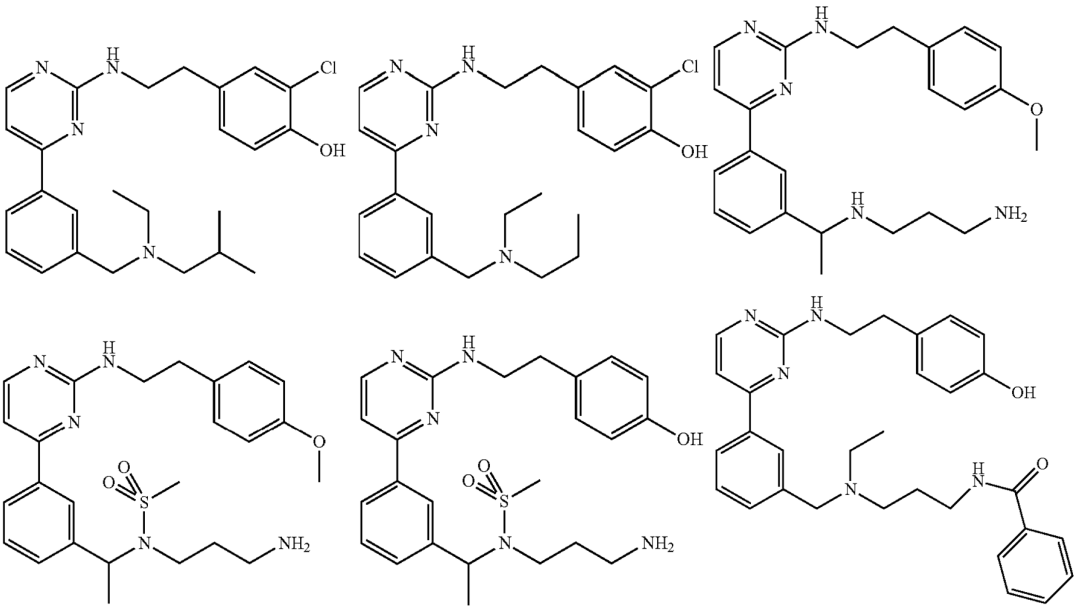


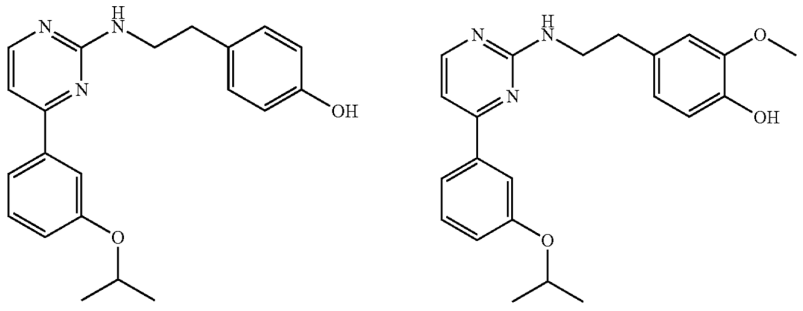


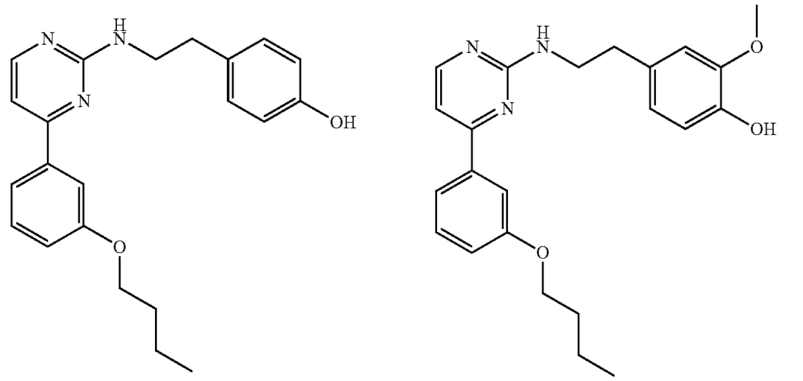




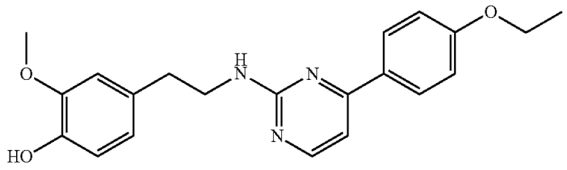
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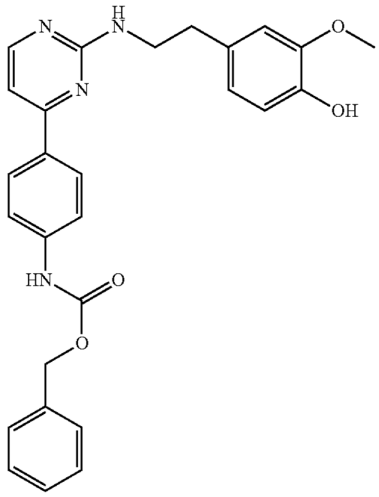


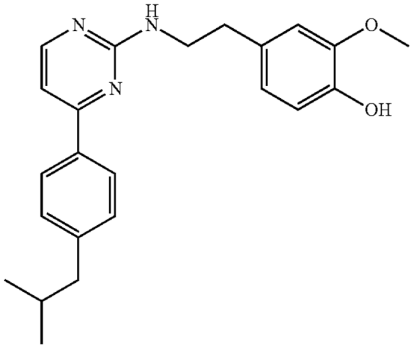


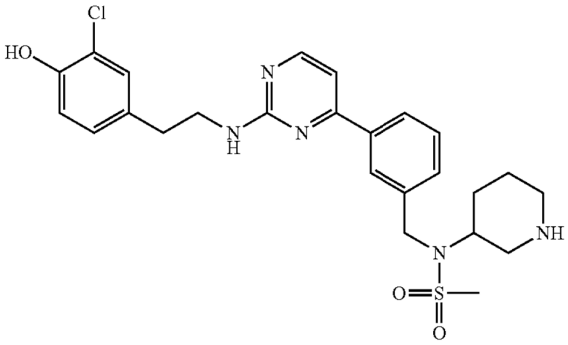


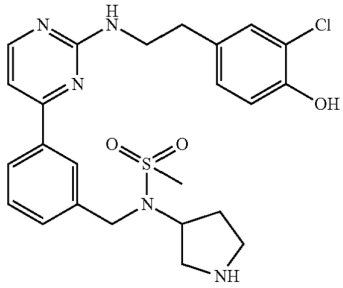
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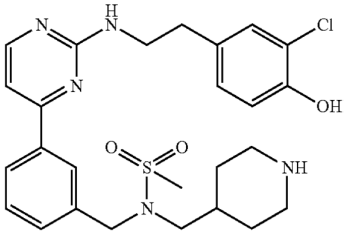


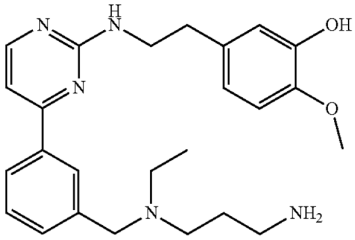


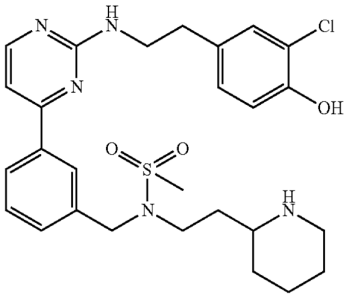


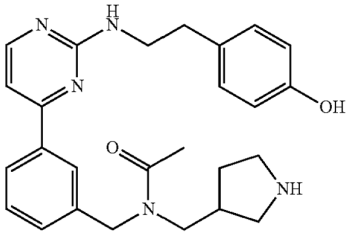


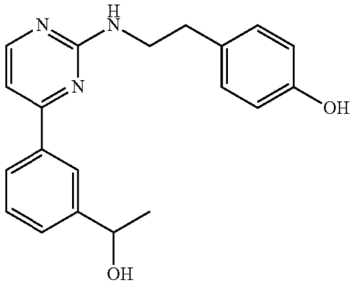


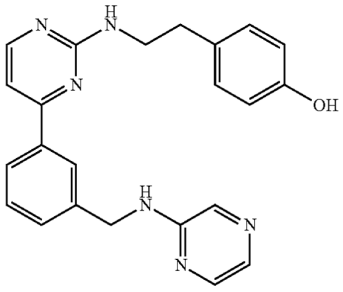


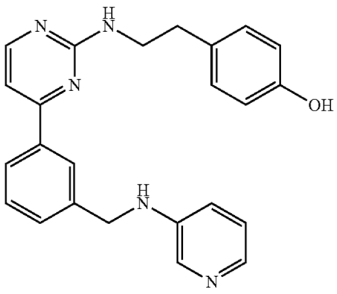


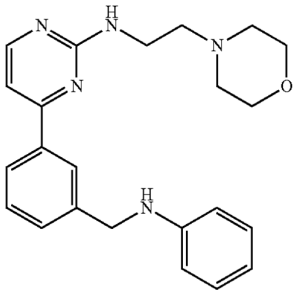


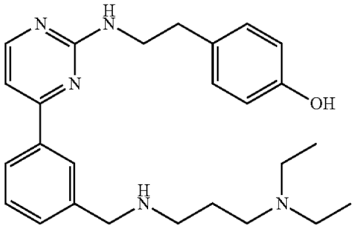




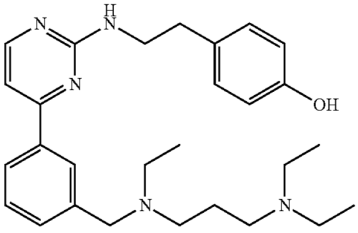
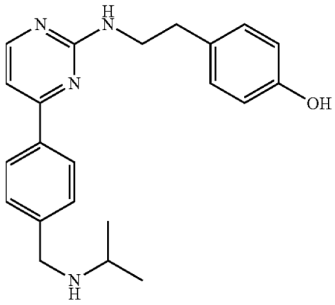
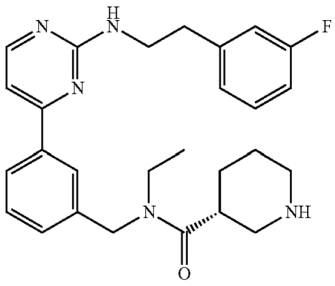
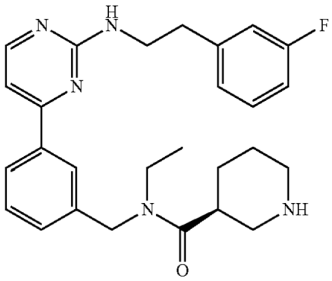
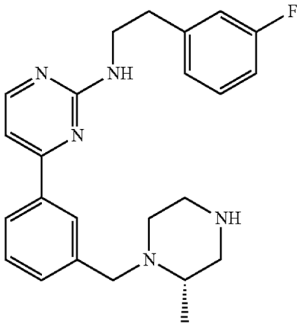
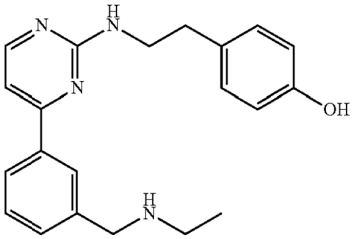
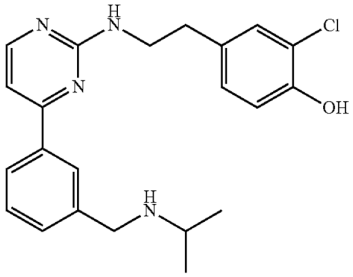
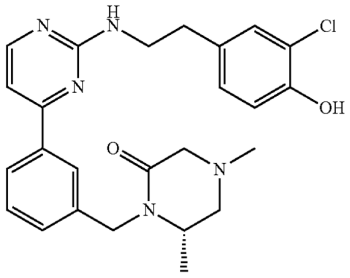
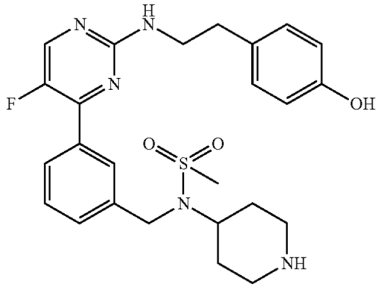
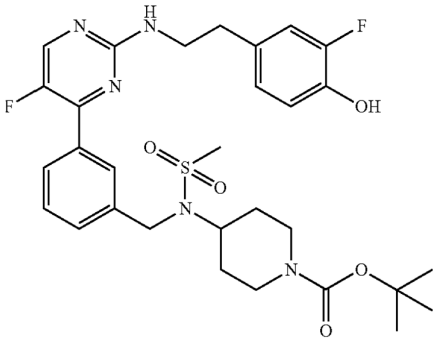
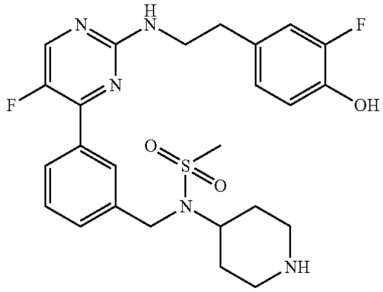
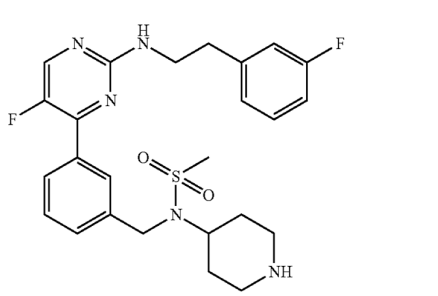


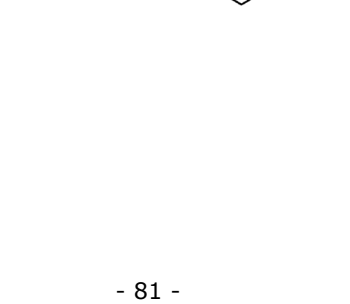




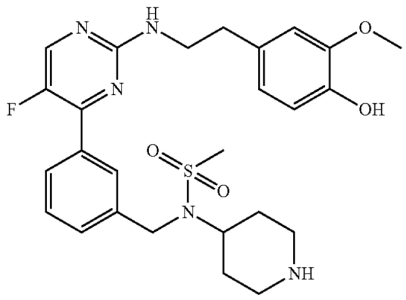
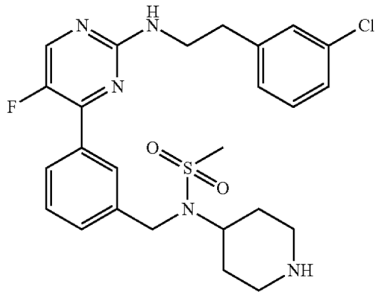
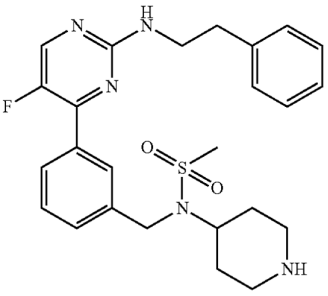
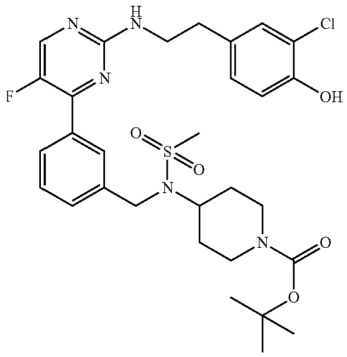
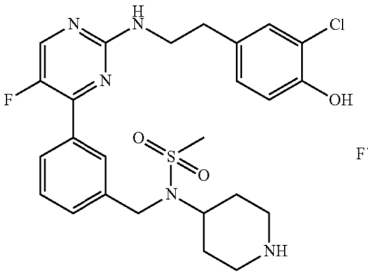
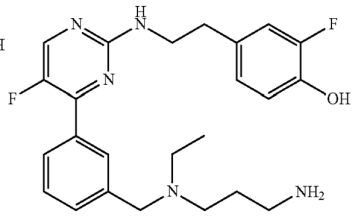
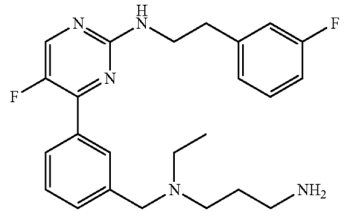
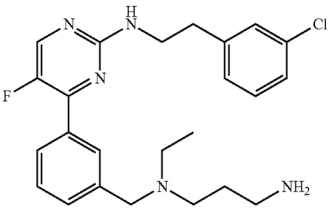
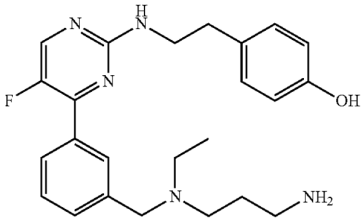
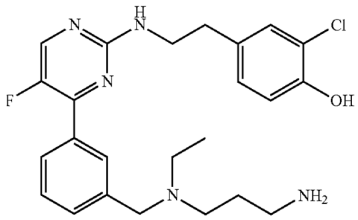
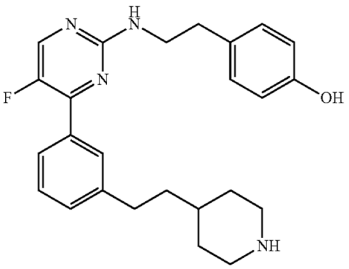
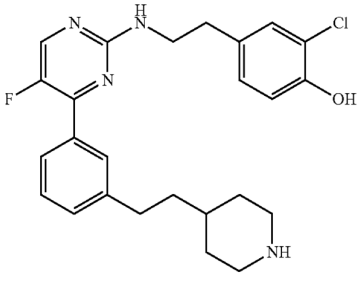
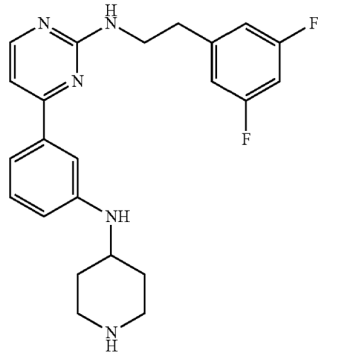
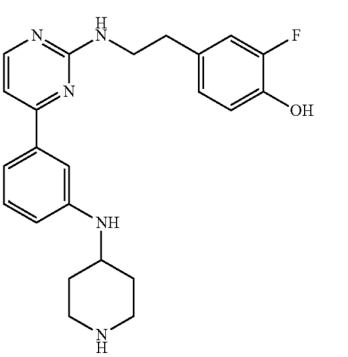
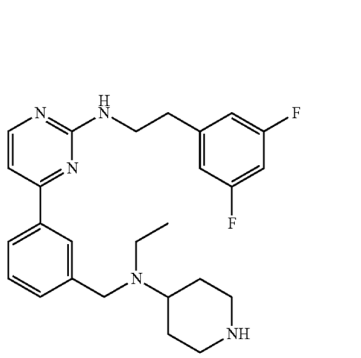


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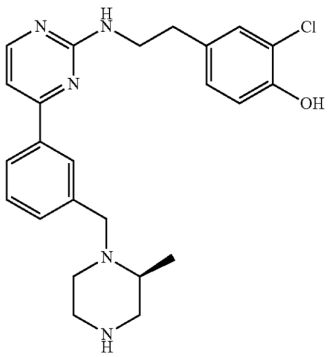
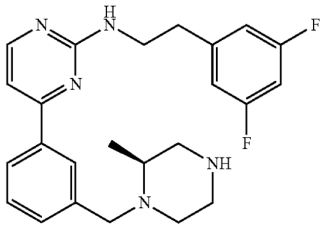
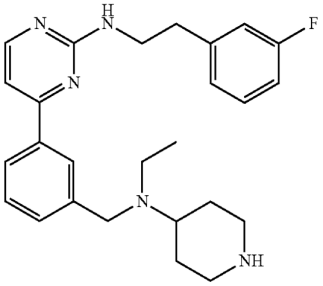
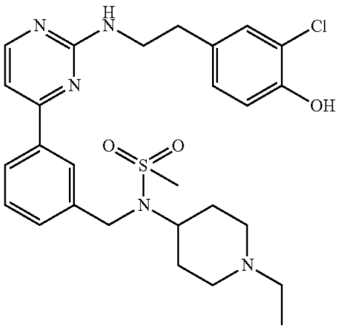
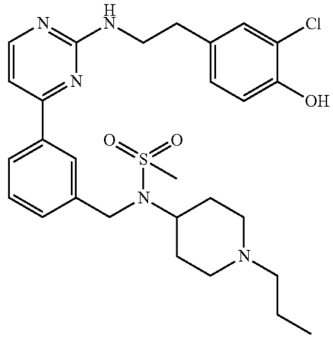
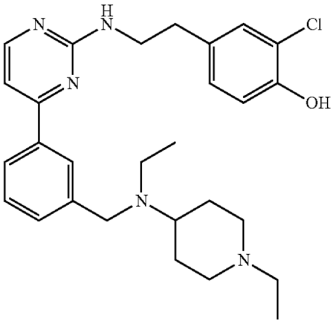
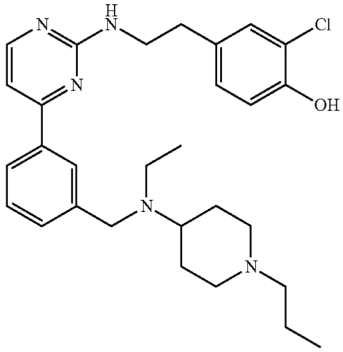
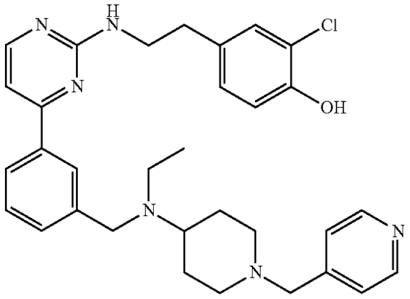
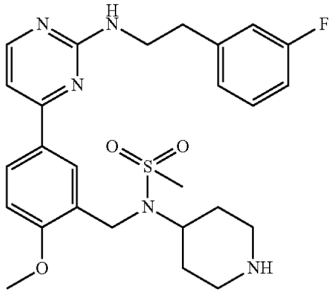
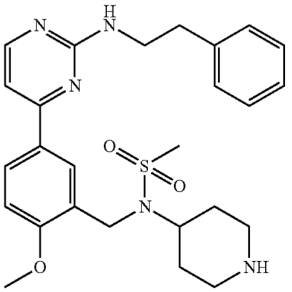
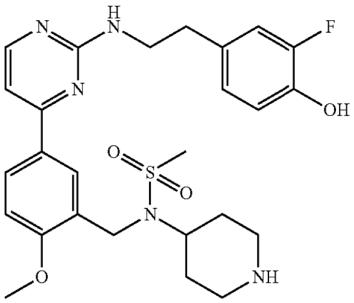
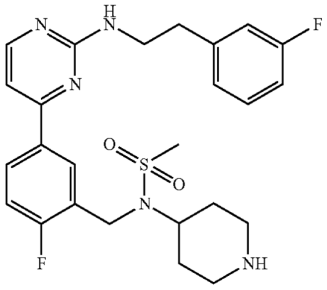
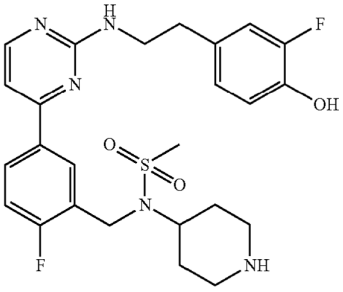
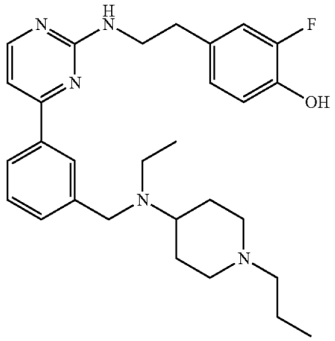
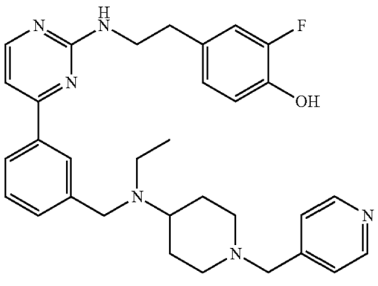


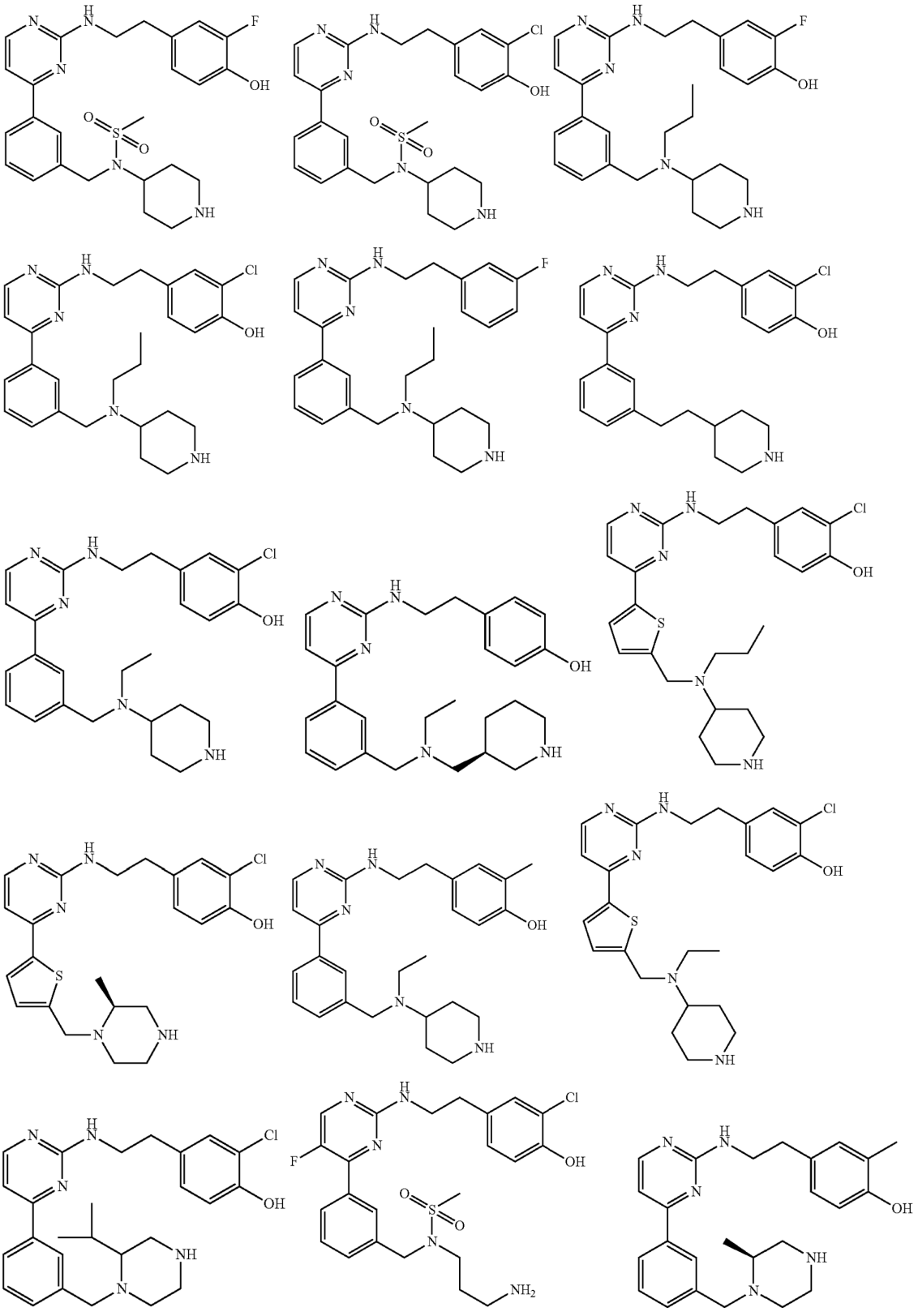
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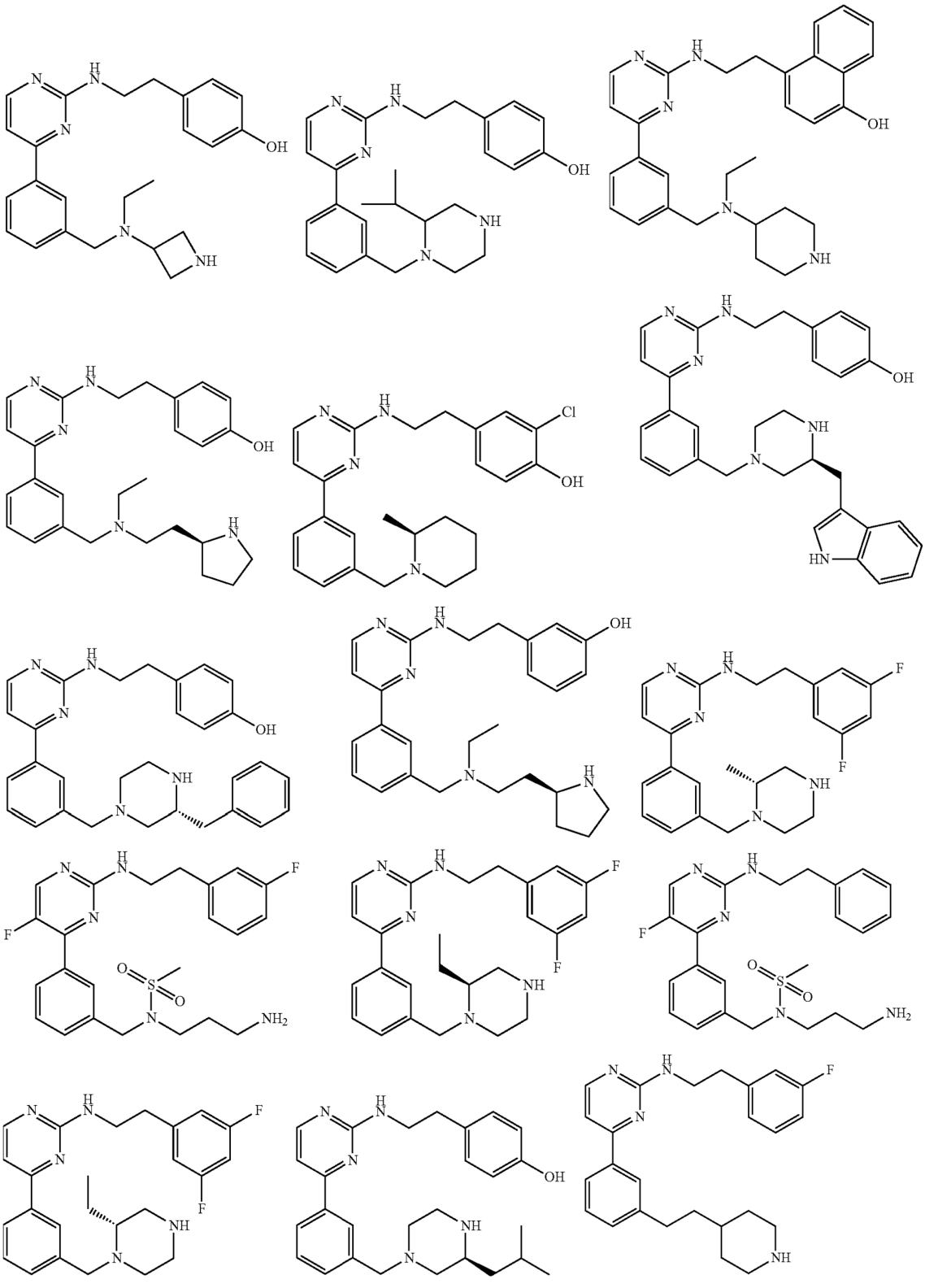
















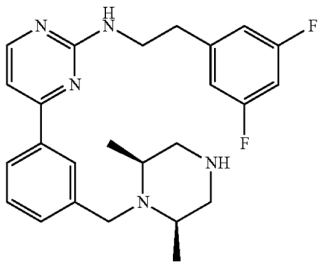
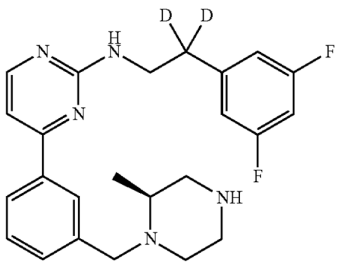
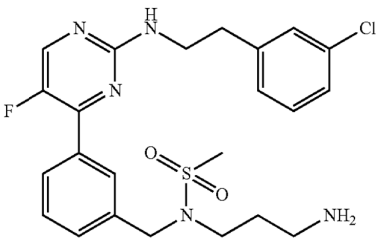
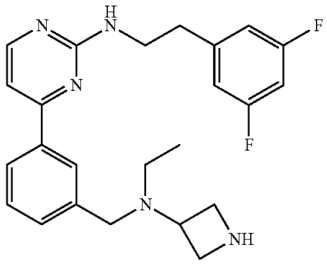
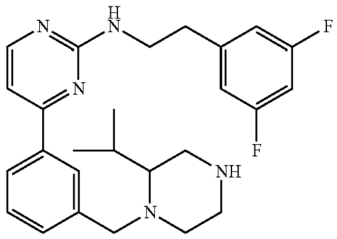
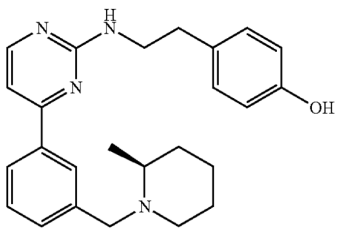
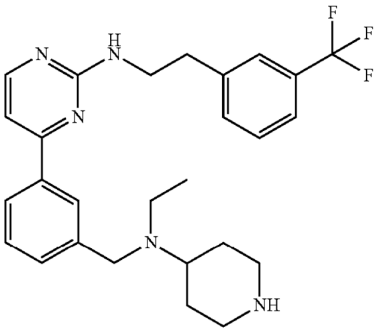
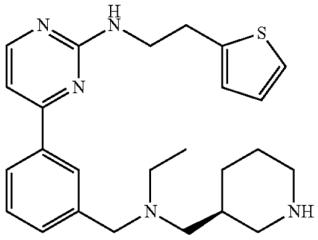
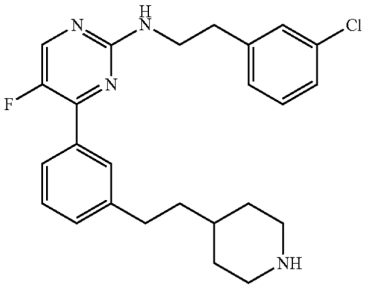
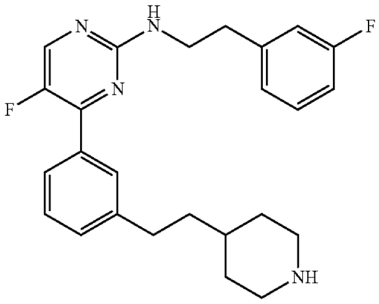
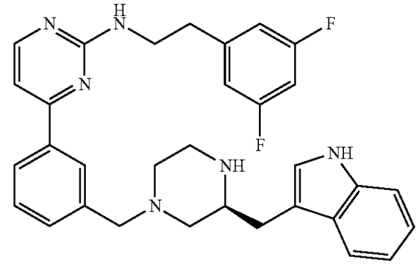
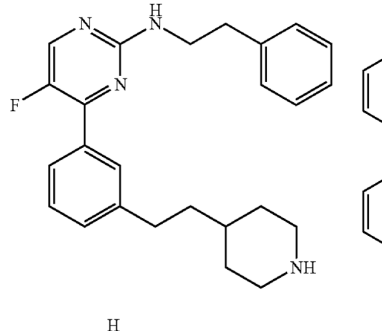
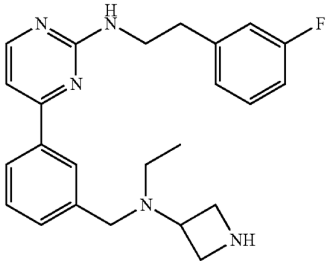
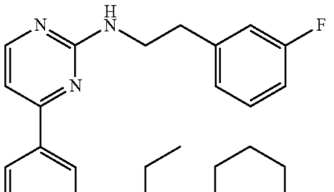
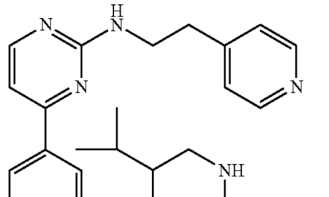
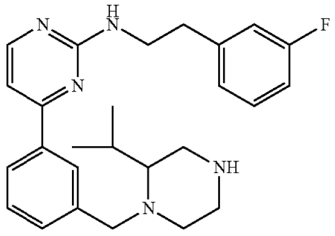


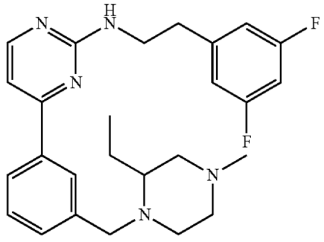
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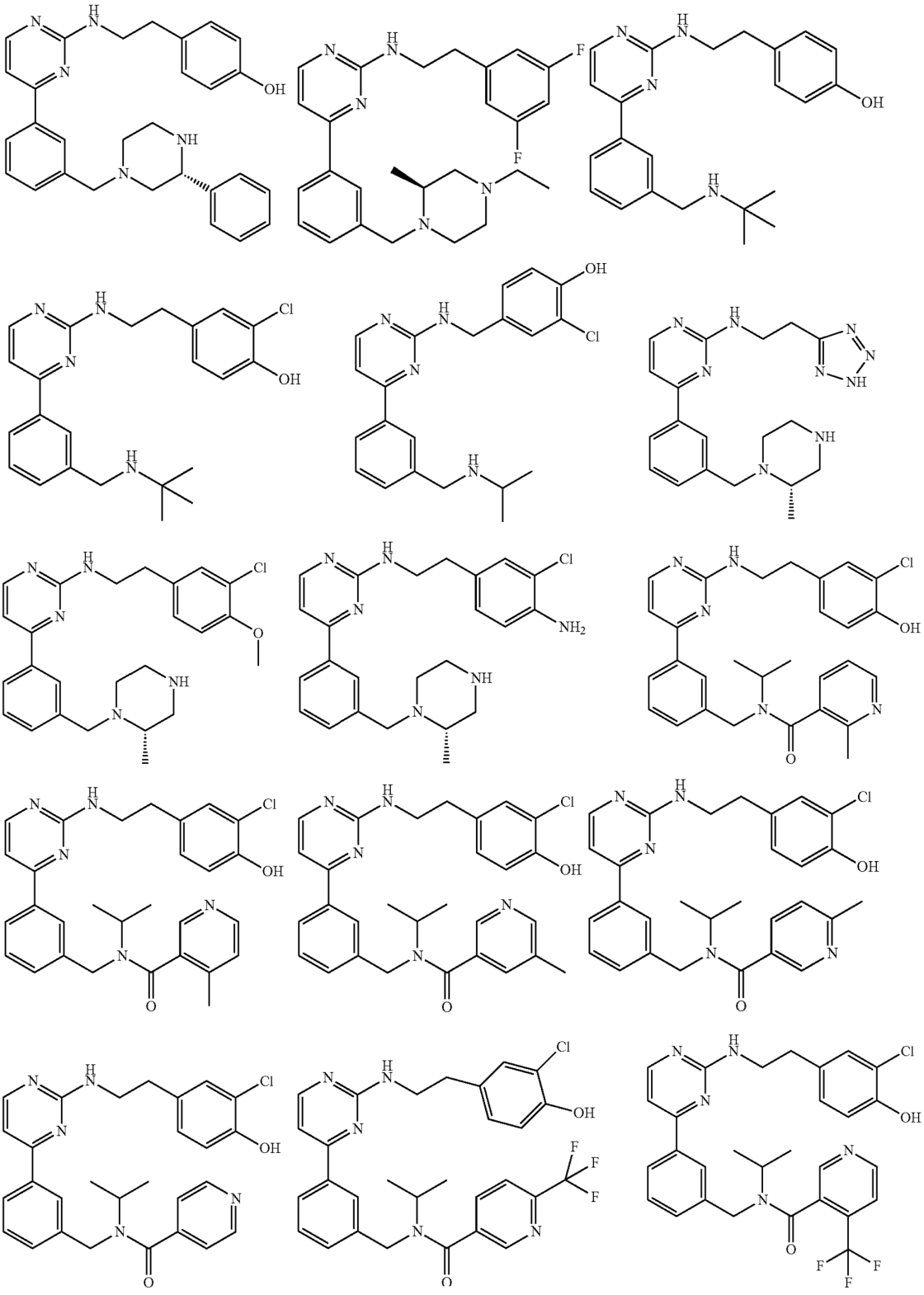
















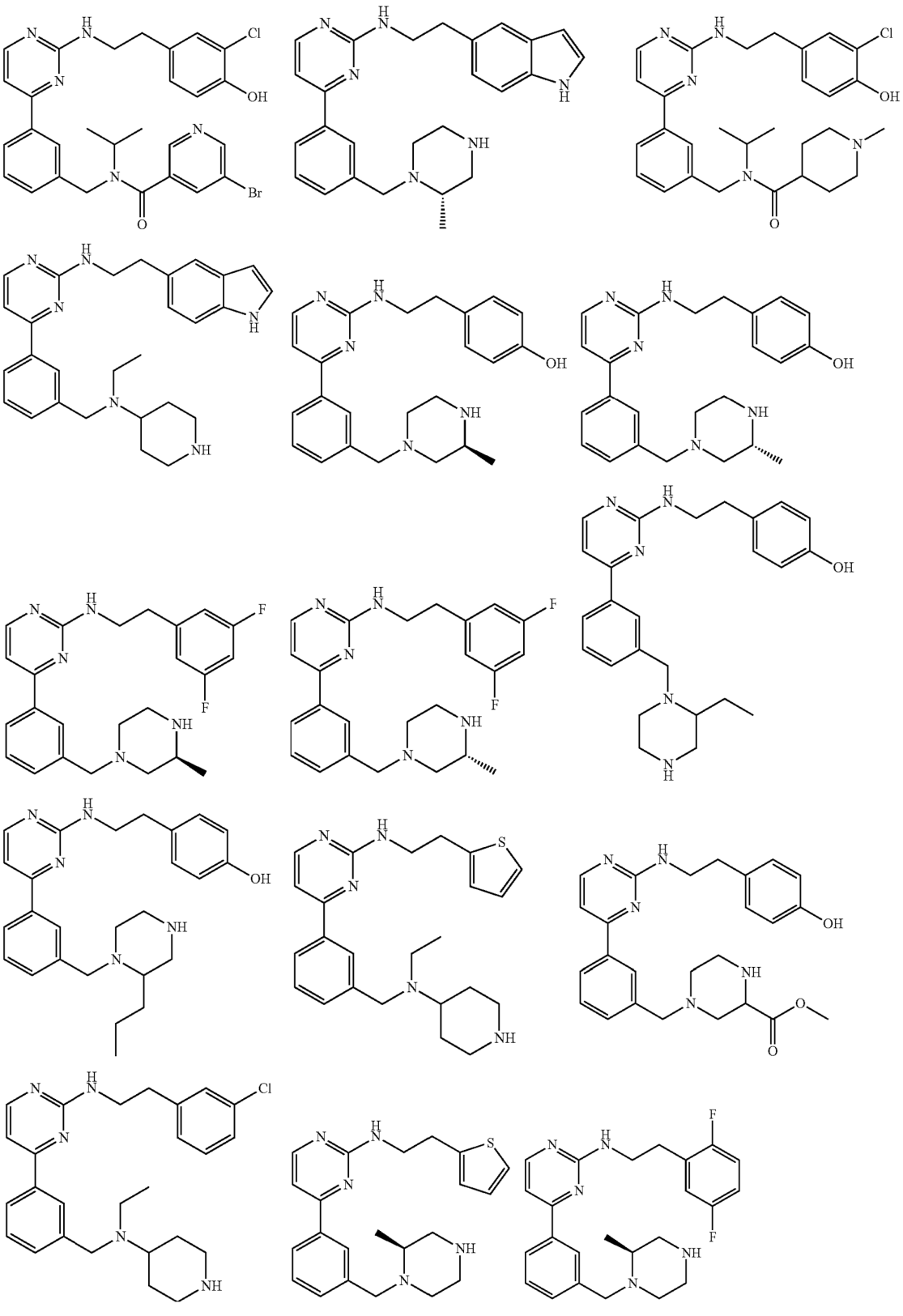


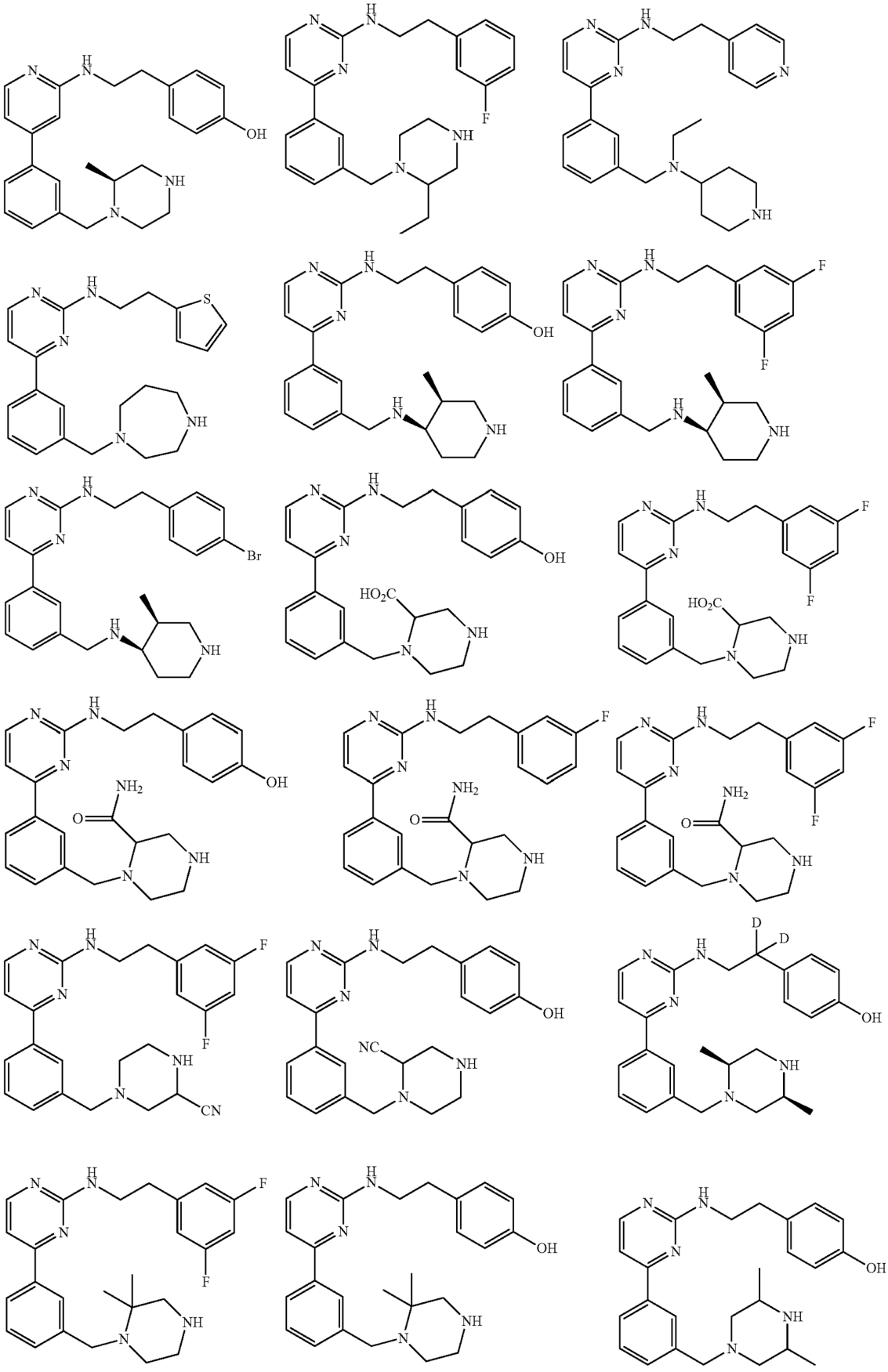


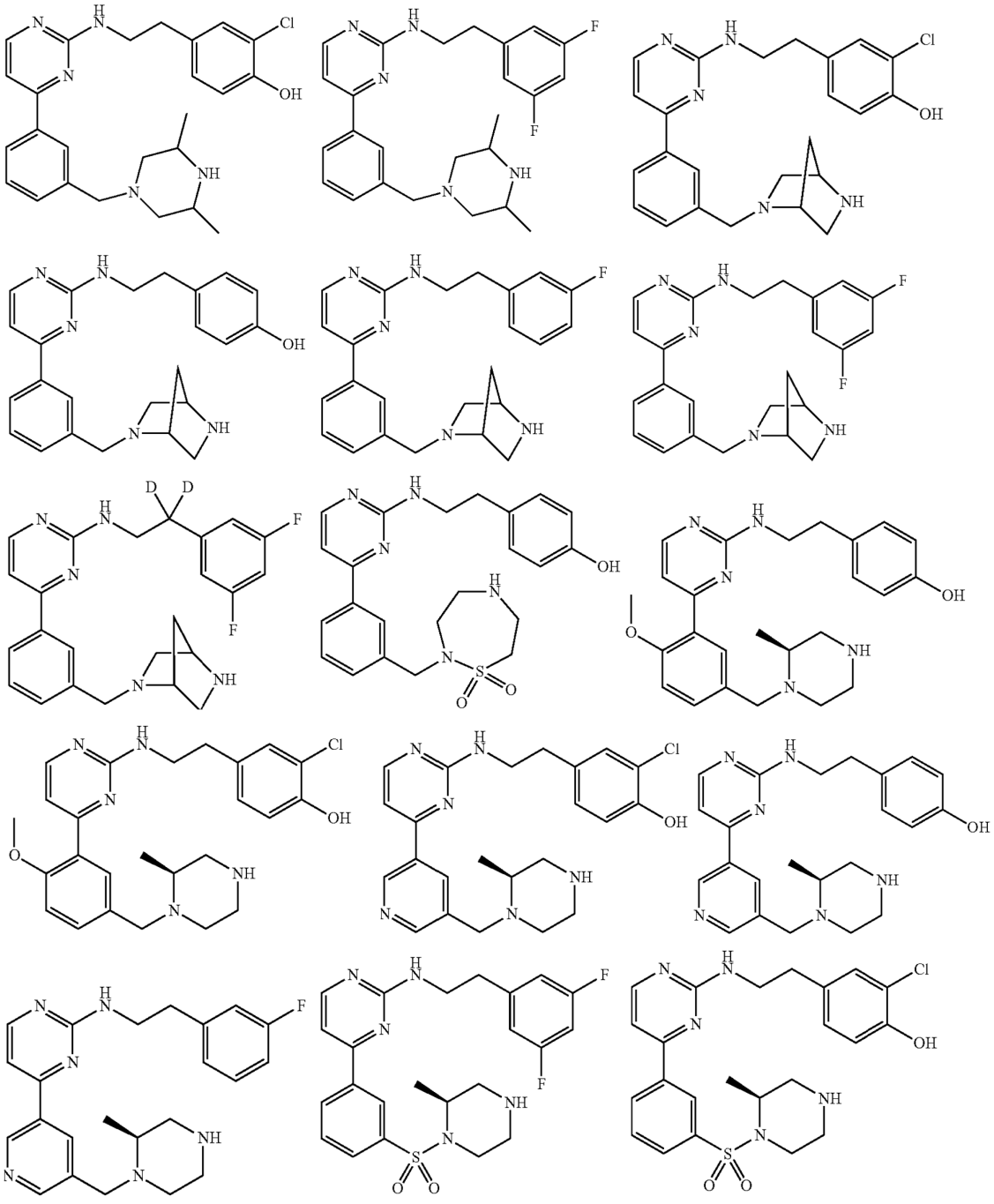











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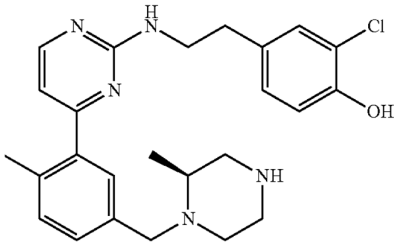
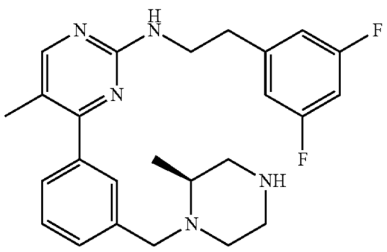
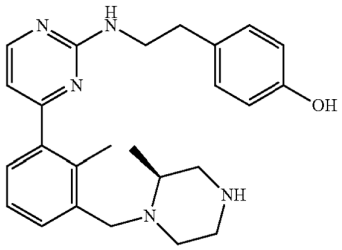
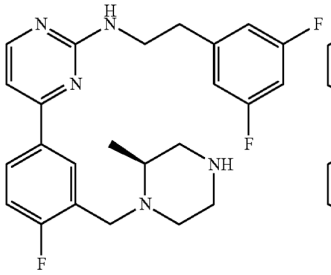
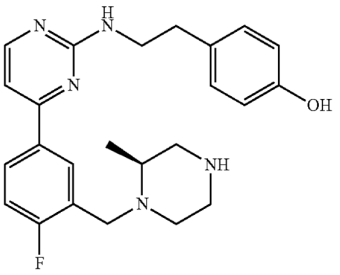
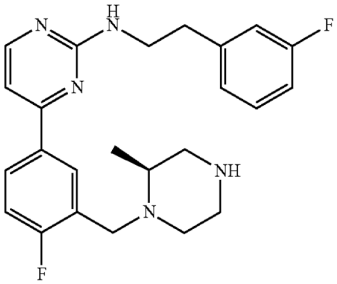
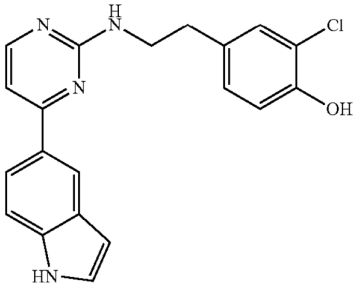
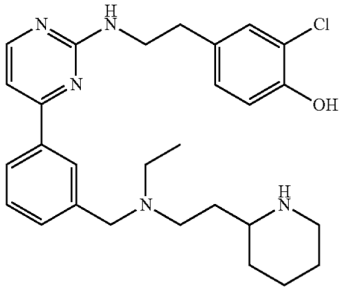
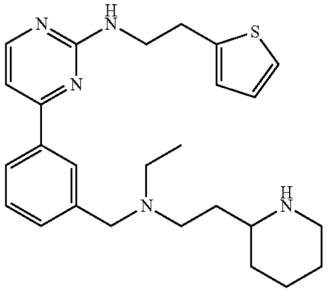
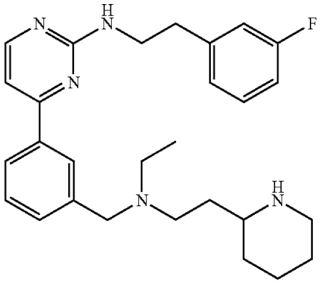
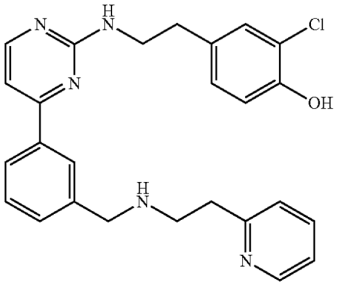
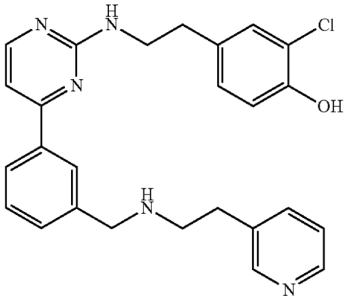
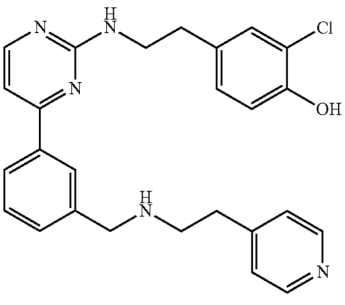
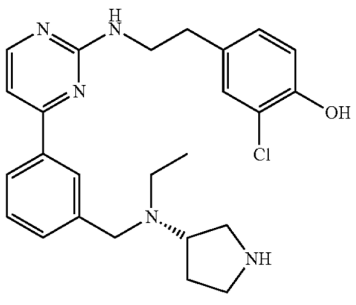
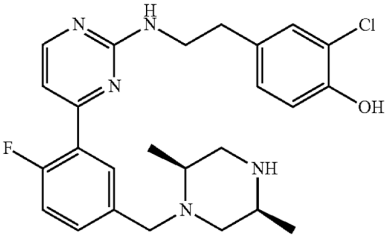
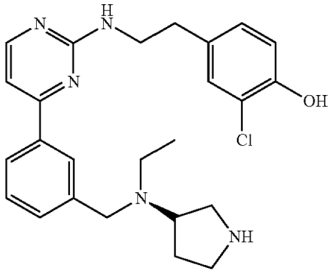
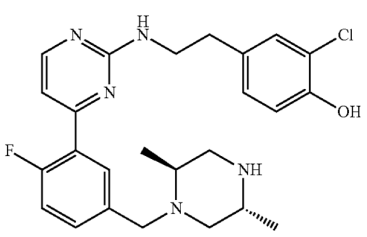


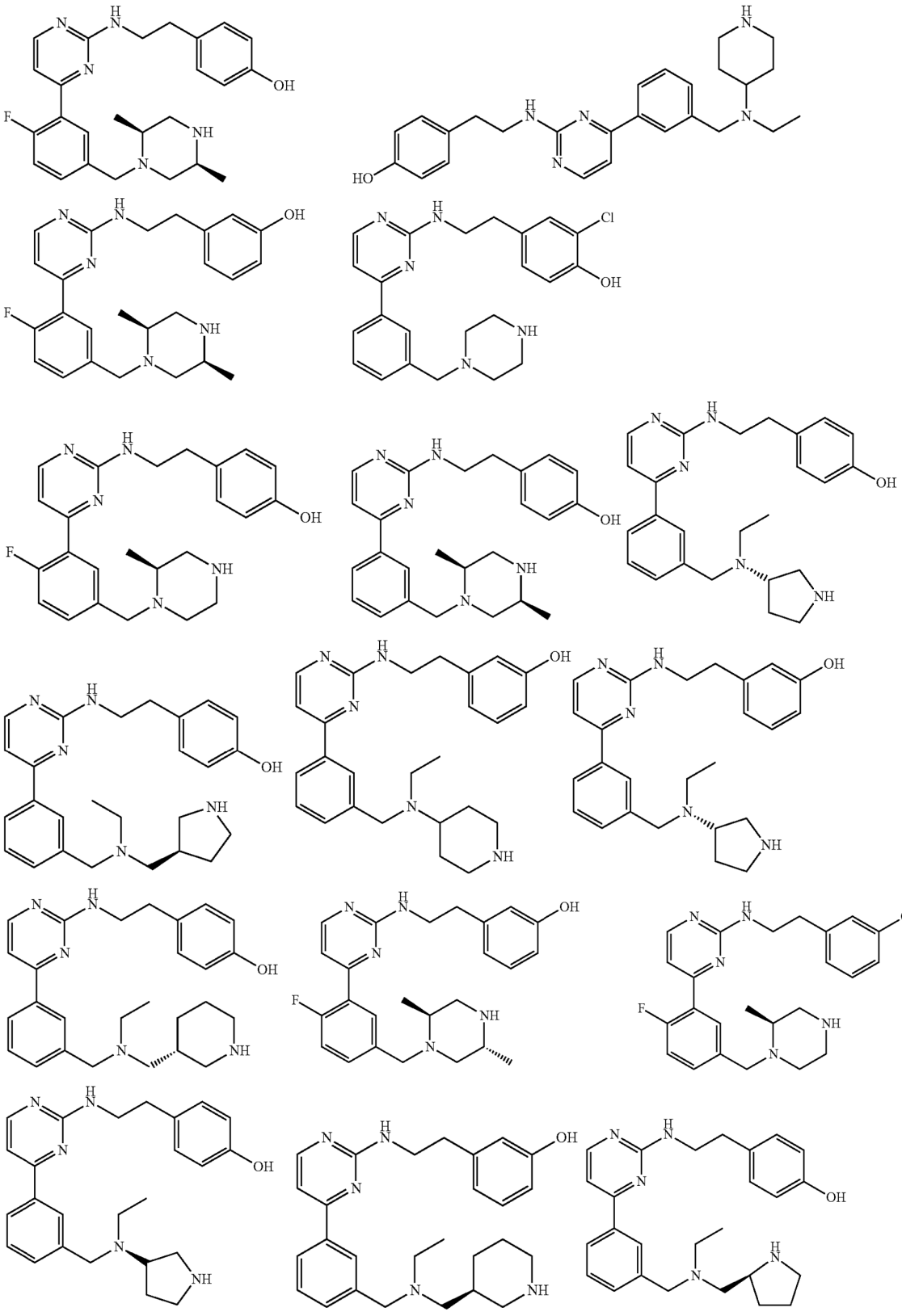


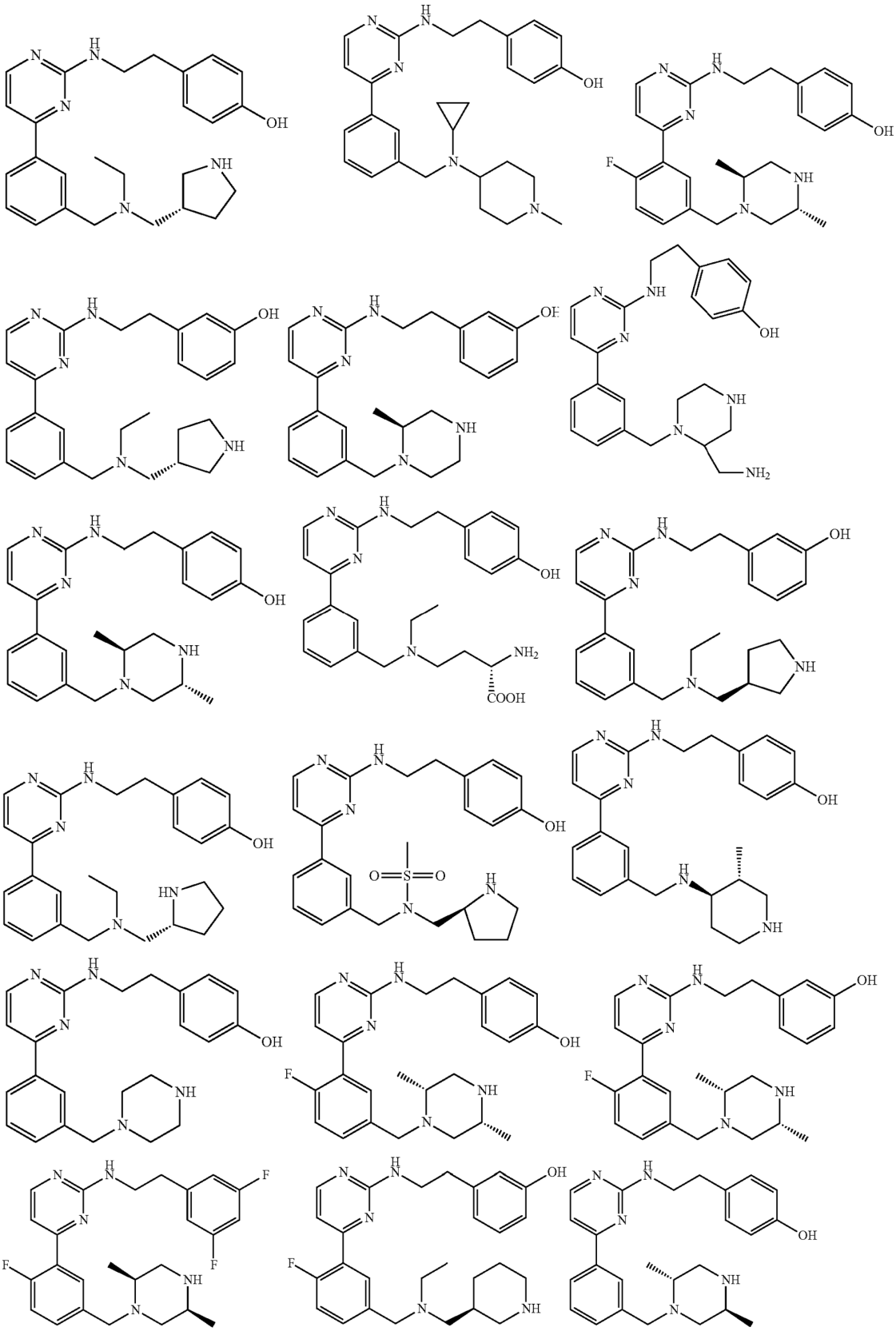


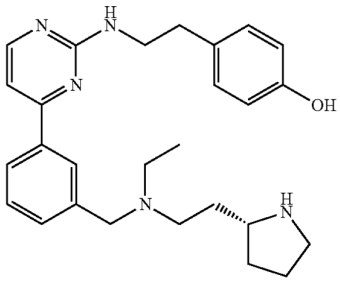
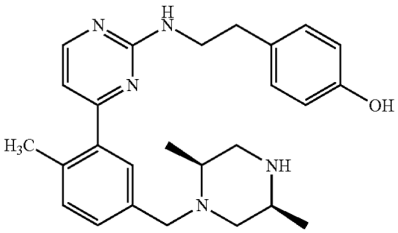
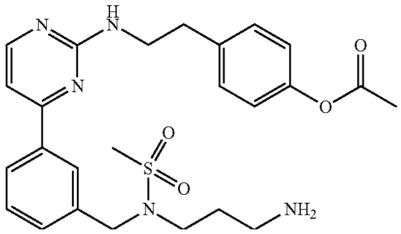
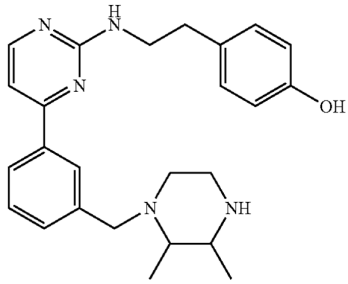
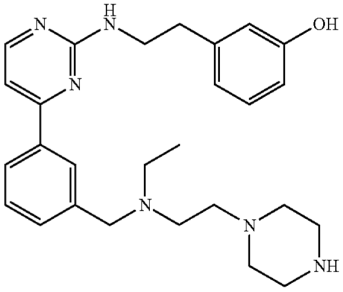
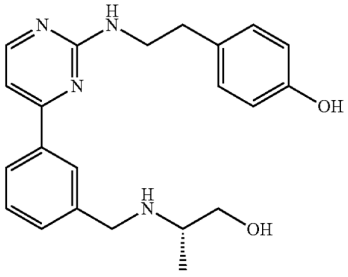
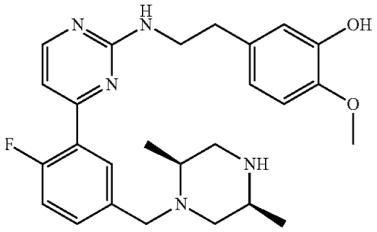
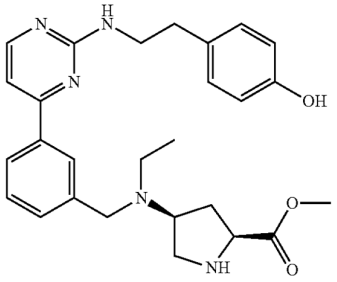
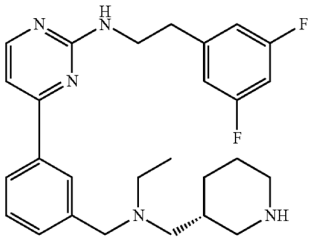
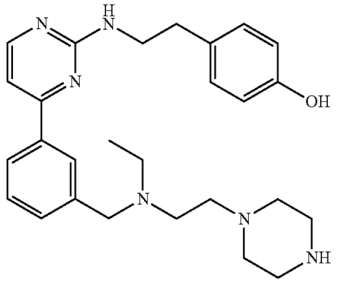
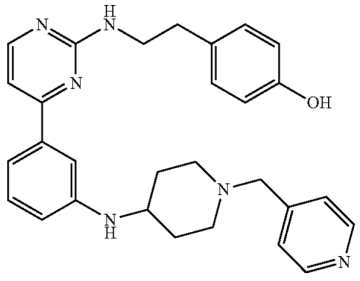
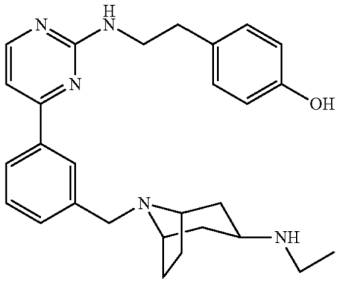
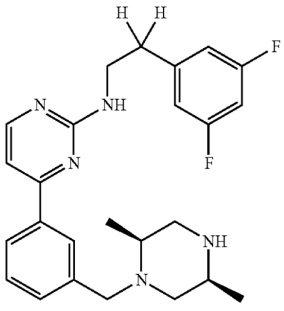
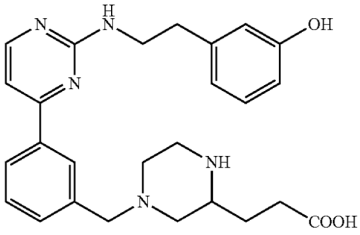
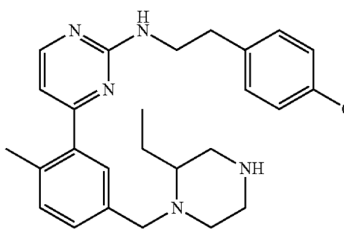




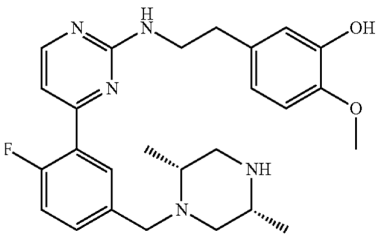
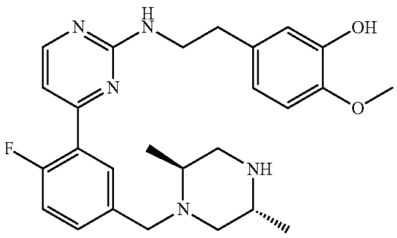
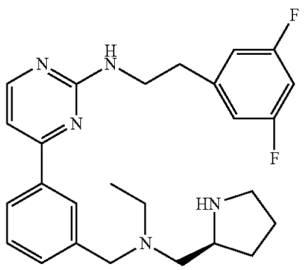
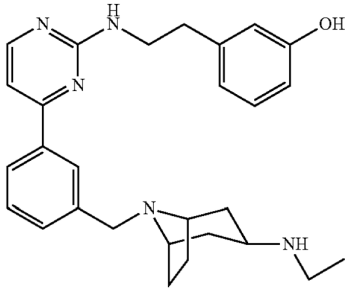
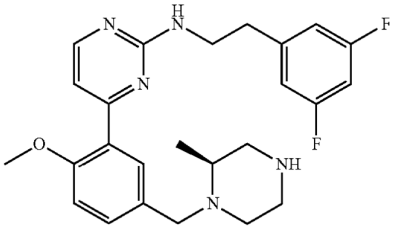
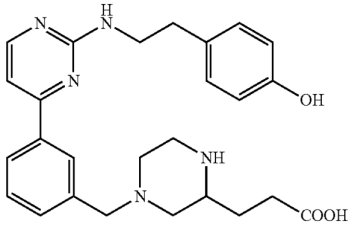
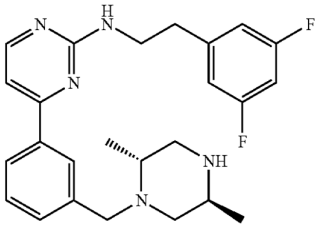
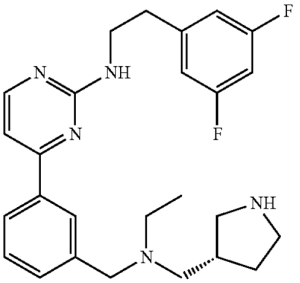
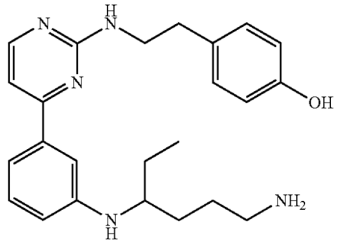
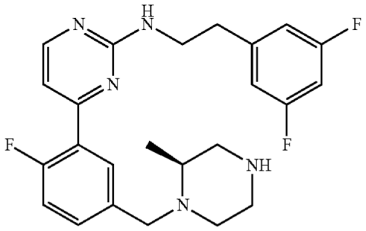
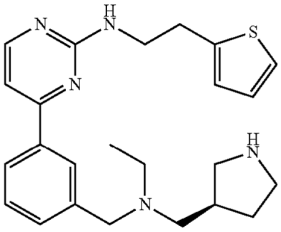
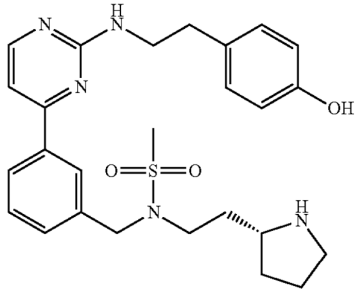
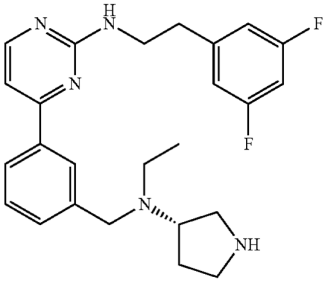
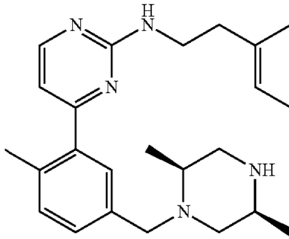
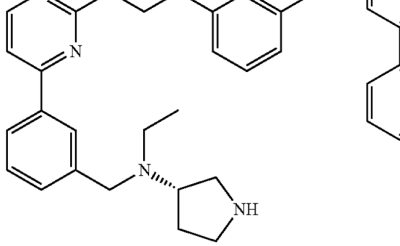














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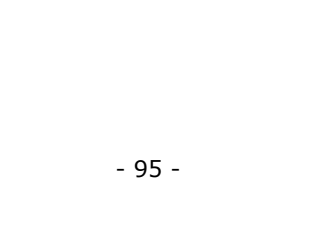


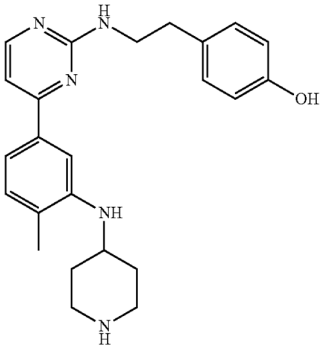
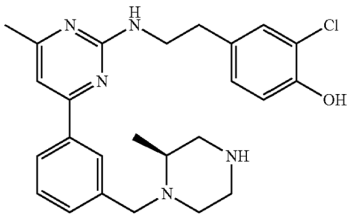
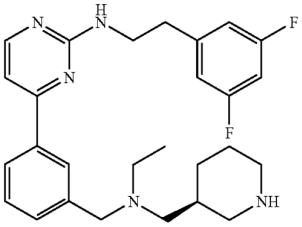
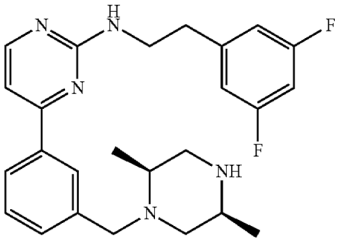
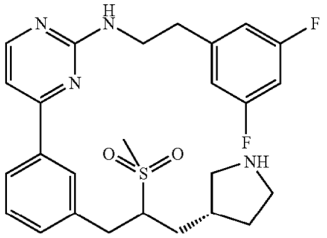
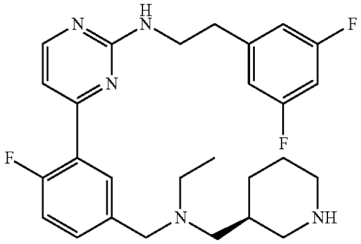
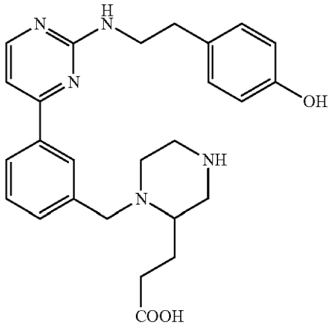
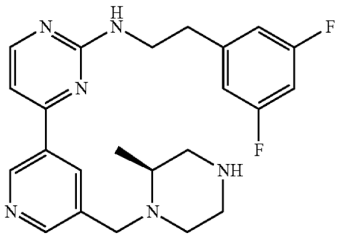
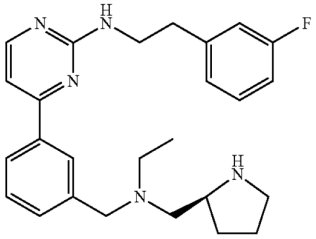
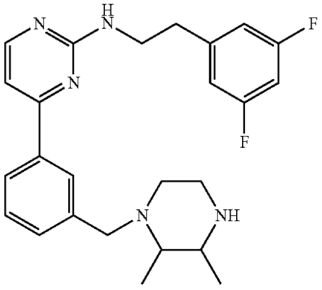
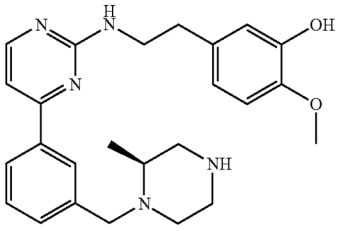
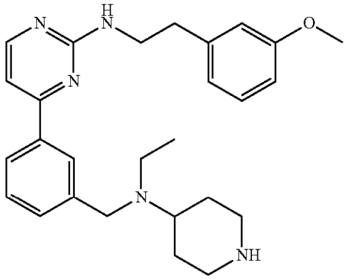
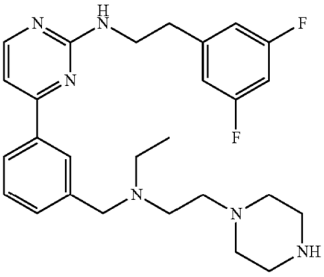
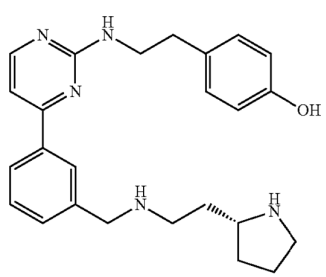
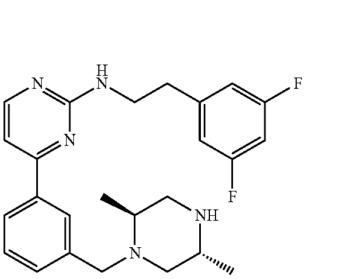
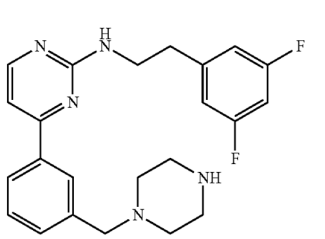
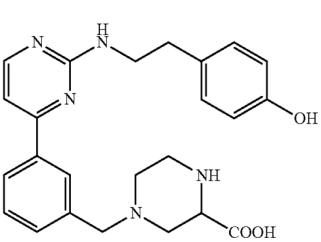


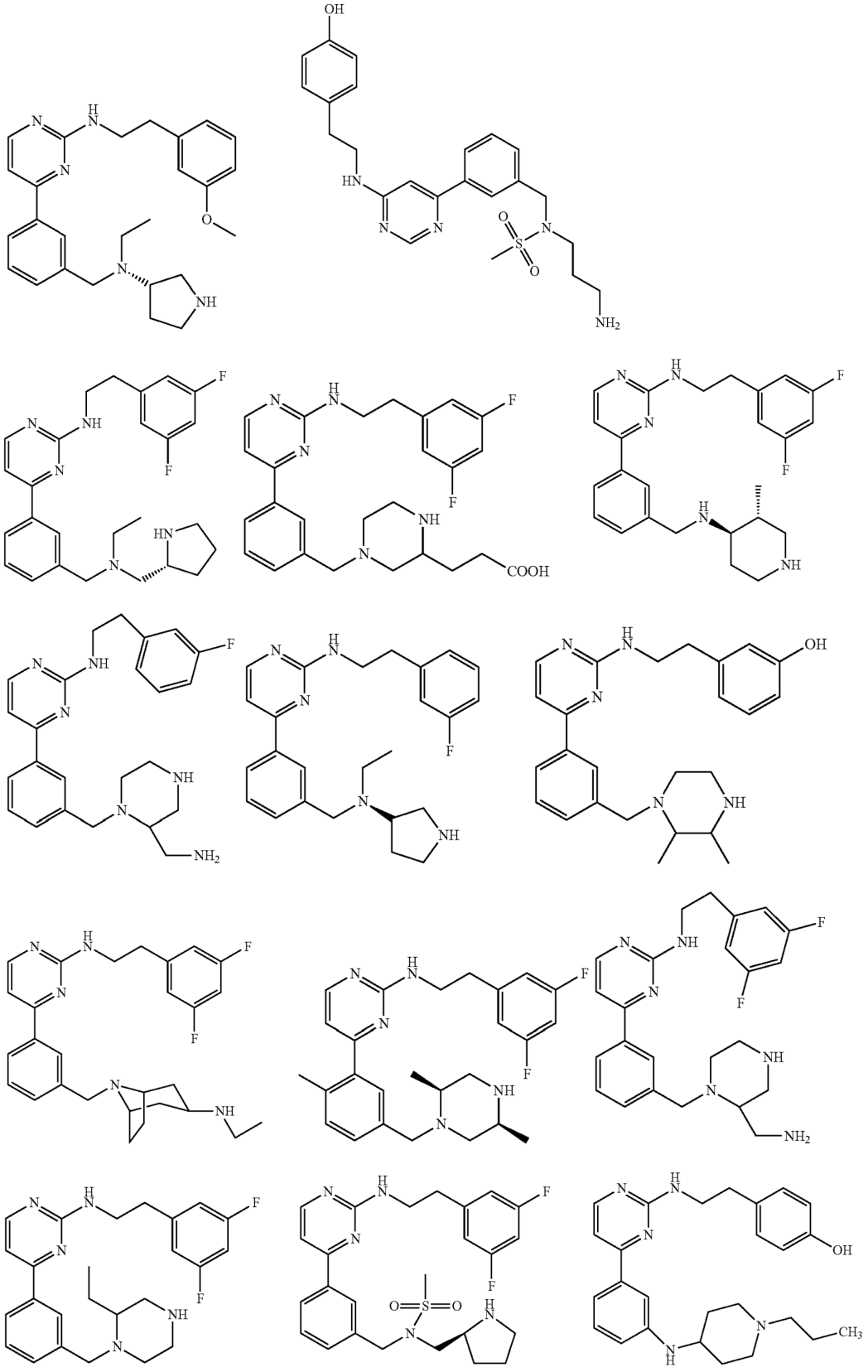
















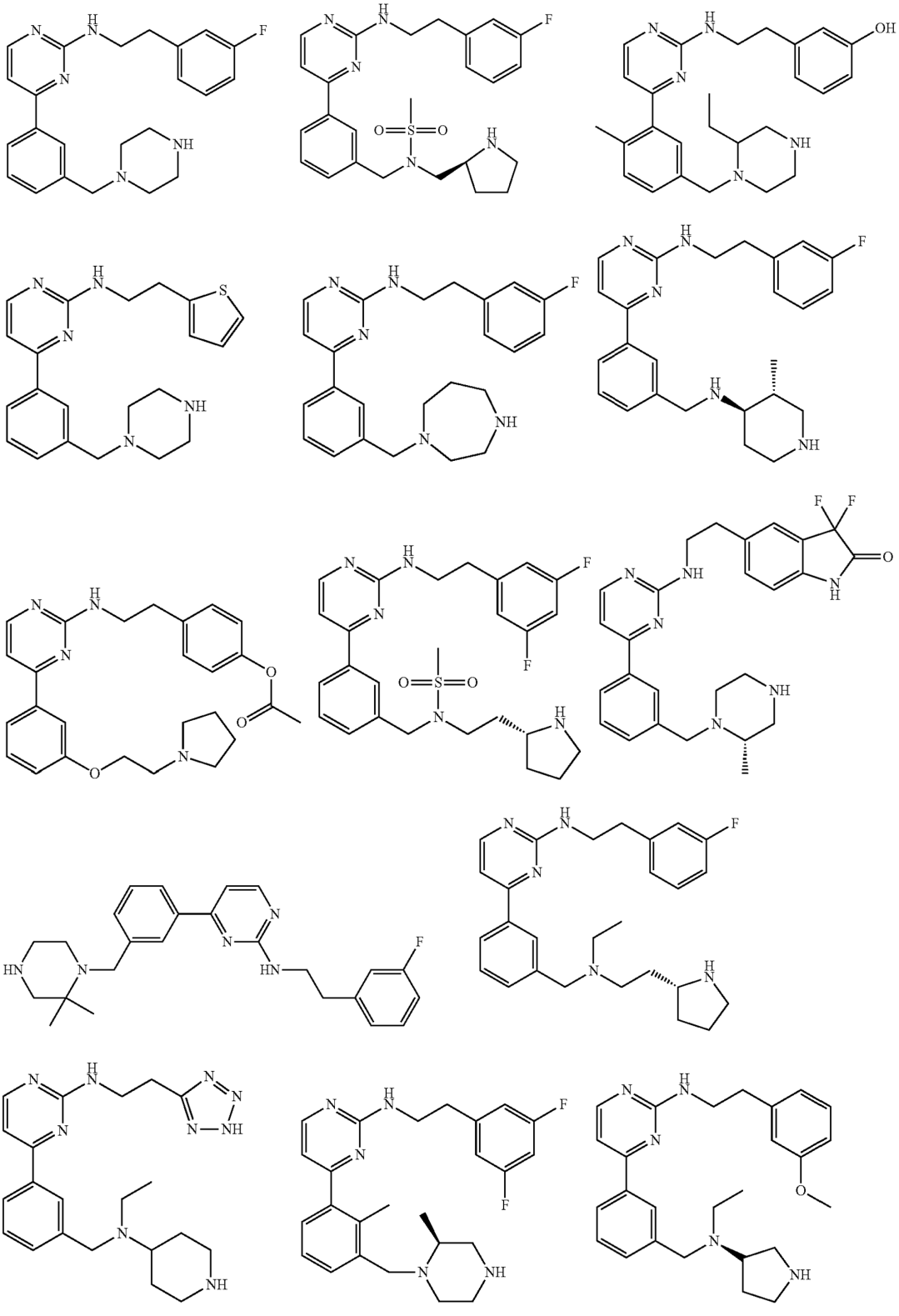


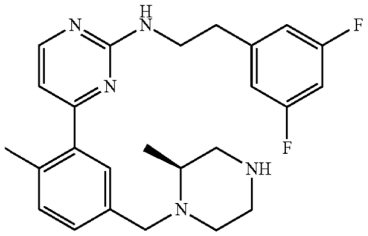
















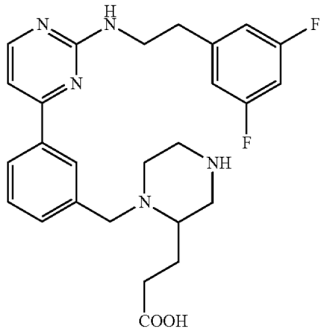


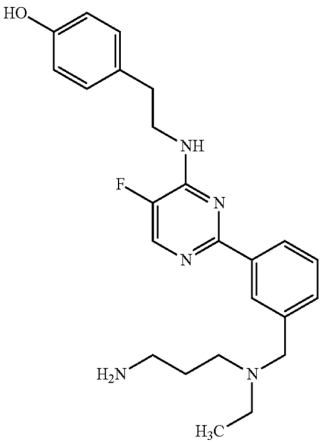


















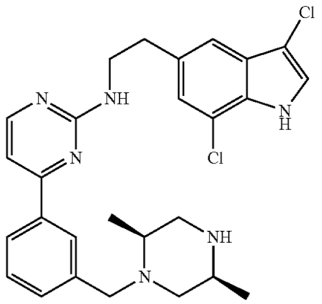


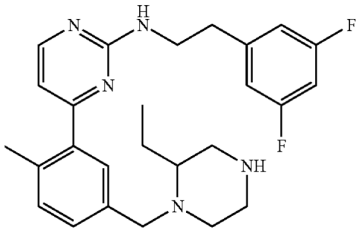


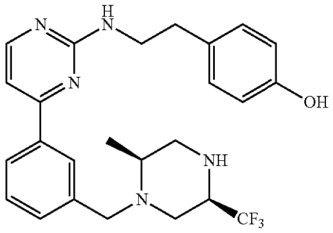


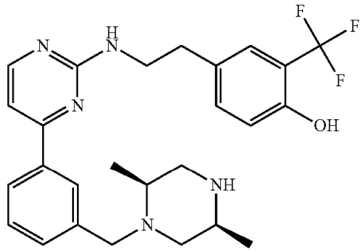


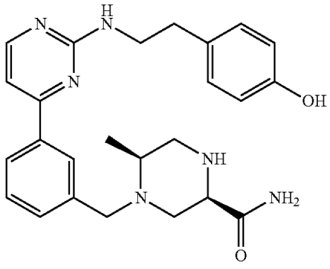


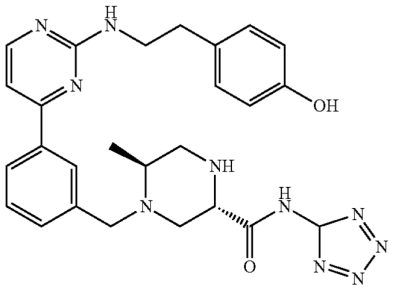


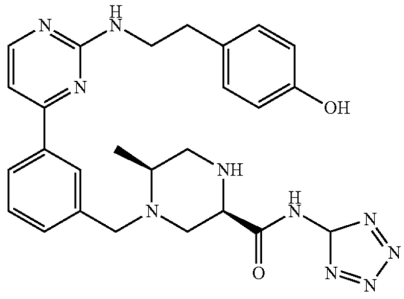


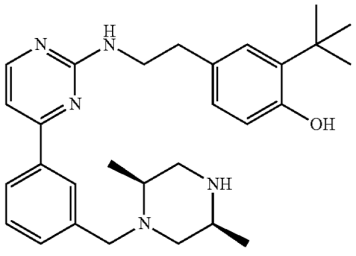


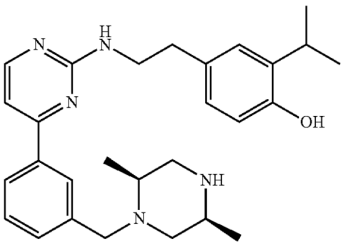


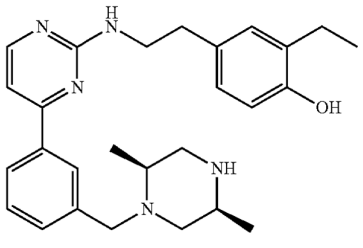


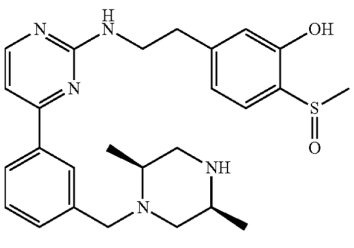


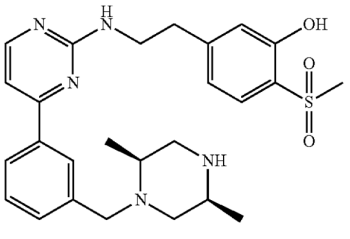


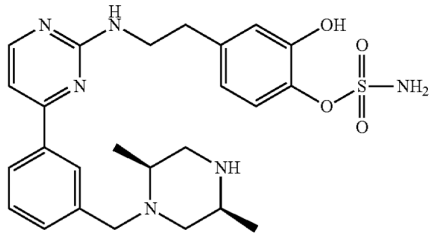


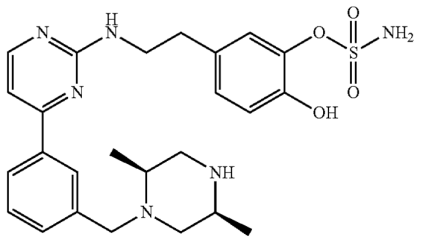


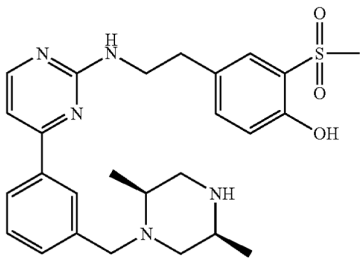


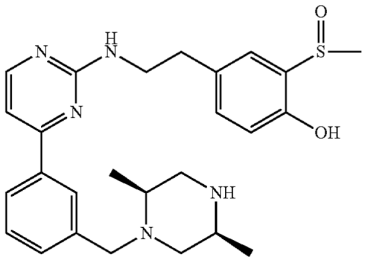


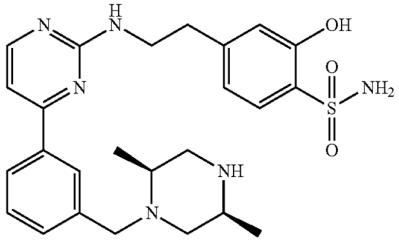


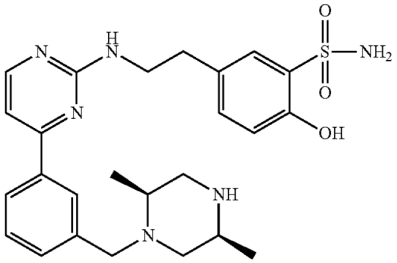


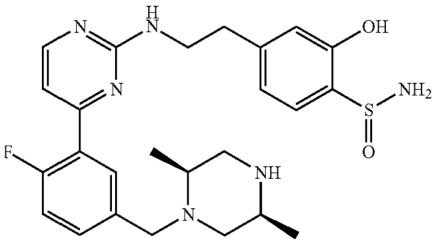


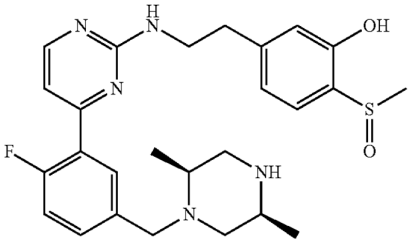


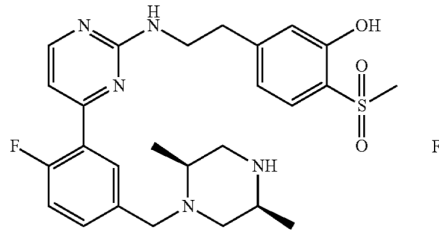


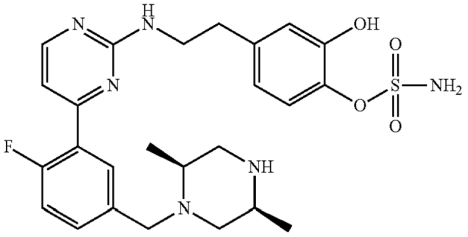




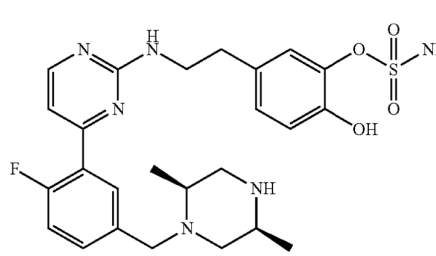


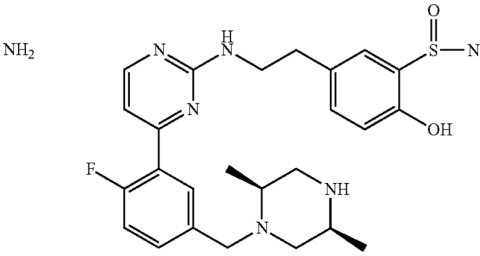


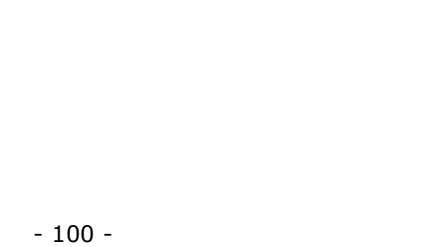


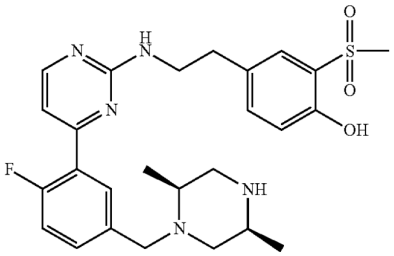
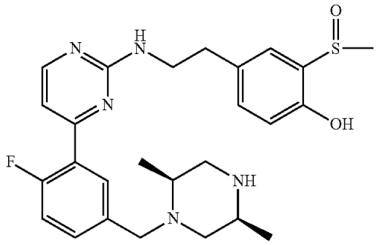
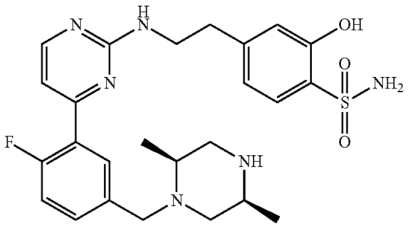


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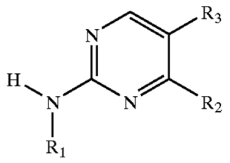








and


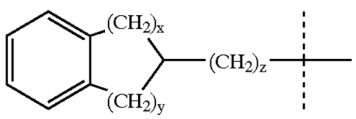
[0362] In yet other embodiments, small molecule PKC-θ inhibitors include pyrimidine derivatives as described for example by Cardozo *et al.* in US Publication No. 2005/0124640, which is incorporated herein by reference in its entirety. Representative compounds of this type are represented by formula (XVIII):


(XVIII)

[0363] wherein:

[0364] R_1 is C_{1-8}alkyl, C_{3-7}cycloalkyl, C_{3-7}cycloalkyl-C_{1-8}alkyl, naphthyl, quinolinyl, aryl-C_{1-8}alkyl, or heteroaryl-C_{1-8}alkyl, wherein in each of the C_{1-8}alkyl groups a methylene group may optionally be replaced by –NHC(O)– or –C(O)NH–, and wherein each of the C_{1-8}alkyl groups is optionally substituted by an oxo group or one or more C_{1-3}alkyl groups wherein two alkyl substituents on the same carbon atom of a C_{1-8}alkyl group may optionally be combined to form a C_{2-5} alkylene bridge, and wherein the aryl group is optionally substituted on adjacent carbon atoms by a C_{3-6} alkylene bridge group wherein a methylene group is optionally replaced by an oxygen, –S–, –S(O)–, $–SO_2–$ or $–N(R_6)–$;

[0365] or R_1 has the following structure:

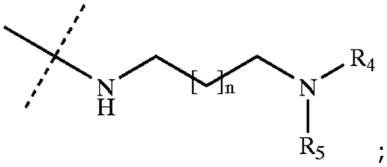


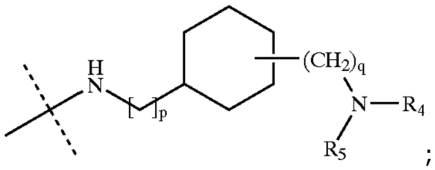
[0366] wherein x and y are independently 0, 1, 2, 3 or 4, provided that x+y is 2 to 4, z is 0, 1 or 2, and one or two CH_2 groups in the ring may optionally be replaced by –O–, –S–, –S(O)–, $–SO_2–$ or $–N(R_6)$;

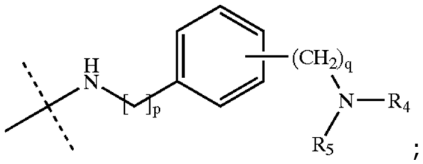
[0367] wherein each R_1 group is optionally substituted by one or more of the following groups: C_{1-6}alkyl, C_{3-6}cycloalkyl, halogen, nitro, hydroxy, C_{1-6}alkyloxy, C_{1-6}alkylthio, aryl, arylC_{1-6}alkyl, aryloxy, arylthio, aminosulfonyl, or amino optionally substituted by one or two C_{1-6}alkyl groups, wherein each aryl group is optionally substituted by one or more C_{1-6}alkyl, halogen, nitro,

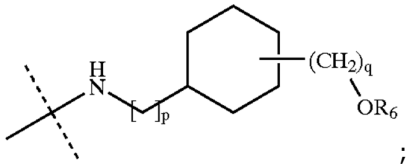
hydroxy or amino optionally substituted by one or two C_{1-6}alkyl groups, and wherein in each of the C_{1-6}alkyl groups a methylene group may optionally be replaced by –NHC(O)– or –C(O)NH–, and wherein each of the C_{1-6}alkyl groups is optionally substituted by one or more halogens;

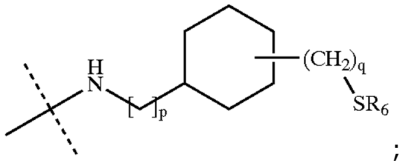
[0368] R_2 is selected from the following groups:

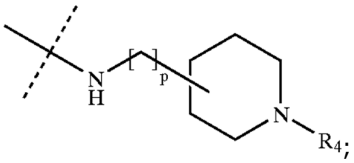

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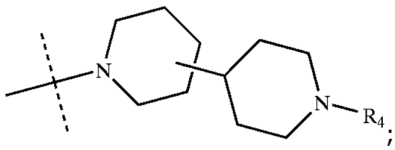

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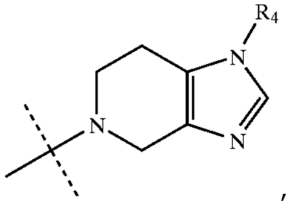

;


;


;


;


; and


,

[0369] wherein:

[0370] n is an integer from 3 to 8;

[0371] p is an integer from 1 to 3;

[0372] q is an integer from 0 to 3;

[0373] R_4 and R_5 are each independently selected from hydrogen, C_{1-6}alkyl, arylC_{1-6}alkyl, or amidino, wherein each aryl group is optionally substituted by one or more C_{1-6}alkyl, halogen, nitro, hydroxy or amino optionally substituted by one or two C_{1-6}alkyl groups, and wherein each of the C_{1-6}alkyl groups is optionally substituted by one or more halogens, and wherein the amidino is optionally substituted by one to three C_{1-6}alkyl;

[0374] R_6 is hydrogen or C_{1-6}alkyl;

[0375] wherein each R_2 group is optionally substituted by one or more C_{1-6}alkyl, C_{1-6}alkoxy, CN, –OH, $–NH_2$ or halogen;

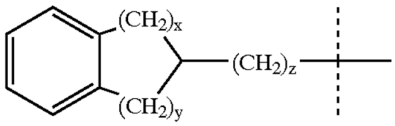
[0376] R_3 is halogen, cyano, nitro, C_{1-6}alkyl, C_{1-6}alkyloxycarbonyl or aminocarbonyl, wherein each of the C_{1-6}alkyl groups is optionally substituted by one or more halogens;

[0377] or a tautomer, pharmaceutically acceptable salt, solvate, or amino-protected derivative thereof,

[0378] In some embodiments of the pyrimidine derivative compounds of formula (XVIII):

[0379] R_1 is aryl-C_{1-4}alkyl or heteroaryl-C_{1-4}alkyl, wherein in each of the C_{1-4}alkyl groups a methylene group may optionally be replaced by –NHC(O)– or –C(O)NH–, and wherein each of the C_{1-4}alkyl groups is optionally substituted by an oxo group or one or more C_{1-3}alkyl groups wherein two alkyl substituents on the same carbon atom of a C_{1-4}alkyl group may optionally be combined to form a C_{2-5} alkylene bridge, and wherein the aryl group is optionally substituted on adjacent carbon atoms by a C_{3-6}alkylene bridge group wherein a methylene group is optionally replaced by an oxygen, sulfur or –N(R_6)–;

[0380] or R_1 has the following structure:

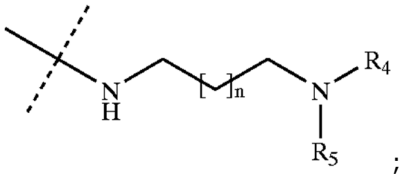
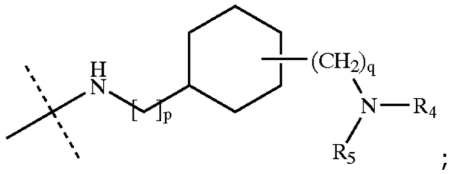
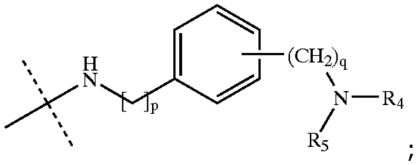
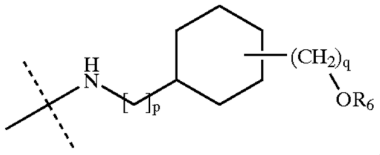
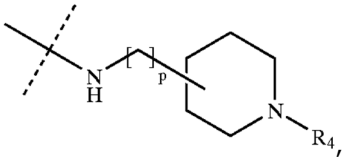


[0381] wherein x and y are independently 0, 1, 2 or 3, provided that x+y is 2 to 3, and z is 0 or 1;

[0382] wherein "heteroaryl" is defined as pyridyl, furyl, thienyl, pyrrolyl, imidazolyl, or indolyl;

[0383] wherein each R_1 group is optionally substituted by one or more of the following groups: C_{1-6}alkyl, Cl, Br, F, nitro, hydroxy, CF_3, $–OCF_3$, $–OCF_2H$, $–SCF_3$, C_{1-4}alkyloxy, C_{1-4}alkylthio, phenyl, benzyl, phenyloxy, phenylthio, aminosulfonyl, or amino optionally substituted by one or two C_{1-3}alkyl groups;

[0384] R_2 is selected from the following groups:


;

;

;

; and

,

[0385] wherein:

[0386] n is an integer from 5 to 7;

[0387] p is an integer from 1 to 2;

[0388] q is an integer from 1 to 2;

[0389] R_4 and R_5 are each independently selected from hydrogen, C_{1-6}alkyl, arylC_{1-6}alkyl, or amidino;

[0390] R_6 is hydrogen;

[0391] R_3 is Br, Cl, F, cyano or nitro;

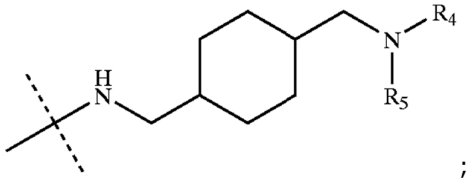
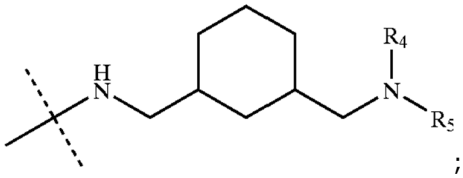
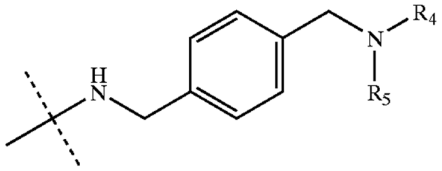
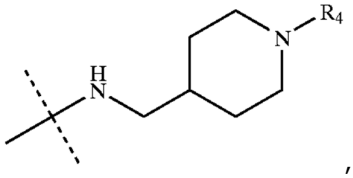
[0392] or a tautomer, pharmaceutically acceptable salt, solvate, or amino-protected derivative thereof;

[0393] In other embodiments of the pyrimidine derivative compounds of formula (XVIII):

[0394] R_1 is phenyl-C_{1-4}alkyl or naphthylC_{1-2}alkyl,

[0395] wherein each R_1 group is optionally substituted by one or more of the following groups: methyl, Cl, Br, F, nitro, hydroxy, CF_3, $-OCF_3$, $-SCF_3$, C_{1-4}alkyloxy or C_{1-4}alkylthio;

[0396] R_2 is selected from the following groups:


;

;

; and

,

[0397] wherein:

[0398] R_4 and R_5 are each independently selected from hydrogen, C_{1-3} alkyl, or amidino;

[0399] R_3 is Br, Cl, cyano or nitro;

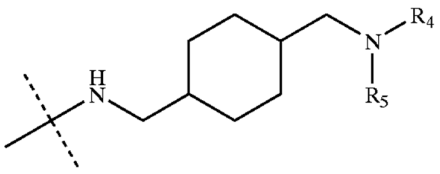
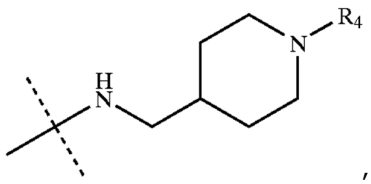
[0400] or a tautomer, pharmaceutically acceptable salt, solvate, or amino-protected derivative thereof;

[0401] In still other embodiments of the pyrimidine derivative compounds of formula (XVIII):

[0402] R_1 is phenylCH_2–

[0403] wherein the phenyl group is optionally substituted by one or more of the following groups: methyl, Cl, Br, F, nitro, hydroxy, CF_3, $-OCF_3$, $-SCF_3$, C_{1-4}alkyloxy or C_{1-4}alkylthio;

[0404] R_2 is selected from the following groups:


; and

,

[0405] R_3 is nitro;

[0406] R_4 and R_5 are each independently selected from hydrogen, methyl, or amidino;

[0407] or a tautomer, pharmaceutically acceptable salt, solvate, or amino-protected derivative thereof.

[0408] Non-limiting examples of the pyrimidine derivative compounds of formula (XVIII) are selected from:

[0409] ethyl 4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-2-[(2-chlorobenzyl)amino]pyrimidine-5-carboxylate; N^4-{[4-(aminomethyl)cyclohexyl]- methyl}-5-nitro-N^2-[(2R)-1,2,3,4-tetrahydronaphthal- en-2-yl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[(2S)-1,2,3,4-tetrahydronaphthalen-2-yl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[(1R)-1,2,3,4-tetrahydronaphthalen-1-yl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]- methyl}-5-nitro-N^2-[(1S)-1,2,3,4-tetrahydronaphthal- en-1-yl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(4-chlorophenyl)ethyl]-5-nitropynmidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2- -[2-(2-methylphenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(3-methylphenyl)ethyl]-5-nitropyri-midine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(4-methylphenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2- -(2-fluorophenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]- methyl}-N^2-[2-(3-fluorophenyl)ethyl]-5-nitropyrimid- ine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(4-fluorophenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^2-(2-aminobenzyl)-N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]m- ethyl}-N^2-(3,5-dimethoxybenzyl)-5-nitropyrimidine-2-,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[3,5-bis(trifluoromethyl)benzyl]-5-nitropyrimidine-2,4-diamine; {3-[({2-[(2-chlorobenzyl)amino]-5-nitropynmidin-4-yl}amino)methyl]phenyl}methane amine; 2-({[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5- -nitropyrimidin-2-yl]amino}methyl)phenol; N^2-(5-amino-2-chlorobenzyl)-N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitropynmidine-2,4-diamine; 4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-2-[(2-chlorobenzyl)amino]pyrimidine-5-carboxamide; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chlorobenzyl)-5-fluoropyrimidine-2,4-diamine; 3-({[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5-nitropyrimidin-2-yl]amino}methyl)-N-[2-(2-methylphenyl)ethyl]benzamide; (1S,2R)-2-({[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5-nitropyrimidin-2-yl]amino}methyl)cyclohexanol; (1R,2R)-2-({[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5- nitropyrimidin-2-yl]amino}methyl)cyclohexanol; methyl 4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-2-[(2-chlorobenzyl)amino]pyrimidine-5-carboxylate; 4-{[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5-nitropyrimidin-2-yl]amino}-N-[2-(2-methylphenyl)ethyl]butanamide; 5-{[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5-nitropyrimidin-2-yl]amino}-N-[2-(2-methylphenyl)ethyl]pentanamide; 6-{[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5-nitropyrimidin-2-yl]amino}-N-[2-(2-methylphenyl)ethyl]hexanamide; (1R,3R)-3-({[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5-nitropyrimidin-2-yl]amino}methyl)-4,4-dimethylcyclohexanol; N^4-({4-cis-[(dimethyl-amino)methyl]cyclohexyl}methyl)-N^2-(1-naphthylmethyl)-5-nitropyrimidine-2,4-diamine; N^2-[2-(methylthio)benzyl]-5-nitro-N^4-(piperidin-4-ylmethyl)pyrimidine-2,4-diamine; 5-nitro-N^4-(piperidin-4-ylmethyl)-N^2-{2-[(trifluoromethyl)thio]benzyl}pyrimidine-2,4-diamine; N^2-(1-naphthylmethyl)-5-nitro-N^4-(piperidin-4-ylmethyl)pyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)– N^2-[2-(methylthio)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-5-nitro-N^2-{2-[(trifluoromethyl)thio]benzyl}pyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-N^2-(1-naphthylmethyl)-5-nitropyrimidine-2,4-

diamine; N^4-{4-[(dimethylamino)methyl]benzyl}-N^2-[2-(methylthio)benzyl]-5-nitropyrimidine–2,4-diamine; N^4-{4-[(dimethylamino)methyl]benzyl}-5-nitro-N^2-{2-[(trifluoromethyl)thio]benzyl}pyrimidine-2,4-diamine; N^4-{4-[(dimethylamino)methyl]benzyl}- N^2-(1-naphthylmethyl)-5-nitropyrimidine-2,4-diamine; N^4-[(1-methylpiperidin-4-yl)methyl]-N^2-[2-(methylthio)benzyl]-5-nitropyrimidine-2- ,4-diamine; N^4-[(1-methylpiperidin-4-yl)methyl]-5-nitro-N^2-{2-[(trifluoromethyl)thio]benzyl}pyrimidine-2,4-diamine; N^4-[(1-methylpiperidin-4-yl)methyl]-N^2-(1-naphthylmethyl)-5-nitropynmidine-2,4-diamine; N^2-(2-chlorobenzyl)-N^4-[(1-m-ethylpiperidin-4-yl)methyl]-5-nitropynmidine-2,4-diamine; N^2-(2-methoxybenzyl)-N^4- -[(1-methylpiperidin-4-yl)methyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-methoxybenzyl)-5-nitropyrim- idine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[2-(trifluoromethyl)benzyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(- 2,4-dichlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-methoxybenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[4-fluoro-2-(trifluoromethyl)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2– (3-methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(pyridin-2-ylmethyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)- cyclohexyl]methyl}-N^2-(3-chlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-chlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N-(4-bromobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,4-dimethoxybenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N.s- up.2-[2-chloro-5-(trifluoromethyl)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,5-dichlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl }-5-nitro-N^2-[2-(trifluoromethoxy)benzyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chloro-6-methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,3-dichlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]- methyl}-N^2-(2-furylmethyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(thien-2-ylmethyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chlorobenzyl)-5-methylpyrimidine-2,4-diamine; N^4-(6-aminohexyl)-N^2-(2-chlorobenzyl)-5-nitropyrimidine-2,4-diamine; N-[4-(aminomethyl)benzyl]-N^2-(2-chlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-(7-aminoheptyl)-N^2-(2-chlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[3-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(1-methyl-1-phenylethyl)-5-nitropyrimidine-2,4-diamine; 4-(4,4'-bipiperidin-1-yl)-N-(2-chlorobenzyl)-5-nitropyrimidin-2-amine; N^2-(2-chlorobenzyl)-N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-5-nitropyri; N^4-{[4-(aminomethyl)cyclohexyl]m- ethyl}-N^2-(2,5-difluorobenzyl)-5-nitropyrimidine-2,- 4-diamine; N^4-{[4-(aminomethyl-)cyclohexyl]methyl}-N^2-[4-(difluoromethoxy)benzyl]– 5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-ethoxybenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[(1S)-1-phenylethyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N.s- up.2-(2-methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-fluorobenzyl)-5-nitropyrimidine-2,4-dia- mine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-chloro-2-fluorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(4-pentylbenzyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-butoxybenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,3-

dimethoxybenzyl)-5-nitropynmidine-2,4-diamine; N^4-{[4-(aminomethyl)- cyclohexyl]methyl}-N^2-(2,5-dimethoxybenzyl)-5-nitro- pyrimidine-2,4-diamine; N^2-(2-chlorobenzyl)-N^4-[7-(dimethylamino)heptyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(1,1'-biphenyl-2-ylmethyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(- 4-fluorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]me- thyl}-N^2-(2,4-difluorobenzyl)-5-nitropyrimidine-2,- 4-diamine; N^4-{[4-(aminomethyl-)cyclohexyl]methyl}-N^2-(3-fluoro-4-methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,3-difluorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-(2-chlorobenzyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]- methyl}-N^2-(2,6-dimethoxybenzyl)-5-nitropyrimidine–2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,6-difluorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-fluor- o-3-(trifluoromethyl)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-chloro-2-fluorobenzyl)-5-nitropy- rimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(1-phenylcyclopropyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2- -[1-(2-chlorophenyl)-1-methylethyl]-5-nitropyrimidine-2- ,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,3-dihydro-1-benzofuran-5-ylmethyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[(1,5-dimethyl-1H-pyrrol-2-yl)methyl]–5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-bromobenzyl)-5-nitronyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,3-dimethylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,4-dimethylbenzyl)-5-nitropyrimidine-2-,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,5-dimethylbenzyl)-5-nitropyrimidine-2,4-diamine; 2 N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N2-[2-fluoro– 5-(trifluoromethyl)benzyl[-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N2-[2- (methylthio)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5- -nitro-N^2-{2- [(trifluoromethyl)thio]- benzyl}pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-fluorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2- -(6-chloro-2- fluoro-3-methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chloro-6- fluoro-3-methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-2-naphthyl- 5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(1-naphthylmethyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N N^2-[2-fluoro-4- (trifluoromethyl)benzyl]-5– nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-chloro-2- methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]meth- yl}-N^2-(5-chloro-2-) methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-chloro-2- methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]- methyl}-N^2-[5-fluoro-2-(trifluoromethyl)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(- 5-chloro-2-fluorobenzyl)-5-nitropyrimidin- e-2,4-diamine; N^4-{[4-(aminomethyl-)cyclohexyl]methyl}-N^2-(2,3- difluoro-4-methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(- 5-fluoro-2- methylbenzyl)-5-nitropyrimidin- e-2,4-diamine; N^4-{[4-(aminomethyl-)cyclohexyl]methyl}-N^2-1-naphthyl- 5-nitropyrimidine-2,4-diamine; {4-trans-[({2-[(2-chlorobenzyl)amino]-5-nitropyrimidin- 4-yl}amino)methyl]cyclohexyl}methanol; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-(2,5-dichlorobenzyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-

N^2- (2,4-dichlorobenzyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-(2-bromobenzyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N.su- p.2- (cyclohexylmethyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-naphthylmethyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-[2- (trifluoromethoxy)benzyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-[2-(trifluoromethyl)benzyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(difluoromethoxy)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl-]methyl}-N^2-[3- (difluoromethoxy)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(- 2-chloro-4-fluorobenzyl)-5-nitropyrimidine- -2,4-diamine; N^4-{[4-(aminomethyl)- cyclohexyl]methyl}-N^2-(2-chloro- 3,6-difluorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2- (2,3,5-trifluorobenzyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(2,3,4,5-tetrafluorobenzyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2- [(1R)-1-phenylethyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]m- ethyl}-N^2-2,3-dihydro- 1H-inden-2-yl-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[(1S)-2,3- dihydro-1H-inden-1-yl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[(1R)-2,3- dihydro-1H-inden-1-yl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-ch- loro-1-naphthyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-methoxy- 2-naphthyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2- quinohn-6-ylpyrimidine-2,4-diamine; N^4-{[4-trans-(aminomethyl)cyclohexyl]methyl}- -N^2-(2,5-dichlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-trans-(aminomethyl)cyclohexyl]methyl}-N^2-(2,3-dichlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}– N^2-[2-(2-chlorophenyl)ethyl]-5-nitropyrimidi- ne-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(3-chlorophenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chloro- -6-phenoxybenzyl)-5-nitropyrimidine-2,4-di- amine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-2-naphthylpyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-(1-naphthylmethyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5- -nitro-N^2-(pyridin-3-ylmethyl)pyrimidine-2,4-diamine; 4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-2-[(2- chlorobenzyl)amino]pyrimidine-5-carbonitrile; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[4- (dimethylamino)benzyl]-5-nitropyrimid- ine-2,4-diamine; N^4-{[4-trans-(aminomethyl)cyclohexyl]methyl}-N^2-(2-bromobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-(7-aminoheptyl)-N^2-(2-bromobenzyl-)-5- nitropyrimidine-2,4-diamine; N^4-(7-aminoheptyl)-N^2-(2,5-dichlorobenzyl)-5- nitropyrimidine-2,4-diamine; N-({4-[({2-[(2-chlorobenzyl)amino]-5-nitropyrimidin-4- yl}amino)methyl]cyclohexyl}methyl-)guanidine; N^2-(3-aminobenzyl)-M-{-[4(aminomethyl)cyclohexyl]methyl}-5-nitro- pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(2-nitrobenzyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[- 2-(2-bromophenyl)ethyl]-5-nitropyrimidine– 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-bromobenzyl)-5-chloropyrimidine-2,4-diamine; (4-{[(2-{[2-(1H-indol-3-yl)ethyl]amino}-5-nitropyrimidin-) 4-yl)amino]methyl}cyclohexyl)methanaminium chloride; N-({3-[({2-[(2-chlorobenzyl)- amino]-5-nitropyrimidin-4- yl}amino)methyl]cyclohexyl}methyl)guanidine; 3-({[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5- nitropyrimidin-2-yl]amino}methyl)phenol; (4-{[(2-

{[2-(1H-imidazol-4-yl)ethyl]amino}-5- nitropyrimidin-4- yl)amino]methyl}cyclohexyl)-methanaminium chloride; N^2-(2-chlorobenzyl)-M-({4-cis-[(dimethylamino)methyl]cyclohexyl}methyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-chloro-N^2-(2- chlorobenzyl)pyrimidine-2,4-diamine; N^2-(2-chlorobenzyl)-5-nitro-N^4-(pipe- ridin-4-ylmethyl)pyrimidine-2.4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]meth- yl}-5-nitro-N^2-(2-phenylethyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(3-phenylpropyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N- ^{2}-(4-phenylbutyl)pyrimidine-2,4-diami- ne; N^4-{[4-(aminomethyl)cyclohexyl-]methyl}-5-nitro-N^2-(2-phenylpropyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(4-methoxyphenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}– N^2-[2-(3-methoxyphenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(2-methoxyphenyl)ethyl]-5-nitropyrimidine-2,4-diamine; 4-[({2-[(2-chlorobenzyl)amino]-5-nitropyrimidin-4-yl}amino)methyl]piperidine-1-carboximidamide; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3,5-dichlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-(5-aminopentyl)-N2-(2-chlorobenzyl)-5-nitropyrimidine-2,4-diamine; 2-(benzylamino)-4-(1,4,6,7-tetrahydro-imidazo[4,5- c]pyridin-5-yl)-5-trifluoromethyl-pyrimidine; 2-(4-chlorobenzylamino)-4-(1,4,6,7-tetrahydro-imidazo[4,5-c]pyridin-5-yl)-5-nitro-pyrimidine; 2-(2-chlorobenzylamino)-4-(1,4,6,7-tetrahydro-imidazo[4,5-c]pyridin-5-yl)-5-nitro-pyrimidine; 2-(benzylamino)-4-(1,4,6,7-tetrahydro-imidazo[4,5-c]pyridin-5-yl)-5-nitro-pyrimidine; or N^4-{[trans-4-(aminomethyl)cyclohexyl]methyl}-5-nitro- N^2-[2-(trifluoromethoxy)benzyl]pyrimidine-2,4-diamine.

[0410] In some embodiments, the pyrimidine derivative compounds of formula (XVIII) are selected from:

[0411] N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[(2R)-1,2,3,4-tetrahydronaphthalen-2-yl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(4-chlorophenyl)ethyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(3-methylphenyl)ethyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl-)cyclohexyl]methyl}-N^2-[2-(4-methylphenyl)ethyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(3-fluorophenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(4-fluorophenyl)ethyl]-5- nitropyrimidine-2,4-diamine; (1R,3R)-3-({[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5-nitropyrimidi- n-2-yl]amino}methyl)-4,4-dimethylcyclohexanol; N^4-({4-cis-[(dimethylamino)methyl]cyclohexyl}methyl)-N^2-(1-naphthylmethyl)-5- nitropyrimidine-2,4-diamine; N^2-[2-(methylthio)benzyl]-5-nitro-N^4-(piperidin-4-ylmethyl)pyrimidine-2,4-diamine; 5-nitro-N^4-(piperidin-4-ylmethyl)-N^2-{- 2-[(trifluoromethyl)thio] benzyl} pyrimidine-2,4-diamine; N^2-(1-naphthylmethyl)-5-nitro-N^4-(piperidin-4-ylmethyl)pyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl-)-N^2-[2-(methylthio)benzyl]-5- nitropyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-5-nitro-N^2– {2-[(trifluoromethyl)thio]benzyl}pyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-N^2-(1-naphthyl- methyl)-5- nitropyrimidine-2,4-diamine; N^4-{4-[(dimethylamino)methyl]benzyl}-N^2-[2-(methylthio)benzyl]– 5-nitropyrimidine-2,4-diamine; N^4-{4-[(dimethylamino)methyl]benzyl}-5-nitro-N^2-{2-[(trifluoromethyl)thio] benzyl}pyrimidine-2,4-diamine; N^4-[(1-methylpiperidin-4-yl)methyl]-N^2-[2-(methylthio)benzyl]-5-

nitropyrimidine-2,4-diamine; N^4-[(1-methylpiperidin-4-yl)methyl]-5-nitro-N^2-{2-[(trifluoromethyl)thio] benzyl}pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-methoxybenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[2- (trifluoromethyl)benzyl]pyrimidine– 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,- 4-dichlorobenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[4-fluoro-2-(trifluoromethyl) benzyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-methylbenzyl)-5-ni- tropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-chlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-bromobenzyl-)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-chloro-5-(trifluoromethyl) benzyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,5-dichlorobenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[2-(trifluoromethoxy) benzyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chloro-6-methylbenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl-)cyclohexyl]methyl}-N^2-(2,3-dichlorobenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[3-(aminomethyl)cyclohexyl]methyl}-N^2-(2- -chlorobenzyl)-5-nitropyrimidine-2,4-diamine; N^2-(2-chlorobenzyl)-N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,5-difluorobenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(- 2-ethoxybenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-methylbenzyl)-5-ni- tropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-fluorobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-chloro-2-fluorobenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(1,1'-biphenyl-2-yl methyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,4-difluorobenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(- 2,3-difluorobenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,6-difluorobenzyl)–5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-fluoro-3-(trifluoromethyl) benzyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-chloro-2-fluorobenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-bromobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl-)cyclohexyl]methyl}-N^2-(2,3-dimethylbenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2- -(methylthio)benzyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-{2-[(trifluoromethyl)thio]benzyl} pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-fluorobenzyl)-5-ni-tropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(6-chloro-2-fluoro-3-methylbenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chloro-6-fluoro-3-methylbenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-2-naphthyl-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(1-naphthylmethyl)-5– nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-chloro-2-methylbenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(5-chloro-2-methylbenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(3-chloro-2- -methylbenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[5-fluoro-2-(trifluoromethyl)benzyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(5-chloro-2-fluorobenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl-

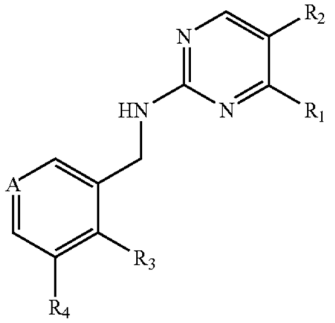
)cyclohexyl]methyl}-N^2-(2,3-difluoro-4-methylbenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(5-fluoro-2-methylbenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-(2,- 5-dichlorobenzyl)pyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-(2-bromobenzy- l) pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(cyclohexylmethyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-[2-(trifluoromethyl)benzyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(difluoromethoxy)benzyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chloro-4-fluoroben- zyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl-)cyclohexyl]methyl}-N^2-(2-chloro-3,6-difluorobenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(2,3,5-trifluorobenzyl)pyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-2,- 3-dihydro-1H-inden-2-yl-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-chloro-1-naphthyl)- -5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(4-methoxy-2-naphthyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-quinolin-6-ylpyrimidine-2,4-diamine; N^4-{[4-trans-(aminomethyl)cyclohexyl]methyl}-N^2-(2,3-dichlorobenzyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(2-chlorophenyl)ethyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(3-chlorophenyl)ethyl]-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-bromo-N^2-2-n-aphthylpyrimidine-2,4-diamine; 4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-2-[(2-chlorobenzyl) amino]pyrimidine-5-carbonitrile; N^4-{[4–trans-(aminomethyl)cyclohexyl]methyl}-N^2-(2-brom-obenzyl)-5- nitropyrimidine-2,4-diamine; N^4-(7-aminoheptyl)-N^2-(2-bromobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-(7-aminoheptyl)-N^2-(2,5-dichlorobenzyl)-5-nitropy- rimidine-2,4-diamine; N-({4-[({2-[(2-chlorobenzyl)amino]-5-nitropyrimidin-4-yl}amino)methyl]cyclohexyl}methyl)guanidine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(2-nitrobenzy- l)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(2-bromophenyl)ethyl]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-bromobenzyl)-5-chloropyrimidine-2,4-diamine; N-({3-[({2-[(2-chlorobenzyl)amino]-5-nitropyrimidin-4-yl}amino)methyl]cyclohexyl}methyl)guanidine 3-({[4-({[4-(aminomethyl)cyclohexyl]methyl}amino)-5-nitropyrimidin-2-yl]amino}methyl)phenol; N^2-(2-chlorobenzyl)-N^4-({4-cis-[(dimethylamino)methyl]cyclohexyl}methyl)-5- nitropyrimidine-2,4-diamine; N^2-(2-chlorobenzyl)-5-nitro-N^4-(piperidin-4-ylmethyl)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(2-phenylethy- l)pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-(4-phenylbutyl)pyrimidine-2,4-diamine; or N^4-{[trans-4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[2– (trifluoromethoxy)- benzyl]pyrimidine-2,4-diamine.

[0412] In yet other embodiments, the pyrimidine derivative compounds of formula (XVIII) are selected from:

[0413] N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-N^2-(1-naphthylmethyl)-5-nitropyrimidine-2,4-diamine; N^2-[2-(methylthio)benzyl]-5-nitro-N^4-(piperidin-4-ylmethyl)pyrimidine-2,4-diamine; 5-nitro-N^4-(piperidin-4-ylmethyl)-N^2-{- 2-[(trifluoromethyl)thio] benzyl}pyrimidine-2,4-diamine; N^2-(1-naphthylmethyl)-5-nitro-N^4-(piperidin-4-ylmethyl)pyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl-)-N^2-[2-

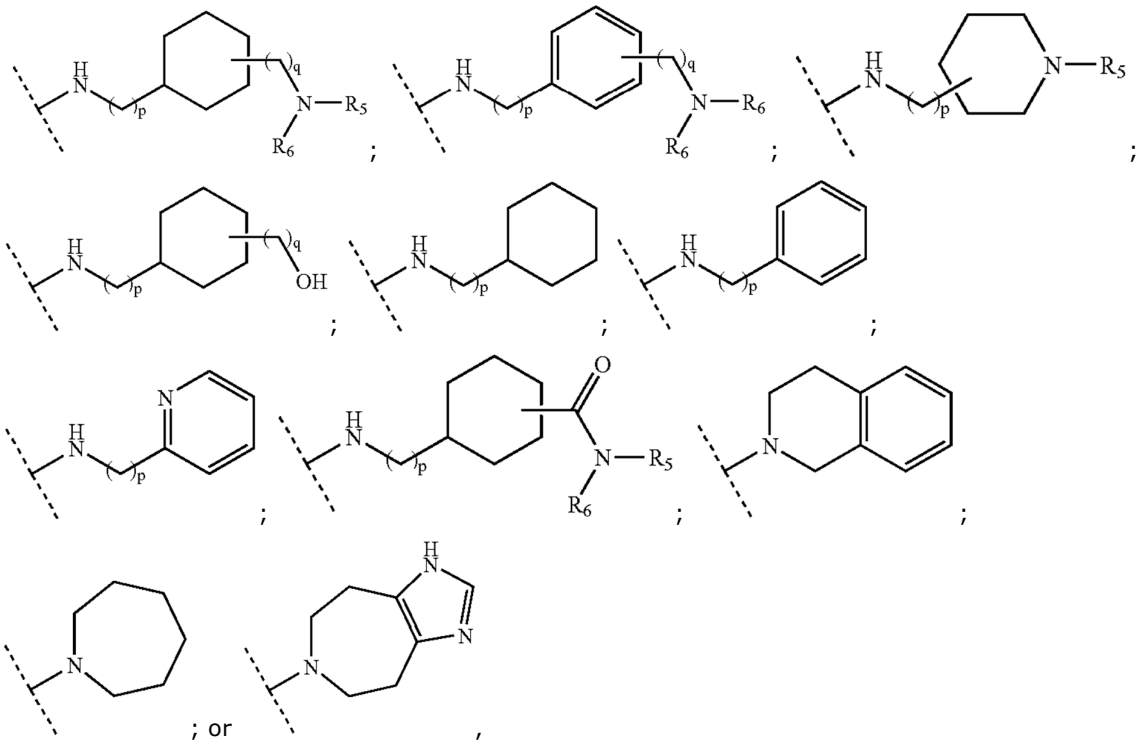
(methylthio)benzyl]-5- nitropyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-5-nitro-N^2– {2-[(trifluoromethyl)thio]benzyl}pyrimidine-2,4-diamine; N^4-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-N^2-(1-naphthyl- methyl)-5- nitropyrimidine-2,4-diamine; N^4-{4-[(dimethylamino)methyl]benzyl}-N^2-[2-(methylthio)benzyl]– 5-nitropyrimidine-2,4-diamine; N^4-{4-[(dimethylamino)methyl]benzyl}-5-nitro-N^2-{2-[(trifluoromethyl)thio] benzyl}pyrimidine-2,4-diamine; N^4-[(1-methylpiperidin-4-yl)methyl]-N^2-[2-(methylthio)benzyl]-5-nitropyrimidine-2,4-diamine; N^4-[(1-methylpiperidin-4-yl)methyl]-5-nitro-N^2-{2-[(trifluoromethyl)thio] benzyl}pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-methoxybenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[2-(trifluoromethoxy) benzyl]pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]-methyl}-N^2-(2,3-dichlorobenzyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[3-(aminomethyl)cyclohexyl]methyl}-N^2-(2-chlorobenzy- l)-5-nitropyrimidine-2,4-diamine; N^2-(2-chlorobenzyl)-N- ^{4}-({4-[(dimethylamino)methyl]cyclohexyl}methyl)-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-bromobenzyl)-5-nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-[2-(methylthio)benzyl-]-5- nitropyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-{2-[(trifluoromethyl)thio]benzyl}- pyrimidine-2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}–N^2-(1-naphthylmethyl)-5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2,3-dichlorobenzyl)– 5-nitropyrimidine- 2,4-diamine; N^4-{[4-(aminomethyl)cyclohexyl]methyl}-N^2-(2-bromobenzyl)-5-nitropyrimidine-2,4-diamine; N^2-(2-chlorobenzyl)-5-nitro-N^4-(piperidin-4-ylmethyl)pyrimidine-2,4-diamine; or N^4-{[trans-4-(aminomethyl)cyclohexyl]methyl}-5-nitro-N^2-[2-(trifluoromethoxy)- benzyl]pyrimidine-2,4-diamine.

[0414] Alternative PKC-θ inhibitor pyrimidine derivatives include the compounds described by Barbosa *et al.* in US Publication No. 2010/0318929, which is incorporated herein by reference in its entirety. These compounds are represented by formula (XIX):



(XIX)

[0415] R_1 is selected from the following groups:

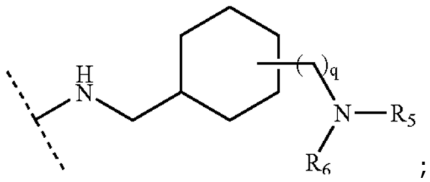
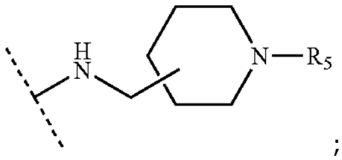
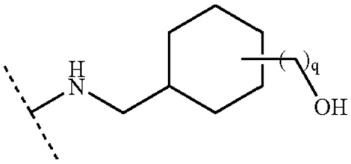
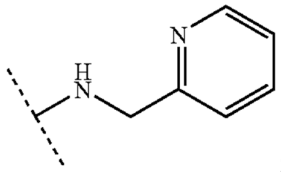
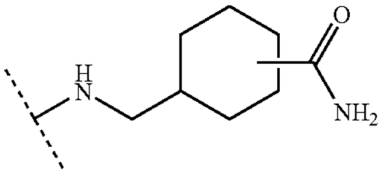
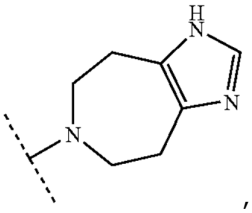


; or ,

[0416] wherein: p is 1, 2 or 3; q is 0 or 1, R_5, R_6 are each independently selected from: (A) hydrogen, (B) C_{1-6}alkyl, or wherein R_5 and R_6 together constitute a methylene bridge which together with the nitrogen atom between them forms a four to six-membered ring wherein one of the methylene groups is optionally replaced by an oxygen or nitrogen atom, and which ring is optionally and independently substituted by one or more of the following groups: (i) C_{1-6}alkyl (ii) COR_7, wherein R_7 is: (a) C_{1-6}alkyl, (b) C_{1-6}alkyloxy, (C) C_{1-6}alkylcarbonyl, (D) C_{1-6}alkylsulfonyl, (E) $-CONR_8R_9$, wherein R_8 and R_9 are each independently selected from: (i) hydrogen (ii) C_{1-6}alkyl; R_2 is selected from the following groups: (F) CF_3, (G) cyano, (H) $CONH_2$ (I) halogen, or (J) nitro; R_3 is selected from the following groups: (A) hydrogen, (B) C_{1-6}alkyl, which is optionally substituted with halogen, (C) C_{1-6}alkyloxy, which is optionally substituted with halogen, (D) halogen, R_4 is selected from the following groups: (A) heteroaryl, which is optionally substituted with C_{1-6}alkyl; (B) aryl or heteroaryl, which is substituted with one or more of the following groups: (i) C_{1-6}alkyl, which is substituted with hydroxyl, oxo, or $NR_{10}R_{11}$, wherein R_{10} and R_{11} are each independently selected from the following groups: (a) hydrogen, (b) C_{1-6}alkyl, which is optionally substituted with hydroxyl or $CONH_2$, (c) C_{1-6}alkylcarbonyl, which is optionally substituted with one or more halogens, (d) C_{1-6}alkylsulfonyl, (e) or wherein R_{10} and R_{11} constitute a methylene bridge which together with the nitrogen atom between them forms a four to six-membered ring, (ii) $CONR_{12}R_{13}$, wherein R_{12} and R_{13} are each independently selected from hydrogen or C_{1-6}alkyl, (iii) $SO_2NR_{12}R_{13}$, wherein R_{12} and R_{13} are each independently selected from hydrogen or C_{1-6}alkyl, (C) $-NR_{14}R_{15}$, wherein R_{14} and R_{15} are each independently selected from: (i) C_{1-6}alkylcarbonyl, which is substituted with amino, (ii) or wherein R_{14} and R_{15} constitute a methylene bridge which together with the nitrogen atom between them forms a four to seven-membered ring, wherein one of the methylene groups is substituted with C_{1-6}alkyl, and wherein each C_{1-6}alkyl is optionally substituted with hydroxyl or $NR_{10}R_{11}$, wherein R_{10} and R_{11} are as defined previously, (D)-$CONR_{16}R_{17}$, wherein R_{16} and R_{17} are each independently selected from: (i) C_{1-6}alkyl, which is substituted with hydroxyl or $NR_{18}R_{19}$, wherein R_{18} and R_{19} are each independently selected from hydrogen or C_{1-6}alkyl, or wherein R_{18} and R_{19}

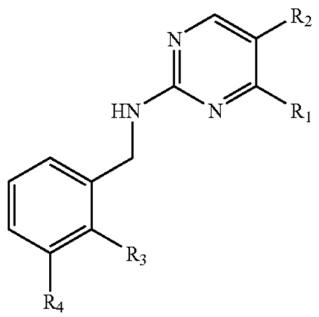
constitute a methylene bridge which together with the nitrogen atom between them forms a four to six-membered ring, wherein one of the methylene groups is optionally replaced by an oxygen; (E) C_6alkynyl group optionally substituted by amino, C_{1-3}alkylamino, or di-(C_{1-3}alkyl)amino; and A is independently selected from carbon or nitrogen; or a tautomer, pharmaceutically acceptable salt, solvate or amino-protected derivative thereof.

[0417] In illustrative examples of this type: R_1 is selected from the following groups:


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; or

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[0418] wherein: q is 0 or 1, R_5, R_6 are each independently selected from: (A) hydrogen, (B) or wherein R_5 and R_6 together constitute a methylene bridge which together with the nitrogen atom between them forms a five to six-membered ring wherein one of the methylene groups is optionally replaced by a nitrogen atom, and which ring is optionally and independently substituted by one or more of the following groups: (iv) C_{1-6}alkyl (v) COR_7, wherein R_7 is C_{1-6}alkyloxy, (C) C_{1-6}alkylcarbonyl (D) C_{1-6}alkylsulfonyl; R_2 is selected from the following groups: (A) cyano, or (B) nitro; R_3 is selected from the following groups: (A) C_{1-3}alkyl, (B) C_{1-3}alkyloxy, which is optionally substituted with fluorine, (C) halogen; R_4 is selected from the following groups: (A) aryl, which is substituted with one or more of the following groups: (i) C_{1-3}alkyl, which is substituted with hydroxyl or $NR_{20}R_{21}$, wherein R_{20} and R_{21} are each independently selected from the following groups: (f) hydrogen, (g) C_{1-3}alkyl, which is optionally substituted with hydroxyl or $CONH_2$, (h) or wherein R_{20} and R_{21} constitute a methylene bridge which together with the nitrogen atom between them forms a five to six-membered ring, (ii) $CONH_2$ (iii) SO_2NH_2, (B) 3-pyridyl, which is optionally substituted with C_{1-3}alkyl, wherein each alkyl group is optionally substituted with amino, (C) –$NR_{22}R_{23}$, wherein R_{22} and R_{23} constitute a methylene bridge which together with the nitrogen atom between them forms a five to six-membered ring, wherein one of the methylene groups is substituted with C_{1-3}alkyl, and wherein each C_{1-3}alkyl is optionally substituted with OH or $NR_{20}R_{21}$, where R_{20} and R_{21} are as defined previously, (D) –$CONR_{24}R_{25}$, wherein R_{24} and R_{25} are each independently selected from: (i) C_{1-3}alkyl, which is substituted with C_{1-3}alkylamino; and A is independently selected from carbon or nitrogen; or a tautomer, pharmaceutically acceptable salt, solvate or amino-protected derivative thereof.

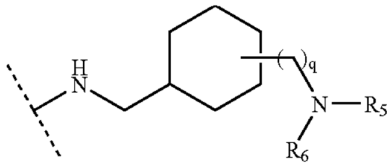
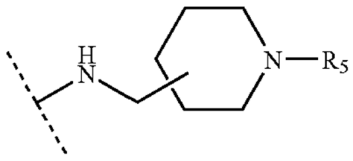
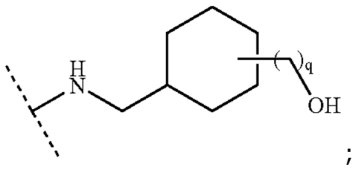
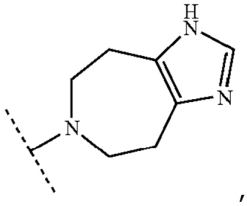
[0419] In other illustrative examples, the compounds of formula (XIX) are represented by formula (XIXa):



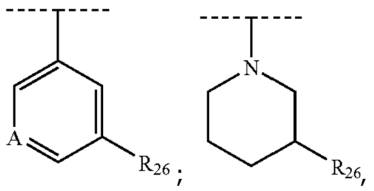
(XIXa)

[0420] wherein:

[0421] R_1 is selected from the following groups:


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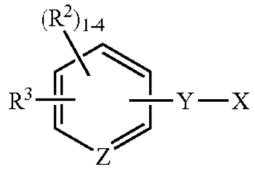
[0422] wherein: q is 0 or 1 R_5, R_6 are each independently selected from: (A) hydrogen, (B) $C_{1\text{-}6}$alkylcarbonyl, (C) $C_{1\text{-}6}$alkylsulfonyl; R_2 is selected from the following groups: (A) cyano, or (B) nitro; R_3 is selected from the following groups: (A) CH_3, (B) OCF_3, (C) Cl; R_4 is selected from the following groups:


;

,

[0423] wherein: R_{26} is selected from the following groups: (A) $C_{1\text{-}3}$alkyl, which is substituted with hydroxyl or $NR_{27}R_{28}$, wherein R_{27} and R_{28} are each independently selected from the following groups: (i) hydrogen, (ii) $C_{1\text{-}3}$alkyl, which is optionally substituted with hydroxyl or $CONH_2$, (B) $CONH_2$ (C) SO_2NH_2; and A is carbon or nitrogen; or a tautomer, pharmaceutically acceptable salt, solvate or amino-protected derivative thereof.

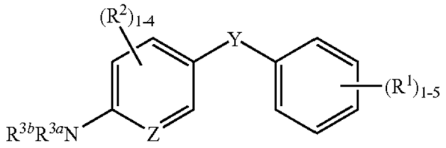
[0424] Also contemplated as small molecule PKC-θ inhibitors are aniline compounds as described for example by Ajioka *et al.* in US Publication No. 2010/0120869, which is incorporated herein by reference in its entirety. Representative compounds of this type are represented by formula (XX):



(XX)

[0425] wherein X of formula XX is aryl or heteroaryl, each substituted with 1-5 R^1 groups. Y of formula XX is –O–, $–S(O)_n–$, $–N(R^4)–$ and $–C(R^4)_2–$, wherein subscript n is 0-2. Z of formula XX is –N= or –CH=. Each R^1 of formula XX is independently from the group consisting of H, halogen, C_{1-8} alkyl, C_{1-6} heteroalkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkoxy, $–OR^{1a}$, $–C(O)R^{1a}$, $–C(O)OR^{1a}$, $–C(O)NR^{1a}R^{1b}$, $–NR^{1a}R^{1b}$, $–SR^{1a}$, $–N(R^{1a})C(O)R^{1b}$, $–N(R^{1a})C(O)OR^{1b}$, $–N(R^{1a})C(O)NR^{1a}R^{1b}$, $–OP(O)(OR^{1a})_2$, $–S(O)_2OR^{1a}$, $–S(O)_2NR^{1a}R^{1b}$, $–S(O)_2–C_{1-6}$ haloalkyl, –CN, cycloalkyl, heterocycloalkyl, aryl or heteroaryl. Each of R^{1a} and R^{1b} of formula XX is independently H or C_{1-6} alkyl. Each R^2 of formula XX is independently H, halogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $–NR^{1a}R^{1b}$, $–NR^{1a}C(O)–C_{1-6}$ alkyl, $–NR^{1a}C(O)–C_{1-6}$ haloalkyl, $–NR^{1a}–(CH_2)–NR^{1a}R^{1b}$, $–NR^{1a}–C(O)–NR^{1a}R^{1b}$, or $–NR^{1a}–C(O)OR^{1a}$, alternatively, adjacent R^1 groups and adjacent R^2 groups can be combined to form a cycloalkyl, heterocycloalkyl, aryl or heteroaryl. R^3 of formula XX is $–NR^{3a}R^{3b}$ or –NCO. Each of R^{3a} and R^{3b} of formula XX are independently H, C_{1-6} alkyl, $–C(O)–C_{1-6}$ alkyl, $–C(O)–C_{1-6}$ haloalkyl, $–(CH_2)–NR^{1a}R^{1b}$, $–C(O)–NR^{1a}R^{1b}$, $–C(O)OR^{1a}$, –C(S)CN, an amino acid residue, a peptide or an oligopeptide. Each R^4 of formula XX is independently H or C_{1-6} alkyl, or when more than one R^4 group is attached to the same atom, the R^4 groups are optionally combined to form a C_{5-8} cycloalkyl. The compounds of formula XX also include the salts, hydrates and prodrugs thereof.

[0426] In some embodiments, the aniline compounds of formula XX are represented by formula XXa:

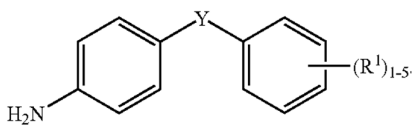


(XXa)

[0427] wherein each R^1 of formula XXa is independently H, halogen, C_{1-8} alkyl, C_{1-6} heteroalkyl, C_{1-6} haloalkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} haloalkoxy, $–OR^{1a}$, –CN, cycloalkyl, heterocycloalkyl, aryl or heteroaryl, and each of R^{3a} and R^{3b} of formula XXa are independently H, $–C(O)–C_{1-6}$ alkyl, an amino acid residue, a peptide or an oligopeptide.

[0428] In still other embodiments, each R^1 of formula XXa is independently H, halogen, C_{1-8} alkyl, C_{1-6} haloalkyl, C_{1-6} haloalkoxy, $–C(O)OR^{1a}$, cycloalkyl, or heteroaryl. Furthermore, each R^2 of formula XXa is independently H, halogen, or $–NR^{1a}C(O)–C_{1-6}$ alkyl. In yet other embodiments, each R^1 of formula XXa is independently H, methyl, n-propyl, isopropyl, t-butyl, t-pentyl, Cl, Br, CF_3, OCF_3, cyclopentyl, pyrrolyl, or CO_2H, and each R^2 is independently H or Cl. In other embodiments, R^{3a} of formula XX is an amino acid residue, and R^{3b} is H. Suitably, the amino acid residue is an arginine residue.

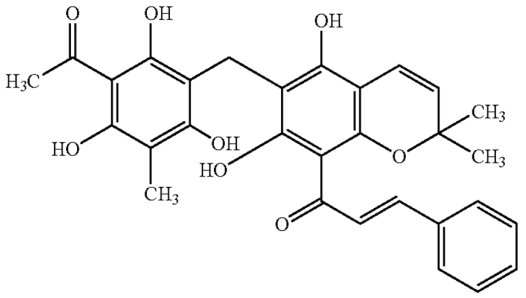
[0429] In still other embodiments, the aniline compounds of formula XX have the formula XXb:



[0430] In some other embodiments, Y of formula XXb is S. In still other embodiments, Y of formula XXb is O. In some embodiments, each R^1 of formula XXb is independently H, methyl, n-propyl, isopropyl, t-butyl, t-pentyl, Cl, Br, CF_3, OCF_3, cyclopentyl, pyrrolyl, or CO_2H. In yet other

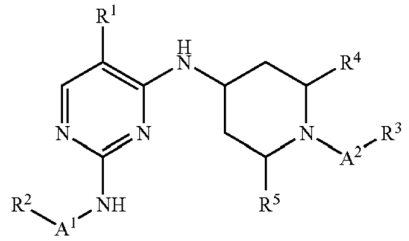
embodiments, each R^1 of formula XXb is independently C_{1-8} alkyl or cycloalkyl. In still yet other embodiments, each R^1 of formula XXb is independently 4-t-butyl, 4-cyclopentyl or 4-t-pentyl.

[0431] In other embodiments, small molecule PKC-θ inhibitors are selected from rottlerin (also known as mallotoxin or 1-[6-[(3-acetyl-2,4,6-trihydroxy-5-methylphenyl)methyl]-5,7-dihydroxy-2,2- -dimethyl-2H-1-benzopyran-8-yl]-3-phenyl-2-propen-1-one, available from Calbiochem, San Diego, Calif.) having formula (XXI), or a derivative or analogue thereof.



(XXI)

[0432] In still other embodiments, small molecule PKC-θ inhibitors include substituted diaminopyrimidines as disclosed for example by Baudler in US Patent Application Publication US 2005/0222186 A1, which is incorporated herein by reference in its entirety. These compounds are represented by formula (XXII):



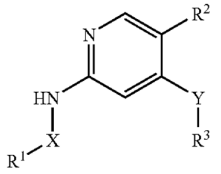
(XXII)

[0433] wherein R^1, R^2 and R^3 are independently selected from the group consisting of substituted or unsubstituted phenyl, naphthyl, pyrrolyl, pyrazolyl, imidazolyl, 1,2,3-triazolyl, indolyl, benzimidazolyl, furanyl(furyl), benzofuranyl(benzofuryl), thiophenyl(thienyl), benzothiophenyl(benzothienyl), thiazolyl, isoxazolyl, pyridinyl, pyrimidinyl, quinolinyl and isoquinolinyl; R^4 is hydrogen or methyl; R^5 is hydrogen or methyl; A^1 is C_{1-3} alkylene or ethyleneoxy ($–CH_2–CH_2–O–$); and A^2 is C_{1-3} alkylene or ethyleneoxy ($–CH_2–CH_2–O–$); and hydrates, solvates, salts, or esters thereof.

[0434] Non-limiting examples of such compounds include [1-benzyl(4-piperidyl)]{2-[(2-pyridylmethyl)amino]-5-(3-thienyl)pyrimidin-4-yl}amine; {5-(4-methoxyphenyl)-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl}[1-benzyl-(- 4-piperidyl)]amine; {5-phenyl-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl}[1-benzyl(4-piperidy-1-)]amine; {5-(4-chlorophenyl)-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl}[1-benzyl(4-piperidyl)]amine; {5-(4-(N,N-dimethylamino)phenyl)-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl- }[1-benzyl(4-piperidyl)]amine; {5-(phenyl-4-carboxamido)-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl}[1-benzyl(4-piperidyl)]-amine; {5-(4-carboxyphenyl)-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl}[1-benzyl-(- 4-piperidyl)]amine; {5-(2-thienyl)-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl}[1-benzyl(4-piperidyl)]amine; {5-(2-furanyl)-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl}[1-benzyl(4-piperidyl)]amine; {5-(3-furanyl)-2-[(4-pyridylmethyl)amino)pyrimidin-4-yl}[1-benzyl(4-piperidyl)]amine; N(4)-(1-benzyl-piperidin-4-yl)-5-(3-chloro-4-fluoro-phenyl)-N(2)-pyridin- 2-

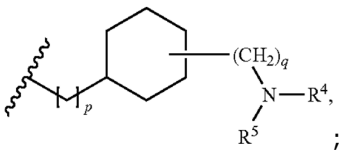
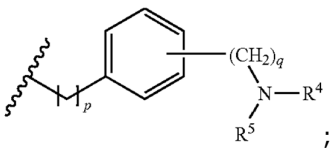
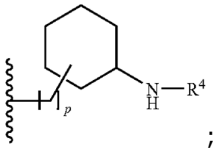
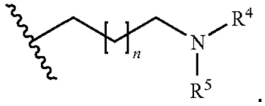
ylmethyl-pyrimidine-2,4-diamine; N-(3-[4-(1-benzyl-piperidin-4-ylamino)-2-[(pyridin-2-ylmethyl)-amino]-pyrimidin-5-yl}phenyl)-acetamide; 3-[4-(1-benzyl-piperidin-4-ylamino)-2-[(pyridin-2-ylmethyl)-amino]-pyrimidin-5-yl]-phenol; and 4-{4-(1-benzyl piperidin-4-ylamino)-2-[(pyridin-2-ylmethyl)-amino]-pyrimidin-5-yl}N,N-di- methyl-benzamide.

[0435] In still other embodiments, small molecule PKC-θ inhibitors are selected from substituted pyridine compounds as disclosed for example by Brunette in US Patent Application Publication US 2006/0217417, which is incorporated herein by reference in its entirety. These compounds are represented by formula (XXIII):



(XXIII)

[0436] wherein X is a bond or C_{1-6} substituted or unsubstituted alkyl wherein one or two of the methylene units can be replaced by an oxygen or sulfur atom; Y is –NH–, –O– or –S–; R^1 is a C_{3-6} substituted or unsubstituted cycloalkyl, substituted or unsubstituted aryl or substituted or unsubstituted heteroaryl; R^2 is selected from the following group consisting of trifluoromethyl, cyano, $–CONH_2$, halogen, and nitro; and R^3 is

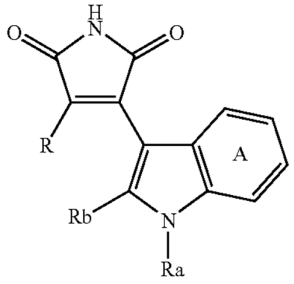

;

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; or

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[0437] wherein p is an integer from 1 to 3, inclusive; q is an integer from 0 to 3, inclusive; n is an integer from 0 to 5, inclusive; R^4 and R^5 are each independently selected from the group consisting of hydrogen, C_{1-6} substituted or unsubstituted alkyl, or wherein R^4 and R^5 together constitute methylene bridges which together with the nitrogen atom between them form a four to six-membered substituted or unsubstituted ring wherein one of the methylene groups is optionally replaced by an oxygen, sulfur or NR group, wherein R is hydrogen or C_{1-6} substituted or unsubstituted alkyl; tautomers; and pharmaceutically acceptable salts, solvates or amino-protected derivatives thereof.

[0438] Non-limiting examples of the compounds having formula (XXIII) include 5-nitro-N4-piperidin-4-ylmethyl-N2-(2-trifluoromethoxy-benzyl)-pyridine-2- ,4-diamine; N2-(2,3-dichloro-benzyl)-5-nitro-N4-piperidin-4-ylmethyl-pyridine-2,4-diamine; N2-[2-(3-chloro-phenyl)-ethyl]-5-nitro-N4-piperidin-4-ylmethyl-pyridine-2,4-diamine; 5-nitro-N2-phenethyl-N4-piperidin-4-ylmethyl-pyridine-2,4-diamine; N4-(4-aminomethyl-cyclohexylmethyl)-5-nitro-N2-(2-trifluoromethoxy-benzyl-)-pyridine-2,4-diamine; N4-(4-aminomethyl-cyclohexylmethyl)-N2-(2,3-dichloro-benzyl)-5-nitro-pyri- dine-2,4-diamine; N4-(4-aminomethyl-cyclohexylmethyl)-5-nitro-N2-phenethyl-pyridine-2,4-dia- mine; N4-(4-aminomethyl-cyclohexylmethyl)-N2-[2-(3-chloro-phenyl)-ethyl]-5-nitro-1-pyridine-2,4-diamine; N4-(4-aminomethyl-cyclohexylmethyl)-5-nitro-N2-(2-chloro-benzyl)-pyridine- -2,4-diamine; N4-(4-trans-aminomethyl-cyclohexylmethyl)-5-nitro-N-2-(2-

trifluoromethoxy- benzyl)-pyridine-2,4-diamine; N4-(4-trans-amino-cyclohexylmethyl)-5-nitro-N2-(2-trifluoromethoxy-benzyl-)-pyridine-2,4-diamine; 4-[(4-aminomethyl-cyclohexylmethyl)-amino]-6-(2-chloro-benzylamino)-nicotinamide; and 4-[(4-aminomethyl-cyclohexylmethyl)-amino]-6-(2-chloro-benzylamino)-nicotinonitrile.

[0439] In still other embodiments, small molecule PKC-θ inhibitors are selected from indolyl-pyrroledione derivatives as disclosed for example by Auberson in US Patent Application Publication US 2007/0142401, which is incorporated herein by reference in its entirety. These compounds are represented by formula (XXIV):



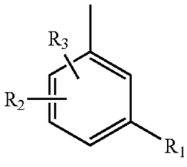
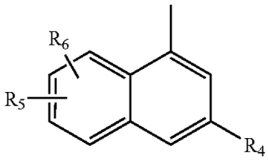
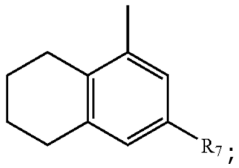
(XXIV)

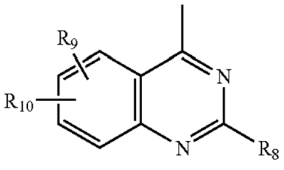
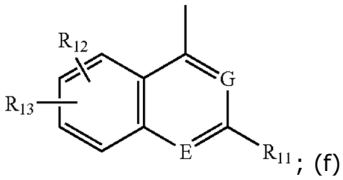
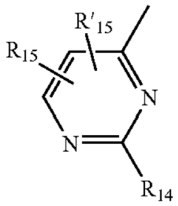
[0440] wherein

[0441] R_a is H; C_{1-4}alkyl; or C_{1-4}alkyl substituted by OH, NH_2, NHC_{1-4}alkyl or $N(di\text{-}C_{1-4}alkyl)_2$;

[0442] R_b is H; or C_{1-4}alkyl;

[0443] R is a radical of formula (a), (b), (c), (d), (e) or (f)

(a) ; (b) ; (c) ;

(d) ; (e) ; (f) 

[0444] wherein each of R_1, R_4, R_7, R_8, R_{11} and R_{14} is OH; SH; a heterocyclic residue; $NR_{16}R_{17}$ wherein each of R_{16} and R_{17}, independently, is H or C_{1-4}alkyl or R_{16} and R_{17} form together with the nitrogen atom to which they are bound a heterocyclic residue; or a radical of formula α – X–R_c–Y (α) wherein X is a direct bond, O, S or NR_{18} wherein R_{18} is H or C_{1-4}alkyl, R_c is C_{1-4}alkylene or C_{1-4}alkylene wherein one CH_2 is replaced by CR_xR_y wherein one of R_x and R_y is H and the other is CH_3, each of R_x and R_y is CH_3 or R_x and R_y form together –CH_2–CH_2–, and Y is bound to the terminal carbon atom and is selected from OH, a heterocyclic residue and –$NR_{19}R_{20}$ wherein each of R_{19} and R_{20} independently is H, C_{3-6}cycloalkyl, C_{3-6}cycloalkyl-C_{1-4}alkyl, aryl-C_{1-4}alkyl or C_{1-4}alkyl optionally substituted on the terminal carbon atom by OH, or R_{10} and R_{20} form together with the nitrogen atom to which they are bound a heterocyclic residue;

[0445] each of R_2, R_3, R_5, R_6, R_9, R_{10}, R_{12}, R_{13}, R_{15} and R'_{15}, independently, is H, halogen, C_{1-4}alkyl, CF_3, OH, SH, NH_2, C_{1-4}alkoxy, C_{1-4}alkylthio, NHC_{1-4}alkyl, $N(di\text{-}C_{1-4}alkyl)_2$ or CN;

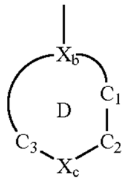
[0446] either E is –N= and G is –CH= or E is –CH= and G is –N=; and

[0447] ring A is optionally substituted,

[0448] or a salt thereof.

[0449] In illustrative examples, the heterocyclic residue as R_1, R_4, R_7, R_8, R_{11}, R_{14} or Y or formed, respectively, by $NR_{16}R_{17}$ or $NR_{19}R_{20}$, is a three to eight membered saturated, unsaturated or aromatic heterocyclic ring comprising 1 or 2 heteroatoms, and optionally substituted on one or more ring carbon atoms and/or on a ring nitrogen atom when present.

[0450] In specific embodiments, the heterocyclic residue is R_1, R_4, R_7, R_8, R_{11}, R_{14} or Y or formed, respectively, by $NR_{16}R_{17}$ or $NR_{19}R_{20}$, is a residue of formula (γ).



(γ)

[0451] wherein

[0452] the ring D is a 5, 6 or 7 membered saturated, unsaturated or aromatic ring;

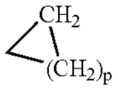
[0453] X_b is –N–, –C– or –CH–;

[0454] X_c is –N=, $-NR_f-$, $-CR_{f'}=$ or $-CHR_{f'}-$ wherein R_f is a substituent for a ring nitrogen atom and is selected from C_{1-6}alkyl; acyl; C_{3-6}cycloalkyl; C_{3-6}cycloalkyl-C_{1-4}alkyl; phenyl; phenyl-C_{1-4}alkyl;

[0455] a heterocyclic residue; and a residue of formula β $-R_{21}-Y'$ (β)

[0456] wherein R_{21} is C_{1-4}alkylene or C_{2-4}alkylene interrupted by O and Y' is OH, NH_2, $NH(C_{1-4}alkyl)$ or $N(C_{1-4}alkyl)_2$; and $R_{f'}$ is a substituent for a ring carbon atom and is selected from C_{1-4}alkyl;

[0457] C_3-cycloalkyl optionally further substituted by C_{1-4}-alkyl;



[0458] wherein p is 1, 2 or 3; CF_3;

[0459] halogen; OH; NH_2; $-CH_2-NH_2$; $-CH_2-OH$; piperidin-1-yl; and pyrrolidinyl;

[0460] the bond between C_1 and C_2 is either saturated or unsaturated;

[0461] each of C_1 and C_2, independently, is a carbon atom which is optionally substituted by one or two substituents selected among those indicated above for a ring carbon atom; and

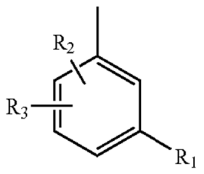
[0462] the line between C_3 and X_b and between C_1 and X_b, respectively, represents the number of carbon atoms as required to obtain a 5, 6 or 7 membered ring D.

[0463] In other non-limiting examples of compounds according to formula (XXIV)

[0464] Ra is H; CH_3; CH_2–CH_3; or isopropyl,

[0465] Rb is H; halogen; C_{1-6}-alkoxy; or C_{1-6}alkyl, and either

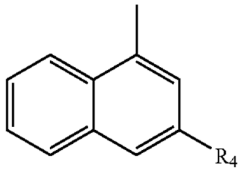
[0466] I. R is a radical of formula (a)



(a)

[0467] wherein R_1 is piperazin-1-yl optionally substituted by CH_3 in position 3 or 4; or 4,7-diaza-spiro (2.5] oct-7-yl; R_2 is Cl; Br; CF_3; or CH_3; and R_3 is H; CH_3; or CF_3; R_3 being other than H when Ra is H or CH_3, Rb is H and R_1 is 4-methyl-1-piperazinyl; or

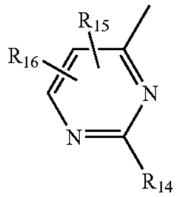
[0468] II. R is a radical of formula (b)



(b)

[0469] wherein R_4 is piperazin-1-yl substituted in positions 3 and/or 4 by CH_3; or 4,7-diaza-spiro [2.5] oct-7-yl; Ra being other than H or CH_3 when R_4 is 4-methyl-1-piperazinyl; or

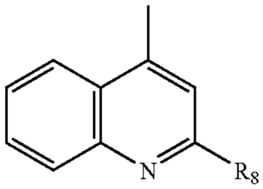
[0470] III. R is a residue of formula (c)



(c)

[0471] wherein R_{14} is piperazin-1-yl optionally substituted by CH_3 in position 3 and/or 4 or in position 3 by ethyl, phenyl-C_{1-4}alkyl, C_{1-4}alkoxy-C_{1-4}alkyl or halogeno-C_{1-4}alkyl; or 4,7-diaza-spiro [2.5] oct-7-yl; R_{15} is halogen; CF_3; or CH_3; R_{15} being other than CH_3 when Ra is H or CH_3, Rb is H and R_{14} is 4-methyl-1-piperazinyl; and R_{16} is H; CH_3; or CF_3; R_{16} being other than H when R_{15} is Cl, Ra is H or CH_3, Rb is H and R_{14} is 4-methyl-1-piperazinyl; or

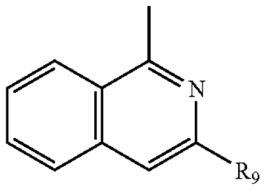
[0472] IV. R is a radical of formula (d)



(d)

[0473] wherein R_8 is piperazin-1-yl, 3-methyl-piperazin-1-yl or 4-benzyl-piperazin-1-yl; or

[0474] V. R is a radical of formula (e)



(e)

[0475] wherein R_9 is 4,7-diaza-spiro [2.5] oct-7-yl; or piperazin-1-yl substituted in position 3 by methyl or ethyl and optionally in position 4 by methyl.

[0476] In some embodiments of compounds according to formula (XXIV)

[0477] when R is of formula (a)

[0478] R_1 is -(4-methyl-piperazin-1-yl), 1-piperazinyl, 3-methyl-piperazin-1-yl or -(4,7-diaza-spiro[2.5]oct-7-yl)

[0479] R_2 is 2-Cl or 2-CH_3

[0480] R_3 is 3-CH_3, 3-CF_3or H

[0481] R_a is H or CH_3

[0482] and when,

[0483] R is of formula (b)

[0484] R_4 is -(4,7-diaza-spiro[2.5]oct-7-yl), 3-methyl-piperazin-1-yl or 4-methyl-3-methyl-piperazin-1-yl

[0485] R_a is H or CH_3

[0486] and when

[0487] R is of formula (c)

[0488] R_{14} is -4-methyl-piperazin-1-yl, 3-methyl-piperazin-1-yl, -4,7-diaza-spiro[2.5]oct-7-yl, 1-piperazinyl, 4-methyl-3-methyl-piperazin-yl, 3-methoxyethyl-piperazin-1-yl, 3-ethyl-piperazin-1-yl, 3-benzyl-piperazin-1-yl or 3-CH_2F-piperazin-1-yl

[0489] R_{15} is Cl, Br, CF_3, F

[0490] R_{16} is CH_3, H, CH_2–CH_3

[0491] R_a is H or CH_3

[0492] R_b is H, CH_2–CH_2–CH_3, F, $CH(CH_3)_2$, Cl, OCH_3, CH_3 or CH_2–CH_3

[0493] and when

[0494] R is of formula (d)

[0495] R_8 is 3-methyl-piperazin-1-yl, 4-benzyl-1-piperazinyl or 1-piperazinyl

[0496] R_a is CH_3 or H

[0497] and when

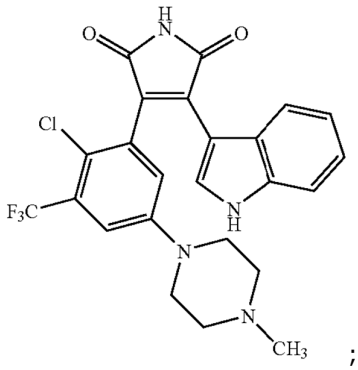
[0498] R is of formula (e)

[0499] R_9 is -4,7-diaza-spiro[2.5]oct-7-yl, 3-ethyl-piperazin-1-yl, 3-methyl-piperazin-1-yl, 4-methyl-3-methyl-piperazin-1-yl or 3-ethyl-piperazin-1-yl

[0500] R_a is H, CH_2–CH_3 or $CH(CH_3)_2$

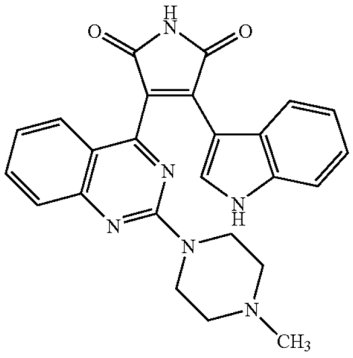
[0501] R_b is CH_3, F, $CH(CH_3)_2$, OCH_3, CH_2–CH_3 or Cl.

[0502] Specific embodiments of compounds according to formula (XXIV) include 3-[2-Chloro-5-(4-methyl-piperazin-1-yl)-3-trifluoromethyl-phenyl]-4-(1H-indol-3-yl)-pyrrole-2,5-dione having the formula

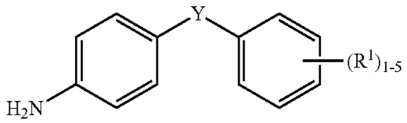


; and

[0503] 3-(1H-Indol-3-yl)-4-[2-(4-methyl-piperazin-1-yl)-quinazolin-4-yl]–pyrrole-2,5-dione having the formula



[0504] In other embodiments, PKC-θ inhibitors are selected from selective PKC-θ small molecule compounds disclosed by Ajioka in US Patent Application Publication US 2013/0225687, which is incorporated herein by reference in its entirety. These compounds are represented by formula (XXV):



(XXV)

[0505] wherein:

[0506] Y is selected from the group consisting of –O–, and –S–; and

[0507] each R^1 is independently selected from the group consisting of n-propyl, isopropyl, t-butyl, t-pentyl, CF_3, OCF_3, cyclopentyl, pyrrolyl, and CO_2H and salts, hydrates and prodrugs thereof, thereby selectively inhibiting PKC-θ.

[0508] In preferred embodiments, the PKC-θ inhibitor is an inhibitor of nuclear translocation/localization of PKC-θ. Representative inhibitors of this type include those disclosed by Rao *et al.* in International Publication No. WO 2017/132728 A1, which is incorporated herein by

reference in its entirety. These compounds are proteinaceous molecules represented by formula (XXVI):

[0509] $Z_1X_1X_2X_3X_4IDX_5PPX_6X_7X_8X_9X_{10}X_{11}Z_2$ (XXVI)

[0510] wherein:

[0511] "Z_1" and "Z_2" are independently absent or are independently selected from at least one of a proteinaceous moiety comprising from about 1 to about 50 amino acid residues (and all integer amino acid residues therebetween), and a protecting moiety;

[0512] "X_1" is absent or is selected from basic amino acid residues including R, K and modified forms thereof;

[0513] "X_2" and "X_3" are independently selected from basic amino acid residues including R, K and modified forms thereof;

[0514] "X_4" is selected from charged amino acid residues including R, K, D, E and modified forms thereof;

[0515] "X_5" is absent or is W or modified forms thereof;

[0516] "X_6" is selected from aromatic or basic amino acid residues including F, Y, W, R, K and modified forms thereof;

[0517] "X_7" is selected from basic amino acid residues including R, K and modified forms thereof;

[0518] "X_8" is absent or is P or modified forms thereof;

[0519] "X_9" is selected from basic amino acid residues including R, K and modified forms thereof;

[0520] "X_{10}" is selected from hydrophobic residues including V, L, I, M and modified forms thereof and P and modified forms thereof;

[0521] "X_{11}" is selected from basic amino acid residues including R, K and modified forms thereof.

[0522] In some embodiments, "X_1" to "X_{11}" are selected from a combination of one or more of the following:

[0523] "X_1" is absent or is R;

[0524] "X_2" is R;

[0525] "X_3" is K;

[0526] "X_4" is E or R;

[0527] "X_5" is absent or is W;

[0528] "X_6" is F or R;

[0529] "X_7" is R;

[0530] "X_8" is absent or is P;

[0531] "X_9" is K;

[0532] "X_{10}" is V or P; and

[0533] "X_{11}" is K.

[0534] In some embodiments, "Z_1" consists of 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues. In some embodiments, "Z_2" consists of 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues. In some embodiments, the amino acid residues in "Z_1" and "Z_2" are selected from any amino acid residues.

[0535] In some embodiments, "Z_1" is a proteinaceous molecule represented by Formula XXVII:

[0536] $X_{12}X_{13}X_{14}X_{15}X_{16}$ (XXVII)

[0537] wherein:

[0538] "X_{12}" is absent or is a protecting moiety;

[0539] "X_{13}" is absent or is selected from P and basic amino acid residues including R, K and modified forms thereof;

[0540] "X_{14}" is absent or is selected from P and basic amino acid residues including R, K and modified forms thereof;

[0541] "X_{15}" is absent or is selected from P and basic amino acid residues including R, K and modified forms thereof;

[0542] "X_{16}" is absent or is selected from P and basic amino acid residues including R, K and modified forms thereof.

[0543] In some embodiments, "Z_2" is a proteinaceous molecule represented by Formula XXVIII:

[0544] $X_{17}X_{18}X_{19}X_{20}$ (XXVIII)

[0545] wherein:

[0546] "X_{17}" is absent or is selected from any amino acid residue;

[0547] "X_{18}" is absent or is selected from any amino acid residue;

[0548] "X_{19}" is absent or is selected from any amino acid residue;

[0549] "X_{20}" is absent or is a protecting moiety.

[0550] In some embodiments, "Z_1" and "Z_2" are absent.

[0551] In particular embodiments, the proteinaceous molecule of Formula XXVI comprises, consists or consists essentially of an amino acid sequence represented by SEQ ID NO: 4 or 5 as shown below:

[0552] RKEIDPPFRPKVK [SEQ ID NO: 4]

[0553] RRKRIDWPPRRKPK [SEQ ID NO: 5].

[0554] The proteinaceous molecule of SEQ ID NO: 4 and SEQ ID NO 5 are referred to in WO 2017/132728 A1 as "importinib4759" and "importinib4759_O1", respectively.

[0555] In some embodiments of the proteinaceous molecules according to formula (XXVI, the molecules comprise at least one membrane permeating moiety. The membrane

permeating moiety may be conjugated at any point of the proteinaceous molecule. Suitable membrane permeating moieties include lipid moieties, cholesterol and proteins, such as cell-penetrating peptides and polycationic peptides; especially lipid moieties.

[0556] Non-limiting examples of cell penetrating peptides include the peptides described in, for example, US 20090047272, US 20150266935 and US 20130136742. Accordingly, suitable cell penetrating peptides may include, but are not limited to, basic poly(Arg) and poly(Lys) peptides and basic poly(Arg) and poly(Lys) peptides containing non-natural analogues of Arg and Lys residues such as YGRKKRPQRRR (HIV TAT_{47-57}), RRWRRWWRRWWRRWRR (W/R), CWK_{18} ($AlkCWK_{18}$), $K_{18}WCCWK_{18}$ (Di-CWK_{18}), WTLNSAGYLLGKINLKALAALAKKIL (Transportan), GLFEALEELWEAK (DipaLytic), K_{16}GGCRGDMFGCAK_{16}RGD (K_{16}RGD), K_{16}GGCMFGCGG (P1), K_{16}ICRRARGDNPDDRCT (P2), KKWKMRRNQFWVKVQRbAK (B) bA (P3), VAYISRGGVSTYYSDTVKGRFTRQKYNKRA (P3a), IGRIDPANGKTKYAPKFQDKATRSNYYGNSPS (P9.3), KETWWETWWTEWSQPKKKRKV (Pep-1), PLAEIDGIELTY (Plae), K_{16}GGPLAEIDGIELGA (Kplae), K_{16}GGPLAEIDGIELCA (cKplae), GALFLGFLGGAAGSTMGAWSQPKSKRKV (MGP), WEAK$(LAKA)_2$-LAKH$(LAKA)_2$LKAC (HA2), $(LARL)_6NHCH_3$ ($LARL4_6$), KLLKLLLKLWLLKLLL (Hel-11-7), $(KKKK)_2$GGC (KK), $(KWKK)_2$GCC (KWK), $(RWRR)_2$GGC (RWR), PKKKRKV (SV40 NLS7), PEVKKKRKPEYP (NLS12), TPPKKKRKVEDP (NLS12a), GGGGPKKKRKVGG (SV40 NLS13), GGGFSTSLRARKA (AV NLS13), CKKKKKKSEDEYPYVPN (AV RME NLS17), CKKKKKKKSEDEYPYVPNFSTSLRARKA (AV FP NLS28), LVRKKRKTEEESPLKDKDAKKSKQE (SV40 N1 NLS24), and $K_9K_2K_4K_8GGK_5$ (Loligomer); HSV-1 tegument protein VP22; HSV-1 tegument protein VP22r fused with nuclear export signal (NES); mutant B-subunit of *Escherichia coli* enterotoxin EtxB (H57S); detoxified exotoxin A (ETA); the protein transduction domain of the HIV-1 Tat protein, GRKKRRQRRRPPQ; the *Drosophila melanogaster* Antennapedia domain Antp (amino acids 43-58), RQIKIWFQNRRMKWKK; Buforin II, TRSSRAGLQFPVGRVHRLLRK; hClock-(amino acids 35-47) (human Clock protein DNA-binding peptide), KRVSRNKSEKKRR; MAP (model amphipathic peptide), KLALKLALKALKAALKLA; K-FGF, AAVALLPAVLLALLAP; Ku70-derived peptide, comprising a peptide selected from the group comprising VPMLKE, VPMLK, PMLKE or PMLK; Prion, Mouse Prpe (amino acids 1-28), MANLGYWLLALFVTMWTDVGLCKKRPKP; pVEC, LLIILRRRIRKQAHAHSK; Pep-I, KETWWETWWTEWSQPKKKRKV; SynBl, RGGRLSYSRRRFSTSTGR; Transportan, GWTLNSAGYLLGKINLKALAALAKKIL; Transportan-10, AGYLLGKINLKALAALAKKIL; CADY, Ac-GLWRALWRLLRSLWRLLWRA-cysteamide; Pep-7, SDLWEMMMVSLACQY; HN-1, TSPLNIHNGQKL; VT5, DPKGDPKGVTVTVTVTVTGKGDPKPD; or pISL, RVIRVWFQNKRCKDKK.

[0557] In preferred embodiments, the membrane permeating moiety is a lipid moiety, such as a C_{10}-C_{20} fatty acyl group, especially octadecanoyl (stearoyl; C_{18}), hexadecanoyl (palmitoyl; C_{16}) or tetradecanoyl (myristoyl; C_{14}); most especially tetradecanoyl. In preferred embodiments, the membrane permeable moiety is conjugated to the N- or C-terminal amino acid residue or through the amine of a lysine side-chain of the proteinaceous molecule, especially the N-terminal amino acid residue of the proteinaceous moiety.

2.2 PD-1 binding antagonists

[0558] PD-1 binding antagonists are suitably molecules that inhibit signaling through PD-1 and include molecules that inhibit the binding of PD-1 to its ligand binding partners. In some embodiments, the PD-1 ligand binding partners are PD-L1 and/or PD-L2. The antagonist may be an antibody, an immunoadhesin, a fusion protein, or oligopeptide.

[0559] The PD-1 binding antagonist is preferably an anti-PD-1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of MDX-1106 (nivolumab, OPDIVO), Merck 3475 (MK-3475, pembrolizumab, KEYTRUDA), CT-011 (pidilizumab), MEDI-4736 (durvalumab) MEDI-0680 (AMP-514), PDR001, REGN2810, BGB-108, and BGB-A317. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PD-L1 or PD-L2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT, hBAT-1 or Pidilizumab, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PD-L2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0560] In some embodiments, the anti-PD-1 antibody is nivolumab (CAS Registry Number: 946414-94-4). In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain variable region comprising the heavy chain variable region amino acid sequence from SEQ ID NO:6 and/or a light chain variable region comprising the light chain variable region amino acid sequence from SEQ ID NO:7. In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

[0561] (a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the heavy chain sequence:

[0562] QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWYDGSKR YYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGTLVTVSSASTKGPSVFPLAPCSRSTS ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT QKSLSLSLGK [SEQ ID NO: 6],

[0563] or (b) the light chain sequences has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the light chain sequence:

[0564] EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPAR FSGSGSGTDFTLTISSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC [SEQ ID NO: 7].

[0565] In some embodiments, the anti-PD-1 antibody is pembrolizumab (CAS Registry Number: 1374853-91-4). In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain variable region comprising the heavy chain variable region amino acid sequence from SEQ ID NO:8 and/or a light chain variable region comprising the light chain variable region amino acid sequence from SEQ ID NO:9. In a still further embodiment, provided is an isolated anti-PD-1 antibody comprising a heavy chain and/or a light chain sequence, wherein:

[0566] (a) the heavy chain sequence has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the heavy chain sequence:

[0567] QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGT NFNEKFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGPSVFPLA PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDH KPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE ALHNHYTQKSLSLSLGK [SEQ ID NO: 8],

[0568] or (b) the light chain sequences has at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to the light chain sequence:

[0569] EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGV PARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC [SEQ ID NO: 9].

[0570] The present invention also contemplates antibody fragments comprising heavy and light chain HVRs of a full-length anti-PD-1 antagonist antibody.

[0571] In a still further aspect, provided herein are nucleic acids encoding any of the antibodies described herein. In some embodiments, the nucleic acid further comprises a vector suitable for expression of the nucleic acid encoding any of the previously described anti-PDL1, anti-PD-1, or anti-PDL2 antibodies. In a still further specific aspect, the vector further comprises a host cell suitable for expression of the nucleic acid. In a still further specific aspect, the host cell is a eukaryotic cell or a prokaryotic cell. In a still further specific aspect, the eukaryotic cell is a mammalian cell, such as Chinese Hamster Ovary (CHO).

[0572] The antibody or antigen binding fragment thereof, may be made using methods known in the art, for example, by a process comprising culturing a host cell containing nucleic acid encoding any of the previously described anti-PD-1, or antigen-binding fragment in a form suitable for expression, under conditions suitable to produce such antibody or fragment, and recovering the antibody or fragment.

[0573] In some embodiments, the isolated anti-PD-1 antibody is aglycosylated. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Removal of glycosylation sites form an antibody is conveniently accomplished by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) is removed. The alteration may be made by substitution of an asparagine, serine or

threonine residue within the glycosylation site another amino acid residue (*e.g.*, glycine, alanine or a conservative substitution).

2.3 Ancillary agents

[0574] In some embodiments, the PKC-θ inhibitor and PD-1 binding antagonist are administered concurrently with an ancillary agent for treating, or for aiding in the treatment of, a T-cell dysfunctional disorder. Non-limiting examples of ancillary agents include cytotoxic agents, gene therapy agents, DNA therapy agents, viral therapy agents, RNA therapy agents, immunotherapeutic agents, bone marrow transplantation agents, nanotherapy agents, or a combination of the foregoing. The ancillary agent may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the ancillary agent is a small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the ancillary agent is a side-effect limiting agent (e.g., agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, *etc.*). In some embodiments, the ancillary agent is a radiotherapy agent. In some embodiments, the ancillary agent is an agent that targets PI3K/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or chemopreventative agent. In some embodiments, the ancillary agent is an immunotherapeutic, *e.g.*, a blocking antibody, ipilimumab (also known as MDX-010, MDX-101, or Yervoy®), tremelimumab (also known as ticilimumab or CP-675,206), an antagonist directed against B7-H3 (also known as CD276), *e.g.*, a blocking antibody, MGA271, an antagonist directed against a TGF-β, *e.g.*, metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), or LY2157299, a T cell (e.g., a cytotoxic T cell or CTL) expressing a chimeric antigen receptor (CAR), a T cell comprising a dominant-negative TGF-β receptor, *e.g.,* a dominant-negative TGF-β type II receptor, an agonist directed against CD137 (also known as TNFRSF9, 4-1BB, or ILA), *e.g.*, an activating antibody, urelumab (also known as BMS-663513), an agonist directed against CD40, *e.g.*, an activating antibody, CP-870893, an agonist directed against OX40 (also known as CD134), *e.g.*, an activating antibody, administered in conjunction with an anti-OX40 antibody (*e.g.*, AgonOX), an agonist directed against CD27, *e.g.*, an activating antibody, CDX-1127, indoleamine-2,3-dioxygenase (IDO), 1-methyl-D-tryptophan (also known as 1-D-MT), an antibody-drug conjugate (in some embodiments, comprising mertansine or monomethyl auristatin E (MMAE)), an anti-NaPi2b antibody-MMAE conjugate (also known as DNIB0600A or RG7599), trastuzumab emtansine (also known as T-DM1, ado-trastuzumab emtansine, or KADCYLA®, Genentech), DMUC5754A, an antibody-drug conjugate targeting the endothelin B receptor (EDNBR), *e.g.*, an antibody directed against EDNBR conjugated with MMAE, an angiogenesis inhibitor, an antibody directed against a VEGF, *e.g.*, VEGF-A, bevacizumab (also known as AVASTIN®, Genentech), an antibody directed against angiopoietin 2 (also known as Ang2), MEDI3617, an antineoplastic agent, an agent targeting CSF-1R (also known as M-CSFR or CD115), anti-CSF-1R (also known as IMC-CS4), an interferon, for example IFN-α or IFN-γ, Roferon-A, GM-CSF (also known as recombinant human granulocyte macrophage colony stimulating factor, rhu GM-CSF, sargramostim, or Leukine®), IL-2 (also known as aldesleukin or Proleukin®), IL-12, an antibody targeting CD20 (in some embodiments, the antibody targeting CD20 is obinutuzumab (also known as GA101 or Gazyva®) or rituximab), an antibody targeting GITR (in some embodiments, the antibody targeting GITR is TRX518), in conjunction with a cancer vaccine (in some embodiments, the cancer vaccine is a peptide cancer vaccine, which in some embodiments is a personalized peptide vaccine; in some embodiments the peptide cancer vaccine is a multivalent long peptide, a multi-peptide, a peptide cocktail, a hybrid peptide, or a peptide-pulsed dendritic cell vaccine (see, e.g., Yamada et al., Cancer Sci, 104:14-21, 2013)), in

conjunction with an adjuvant, a TLR agonist, e.g., Poly-ICLC (also known as Hiltonol®), LPS, MPL, or CpG ODN, TNF-α , IL-1, HMGB1, an IL-10 antagonist, an IL-4 antagonist, an IL-13 antagonist, an HVEM antagonist, an ICOS agonist, *e.g.*, by administration of ICOS-L, or an agonistic antibody directed against ICOS, an agent targeting CX3CL1, an agent targeting CXCL10, an agent targeting CCL5, an LFA-1 or ICAM1 agonist, a Selectin agonist, a targeted therapeutic agent, an inhibitor of B-Raf, vemurafenib (also known as Zelboraf®, dabrafenib (also known as Tafinlar®), erlotinib (also known as Tarceva®), an inhibitor of a MEK, such as MEK1 (also known as MAP2K1) or MEK2 (also known as MAP2K2). cobimetinib (also known as GDC-0973 or XL-518), trametinib (also known as Mekinist®), an inhibitor of K-Ras, an inhibitor of c-Met, onartuzumab (also known as MetMAb), an inhibitor of Alk, AF802 (also known as CH5424802 or alectinib), an inhibitor of a phosphatidylinositol 3-kinase (PI3K), BKM120, idelalisib (also known as GS-1101 or CAL-101), perifosine (also known as KRX-0401), an Akt, MK2206, GSK690693, GDC-0941, an inhibitor of mTOR, sirolimus (also known as rapamycin), temsirolimus (also known as CCI-779 or Torisel®), everolimus (also known as RAD001), ridaforolimus (also known as AP-23573, MK-8669, or deforolimus), OSI-027, AZD8055, INK128, a dual PI3K/mTOR inhibitor, XL765, GDC-0980, BEZ235 (also known as NVP-BEZ235), BGT226, GSK2126458, PF-04691502, PF-05212384 (also known as PKI-587). The ancillary agent may be one or more of the cytotoxic or chemotherapeutic agents described herein.

[0575] In some embodiments, the ancillary agent is an anti-infective drug. The anti-infective drugs is suitably selected from antimicrobials, which include without limitation compounds that kill or inhibit the growth of microorganisms such as viruses, bacteria, yeast, fungi, protozoa, etc. and thus include antibiotics, amebicides, antifungals, antiprotozoals, antimalarials, antituberculotics and antivirals. Anti-infective drugs also include within their scope anthelmintics and nematocides. Illustrative antibiotics include quinolones (*e.g.*, amifloxacin, cinoxacin, ciprofloxacin, enoxacin, fleroxacin, flumequine, lomefloxacin, nalidixic acid, norfloxacin, ofloxacin, levofloxacin, lomefloxacin, oxolinic acid, pefloxacin, rosoxacin, temafloxacin, tosufloxacin, sparfloxacin, clinafloxacin, gatifloxacin, moxifloxacin; gemifloxacin; and garenoxacin), tetracyclines, glycylcyclines and oxazolidinones (*e.g.*, chlortetracycline, demeclocycline, doxycycline, lymecycline, methacycline, minocycline, oxytetracycline, tetracycline, tigecycline; linezolide, eperozolid), glycopeptides, aminoglycosides (*e.g.*, amikacin, arbekacin, butirosin, dibekacin, fortimicins, gentamicin, kanamycin, meomycin, netilmicin, ribostamycin, sisomicin, spectinomycin, streptomycin, tobramycin), β-lactams (*e.g.*, imipenem, meropenem, biapenem, cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefazolin, cefixime, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefpimizole, cefpiramide, cefpodoxime, cefsulodin, ceftazidime, cefteram, ceftezole, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, cefuzonam, cephaacetrile, cephalexin, cephaloglycin, cephaloridine, cephalothin, cephapirin, cephradine, cefinetazole, cefoxitin, cefotetan, azthreonam, carumonam, flomoxef, moxalactam, amidinocillin, amoxicillin, ampicillin, azlocillin, carbenicillin, benzylpenicillin, carfecillin, cloxacillin, dicloxacillin, methicillin, mezlocillin, nafcillin, oxacillin, penicillin G, piperacillin, sulbenicillin, temocillin, ticarcillin, cefditoren, SC004, KY-020, cefdinir, ceftibuten, FK-312, S-1090, CP-0467, BK-218, FK-037, DQ-2556, FK-518, cefozopran, ME1228, KP-736, CP-6232, Ro 09-1227, OPC-20000, LY206763), rifamycins, macrolides (*e.g.*, azithromycin, clarithromycin, erythromycin, oleandomycin, rokitamycin, rosaramicin, roxithromycin, troleandomycin), ketolides (*e.g.*, telithromycin, cethromycin), coumermycins, lincosamides (*e.g.*, clindamycin, lincomycin) and chloramphenicol. Illustrative antivirals include abacavir sulfate, acyclovir sodium, amantadine

hydrochloride, amprenavir, cidofovir, delavirdine mesylate, didanosine, efavirenz, famciclovir, fomivirsen sodium, foscarnet sodium, ganciclovir, indinavir sulfate, lamivudine, lamivudine/zidovudine, nelfinavir mesylate, nevirapine, oseltamivir phosphate, ribavirin, rimantadine hydrochloride, ritonavir, saquinavir, saquinavir mesylate, stavudine, valacyclovir hydrochloride, zalcitabine, zanamivir, and zidovudine. Non-limiting examples of amebicides or antiprotozoals include atovaquone, chloroquine hydrochloride, chloroquine phosphate, metronidazole, metronidazole hydrochloride, and pentamidine isethionate. Anthelmintics can be at least one selected from mebendazole, pyrantel pamoate, albendazole, ivermectin and thiabendazole. Illustrative antifungals can be selected from amphotericin B, amphotericin B cholesteryl sulfate complex, amphotericin B lipid complex, amphotericin B liposomal, fluconazole, flucytosine, griseofulvin microsize, griseofulvin ultramicrosize, itraconazole, ketoconazole, nystatin, and terbinafine hydrochloride. Non-limiting examples of antimalarials include chloroquine hydrochloride, chloroquine phosphate, doxycycline, hydroxychloroquine sulfate, mefloquine hydrochloride, primaquine phosphate, pyrimethamine, and pyrimethamine with sulfadoxine. Antituberculotics include but are not restricted to clofazimine, cycloserine, dapsone, ethambutol hydrochloride, isoniazid, pyrazinamide, rifabutin, rifampin, rifapentine, and streptomycin sulfate.

3. Pharmaceutical compositions and formulations

[0576] Also provided herein are pharmaceutical compositions and formulations comprising a PKC-θ inhibitor, a PD-1 binding antagonist and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical compositions and formulations further comprise an ancillary agent as described for example herein.

[0577] Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (*e.g.*, a small molecule, nucleic acid, or polypeptide) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0578] In some embodiments, especially relating to peptide and polypeptide active agents (*e.g.*, antibodies, inhibitory peptides and immunoadhesins), the active agents and optional pharmaceutically acceptable carriers are in the form of lyophilized formulations or aqueous solutions. Exemplary lyophilized antibody formulations are described in U.S. Pat. No. 6,267,958. Aqueous antibody formulations include those described in U.S. Pat. No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0579] The compositions and formulations herein may also contain further active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0580] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (*e.g.*, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0581] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

[0582] Depending on the specific conditions being treated, the formulations may be administered systemically or locally. Suitable routes may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Techniques for formulation and administration may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition.

4. Therapeutic uses

[0583] The present invention discloses that a PKC-θ inhibitor and a PD-1 binding antagonist (also referred to herein as the "therapeutic combination" or "combination treatment") are useful for treating a T-cell dysfunctional disorder, or for enhancing immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, for treating or delaying the progression of cancer, or for treating infection in an individual. In specific embodiments, the therapeutic combination is disclosed for treating or delaying the progression of cancer, including metastatic cancer, and for preventing cancer recurrence. Any of the PKC-θ inhibitors and PD-1 binding antagonists known in the art or described herein may be used in this regard.

[0584] In some embodiments, the combination therapy further comprises the use or administration of an ancillary agent (*e.g.*, a chemotherapeutic agent), as described for example herein.

[0585] Suitably, the individual to be treated with the combination therapy comprises a T-cell (*e.g.*, a $CD8^+$ T-cell) with a mesenchymal phenotype, for example, a T-cell that expresses nuclear PKC-θ at a higher level than the level of expression of TBET in the same T-cell, and/or at a higher level than in an activated T-cell. The T-cell may be a tumor-infiltrating lymphocyte or a circulating lymphocyte. The T-cell suitably exhibits T-cell exhaustion or anergy and in representative examples of this type, the T-cell expresses a higher level of EOMES than TBET and/or has elevated expression of PD-1. In some embodiments, the T-cell has impaired or repressed immune function and suitably expresses biomarkers of reduced T-cell activation (*e.g.*, reduced production and/or secretion of cytokines such as Il-2, IFN-γ and TNF-α). In these embodiments, the T-cell suitably expresses ZEB1 in the nucleus of the T cell at a higher level than the level of TBET in the same T-cell or the level of ZEB1 in the nucleus of an activated T-cell. Accordingly, nuclear PKC-θ, ZEB1, TBET, PD-1 and EOMES (also referred to herein as "T-cell function biomarkers") can be used to determine the immune function of T cells in a patient for assessing a patient's T-cell immune status, including susceptibility to treatment with PD-1 binding antagonists.

[0586] In some embodiments, the individual is a human.

[0587] In some embodiments, the individual has been treated with a PD-1 binding antagonist before the combination treatment with a PD-1 binding antagonist and a PKC-θ inhibitor (*e.g.*, a nuclear translocation inhibitor of PKC-θ).

[0588] In some embodiments, the individual has cancer that is resistant (has been demonstrated to be resistant) to one or more PD-1 binding antagonists. In some embodiments, resistance to a PD-1 antagonist includes recurrence of cancer or refractory cancer. Recurrence may refer to the reappearance of cancer, in the original site or a new site, after treatment. In some embodiments, resistance to a PD-1 binding antagonist includes progression of the cancer during treatment with the PD-1 binding antagonist. In some embodiments, resistance to a PD-1 binding antagonist includes cancer that does not respond to treatment. The cancer may be resistant at the beginning of treatment or it may become resistant during treatment. In some embodiments, the cancer is at early stage or at late stage.

[0589] In some embodiments of any of the methods, assays and/or kits, any one or more of the T-cell function biomarkers are detected in the sample using a method selected from the group consisting of FACS, Western blot, ELISA, immunoprecipitation, immunohistochemistry, immunofluorescence, radioimmunoassay, dot blotting, immunodetection methods, HPLC, surface plasmon resonance, optical spectroscopy, mass spectrometry, HPLC, qPCR, RT-qPCR, multiplex qPCR or RT-qPCR, RNA-seq, microarray analysis, SAGE, MassARRAY technique, and FISH, and combinations thereof.

[0590] In some embodiments of any of the methods, assays and/or kits, any one or more of the T-cell function biomarkers are detected in the sample by protein expression. In some embodiments, protein expression is determined by immunohistochemistry (IHC). In some embodiments, any one or more of the T-cell function biomarkers are detected using an antibody that binds specifically to a respective biomarker. In some embodiments, nuclear PKC-θ and/or ZEB1 biomarkers are detected in the nucleus of a T-cell, for example using IHC. In some embodiments, a complex comprising nuclear PKC-θ and ZEB1 biomarkers is detected in the nucleus of a T-cell.

[0591] In some embodiments, the combination therapy of the invention comprises administration of a PKC-θ inhibitor and a PD-1 binding antagonist. The PKC-θ inhibitor and PD-1 binding antagonist may be administered in any suitable manner known in the art. For example, The PKC-θ inhibitor and PD-1 binding antagonist may be administered sequentially (at different times) or concurrently (at the same time). In some embodiments, the PKC-θ inhibitor is in a separate composition as the PD-1 binding antagonist. In some embodiments, the PKC-θ inhibitor is in the same composition as the PD-1 binding antagonist. Accordingly, the combination therapy may involve administering the PKC-θ inhibitor separately, simultaneously or sequentially with PD-1 binding antagonist. In some embodiments, this may be achieved by administering a single composition or pharmacological formulation that includes both types of agent, or by administering two separate compositions or formulations at the same time, wherein one composition includes the PKC-θ inhibitor and the other, PD-1 binding antagonist. In other embodiments, the treatment with the PKC-θ inhibitor may precede or follow the treatment with the PD-1 binding antagonist by intervals ranging from minutes to days. In embodiments where the PKC-θ inhibitor is applied separately to the PD-1 binding antagonist, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the PKC-θ inhibitor would still be able to exert an advantageously effect on a functionally repressed T-cell (*e.g.*, a mesenchymal T-cell) as noted above, and in particular, to render the T-cell with enhanced immune function, including susceptibility of the T-cell to reinvigoration by the PD-1 binding antagonist. In such instances, it is contemplated that one would administer both modalities within about 1-12 hours of each other and, more suitably, within about 2-6 hours of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several hours (2, 3, 4, 5, 6 or 7) to several days (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

[0592] It is conceivable that more than one administration of either the PKC-θ inhibitor or the PD-1 binding antagonist will be desired. Various combinations may be employed, where the PKC-θ inhibitor is "A" and the PD-1 binding antagonist is "B", as exemplified below:

[0593] A/B/A B/A/B B/B/A A/A/B B/A/A A/B/B B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A B/A/B/A B/A/A/B B/B/B/A A/A/A/B B/A/A/A A/B/A/A A/A/B/A A/B/B/B B/A/B/B B/B/A/B.

[0594] The PKC-θ inhibitor and PD-1 binding antagonist may be administered by the same route of administration or by different routes of administration. In some embodiments, the PD-1 binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the PKC-θ inhibitor is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. An effective amount of the PKC-θ inhibitor and PD-1 binding antagonist may be administered for prevention or treatment of disease. The appropriate dosage of the PKC-θ inhibitor and PD-1 binding antagonist may be determined based on the type of disease to be treated, the type of the PKC-θ inhibitor and PD-1 binding antagonist, the severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician. In some embodiments, combination treatment with PKC-θ inhibitor (*e.g.*, a nuclear translocation inhibitor of PKC-θ) and PD-1 binding antagonists (*e.g.*, anti-PD-1 antibody) are synergistic, whereby an efficacious dose of a PD-1 binding antagonists (*e.g.*,

anti-PD-1 antibody) in the combination is reduced relative to efficacious dose of the PD-1 binding antagonists (*e.g.*, anti-PD-1 antibody) as a single agent.

[0595] As a general proposition, the therapeutically effective amount of a peptide or polypeptide active agent (*e.g.*, an antibody, peptide inhibitor, immunoadhesin, *etc.*) administered to a human will be in the range of about 0.01 to about 50 mg/kg of patient body weight whether by one or more administrations. In some embodiments, the antibody used is about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg administered daily, for example. In some embodiments, the peptide or polypeptide active agent (*e.g.*, an antibody, peptide inhibitor, immunoadhesin, *etc.*) is administered at 15 mg/kg. However, other dosage regimens may be useful. In one embodiment, an anti-PDL1 antibody described herein is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg or about 1400 mg on day 1 of 21-day cycles. The dose may be administered as a single dose or as multiple doses (*e.g.*, 2 or 3 doses), such as infusions. The dose of peptide or polypeptide active agent (*e.g.*, an antibody, peptide inhibitor, immunoadhesin, *etc.*) administered in a combination treatment may be reduced as compared to a single treatment. The progress of this therapy is easily monitored by conventional techniques.

[0596] Small molecule compounds are generally administered at an initial dosage of about 0.0001 mg/kg to about 1000 mg/kg daily. A daily dose range of about 0.01 mg/kg to about 500 mg/kg, or about 0.1 mg/kg to about 200 mg/kg, or about 1 mg/kg to about 100 mg/kg, or about 10 mg/kg to about 50 mg/kg, can be used. The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compound being employed.

[0597] In any event, dosages can be empirically determined considering the type and stage of disease diagnosed in a particular patient. The dose administered to a patient, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the patient over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound in a particular patient. Determination of the proper dosage for a particular situation is within the skill of the practitioner. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired. Doses can be given daily, or on alternate days, as determined by the treating physician. Doses can also be given on a regular or continuous basis over longer periods of time (weeks, months or years), such as through the use of a subdermal capsule, sachet or depot, or *via* a patch or pump. In some embodiments, the PKC-θ inhibitor, PD-1 binding antagonist and optionally an ancillary agent (*e.g.*, a chemotherapeutic agent) are administered on a routine schedule. Alternatively, the combination therapy may be administered as symptoms arise.

[0598] A "routine schedule" as used herein, refers to a predetermined designated period of time. The routine schedule may encompass periods of time which are identical or which

differ in length, as long as the schedule is predetermined. For instance, the routine schedule may involve administration of the PKC-θ inhibitor, PD-1 binding antagonist and optional ancillary agent on a daily basis, every two days, every three days, every four days, every five days, every six days, a weekly basis, a monthly basis or any set number of days or weeks there-between, every two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, *etc.* Alternatively, the predetermined routine schedule may involve concurrent administration of the PKC-θ inhibitor, PD-1 binding antagonist and optional ancillary agent on a daily basis for the first week, followed by a monthly basis for several months, and then every three months after that. Any particular combination would be covered by the routine schedule as long as it is determined ahead of time that the appropriate schedule involves administration on a certain day.

[0599] In some embodiments, the treatment methods and uses may further comprise an additional therapy. The additional therapy may be radiation therapy, surgery (*e.g.*, lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation.

[0600] The efficacy of any of the methods described herein (*e.g.*, combination treatments including administering an effective amount of a combination of PKC-θ inhibitor, PD-1 binding antagonist and optional ancillary agent may be tested in various models known in the art, such as clinical or pre-clinical models. Suitable pre-clinical models are exemplified herein and further may include without limitation ID8 ovarian cancer, GEM models, B16 melanoma, RENCA renal cell cancer, CT26 colorectal cancer, MC38 colorectal cancer, and Cloudman melanoma models of cancer.

[0601] The efficacy of any of the methods described herein (e.g., combination treatments including administering an effective amount of a combination of PKC-θ inhibitor, PD-1 binding antagonist and optional ancillary agent) may be tested in a GEM model that develops tumors, including without limitation GEM models of non-small-cell lung cancer, pancreatic ductal adenocarcinoma, or melanoma. For example, a mouse expressing $Kras^{G12D}$ in a $p53^{null}$ background after adenoviral recombinase treatment as described in Jackson *et al.* (2001 *Genes Dev.* 15(24):3243-8) (description of $Kras^{G12D}$) and Lee *et al.* (2012 *Dis. Model Mech.* 5(3):397-402) (FRT-mediated $p53^{null}$ allele) may be used as a pre-clinical model for non-small-cell lung cancer. As another example, a mouse expressing $Kras^{G12D}$ in a $p16/p19^{null}$ background as described in Jackson *et al.* (2001, *supra*) (description of $Kras^{G12D}$) and Aguirre *et al.* (2003 *Genes Dev.* 17(24):3112-26) ($p16/p19^{null}$ allele) may be used as a pre-clinical model for pancreatic ductal adenocarcinoma (PDAC). As a further example, a mouse with melanocytes expressing $Braf^{V600E}$ in a melanocyte-specific $PTEN^{null}$ background after inducible (*e.g.*, 4-OHT treatment) recombinase treatment as described in Dankort *et al.* (2007 *Genes Dev.* 21(4):379-84) (description of Braf.sup.V600E) and Trotman *et al.* (2003 *PLoS Biol.* 1(3):E59) ($PTEN^{null}$ allele) may be used as a pre-clinical model for melanoma. For any of these exemplary models, after developing tumors, mice are randomly recruited into treatment groups receiving combination PKC-θ inhibitor, PD-1 binding antagonist and

optional ancillary agent treatment or control treatment. Tumor size (*e.g.*, tumor volume) is measured during the course of treatment, and overall survival rate is also monitored.

[0602] In some embodiments of the methods of the present disclosure, the cancer (in some embodiments, a sample of the patient's cancer as examined using a diagnostic test, as described for example herein) comprises tumor-infiltrating lymphocytes (TILs), wherein the TILs are within or otherwise associated with the cancer tissue. In these embodiments, the TILs are assessed for expression of any one or more of the T-cell function biomarkers disclosed herein. For example, nuclear PKC-θ and ZEB1 can be used as biomarkers of mesenchymal phenotype and T-cell activation. In addition, TBET, PD-1 and EOMES can be used as biomarkers of T-cell exhaustion, which is characterized for example by high levels of inhibitory co-receptors and lacking the capacity to produce effector cytokines (Wherry, E. J. 2011 *Nature immunology* 12: 492-499; Rabinovich *et al.*, 2007 *Annual Review of immunology* 25:267-296).

[0603] In some embodiments of the methods of the present disclosure, the individual has a T-cell dysfunction that manifests in a T-cell dysfunctional disorder. The T-cell dysfunctional disorder may be characterized by T-cell anergy or decreased ability to secrete cytokines, proliferate or execute cytolytic activity. In some embodiments of the methods of the present disclosure, the T-cell dysfunctional disorder is characterized by repressed T-cell immune function. In some embodiments of the methods of the present disclosure, the T-cell dysfunctional disorder is characterized by T-cell of a mesenchymal phenotype. In some embodiments of the methods of the present disclosure, the T-cell dysfunctional disorder is characterized by T-cell exhaustion. In some embodiments of the methods of the present disclosure, the T-cells are $CD4^+$ and/or $CD8^+$ T cells. In accordance with the present invention, PKC-θ inhibitor treatment may increase expression of biomarkers of T-cell activation and effector capacity (*e.g.*, IL-2, IFN-γ and TNF-α), decrease expression of biomarkers of T-cell effector inhibition and cancer progression (*e.g.*, ZEB1), decrease expression of biomarkers of T-cell exhaustion (*e.g.*, PD-1 and EOMES) and/or increase expression of the transcription factor TBET, which increases production of IFN-γ in cells of the adaptive and innate immune systems. Notably, PKC-θ inhibitor treatment may confer enhanced susceptibility of exhausted T-cells to reinvigoration by PD-1 binding antagonists. As such, the combination treatment PKC-θ inhibitor and a PD-1 binding antagonist may increase T-cell (*e.g.*, $CD4^+$ T-cell, $CD8^+$ T-cell, memory T-cell) priming, activation and/or proliferation relative to prior to the administration of the combination. In some embodiments, the T cells are $CD4^+$ and/or $CD8^+$ T cells.

[0604] In some embodiments of the methods of the present disclosure, activated $CD4^+$ and/or $CD8^+$ T-cells in the individual are characterized by IFN-γ producing $CD4^+$ and/or $CD8^+$ T cells and/or enhanced cytolytic activity as compared to before the administration of the combination. .gamma. IFN-γ may be measured by any means known in the art, including, *e.g.*, intracellular cytokine staining (ICS) involving cell fixation, permeabilization, and staining with an antibody against IFN-γ. Cytolytic activity may be measured by any means known in the art, *e.g.*, using a cell killing assay with mixed effector and target cells.

[0605] In some embodiments, $CD8^+$ T-cells are characterized, e.g., by presence of CD8b expression (*e.g.*, by RT-PCR using *e.g.*, Fluidigm) (Cd8b is also known as T-cell surface glycoprotein CD8 beta chain; CD8 antigen, alpha polypeptide p3'7; Accession No. is NM_172213). In some embodiments, $CD8^+$ T cells are from peripheral blood. In some embodiments, $CD8^+$ T cells are from tumor.

[0606] In some embodiments, Treg cells are characterized, *e.g.*, by presence of Fox3p expression (*e.g.*, by RT-PCR *e.g.*, using Fluidigm) (Foxp3 is also known as Forkhead box protein P3; scurfin; FOXP3delta7; immunodeficiency, polyendocrinopathy, enteropathy, X-linked; the accession no. is NM_014009). In some embodiments, Treg are from peripheral blood. In some embodiments, Treg cells are from tumor.

[0607] In some embodiments, inflammatory or activated T-cells are characterized, *e.g.*, by presence of TBET and/or CXCR3 expression or by a TBET:EOMES ratio that correlates with inflammatory or activated T-cells (e.g., by RT-PCR using, e.g., Fluidigm). In some embodiments, inflammatory or activated T cells are from peripheral blood. In some embodiments, inflammatory or activated T cells are from tumor.

[0608] In some embodiments of the methods of the present disclosure, $CD4^+$ and/or $CD8^+$ T cells exhibit increased release of cytokines selected from the group consisting of IFN-γ, TNF-α and interleukins such as IL-2. Cytokine release may be measured by any means known in the art, *e.g.*, using Western blot, ELISA, or immunohistochemical assays to detect the presence of released cytokines in a sample containing $CD4^+$ and/or $CD8^+$ T-cells.

[0609] In some embodiments of the methods of the present disclosure, the $CD4^+$ and/or $CD8^+$ T cells are effector memory T cells. In some embodiments of the methods of the present disclosure, the $CD4^+$ and/or $CD8^+$ effector memory T cells are characterized by having the expression of $CD44^{high}$ $CD62L^{low}$. Expression of $CD44^{high}$ $CD62L^{low}$ may be detected by any means known in the art, *e.g.*, by preparing single cell suspensions of tissue (*e.g.*, a cancer tissue) and performing surface staining and flow cytometry using commercial antibodies against CD44 and CD62L. In some embodiments of the methods of the present disclosure, the $CD4^+$ and/or $CD8^+$ effector memory T cells are characterized by having expression of CXCR3 (also known as C-X-C chemokine receptor type 3; Mig receptor; IP10 receptor; G protein-coupled receptor 9; interferon-inducible protein 10 receptor; Accession No. NM_001504). In some embodiments, the $CD4^+$ and/or $CD8^+$ effector memory T cells are from peripheral blood. In some embodiments, the $CD4^+$ and/or $CD8^+$ effector memory T cells are from tumor.

[0610] In some embodiments of the methods of the present disclosure, the administration of an effective amount of a PKC-θ inhibitor and a PD-1 binding antagonist and optionally an ancillary agent to an individual is characterized by increased levels of inflammatory markers (*e.g.*, CXCR3) on $CD8^+$ T cells as compared to before administration of the combination therapy. $CXCR3/CD8^+$ T cells may be measured by any means known the art. In some embodiments, $CXCR3/CD8^+$ T cells are from peripheral blood. In some embodiments, $CXCR3/CD8^+$ T cells are from tumor.

[0611] In some embodiments of the methods of the invention, Treg function is suppressed as compared to before administration of the combination. In some embodiments, T-cell exhaustion is decreased as compared to before administration of the combination.

[0612] In some embodiments, number of Treg is decreased as compared to before administration of the combination. In some embodiments, the levels of plasma IFN-γ is increased as compared to before administration of the combination. Treg number may be assessed, *e.g.*, by determining percentage of $CD4^+Fox3p^+CD45^+$ cells (*e.g.*, by FACS analysis). In some embodiments, absolute number of Treg, *e.g.*, in a sample, is determined. In some embodiments, Treg are from peripheral blood. In some embodiments, Treg are from tumor.

[0613] In some embodiments, T-cell priming, activation and/or proliferation is increased as compared to before administration of the combination. In some embodiments, the T-cells are $CD4^+$ and/or $CD8^+$ T cells. In some embodiments, T-cell proliferation is detected by determining percentage of $Ki67^+CD8^+$ T cells (*e.g.*, by FACS analysis). In some embodiments, T-cell proliferation is detected by determining percentage of $Ki67^+CD4^+$ T cells (*e.g.*, by FACS analysis). In some embodiments, the T-cells are from peripheral blood. In some embodiments, the T-cells are from tumor.

5. Methods of detection and diagnosis

[0614] In accordance with the present invention, nuclear PKC-θ and ZEB1 can be employed as biomarkers of T-cell mesenchymal phenotype and impaired T-cell function. Additionally, PD-1, TBET and EOMES may be used as known in the art to assess T-cell exhaustion. T-cells can be obtained from T-cell containing patient samples which are suitably selected tissue samples such as tumors and fluid samples such as peripheral blood. In some embodiments, the sample is obtained prior to treatment with the therapeutic combination. In some embodiments, the tissue sample is formalin fixed and paraffin embedded, archival, fresh or frozen. In some embodiments, the sample is whole blood. In some embodiments, the whole blood comprises immune cells, circulating tumor cells and any combinations thereof.

[0615] Presence and/or expression levels/amount of a biomarker (*e.g.*, any one or more of PKC-θ, ZEB1, TBET and EOMES, also referred to herein collectively as "T-cell function biomarkers") can be determined qualitatively and/or quantitatively based on any suitable criterion known in the art, including but not limited to DNA, mRNA, cDNA, proteins, protein fragments and/or gene copy number. In certain embodiments, presence and/or expression levels/amount of a biomarker in a first sample is increased or elevated as compared to presence/absence and/or expression levels/amount in a second sample (*e.g.*, before treatment with the therapeutic combination). In certain embodiments, presence/absence and/or expression levels/amount of a biomarker in a first sample is decreased or reduced as compared to presence and/or expression levels/amount in a second sample. In certain embodiments, the second sample is a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. Additional disclosures for determining presence/absence and/or expression levels/amount of a gene are described herein.

[0616] In some embodiments of any of the methods, elevated expression refers to an overall increase of about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of biomarker (*e.g.*, protein or nucleic acid (*e.g.*, gene or mRNA)), detected by standard art known methods such as those described herein, as compared to a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In certain embodiments, the elevated expression refers to the increase in expression level/amount of a biomarker in the sample wherein the increase is at least about any of 1.5x, 1.75x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 25x, 50x, 75x, or 100x the expression level/amount of the respective biomarker in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In some embodiments, elevated expression refers to an overall increase of greater than about 1.5-fold, about 1.75-fold, about-2 fold, about 2.25-fold, about 2.5-fold, about 2.75-fold, about 3.0-fold, or about 3.25-fold as compared to a reference sample, reference cell, reference tissue, control sample, control cell, control tissue, or internal control (*e.g.*, housekeeping gene).

[0617] In some embodiments of any of the methods, reduced expression refers to an overall reduction of about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of biomarker (*e.g.*, protein or nucleic acid (*e.g.*, gene or mRNA)), detected by standard art known methods such as those described herein, as compared to a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In certain embodiments, reduced expression refers to the decrease in expression level/amount of a biomarker in the sample wherein the decrease is at least about any of 0.9x, 0.8x, 0.7x, 0.6x, 0.5x, 0.4x, 0.3x, 0.2x, 0.1x, 0.05x, or 0.01x the expression level/amount of the respective biomarker in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue.

[0618] Presence and/or expression level/amount of various biomarkers in a sample can be analyzed by a number of methodologies, many of which are known in the art and understood by the skilled artisan, including, but not limited to, immunohistochemistry ("IHC"), Western blot analysis, immunoprecipitation, molecular binding assays, ELISA, ELIFA, fluorescence activated cell sorting ("FACS"), MassARRAY, proteomics, quantitative blood based assays (as for example Serum ELISA), biochemical enzymatic activity assays, in situ hybridization, Southern analysis, Northern analysis, whole genome sequencing, polymerase chain reaction ("PCR") including quantitative real time PCR ("qRT-PCR") and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like), RNA-Seq, FISH, microarray analysis, gene expression profiling, and/or serial analysis of gene expression ("SAGE"), as well as any one of the wide variety of assays that can be performed by protein, gene, and/or tissue array analysis. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel et al., eds., 1995, Current Protocols In Molecular Biology, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis). Multiplexed immunoassays such as those available from Rules Based Medicine or Meso Scale Discovery ("MSD") may also be used.

[0619] In some embodiments, presence and/or expression level/amount of a biomarker is determined using a method comprising: (a) performing gene expression profiling, PCR (such as rtPCR or qRT-PCR), RNA-seq, microarray analysis, SAGE, MassARRAY technique, or FISH on a sample (such as a subject cancer sample); and b) determining presence and/or expression level/amount of a biomarker in the sample. In some embodiments, the microarray method comprises the use of a microarray chip having one or more nucleic acid molecules that can hybridize under stringent conditions to a nucleic acid molecule encoding a gene mentioned above or having one or more polypeptides (such as peptides or antibodies) that can bind to one or more of the proteins encoded by the genes mentioned above. In one embodiment, the PCR method is qRT-PCR. In one embodiment, the PCR method is multiplex-PCR. In some embodiments, gene expression is measured by microarray. In some embodiments, gene expression is measured by qRT-PCR. In some embodiments, expression is measured by multiplex-PCR.

[0620] Methods for the evaluation of mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as *in situ* hybridization using labeled riboprobes specific for the one or more genes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for one or more of the genes, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like).

[0621] Samples from mammals can be conveniently assayed for mRNAs using Northern, dot blot or PCR analysis. In addition, such methods can include one or more steps that

allow one to determine the levels of target mRNA in a biological sample (*e.g.*, by simultaneously examining the levels a comparative control mRNA sequence of a "housekeeping" gene such as an actin family member). Optionally, the sequence of the amplified target cDNA can be determined.

[0622] Optional methods include protocols which examine or detect mRNAs, such as target mRNAs, in a tissue or cell sample by microarray technologies. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes whose expression correlates with increased or reduced clinical benefit of anti-angiogenic therapy may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene.

[0623] According to some embodiments, presence and/or expression level/amount is measured by observing protein expression levels of an aforementioned gene. In certain embodiments, the method comprises contacting the biological sample with antibodies to a biomarker (*e.g.*, anti-PD-1 antibodies, anti-PKC-θ antibodies, anti-TBET antibodies, anti-ZEB antibodies, anti-EOMES antibodies) described herein under conditions permissive for binding of the biomarker, and detecting whether a complex is formed between the antibodies and biomarker. Such method may be an *in vitro* or *in vivo* method. In some embodiments, one or more anti-biomarker antibodies are used to select subjects eligible for combination therapy with a PKC-θ inhibitor and a PD-1 binding antagonist.

[0624] In certain embodiments, the presence and/or expression level/amount of biomarker proteins in a sample is examined using IHC and staining protocols. IHC staining of tissue sections has been shown to be a reliable method of determining or detecting presence of proteins in a sample. In some embodiments, expression of a T-cell function biomarker in a sample from an individual is elevated protein expression and, in further embodiments, is determined using IHC. In one embodiment, expression level of biomarker is determined using a method comprising: (a) performing IHC analysis of a sample (such as a subject cancer sample) with an antibody; and b) determining expression level of a biomarker in the sample. In some embodiments, IHC staining intensity is determined relative to a reference. In some embodiments, the reference is a reference value. In some embodiments, the reference is a reference sample (*e.g.*, control cell line staining sample or tissue sample from non-cancerous patient).

[0625] In some embodiments, T-cell function biomarker expression is evaluated on a tumor or tumor sample. As used herein, a tumor or tumor sample may encompass part or all of the tumor area occupied by tumor cells. In some embodiments, a tumor or tumor sample may further encompass tumor area occupied by tumor associated intratumoral cells and/or tumor associated stroma (*e.g.*, contiguous peri-tumoral desmoplastic stroma). Tumor associated intratumoral cells and/or tumor associated stroma may include areas of immune infiltrates (*e.g.*, tumor infiltrating immune cells as described herein) immediately adjacent to and/or contiguous with the main tumor mass. In some embodiments, T-cell function biomarker expression is evaluated on tumor cells. In some embodiments, T-cell function biomarker expression is evaluated on immune cells within the tumor area as described above, such as tumor infiltrating immune cells.

[0626] In alternative methods, the sample may be contacted with an antibody specific for said biomarker under conditions sufficient for an antibody-biomarker complex to form, and then detecting said complex. The presence of the biomarker may be detected in a number of ways, such as by Western blotting and ELISA procedures for assaying a wide variety of tissues and samples, including plasma or serum. A wide range of immunoassay techniques using such an assay format are available, see, *e.g.*, U.S. Pat. Nos. 4,016,043, 4,424,279 and 4,018,653. These include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labeled antibody to a target biomarker.

[0627] Presence and/or expression level/amount of a selected T-cell function biomarker in a tissue or cell sample may also be examined by way of functional or activity-based assays. For instance, if the biomarker is an enzyme (*e.g.*, PKC-θ), one may conduct assays (*e.g.*, kinase assays) known in the art to determine or detect the presence of the given enzymatic activity in the tissue or cell sample.

[0628] In certain embodiments, the samples are normalized for both differences in the amount of the biomarker assayed and variability in the quality of the samples used, and variability between assay runs. Such normalization may be accomplished by detecting and incorporating the expression of certain normalizing biomarkers, including well known housekeeping genes. Alternatively, normalization can be based on the mean or median signal of all of the assayed genes or a large subset thereof (global normalization approach). On a gene-by-gene basis, measured normalized amount of a subject tumor mRNA or protein is compared to the amount found in a reference set. Normalized expression levels for each mRNA or protein per tested tumor per subject can be expressed as a percentage of the expression level measured in the reference set. The presence and/or expression level/amount measured in a particular subject sample to be analyzed will fall at some percentile within this range, which can be determined by methods well known in the art.

[0629] In some embodiments, the sample is a clinical sample. In other embodiments, the sample is used in a diagnostic assay. In some embodiments, the sample is obtained from a primary or metastatic tumor. Tissue biopsy is often used to obtain a representative piece of tumor tissue. Alternatively, tumor cells can be obtained indirectly in the form of tissues or fluids that are known or thought to contain the tumor cells of interest. For instance, samples of lung cancer lesions may be obtained by resection, bronchoscopy, fine needle aspiration, bronchial brushings, or from sputum, pleural fluid or blood. Genes or gene products can be detected from cancer or tumor tissue or from other body samples such as urine, sputum, serum or plasma. The same techniques discussed above for detection of target genes or gene products in cancerous samples can be applied to other body samples. Cancer cells may be sloughed off from cancer lesions and appear in such body samples. By screening such body samples, a simple early diagnosis can be achieved for these cancers. In addition, the progress of therapy can be monitored more easily by testing such body samples for target genes or gene products.

[0630] In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a single sample or combined multiple samples from the same subject or individual that are obtained at one or more different time points than when the test sample is obtained. For example, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained at an earlier time point from the same subject or

individual than when the test sample is obtained. Such reference sample, reference cell, reference tissue, control sample, control cell, or control tissue may be useful if the reference sample is obtained during initial diagnosis of cancer and the test sample is later obtained when the cancer becomes metastatic.

[0631] In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a combination of multiple samples from one or more healthy individuals who are not the subject or individual. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a combination of multiple samples from one or more individuals with a disease or disorder (*e.g.*, cancer) who are not the subject or individual. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is pooled RNA samples from normal tissues or pooled plasma or serum samples from one or more individuals who are not the subject or individual. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is pooled RNA samples from tumor tissues or pooled plasma or serum samples from one or more individuals with a disease or disorder (*e.g.*, cancer) who are not the subject or individual.

[0632] In some embodiments, the sample is a tissue sample from the individual. In some embodiments, the tissue sample is a tumor tissue sample (*e.g.*, biopsy tissue). In some embodiments, the tissue sample is lung tissue. In some embodiments, the tissue sample is renal tissue. In some embodiments, the tissue sample is skin tissue. In some embodiments, the tissue sample is pancreatic tissue. In some embodiments, the tissue sample is gastric tissue. In some embodiments, the tissue sample is bladder tissue. In some embodiments, the tissue sample is esophageal tissue. In some embodiments, the tissue sample is mesothelial tissue. In some embodiments, the tissue sample is breast tissue. In some embodiments, the tissue sample is thyroid tissue. In some embodiments, the tissue sample is colorectal tissue. In some embodiments, the tissue sample is head and neck tissue. In some embodiments, the tissue sample is osteosarcoma tissue. In some embodiments, the tissue sample is prostate tissue. In some embodiments, the tissue sample is ovarian tissue, HCC (liver), blood cells, lymph nodes, and/or bone/bone marrow tissue. In some embodiments, the tissue sample is colon tissue. In some embodiments, the tissue sample is endometrial tissue. In some embodiments, the tissue sample is brain tissue (*e.g.*, glioblastoma, neuroblastoma, and so forth).

[0633] In some embodiments, a tumor tissue sample (the term "tumor sample" is used interchangeably herein) may encompass part or all of the tumor area occupied by tumor cells. In some embodiments, a tumor or tumor sample may further encompass tumor area occupied by tumor associated intratumoral cells and/or tumor associated stroma (*e.g.*, contiguous peri-tumoral desmoplastic stroma). Tumor associated intratumoral cells and/or tumor associated stroma may include areas of immune infiltrates (*e.g.*, tumor infiltrating immune cells as described herein) immediately adjacent to and/or contiguous with the main tumor mass.

[0634] In some embodiments, tumor cell staining is expressed as the percent of all tumor cells showing membranous staining of any intensity. Infiltrating immune cell staining may be expressed as the percent of the total tumor area occupied by immune cells that show staining of any intensity. The total tumor area encompasses the malignant cells as well as tumor-associated stroma, including areas of immune infiltrates immediately adjacent to and contiguous with the

main tumor mass. In addition, infiltrating immune cell staining may be expressed as the percent of all tumor infiltrating immune cells.

[0635] In some embodiments of any of the methods, the disease or disorder is a tumor. In some embodiments, the tumor is a malignant cancerous tumor (*i.e.*, cancer). In some embodiments, the tumor and/or cancer is a solid tumor or a non-solid or soft tissue tumor. Examples of soft tissue tumors include leukemia (*e.g.*, chronic myelogenous leukemia, acute myelogenous leukemia, adult acute lymphoblastic leukemia, acute myelogenous leukemia, mature B-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia, prolymphocytic leukemia, or hairy cell leukemia) or lymphoma (*e.g.*, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, or Hodgkin's disease). A solid tumor includes any cancer of body tissues other than blood, bone marrow, or the lymphatic system. Solid tumors can be further divided into those of epithelial cell origin and those of non-epithelial cell origin. Examples of epithelial cell solid tumors include tumors of the gastrointestinal tract, colon, colorectal (*e.g.*, basaloid colorectal carcinoma), breast, prostate, lung, kidney, liver, pancreas, ovary (*e.g.*, endometrioid ovarian carcinoma), head and neck, oral cavity, stomach, duodenum, small intestine, large intestine, anus, gall bladder, labium, nasopharynx, skin, uterus, male genital organ, urinary organs (*e.g.*, urothelium carcinoma, dysplastic urothelium carcinoma, transitional cell carcinoma), bladder, and skin. Solid tumors of non-epithelial origin include sarcomas, brain tumors, and bone tumors. In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the cancer is second-line or third-line locally advanced or metastatic non-small cell lung cancer. In some embodiments, the cancer is adenocarcinoma. In some embodiments, the cancer is squamous cell carcinoma. In some embodiments, the cancer is non-small cell lung cancer (NSCLC), glioblastoma, neuroblastoma, melanoma, breast carcinoma (*e.g.* triple-negative breast cancer), gastric cancer, colorectal cancer (CRC), or hepatocellular carcinoma. In some embodiments, the cancer is a primary tumor. In some embodiments, the cancer is a metastatic tumor at a second site derived from any of the above types of cancer.

[0636] In some embodiments of any of the methods, the cancer displays human effector cells (*e.g.*, is infiltrated by human effector cells). Methods for detecting human effector cells are well known in the art, including, *e.g.*, by IHC. In some embodiments, the cancer displays high levels of human effector cells. In some embodiments, human effector cells are one or more of NK cells, macrophages, monocytes. In some embodiments, the cancer is any cancer described herein. In some embodiments, the cancer is non-small cell lung cancer (NSCLC), glioblastoma, neuroblastoma, melanoma, breast carcinoma (*e.g.* triple-negative breast cancer), gastric cancer, colorectal cancer (CRC), or hepatocellular carcinoma.

[0637] In some embodiments of any of the methods, the cancer displays cells expressing FcR (*e.g.*, is infiltrated by cells expressing FcR). Methods for detecting FcR are well known in the art, including, *e.g.*, by IHC. In some embodiments, the cancer displays high levels of cells expressing FcR. In some embodiments, FcR is FcγR. In some embodiments, FcR is activating FcγR. In some embodiments, the cancer is non-small cell lung cancer (NSCLC), glioblastoma, neuroblastoma, melanoma, breast carcinoma (*e.g.* triple-negative breast cancer), gastric cancer, colorectal cancer (CRC), or hepatocellular carcinoma.

[0638] In some embodiments, the T-cell function biomarker is detected in the sample using a method selected from the group consisting of FACS, Western blot, ELISA, immunoprecipitation, immunohistochemistry, immunofluorescence, radioimmunoassay, dot

blotting, immunodetection methods, HPLC, surface plasmon resonance, optical spectroscopy, mass spectrometry, HPLC, qPCR, RT-qPCR, multiplex qPCR or RT-qPCR, RNA-seq, microarray analysis, SAGE, MassARRAY technique, and FISH, and combinations thereof. In some embodiments, the T-cell function biomarker is detected using FACS analysis. In some embodiments, the T-cell function biomarker is PD-1. In some embodiments, the PD-1 expression is detected in blood samples. In some embodiments, the PD-1 expression is detected on circulating immune cells in blood samples. In some embodiments, the circulating immune cell is a $CD3^+/CD8^+$ T cell. In some embodiments, prior to analysis, the immune cells are isolated from the blood samples. Any suitable method to isolate/enrich such population of cells may be used including, but not limited to, cell sorting. In some embodiments, the PD-1 expression is reduced in samples from individuals that respond to treatment with a PKC-θ inhibitor and/or PD-1 binding antagonist, such as an anti-PD-1 antibody. In some embodiments, the PD-1 expression is elevated on circulating immune cells, such as $CD3^+/CD8^+$ T cells, in blood samples.

[0639] Also provided herein are diagnostic methods and kits that are based on the determination that PKC-θ and ZEB1 co-localize in the nucleus and that this co-localization contributes at least in part to EMT of T-cells and repression of their immune function. The diagnostic methods suitably comprise: (i) obtaining a sample from a subject, wherein the sample comprises a T-cell (*e.g.*, $CD8^+$ T-cell); (ii) contacting the sample with a first binding agent that binds to PKC-θ in the sample and a second binding agent that binds to ZEB1 in the sample; and (iii) detecting localization of the first and second binding agents in the nucleus of the T-cell, wherein localization of the first and second binding agents in the nucleus of the T-cell is indicative of the presence of a T-cell dysfunctional disorder in the subject.

[0640] The first and second binding-agents suitably bind to epitopes of PKC-θ and ZEB1 polypeptides, respectively. Any suitable epitope may be chosen in the amino acid sequence of PKC-θ (as set forth for example in GenPept Accession Nos. XP_005252553, XP_005252554, XP_005252555 and XP_005252556), or in the amino acid sequence of ZEB1 (as set forth for example in GenPept Accession Nos. NP_00131058, NP_001310579, NP_001310586 and NP_001310601).

[0641] Localization of PKC-θ and ZEB1 in the nucleus of the T-cell may be performed using any suitable localization technique, *e.g.*, by IHC, typically using an anti-PKC-θ antibody that has a different detectable moiety or label than an anti-ZEB1 antibody. In some embodiments, spatial proximity assays (also referred to as "proximity assays") are employed, which can be used to assess the formation of a complex between PKC-θ and ZEB1. Proximity assays rely on the principle of "proximity probing", wherein an analyte, typically an antigen, is detected by the coincident binding of multiple (*i.e.*, two or more, generally two, three or four) binding agents or probes, which when brought into proximity by binding to the analyte (hence "proximity probes") allow a signal to be generated.

[0642] In some embodiments, at least one of the proximity probes comprises a nucleic acid domain (or moiety) linked to the analyte-binding domain (or moiety) of the probe, and generation of the signal involves an interaction between the nucleic acid moieties and/or a further functional moiety which is carried by the other probe(s). Thus signal generation is dependent on an interaction between the probes (more particularly by the nucleic acid or other functional moieties/domains carried by them) and hence only occurs when both the necessary two (or more) probes have bound to the analyte, thereby lending improved specificity to the detection system.

The concept of proximity probing has been developed in recent years and many assays based on this principle are now well known in the art.

[0643] Proximity assays are typically used to assess whether two particular proteins or portions thereof are in close proximity, e.g., proteins that are bound to each other, fusion proteins, and/or proteins that are positioned in close proximity. One such assay, known as proximity ligation assay (PLA), and which is used in some embodiments of the present invention, features two antibodies (raised in different species) bound to the targets of interest (see Nature Methods 3, 995-1000 (2006)). PLA probes, which are species-specific secondary antibodies with a unique oligonucleotide strand attached, are then bound to the appropriate primary antibodies. In the case of the targets being in close proximity, the oligonucleotide strands of the PLA probes can interact with additional ssDNA and DNA ligase such they can be circulated and amplified via rolling circle amplification (RCA). When highly processive DNA polymerases such as Phi29 DNA polymerase is used, the circular DNA template can be replicated hundreds to thousands of times longer and as a result producing ssDNA molecules from hundreds of nanometers to microns in length (*see*, Angewandte Chemie International Edition, 2008, 47, 6330-6337). After the amplification, the replicated DNA can be detected *via* detection systems. Thus, a visible signal is indicative that the targets of interest are in close proximity. These assays feature the use of several DNA-antibody conjugates as well as enzymes such as DNA ligase and DNA polymerase.

[0644] In other embodiments, a dual binders (DB) assay is employed, which utilizes a bi-specific detection agent consisting of two Fab fragments with fast off-rate kinetics joined by a flexible linker (Van dieck *et al.*, 2014 *Chemistry & Biology* Vol.21(3):357-368). In principle, because the dual binders comprise Fab fragments with fast off-rate kinetics, the dual binders are washed off if only one of the Fab fragments is bound to its epitope (simultaneous cooperative binding of both Fab fragments of the dual binder prevents dissociation of the dual binder and leads to positive staining/visibility).

[0645] According to another approach disclosed in International Publication WO2014/139980, which is encompassed in the practice of the present invention, proximity assays and tools are described, which employ a biotin ligase substrate and an enzyme to perform a proximity assay. The method provides detection of target molecules and proximity while maintaining the cellular context of the sample. The use of biotin ligase such as an enzyme from *E. coli* and peptide substrate such as amino-acid substrate for that enzyme provides for a sensitive and specific detection of protein-protein interactions in FFPE samples. Because biotin ligase can efficiently biotinylate appropriate peptide substrate in the presence of biotin and the reaction can only occur when the enzyme makes physical contact with the peptide substrate, biotin ligase and the substrate can be separately conjugated to two antibodies that recognize targets of interest respectively.

[0646] Also provided herein are methods for monitoring pharmacodynamic activity of a PD-1 binding antagonist treatment by measuring the expression level of one or more T-cell function biomarkers as described herein in a sample comprising leukocytes obtained from the subject, where the subject has been treated with a PD-1 binding antagonist and a PKC-θ inhibitor, and where the one or more T-cell function biomarkers are selected from nuclear PKC-θ, ZEB1, TBET, PD-1 and EOMES, and determining the treatment as demonstrating pharmacodynamic activity based on the expression level of the one or more T-cell function biomarkers in the sample obtained from the subject, as compared with a reference, where an increased expression level of

the one or more T-cell function biomarkers as compared with the reference indicates pharmacodynamic activity to the PD-1 antagonist treatment. These methods may further comprise measuring the expression level of one or more additional biomarkers of T cell function and/or cellular composition (*e.g.*, percentage of Treg and/or absolute number of Treg; *e.g.*, number of CD8+ effector T cells), wherein the additional biomarkers of T cell function include a cytokine, *e.g.*, IFN-γ, a T cell marker, or a memory T cell marker (*e.g.*, a marker of T effector memory cells); and determining the treatment as demonstrating pharmacodynamic activity based on the expression level of the one or more T-cell function biomarkers, the one or more additional biomarkers of T cell function and/or cellular composition in the sample obtained from the subject, as compared with a reference, where an increased expression level of the one or more T-cell function biomarkers, the one or more additional biomarkers of T cell function and/or cellular composition as compared with the reference indicates pharmacodynamic activity to the PD-1 antagonist treatment. Expression level of the biomarker(s) and/or cellular composition may be measured by one or more methods as described herein.

[0647] As used herein, "pharmacodynamic (PD) activity" may refer to an effect of a treatment (*e.g.*, a PKC-θ inhibitor in combination with a PD-1 binding antagonist treatment) to the subject. An example of a PD activity may include modulation of the expression level of one or more genes. Without wishing to be bound to theory, it is thought that monitoring PD activity, such as by measuring expression of one or more T-cell function biomarkers, may be advantageous during a clinical trial examining a PKC-θ inhibitor and PD-1 binding antagonist. Monitoring PD activity may be used, for example, to monitor response to treatment, toxicity, and the like.

[0648] In some embodiments, the expression level of one or more marker genes, proteins and/or cellular composition may be compared to a reference which may include a sample from a subject not receiving a treatment (*e.g.*, a PKC-θ inhibitor treatment in combination with a PD-1 binding antagonist). In some embodiments, a reference may include a sample from the same subject before receiving a treatment (*e.g.*, a PKC-θ inhibitor treatment in combination with a PD-1 binding antagonist). In some embodiments, a reference may include a reference value from one or more samples of other subjects receiving a treatment (*e.g.*, a PKC-θ inhibitor treatment in combination with a PD-1 binding antagonist). For example, a population of patients may be treated, and a mean, average, or median value for expression level of one or more genes may be generated from the population as a whole. A set of samples obtained from cancers having a shared characteristic (*e.g.*, the same cancer type and/or stage, or exposure to a common treatment such as a PKC-θ inhibitor treatment in combination with a PD-1 binding antagonist) may be studied from a population, such as with a clinical outcome study. This set may be used to derive a reference, *e.g.*, a reference number, to which a subject's sample may be compared. Any of the references described herein may be used as a reference for monitoring PD activity.

[0649] Certain aspects of the present disclosure relate to measurement of the expression level of one or more biomarkers (*e.g.*, gene expression products including mRNAs and proteins) in a sample. In some embodiments, a sample may include leukocytes. In some embodiments, the sample may be a peripheral blood sample (*e.g.*, from a patient having a tumor). In some embodiments, the sample is a tumor sample. A tumor sample may include cancer cells, lymphocytes, leukocytes, stroma, blood vessels, connective tissue, basal lamina, and any other cell type in association with the tumor. In some embodiments, the sample is a tumor tissue sample containing tumor-infiltrating leukocytes. In some embodiments, the sample may be processed to

separate or isolate one or more cell types (*e.g.*, leukocytes). In some embodiments, the sample may be used without separating or isolating cell types.

[0650] A tumor sample may be obtained from a subject by any method known in the art, including without limitation a biopsy, endoscopy, or surgical procedure. In some embodiments, a tumor sample may be prepared by methods such as freezing, fixation (*e.g.*, by using formalin or a similar fixative), and/or embedding in paraffin wax. In some embodiments, a tumor sample may be sectioned. In some embodiments, a fresh tumor sample (i.e., one that has not been prepared by the methods described above) may be used. In some embodiments, a tumor sample may be prepared by incubation in a solution to preserve mRNA and/or protein integrity.

[0651] In some embodiments, the sample may be a peripheral blood sample. A peripheral blood sample may include white blood cells, PBMCs, and the like. Any technique known in the art for isolating leukocytes from a peripheral blood sample may be used. For example, a blood sample may be drawn, red blood cells may be lysed, and a white blood cell pellet may be isolated and used for the sample. In another example, density gradient separation may be used to separate leukocytes (*e.g.*, PBMCs) from red blood cells. In some embodiments, a fresh peripheral blood sample (i.e., one that has not been prepared by the methods described above) may be used. In some embodiments, a peripheral blood sample may be prepared by incubation in a solution to preserve mRNA and/or protein integrity.

[0652] In some embodiments, responsiveness to treatment may refer to any one or more of: extending survival (including overall survival and progression free survival); resulting in an objective response (including a complete response or a partial response); or improving signs or symptoms of cancer. In some embodiments, responsiveness may refer to improvement of one or more factors according to the published set of RECIST guidelines for determining the status of a tumor in a cancer patient, *i.e.*, responding, stabilizing, or progressing. For a more detailed discussion of these guidelines, *see*, Eisenhauer *et al.* (2009 *Eur J Cancer* 45: 228-47), Topalian *et al.* (2012 *N Engl J Med* 366:2443-54), Wolchok *et al.* (2009 *Clin Can Res* 15:7412-20) and Therasse *et al.* (2000 *J. Natl. Cancer Inst.* 92:205-16). A responsive subject may refer to a subject whose cancer(s) show improvement, *e.g.*, according to one or more factors based on RECIST criteria. A non-responsive subject may refer to a subject whose cancer(s) do not show improvement, *e.g.*, according to one or more factors based on RECIST criteria.

[0653] Conventional response criteria may not be adequate to characterize the anti-tumor activity of therapeutic agents of the invention, which can produce delayed responses that may be preceded by initial apparent radiological progression, including the appearance of new lesions. Therefore, modified response criteria have been developed that account for the possible appearance of new lesions and allow radiological progression to be confirmed at a subsequent assessment. Accordingly, in some embodiments, responsiveness may refer to improvement of one of more factors according to immune-related response criteria (irRC). See, *e.g.*, Wolchok *et al.* (2009, *supra*). In some embodiments, new lesions are added into the defined tumor burden and followed, *e.g.*, for radiological progression at a subsequent assessment. In some embodiments, presence of non-target lesions is included in assessment of complete response and not included in assessment of radiological progression. In some embodiments, radiological progression may be determined only on the basis of measurable disease and/or may be confirmed by a consecutive assessment ≥4 weeks from the date first documented.

[0654] In some embodiments, responsiveness may include immune activation. In some embodiments, responsiveness may include treatment efficacy. In some embodiments, responsiveness may include immune activation and treatment efficacy.

6. Kits

[0655] In other aspects of the invention, therapeutic kits are provided comprising a PKC-θ inhibitor and a PD-1 binding antagonist. In some embodiments, the therapeutic kits further comprise a package insert comprising instructional material for administering concurrently the PKC-θ inhibitor and the PD-1 binding antagonist to treat a T-cell dysfunctional disorder, or to enhance immune function (*e.g.*, immune effector function, T-cell function *etc.*) in an individual having cancer, or to treat or delay cancer progression, or to treat infection in an individual. Any of PKC-θ inhibitor and PD-1 binding antagonist described herein or known in the art may be included in the kits.

[0656] In some embodiments, the PKC-θ inhibitor and PD-1 binding antagonist are in the same container or separate containers. Suitable containers include, for example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container holds the formulation and the label on, or associated with, the container may indicate directions for use. The kits may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructional material for use. In some embodiments, the kits further include one or more of other agents (*e.g.*, a chemotherapeutic agent, and anti-neoplastic agent). Suitable containers for the one or more agent include, for example, bottles, vials, bags and syringes.

[0657] In other embodiments of the invention, diagnostic kits are provided for determining expression of biomarkers, including the T-cell function biomarkers disclosed herein, which include reagents that allow detection and/or quantification of the biomarkers. Such reagents include, for example, compounds or materials, or sets of compounds or materials, which allow quantification of the biomarkers. In specific embodiments, the compounds, materials or sets of compounds or materials permit determining the expression level of a gene (*e.g.*, T-cell function biomarker gene), including without limitation the extraction of RNA material, the determination of the level of a corresponding RNA, *etc*., primers for the synthesis of a corresponding cDNA, primers for amplification of DNA, and/or probes capable of specifically hybridizing with the RNAs (or the corresponding cDNAs) encoded by the genes, TaqMan probes, proximity assay probes, ligases, antibodies *etc*.

[0658] The kits may also optionally include appropriate reagents for detection of labels, positive and negative controls, washing solutions, blotting membranes, microtiter plates, dilution buffers and the like. For example, a nucleic acid-based detection kit may include (i) a T-cell function biomarker polynucleotide (which may be used as a positive control), (ii) a primer or probe that specifically hybridizes to a T-cell function biomarker polynucleotide. Also included may be enzymes suitable for amplifying nucleic acids including various polymerases (reverse transcriptase, *Taq*, Sequenase™, DNA ligase *etc*. depending on the nucleic acid amplification technique employed), deoxynucleotides and buffers to provide the necessary reaction mixture for amplification. Such kits also generally will comprise, in suitable means, distinct containers for each

individual reagent and enzyme as well as for each primer or probe. Alternatively, a protein-based detection kit may include (i) a T-cell function biomarker polypeptide (which may be used as a positive control), (ii) an antibody that binds specifically to a T-cell function biomarker polypeptide. The kit can also feature various devices (*e.g.*, one or more) and reagents (*e.g.*, one or more) for performing one of the assays described herein; and/or printed instructional material for using the kit to quantify the expression of a T-cell function biomarker gene. The reagents described herein, which may be optionally associated with detectable labels, can be presented in the format of a microfluidics card, a chip or chamber, a microarray or a kit adapted for use with the assays described in the examples or below, *e.g.*, RT-PCR or Q PCR techniques described herein.

[0659] Materials suitable for packing the components of the diagnostic kits may include crystal, plastic (polyethylene, polypropylene, polycarbonate and the like), bottles, vials, paper, envelopes and the like. Additionally, the kits of the invention can contain instructional material for the simultaneous, sequential or separate use of the different components contained in the kit. The instructional material can be in the form of printed material or in the form of an electronic support capable of storing instructions such that they can be read by a subject, such as electronic storage media (magnetic disks, tapes and the like), optical media (CD-ROM, DVD) and the like. Alternatively or in addition, the media can contain Internet addresses that provide the instructional material.

[0660] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

EXAMPLE 1

PKC-θ AS A TARGET FOR THERAPEUTIC INTERVENTION

[0661] The present inventors developed a novel class of peptide inhibitors with specificity in inhibiting entry of PKC-θ into the nucleus, which are disclosed in PCT/AU2017/050083 filed 1 February 2017. One of these peptide inhibitors, RKEIDPPFRPKVK (also referred to herein as "PKCθi"), whose structure and physical properties are shown in Figures 1A, B and C, was tested in a MCF7 breast cancer cell line to determine its effect on a variety of PKC isoforms (β2, β1, α, ε and γ) as well as PKC-θ. The PKCθi peptide was shown to markedly inhibit nuclear localization of PKC-θ, with no effect on the other PKC isoforms, demonstrating its target specificity (Figure 1D). This peptide inhibitor was also able to significantly inhibit the proliferation of MCF7 cells (Figure 1E), without impacting PKC-θ catalytic activity, indicating that its mode of action is through inhibiting the nuclear axis of PKC-θ (Figure 1F).

Methods

Treatment of cells:

[0662] Treated cells were permeabilized by incubating with 1% Triton X-100 for 20 min and probed with primary rabbit antibodies to PKC-θ (T538p), PKC- β1, PKC-β2, PKC-α, PKC-ε and PKC-γ, and visualized with a donkey anti-rabbit AF 488. Cover slips were mounted on glass microscope slides with ProLong Diamond Antifade reagent (Life Technologies). Protein targets were localized by confocal laser scanning microscopy. Single 0.5 µm sections were obtained using a Leica DMI8 microscope using 100x oil immersion lens running LAX software. The final image was

obtained by averaging four sequential images of the same section. Digital images were analysed using ImageJ software (ImageJ, NIH, Bethesda, MD, USA) to determine Total Nuclear Fluorescent Intensity (TNFI), the Total Cytoplasmic Fluorescent Intensity (TCFI) or total Fluorescent Intensity (TFI). The nuclear to cytoplasmic fluorescence ratio (Fn/c) using the equation: Fn/c = (Fn − Fb)/(Fc − Fb), where Fn is nuclear fluorescence, Fc is cytoplasmic fluorescence, and Fb is background fluorescence was used to determine the impact on nuclear localisation.

Mouse Model MDA-MB-231 mouse xenografts:

[0663] Five-week-old female nude mice were acquired from the Animal Resources Centre (Perth) and allowed to acclimatize for one week in the animal facility at the John Curtin School of Medical Research (JCSMR) before experimentation. All experimental procedures were accessed and approved by The Australian National University Animal Experimental Ethics Committee (Ethics ID A2014/30). MDA-MB-231 human breast carcinoma cells were injected subcutaneously into the right mammary gland (2 x 106 cells in 1:1 PBS and BD Matrigel Matrix). Tumors were measured using external calipers and calculated using the modified ellipsoidal formula: ½ (a/b2), where a = longest diameter and b = shortest diameter. Tumors were allowed to grow to around 50 mm3 before commencing treatments (around 15 days). All treatments were given by IP injections of 40mg/kg PKC nuclear Inhibitor. Tumors were excised and collected in DMEM supplemented with 2.5% FCS. Tumors were then finely minced using a surgical blade and incubated at 37°C for 1 hour in DMEM 2.5% FCS and collagenase type 4 (Worthington-Biochem) (1 mg of collagenase/1 g of tumor). Digested tumors were spun and resuspended in DMEM 2.5% FCS before being passed through a 0.2 μM filter and processed for analysis on the Nanostring platform.

EXAMPLE 2

PKC-θ EXPRESSION SIGNATURE IN CD8$^+$ T-CELLS FROM BRAF NEGATIVE MELANOMA PATIENTS

[0664] The inventors examined PKC-θ expression in CD8$^+$ T-cells of BRAF negative melanoma patients receiving PD-1 immunotherapy to determine whether PKC-θ has a role in resistance to treatment with this immunecheckpoint inhibitor. To characterize the profile of PKC-θ in CD8$^+$ T-cells, expression of this biomarker was examined in FFPE tissue from BRAF negative melanoma tissue biopsies obtained from melanoma patients divided into 4 cohorts based on RECIST 1.1 responses to immunotherapy, as summarized in Table 1.

Table 1

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.
Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, as well as an absolute increase of at least 5 mm. (Appearance of one or more new lesions is also considered progression).

[0665] FFPE tissue from BRAF negative melanoma tissue biopsies was examined for PKC-θ and PD1 expression in CD8$^+$ T-cells and found that only complete responder (CR) and stable

disease (SD) cohorts expressed PD1, with PKC-θ highly expressed in PD cohort (Figure 2A). IL-2, IFN-γ and TNF-α are markers of T-cell activation and effector capacity whereas ZEB1 is a negative regulator of T-cell responses and is linked with T-cell effector inhibition as well as cancer progression. Accordingly, the expression of these biomarkers was investigated in the context of modulating the PKC-θ pathway. Employing RT-PCR, the present inventors profiled expression of the effect of PKCθi in PBMC cells isolated from CR and PD melanoma liquid biopsies. They found that expression of IL-2, IFN-γ and TNF-α was significantly induced in the PD patient cohort with some induction seen in the CR patient cohort, indicating a significant role of PKC-θ in T-cell modulation in PD patient cohorts (Figure 2B).

[0666] Next, expression of ZEB1 repressor protein and PKC-θ was analyzed in melanoma patient FFPE samples, and the results presented in Figure 2C show that only the PD cohort had significant expression of ZEB1 and within this cohort, dual immunotherapy resistant patient samples had the highest expression of ZEB1.

[0667] These data indicate that in patients with resistance to immunotherapy, expression of IL-2, IFN-γ and TNF-α is low and ZEB1 is upregulated in $CD8^+$ T-cells.

[0668] The present inventors next examined the expression signature of PKC-θ in $CD8^+$ T-cells isolated from melanoma patient's bloods (Figure 3). $CD8^+$ T-cells were treated either with vehicle control or PMA/CI and then treated with mock or PKCθi. The cells were then probed with antibodies to CD8 (to target $CD8^+$ T-cells), ZEB1 and PKC-θ. Interestingly, it was found that ZEB1 and PKC-θ had highest expression in the PD Cohort, which is a primary resistant patient to both mono and dual immunotherapy whereas responder SD and CR cohort patients had lower expression of ZEB1 and PKC-θ. Of note, the PD/PR/PD Cohort sample that transitioned to a PR cohort and then relapsed, had intermediate to high expression of ZEB1 and PKC- θ in between the SD and PD cohorts (Figure 3A). The Pearson's Correlation Co-efficient (PCC) for PKC-θ and ZEB1 was also evaluated to judge the degree of co-localization and the data strongly indicate that those two proteins co-localize significantly in the nucleus of melanoma patients with the PD cohort having the highest PCC. Treatment with the PKCθi peptide inhibitor in all patient samples strikingly abrogated the expression of both nuclear PKC-θ and ZEB1.

[0669] Next, markers for T-cell activity, IFN-γ and TNF-α were examined in $CD8^+$ T-cells treated as above (Figure 3B). The inventors found that in line with Figure 2A's data that Cohort PD had the lowest expression of IFN-γ and TNF-α, whereas the CR/SD cohort (responders to immunotherapy) and the PD/PR/PD cohort had higher levels of IFN-γ and TNF-α. Upon treatment with PKCθi, which targets the nuclear axis of PKC-θ, they observed across all samples and particularly in the stimulated samples a significant increase in both the expression of IFN-γ and TNF-α.

[0670] This data set clearly indicates the strong role that PKC-θ overexpression plays in inhibiting the T-cell based immune response in metastatic melanoma patients and that one of the primary mechanism by which this inhibition is mediated is the repressor ZEB1. Inhibition of the nuclear axis of PKC-θ and consequently ZEB1 rescues expression of markers of T-cell activation IFN-γ and TNF-α, which again suggests that the PKCθi peptide inhibitor has strong potential to directly target cancer stem cells (CSC) and circulating tumor cells (CTCs) and simultaneously rescue immune responses mediated by $CD8^+$ T-cells that may lead to improved outcomes for patients with metastatic cancer, including advanced metastatic melanoma patients.

Methods

Patient categories:

[0671] Melanoma patients were selected for this biomarker study and classified into 4 groups based on response to immunotherapy using the RECIST 1.1 analysis as indicated (either mono or dual therapy using Pembrolizumab, Nivolumab and/or Ipilimumab) into the following treatment response cohorts, based on evaluation of target lesions: **Complete Response (CR)**: Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. **Partial Response (PR)**: At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. **Stable Disease (SD)**: Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. **Progressive Disease (PD)**: At least a 20% increase in the sum of diameters of target lesions, as well as an absolute increase of at least 5 mm. (Appearance of one or more new lesions is also considered progression). Samples were taken every 3 months after baseline bleed for 24 months.

Cell preparation and treatment:

[0672] Metastatic melanoma biopsies were pre-enriched using the RosetteSep™ method to isolate CTCs by employing the RosetteSep™ Human CD45 Depletion Kit (15162, Stemcell Technologies) to remove CD45+ cells and red blood cells, using density gradient centrifugation with SepMate™-50 (IVD) density gradient tubes (85450, Stemcell Technologies) and Lymphoprep™ density gradient medium (07861, Stemcell Technologies). Enriched CTC cells were then either pre-clinically screened with either a control or PKCθi peptide inhibitor at a concentration of 8 µm. Enriched cells where then cytospun onto a coverslip pre-treated with poly-l-lysine and fixed then stored in PBS for staining. To examine the dynamics of PKC-Theta (T538p) in melanoma CTCs treated with the PKCθi peptide inhibitor, CTCs were permeabilized by incubating with 1% Triton X-100 for 20 min and were probed with rabbit anti PKC-Theta (T538p); mouse anti CSV and goat anti-ABCB5 and visualized with a donkey anti-rabbit AF 488, anti-mouse 568 and anti-goat 633. To examine the dynamics of PKC-Theta (T538p), ZEB1, IFN-γ and TNF-α in melanoma CD8 T-cells treated with PKCθi, CD8 T-cells were permeabilized by incubating with 1% Triton X-100 for 20 min and were probed with rabbit anti PKC-Theta (T538p); mouse anti ZEB1 and goat anti CD8 or IFNγ(mouse), TNF-α (rabbit) and goat anti CD8 and visualized with a donkey anti-rabbit AF 488, anti-mouse 568 and anti-goat 633.Cover slips were mounted on glass microscope slides with ProLong Diamond Antifade reagent (Life Technologies). Protein targets were localised by confocal laser scanning microscopy. Single 0.5 µm sections were obtained using a Leica DMI8 microscope using 100x oil immersion lens running LAX software. The final image was obtained by averaging four sequential images of the same section. Digital images were analysed using ImageJ software (ImageJ, NIH, Bethesda, MD, USA) to determine TNFI, TCFI or TFI. The plot-profile feature of ImageJ was used to plot the fluorescence signal intensity along a single line spanning the nucleus (n = 5 lines per a nucleus, 5 individual cells) using the average fluorescent signal intensity for the indicated pair of antibodies was plotted for each point on the line with SE. Signal plotted to compare how the signals for each antibody varied in comparison to the opposite antibody. For each plot-profile the PCC was determined. PCC indicates the strength of relation between the two fluorochrome signals for at least 20 individual cells ± SE. Colours from representative images correspond to plot-profiles.

EXAMPLE 3

CD8 T-CELLS EXHAUSTION BIOMARKER SIGNATURE

[0673] TBET, EOMES and PD1 can define an exhaustive or effector biomarker signature for T-cells. An exhaustive biomarker signature would comprise of TBET-low, EOMES-high, PD1-high whereas an effector biomarker signature would comprise TBET-high, EOMES-low, PD1-low.

[0674] The present inventors examined CR, SD and PD cohorts for expression of these markers and found that: 1) EOMES and PD1 were highly expressed in the PD cohort whereas TBET was significantly lower than CR/SD cohorts; 2) TBET was highly expressed in the CR/SD cohorts with low expression in PD; 3) PKCθi targets both the exhaustion pathway, inhibiting expression of EOMES and PD-1 as well as other T-cell activation pathways. This allows PKC-θ inhibition to simultaneously inhibit the exhaustive pathway while enhancing TBET expression; 4) This allows the PKCθi to epigenetically re-program the T-cell away from an exhaustive biomarker signature to an effector/active T-cell biomarker signature; and 5) Inhibition of PD-1 by PKC-θ inhibitors such as PKCθi suggests that this will further aid PD-1 immunotherapy.

Methods

[0675] Metastatic Melanoma Biopsies were pre-enriched using the RosetteSep™ method to isolate CTCs by employing the RosetteSep™ Human CD8 enrichment Kit (15063, StemCell Technologies) to isolate CD8+ cells and red blood cells, using density gradient centrifugation with SepMate™-50 (IVD) density gradient tubes (85450, StemCell Technologies) and Lymphoprep™ density gradient medium (07861, StemCell Technologies). Enriched CTC cells were then stimulated with vehicle or PMA/CI and either pre-clinically screened with either a control or our proprietary novel nuclear PKC-Theta inhibitor at a concentration of 8mm. Enriched cells where then cytospun onto a coverslip pre-treated with poly-l-lysine and fixed then stored in PBS for staining. To examine the dynamics of EOMES, TBET and PD-1 in melanoma $CD8^+$ T-cell treated with PKCθi. $CD8^+$ T-cells were permeabilized by incubating with 1% Triton X-100 for 20 min and were probed with rabbit anti PKC-Theta (T538p); mouse anti-ZEB1 and goat anti-CD8 or IFN-γ (mouse), TNF-α (rabbit) and goat anti CD8 and visualized with a donkey anti-rabbit AF 488, anti-mouse 568 and anti-goat 633. Cover slips were mounted on glass microscope slides with ProLong Diamond Antifade reagent (Life Technologies). Protein targets were localized by confocal laser scanning microscopy. Single 0.5 µm sections were obtained using a Leica DMI8 microscope using 100x oil immersion lens running LAX software. The final image was obtained by averaging four sequential images of the same section. Digital images were analysed using ImageJ software (ImageJ, NIH, Bethesda, MD, USA) to determine the either the Total Nuclear Fluorescent Intensity (TNFI), the Total Cytoplasmic Fluorescent Intensity (TCFI) or total Fluorescent Intensity (TFI). The plot-profile feature of ImageJ was used to plot the fluorescence signal intensity along a single line spanning the nucleus (n = 5 lines per a nucleus, 5 individual cells) using the average fluorescent signal intensity for the indicated pair of antibodies was plotted for each point on the line with SE. Signal plotted to compare how the signals for each antibody varied in comparison to the opposite antibody. For each plot-profile the PCC was determined. PCC indicates the strength of relation between the two fluorochrome signals for at least 20 individual cells ± SE. Colours from representative images correspond to plot-profiles.

[0676] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0677] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

[0678] Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. Use of a PKC-θ inhibitor and a PD-1 binding antagonist in the manufacture of a medicament for treating a T-cell dysfunctional disorder in a subject, wherein the T-cell dysfunctional disorder is a cancer that is resistant to one or more PD-1 binding antagonists, and wherein the PKC-θ inhibitor is an inhibitor of PKC-θ nuclear translocation.

2. The use of claim 1, wherein the PKC-θ inhibitor is a peptide corresponding to the nuclear localization site of PKC-θ.

3. The use of claim 2, wherein the PKC-θ inhibitor is a proteinaceous molecule represented by formula (XXVI):

$Z_1X_1X_2X_3X_4IDX_5PPX_6X_7X_8X_9X_{10}X_{11}Z_2$ (XXVI)

wherein:

"Z_1" and "Z_2" are independently absent or are independently selected from at least one of a proteinaceous moiety comprising from about 1 to about 50 amino acid residues (and all integer amino acid residues therebetween), and a protecting moiety;

"X_1" is absent or is selected from basic amino acid residues including R, K and modified forms thereof;

"X_2" and "X_3" are independently selected from basic amino acid residues including R, K and modified forms thereof;

"X_4" is selected from charged amino acid residues including R, K, D, E and modified forms thereof;

"X_5" is absent or is W or modified forms thereof;

"X_6" is selected from aromatic or basic amino acid residues including F, Y, W, R, K and modified forms thereof;

"X_7" is selected from basic amino acid residues including R, K and modified forms thereof;

"X_8" is absent or is P or modified forms thereof;

"X_9" is selected from basic amino acid residues including R, K and modified forms thereof;

"X_{10}" is selected from hydrophobic residues including V, L, I, M and modified forms thereof and P and modified forms thereof;

"X_{11}" is selected from basic amino acid residues including R, K and modified forms thereof.

4. The use of claim 3, wherein "X_1" to "X_{11}" are selected from a combination of one or more of the following:

"X_1" is absent or is R;

"X_2" is R;

"X_3" is K;

"X_4" is E or R;

"X_5" is absent or is W;

"X_6" is F or R;

"X_7" is R;

"X_8" is absent or is P;

"X_9" is K;

"X_{10}" is V or P; and

"X_{11}" is K.

5. The use of claim 3 or claim 4, wherein "Z_1" consists of 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues.

6. The use of any one of claims 3 to 5, wherein "Z_2" consists of 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues.

7. The use of any one of claims 3 to 6, wherein the amino acid residues in "Z_1" and "Z_2" are selected from any amino acid residues.

8. The use of claim 3 or claim 4, wherein "Z_1" is a proteinaceous molecule represented by formula XXVII:

$X_{12}X_{13}X_{14}X_{15}X_{16}$ (XXVII)

wherein:

"X_{12}" is absent or is a protecting moiety;

"X_{13}" is absent or is selected from P and basic amino acid residues including R, K and modified forms thereof;

"X_{14}" is absent or is selected from P and basic amino acid residues including R, K and modified forms thereof;

"X_{15}" is absent or is selected from P and basic amino acid residues including R, K and modified forms thereof;

"X_{16}" is absent or is selected from P and basic amino acid residues including R, K and modified forms thereof.

9. The use of any one of claims 3, 4 and 8, wherein "Z_2" is a proteinaceous molecule represented by formula XXVIII:

$X_{17}X_{18}X_{19}X_{20}$ (XXVIII)

wherein:

"X_{17}" is absent or is selected from any amino acid residue;

"X_{18}" is absent or is selected from any amino acid residue;

"X_{19}" is absent or is selected from any amino acid residue;

"X_{20}" is absent or is a protecting moiety.

10. The use of claim 3 or claim 4, wherein "Z_1" and "Z_2" are absent.

11. The use of claim 3, wherein the proteinaceous molecule of formula XXVI comprises, consists or consists essentially of an amino acid sequence represented by SEQ ID NO: 4 or 5 as shown below:

RKEIDPPFRPKVK [SEQ ID NO: 4]

RRKRIDWPPRRKPK [SEQ ID NO: 5].

12. The use of any one of claims 1 to 11, wherein the PD-1 binding antagonist is an anti-PD-1 antagonist antibody.

13. The use of claim 12, wherein the anti-PD-1 antagonist antibody is selected from nivolumab, pembrolizumab, lambrolizumab and pidilizumab.

14. The use of any one of claims 1 to 11, wherein the PD-1 binding antagonist is an immunoadhesin (*e.g.*, AMP-224).

15. The use of any one of claims 1 to 14, wherein the medicament is to be concurrently administered with an ancillary agent (*e.g.*, a chemotherapeutic agent) or an ancillary therapy (*e.g.*, ablation or cytotoxic therapy) for treating, or for aiding in the treatment of, a T-cell dysfunctional disorder.

16. The use of claim 15, wherein the ancillary agent is selected from adjuvants, neoadjuvants, anti-infective drugs, cytotoxic agents, radiotherapy agents, gene therapy agents, DNA therapy agents, viral therapy agents, RNA therapy agents, immunotherapeutic agents, bone marrow transplantation agents, nanotherapy agents, or a combination of the foregoing.

17. The use of any one of claims 1 to 16, wherein the T-cell dysfunctional disorder is a disorder or condition of T-cells characterized by any one or more of (1) decreased responsiveness to antigenic stimulation, preferably wherein the decreased responsiveness to antigenic stimulation results in ineffective control of a tumor; (2) increased inhibitory signal transduction through PD-1; (3) decreased ability to secrete cytokines, proliferate, or execute cytolytic activity; and (4) T-cell anergy.

18. The use of any one of claims 1 to 17, wherein T-cell of the T-cell disorder is characterized by any one or more of: (1) expressing nuclear PKC-θ at a higher level than the level of expression of TBET in the same T-cell, and/or at a higher level than in an activated T-cell; (2) exhibiting T-cell exhaustion or anergy; (3) expressing a higher level of EOMES than TBET and/or have elevated expression of PD-1; (4) is a tumor-infiltrating lymphocyte; (5) is a circulating lymphocyte; and (6) is a $CD8^+$ T-cell.

19. The use of any one of claims 1 to 18, wherein the cancer is skin cancer (*e.g.*, melanoma), lung cancer, breast cancer, ovarian cancer, gastric cancer, bladder cancer, pancreatic cancer, endometrial cancer, colon cancer, kidney cancer, esophageal cancer, prostate cancer, colorectal cancer, glioblastoma, neuroblastoma, or hepatocellular carcinoma.

20. The use of claim 19, wherein the cancer is a metastatic cancer.

21. The use of claim 20, wherein the metastatic cancer is metastatic melanoma or metastatic lung cancer.

22. The use of any one of claims 1 to 21, wherein the treatment with the medicament results in any one or more of the following:

- $CD8^+$ T cells having enhanced priming, activation, proliferation and/or cytolytic activity as compared to before the treatment;
- the number of $CD8^+$ T cells is elevated as compared to before the treatment;
- Treg function is suppressed as compared to before the treatment;
- T cell exhaustion is decreased as compared to before the treatment;
- the number of Treg cells is decreased as compared to before the treatment;
- plasma IFN-γ is increased as compared to before the treatment;
- plasma TNF-α is increased as compared to before the treatment;
- plasma IL-2 is increased as compared to before the treatment;
- the number of memory T effector cells is increased as compared to before the treatment;
- memory T effector cell activation and/or proliferation is increased as compared to before the treatment;
- memory T effector cells are detected in peripheral blood, preferably wherein detection of memory T effector cells is by detection of CXCR3.