G PROTEIN-COUPLED RECEPTOR (GPCR) MODULATION BY IMIPRIDONES

BACKGROUND OF THE INVENTION

[0001] Human cells have a variety of receptors on their surfaces. G rotein-coupled receptors ("GPCR" or "GPCRs") form one of the largest protein families of transmembrane receptors. The human genome has approximately 30,000 genes, as many as 1,000 of which encode GPCRs. GPCRs have been grouped into five classes. The first class is the rhodopsin receptor family or "Class A GPCR" with 670 receptor proteins. The rhodopsin receptor family can react with various ligands including amines (alpha group), peptides (beta group), lipid-like substances (gamma group), nucleotides, and glycoproteins (delta group), and comprises a lot of drug target receptors. The second class is the secretin receptor family, and has binding domains for peptide hormones. Receptors in this family are associated with homeostasis and have been arising as important targets for drug development. The third class is the adhesion receptor family, characterized by a GPCR proteolytic site (GPS). Development of drugs targeting this family of GPCRs has not yet taken place because they exhibit various N-terminal moieties and little is known about their ligands. The fourth class is the glutamate receptor family with 22 GPCR members have so far been identified. Relatively little is known about the specificity of each protein. The last class is the Frizzled/Taste2 family that encompasses 10 Frizzled receptors for which Wnt glycoproteins serve as ligands, 5 SMO (smoothened) receptors which need no ligands, and 25 Taste2 receptors which are required for sensing various tastes. Receptors including GPCRs are also classified on the basis of the identification of endogenous ligands. Receptors bind with known endogenous compounds or are classified as orphan receptors whose endogenous ligands have not yet been identified.

[0002] GPCRs are found in a broad range of tissue and cell types and associated with many different physiological mechanisms. They are activated by a wide range of ligands, *e.g.*, hormones such as thyroid-stimulating hormone (TSH), adrenocorticotropic hormone, glucagon and vasopressin, amines such as 5-HT, acetylcholine (muscarinic AchR), and histamines, lipids such as LPA and S1P, and signal transmitters such as amino acids, Ca^{2+}, nucleic acids, peptides and light. The wide distribution and diversity of roles that GPCRs play is evidence of their importance in various pathological diseases. Indeed, GPCRs are involved in various diseases including bronchoconstriction, hypertension, diabetes, inflammation, cell death, hormone disorders, cancer, neurotransmission and behavioral disorders. GPCRs are therefore an important area for the development of pharmaceutical products. Approximately 360 GPCRs are now considered available for drug development. Of these, 46 have already been used for drug development. There are approximately an estimated 150 Orphan GPCRs (oGPCRs). In

the drug development field, cell membrane receptors act as selective sites for drug action and are responsible for 50% of all drug targets; GPCR activity modulating drugs account for 30% of the most frequently used top 100 drugs (40 billion dollars, 9% of the total drug market). Therefore, GPCRs are among the most significant targets for new drug development.

[0003] GPCRs have common structural features. They have seven hydrophobic membrane-spanning domains, each 20-30 amino acids long, which are connected by hydrophilic amino acid sequences of various lengths. The receptors have an extracellular N-terminus while the C-terminus is located in the cytoplasm. GTP-binding proteins (G proteins) act as mediators transmitting to intracellular effectors the signals that are generated by binding hormones or other chemical ligands that stimulate GPCR. After ligand binding, the GPCR intracellular domain undergoes a conformational change to allow the receptor to interact with a G protein, which in turn activates intracellular signal transmitters such as adenylate cyclase, phospholipase C or ion channels. This system generates a signaling cascade in which many secondary transmitters act in response to the binding of one ligand to GPCR. Cells use this mechanism to detect extracellular environmental changes and to properly react in response to the changes. On the whole, endogenous ligands activate receptors with the concomitant generation of a conformational change, which allows association between the receptors and G proteins. Recent studies on the interaction between proteins have revealed that GPCRs associate with various proteins such as GRK or SH2 (Src Homology 2) domain-containing proteins, and adaptor Grb2 as well as G protein to participate in signaling transduction.

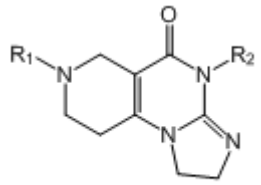
[0004] Under normal conditions, signaling transduction brings about the final result which is cell activation or suppression. In a physiological environment, GPCRs exist in equilibrium between their inactive and active states in the cell membrane. Inactive receptors cannot exert a biological response in conjunction with cellular signal transduction pathways. The receptors exhibit biological responses via a signal transduction pathway (through G proteins) only when they have structurally changed to their active form. The receptor may be stabilized into an active form by compounds such as endogenous ligands or drugs. Therefore, functional studies, such as cloning such gene families, and the identification of new ligands thereof, have the same meaning as the development of new drug candidates, that is, siRNA, antibodies, polypeptides, effectors, inhibitors, agonists, antagonists.

[0005] Development, differentiation, homeostasis, responses to stimuli, control of the cell cycle, as well as the aging and apoptosis of living organisms are mostly a result of selective expression of specific genes within cells. This is true for cellular mechanisms associated with diseases. Particularly, pathological phenomena, such as oncogenesis, are induced by gene mutations that in the end lead to changes in gene expression.

[0006] ONC201 (7-benzyl-4-(2-methylbenzyl)-1,2,6,7,8,9-hexahydroimidazo [1,2-a]pyrido [3,4-e]pyrimidin-5(1H)-one) is the founding member of a class of anti-cancer compounds called imipridones that is in Phase II clinical trials in multiple advanced cancers. Since the discovery of ONC201 as a p53-independent inducer of TRAIL gene transcription, preclinical studies have determined that ONC201 has anti-proliferative and pro-apoptotic effects against a broad range of tumor cells but not normal cells. The mechanism of action of ONC201 involves engagement of PERK-independent activation of the integrated stress response, leading to tumor upregulation of DR5 and dual Akt/ERK inactivation, and consequent Foxo3a activation leading to upregulation of the death ligand TRAIL. ONC201 is orally active with infrequent dosing in animal models, causes sustained pharmacodynamic effects, and is not genotoxic. The first-in-human ONC201 clinical trial in advanced aggressive refractory solid tumors confirmed that it is well-tolerated. In summary, the imipridone family that comprises ONC201 and its chemical analogs represent a new class of therapeutic agents.

BRIEF SUMMARY OF THE INVENTION

[0007] In one aspect, provided herein are compounds of formula (**10**):



(**10**), wherein R_1 and R_2 are independently selected from H, alkyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heterocycloalkylalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, and acyl radicals. In one embodiment, when R_1 is CH_2Ph, R_2 is not CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2-CH_3-Ph) (i.e., ONC201). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2,4-di F-Ph) (i.e., ONC206). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(4-CF_3-Ph) (i.e., ONC212). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(3,4-di F-Ph) (i.e., ONC213). In one embodiment, R_1 is CH_2 (3,4-di-Cl-Ph and R_2 is CH_2-(4-CF_3-Ph) (i.e., ONC234). In one embodiment, R_1 is CH_2-3-thienyl and R_2 is CH_2-(4-CF_3-Ph) (i.e., ONC236).

[0008] In another aspect, provided herein are methods of treating or preventing a disease, disorder, or condition in a subject in need thereof, comprising: administering to the subject in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount a compound of formula (**10**) or an analog thereof, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is selected from the group consisting of ONC201, ONC206, ONC212, ONC213, ONC234 and ONC236. In one embodiment, the subject has, or

is at risk of having, cancer. In one embodiment, the cancer is selected from a central nervous system tumor, a brain tumor, a peripheral nervous system tumor, a pheochromocytoma, a paraganglioma, a neuroendocrine tumor, ewings sarcoma, a pancreatic cancer, a prostate cancer, an endometrial cancer, a hematological malignancy, a bone cancer, and a lymphatic system tumor. In one embodiment, the cancer is selected from meningioma, ependymoma, glioma, neuroblastoma, or diffuse intrinsic pontine glioma. In one embodiment, the cancer is selected from an acute leukemia selected from an acute lymphotyte leukemia, acute myeloid leukemia, myelodysplastic syndrome, or myeloproliferative disease. In one embodiment, In one embodiment, the cancer has a histone H3 mutation (*e.g.*, the mutation H3.3 K27M) or an epigentically silenced unmethylated O(6)-methylguanine-DNA methyltransferase (MGMT) gene. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the psychiatric disorder is selected from a psychosis, schizophrenia, bipolar disorder, or major depressive disorder. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the infection is a bacterial infection. In one embodiment, the infection is a gram-negative bacterial infection. In one embodiment, the infection is a gram-positive bacterial infection. In one embodiment, the bacterial infection is an infection of a bacteria selected from the group consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. In one embodiment, the bacterial infection is a *Staphylococcus* infection. In one embodiment, the *Staphylococcus* infection is an *S. aureus* infection (*e.g.*, a methicillin-resistant *S. aureus* (MRSA) infection).

[0009] In another aspect, provided herein are methods of treating or preventing a disease, disorder, or condition in a subject in need of selective modulation of the activity of a G protein–coupled receptor (GPCR) or of a G protein–coupled receptor (GPCR) signaling pathway. Modulation includes, but is not lmited to, agonism, partial agonism, inverse agonism, partial antagonism, antagonism, bivalent modulation, or bitopic modulation. In one embodiment, the methods comprise administering to the subject in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount a compound of formula (**10**) or an analog thereof, or a pharmaceutically acceptable salt thereof. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the psychiatric disorder is psychosis. In one embodiment, the psychiatric disorder is schizophrenia. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the infection is a bacterial infection. In one embodiment, the infection is a gram-negative bacterial infection. In one embodiment, the infection is a gram-positive bacterial infection. In one embodiment, the bacterial infection is an

infection of a bacteria selected from *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, or *Enterobacter* species. In one embodiment, the bacterial infection is a *Staphylococcus* infection. In one embodiment, the *Staphylococcus* infection is an *S. aureus* infection (*e.g.*, a methicillin-resistant *S. aureus* (MRSA) infection). In one embodiment, the treatment regimen comprises administering an effective amount of a therapeutic, such as compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In one embodiment, the GPCR is a Class A GPCR. In one embodiment, the GPCR is GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof. In one embodiment, the GPCR is GPR132 (also called G2A). In one embodiment, the GPCR is GPR91. In one embodiment, the GPCR is MTNR1A. In one embodiment, the GPCR is CXCR7.

[0010] In another aspect, provided herein are methods of treating or preventing a disease, disorder, or condition in a subject in need of selective modulation of the activity of a dopamine receptor or of a member of a dopamine receptor signaling pathway. In one embodiment, the methods comprise administering to the subject in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount a compound of formula (**10**) or an analog thereof, or a pharmaceutically acceptable salt thereof. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the psychiatric disorder is psychosis. In one embodiment, the psychiatric disorder is schizophrenia. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the infection is a bacterial infection. In one embodiment, the infection is a gram-negative bacterial infection. In one embodiment, the infection is a gram-positive bacterial infection. In one embodiment, the bacterial infection is an infection of a bacteria selected from the group consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. In one embodiment, the bacterial infection is a *Staphylococcus* infection. In one embodiment, the *Staphylococcus* infection is an *S. aureus* infection (*e.g.*, a methicillin-resistant *S. aureus* (MRSA) infection). In one embodiment, the treatment regimen comprises administering an effective amount of a therapeutic, such as compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In one embodiment, the dopamine receptor is from the D2-like family of dopamine receptors.

[0011] In another aspect, provided herein are methods of treating or preventing liver fibrosis or of regenerating liver tissue, comprising: administering to the subject in need of such treatment a pharmaceutical composition comprising a therapeutically effective amount a compound of formula (**10**) or a compound of formula (**100**) (*e.g.*, TIC-10), or an analog

thereof, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is a CXCR7 agonist.

[0012] In another aspect, provided herein are methods of stimulating the immune system (*e.g.*, activating NK cells) in a subject in need thereof, comprising: administering to the subject a pharmaceutical composition comprising a therapeutically effective amount a compound of formula (**10**) or an analog thereof, or a pharmaceutically acceptable salt thereof. In one embodiment, the compound is a GPR91 agonist. In one embodiment, the compound is ONC213. In one embodiment, the subject has cancer and the method is a method of cancer immunotherapy. In one embodiment, the subject has a viral infection (*e.g.*, HIV). In one embodiment, the subject has systemic lupus erythematosus. In one embodiment, the method further comprises administering a vaccine (*e.g.*, a cancer vaccine) to the subject, and the compound is administered as an adjuvant.

[0013] In another aspect, provided herein are methods of identifying whether a subject having a condition is likely to be responsive to a treatment regimen described herein. In one embodiment, the methods comprise (i) obtaining a biological sample from the subject; (ii) measuring expression levels of at least one dopamine receptor or G protein–coupled receptor (GPCR) in the sample; (iii) comparing the levels measured in the sample to those for a pre-determined standard; and (iv) determining whether the subject is likely to be responsive to the treatment regimen, based on the levels measured in the sample to those for the pre-determined standard. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the treatment regimen further comprises administering an effective amount of a therapeutic, such as compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In one embodiment, the dopamine receptor is from the D2-like family of dopamine receptors. In one embodiment, the GPCR is a Class A GPCR. In one embodiment, the GPCR is GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof. In one embodiment, the GPCR is GPR132, GPR91, MTNR1A, CXCR7 or a combination thereof. In one embodiment, the GPCR is GPR132.

[0014] In another aspect, provided herein are methods of assessing the effectiveness of a treatment regimen described herein, monitoring, or providing a prognosis for a subject with a condition. In one embodiment, the methods comprises (i) obtaining a biological sample from the subject; (ii) measuring expression levels of at least one dopamine receptor or G protein–coupled receptor (GPCR) in the sample; (iii) comparing the levels measured in the sample to those for a pre-determined standard; and (iv) determining a prognosis or determining whether

the subject is responsive to the treatment regimen, based on the levels measured in the sample to those for the pre-determined standard. In one embodiment, the methods comprises (i) obtaining a biological sample from the subject; (ii) measuring gene copy number or mutations in at least one dopamine receptor in the sample; (iii) comparing the copy number measured or mutations found in the sample to those for a pre-determined standard; and (iv) determining whether the subject is responsive to the treatment regimen, based on the copy number measured or mutations found in the sample to those for the pre-determined standard. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the treatment regimen comprises administering an effective amount of a therapeutic, such as compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In one embodiment, the dopamine receptor is selected from DRD2, DRD2S, DRD2L, and DRD3. In one embodiment, the dopamine receptor is from the D2-like family of dopamine receptors In one embodiment, the GPCR is a Class A GPCR. In one embodiment, the GPCR is GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof.

[0015] In another aspect, provided herein are methods for screening a potential therapeutic for a condition. In one embodiment, the method comprises (i) contacting at least one G protein–coupled receptor (GPCR) with a test molecule suspected of being a therapeutic for a condition; (ii) measuring the binding affinity, interaction or GPCR signalling of the test compound to the GPCR; and (iii) comparing the binding affinity, interaction or signalling of the test molecule to a pre-determined threshold. In one embodiment, GPCR modulation or GPCR signaling modulation by the test molecule comparable to or greater than the threshold is indicative of a therapeutic for the condition. In one embodiment, the condition is cancer. In one embodiment, the pre-determined threshold is the GPCR modulation or GPCR signaling modulation of a therapeutic, such as a compound of formula (**10**) or a pharmaceutically acceptable salt thereof, or an analog thereof. In one embodiment, the GPCR is a Class A GPCR. In one embodiment, the GPCR is GPR132. In one embodiment, the GPCR is GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof. In one embodiment, the GPCR is GPR132. In one embodiment, the GPCR is GPR91. In one embodiment, the GPCR is MTNR1A. In one embodiment, the GPCR is CXCR7.

[0016] In another aspect, provided herein are methods for screening a potential therapeutic for a condition. In one embodiment, the method comprises (i) contacting at least one dopamine receptor with a test molecule suspected of being a therapeutic for a condition; (ii) measuring the binding affinity, interaction or signalling of the test molecule to the at least one dopamine

receptor; and (iii) comparing the binding affinity or interaction of the test molecule to a pre-determined threshold. In one embodiment, modulation of the dopamine receptor by the test molecule comparable to or greater than the threshold is indicative of a therapeutic for the condition. In one embodiment, the condition is cancer. In one embodiment, the dopamine receptor is a member of the D2-like family of dopamine receptors. In one embodiment, the pre-determined threshold is the modulation of the dopamine receptor or dopamine receptor signalling by a therapeutic, such as a compound of formula (**10**) or a pharmaceutically acceptable salt thereof, or an analog thereof.

[0017] In another aspect, provided herein are methods for screening a potential therapeutic for a condition. In one embodiment, using a processor, the method comprises (i) using a computational docking method to model binding or interaction, if any, of one or more 3-dimensional structures (conformations) of a test molecule suspected of being a therapeutic for the condition to a 3-dimensional structure or model of at least one dopamine receptor; (ii) using the computational method to estimate the binding affinity or interaction of the test molecule structure to the structure or model of the at least one dopamine receptor; and (iii) using the computational method to compare the binding affinity or interaction of the test molecule to a pre-determined threshold, wherein modulation of the dopamine receptor by the test molecule comparable to or greater than the threshold is indicative of a therapeutic for the condition. In one embodiment, the condition is cancer. In one embodiment, the dopamine receptor is a member of the D2-like family of dopamine receptors.

[0018] In another aspect, provided herein are methods of treating a subject having a condition. In one embodiment, the method comprises administering an effective amount of a therapeutic agent that targets at least one dopamine receptor or G protein–coupled receptor (GPCR). In one embodiment, the therapeutic agent is a neutralizing agent. In one embodiment, the therapeutic agent is an antagonist of the receptor. In one embodiment, the therapeutic agent is an agonist of the receptor. In one embodiment, the therapeutic agent is a competitive inhibitor of the receptor with respect to dopamine. In one embodiment, the therapeutic agent is a non-competitive inhibitor of the receptor with respect to dopamine. In one embodiment, the therapeutic agent is selective for the D2-like family of dopamine receptors with respect to the D1-like family of dopamine receptors. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the dopamine receptor is a member of the D2-like family of dopamine receptors. In one embodiment, the GPCR is a Class A GPCR. In one embodiment, the GPCR is GPR132. In one embodiment, the GPCR is GPR91. In one embodiment, the GPCR is MTNR1A. In one

embodiment, the GPCR is CXCR7.In one embodiment, the GPCR is GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7 or a combination thereof. In one embodiment, the therapeutic agent is a monoclonal antibody (*e.g.*, a chimerized or humanized monoclonal antibody), polyclonal antibody (*e.g.*, a chimerized or humanized polyclonal antibody), or a bispecific antibody. In one embodiment, the therapeutic agent is a drug or active agent, such as an anti-cancer agent, conjugated to an antibody. In one embodiment, the therapeutic agent is a radioactively-conjugated antibody or a small molecule-conjugated antibody. In one embodiment, the therapeutic agent is a vector that expresses a recombinant antibody to the dopamine receptor or GPCR. In one embodiment, the therapeutic agent is a fusion protein or a peptide that targets the dopamine receptor or GPCR. In one embodiment, the therapeutic agent is an siRNA, shRNA, or an antisense oligonucleotide that targets the dopamine receptor or GPCR. In one embodiment, the dopamine receptor or GPCR is targeted by CRISPR interference.

[0019] In another aspect, provided herein are methods of treating and assessing the efficacy of a treatment in a subject having a condition. In one embodiment, the method comprises (i) treating the subject according to a treatment method described herein (ii) assessing as decribed herein the treatment's efficacy. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the treatment regimen comprises administering an effective amount of a therapeutic, such as a compound of formula (**10**) or a pharmaceutically acceptable salt thereof or an analog thereof. In one embodiment, the dosage of a therapeutic administered, the frequency of administration of the compound (*e.g.*, a compound of formula (**10**)), or both, is selected or adjusted based on the levels of gene expression or gene copy number measured or mutations found.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The above summary, as well as the following detailed description of embodiments of the invention, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown. In the drawings:

[0021] Figure **1**.Antagonism of dopamine receptors (DRD1, DRD2S, DRD2L, DRD3, DRD4, and DRD5) by ONC201.

[0022] Figure **2** illustrates soluble prolactin detected by ELISA in the peripheral blood of advanced solid tumor patients at baseline and following a single ONC201 dose (PO 125-625 mg). Sampling time points post-treatment include 6 hours, 1, 2, 7, and 21 days post-treatment.

[0023] Figure **3**. Tumor type sensitivity of the Genomic of Drug Sensitivity in Cancer program (GDSC) cell line collection. The average sensitivity was determined by average estimated IC_{50} values from cell viability assays conducted at 72 hours post-treatment. Numbers above the bar indicates indicate the number of cell lines per tumor type.

[0024] Figure **4**. ONC201 is a selective DRD2 antagonist. (A) Agonism of orphan or known GPCRs or antagonism of known GPCRs using an arrestin recruitment reporter assay (10 μM ONC201). (B) Antagonism of ligand-stimulated dopamine receptors by ONC201 using an arrestin recruitment reporter assay. Schild analysis of DRD2L antagonism by ONC201 using (C) arrestin recruitment or (D) cAMP modulation reporters.

[0025] Figure **5**. ONC201 antagonism of DRD2 is highly specific among GPCRs and other cancer drug targets. (A) Antagonism of GPCRs using an arrestin recruitment reporter assay (10 μM ONC201). Competition of ONC201-mediated antagonism of DRD2L by dopamine in (B) arrestin recruitment or (C) cAMP modulation reporters. (D) Antagonism or agonism of nuclear hormone receptors by ONC201 (2 or 20 μM) with a nuclear translocation reporter assay. (E) *In vitro* inhibition of kinase enzymatic activity by ONC201 (1 μM). (F) DRD2L antagonistic activity of ONC201 or an ONC201 linear isomer with no biological activity using an arrestin recruitment reporter assay.

[0026] Figure **6**. GBM cell lines with higher DRD2 expression are more responsive to ONC201. (A) Inhibition of NCI60 GBM cell lines as a function of ONC201 concentration. (B) Log ONC201 GI_{50} (M) vs DRD2 expression for each GBM cell line. $R^2 = 0.8707$.

[0027] Figure **7**. ONC201 exhibits superior selectivity among GPCRs for DRD2 compared to other DRD2 antagonists, such as risperidone.

[0028] Figure **8**. ONC201 has a higher selectivity for tumor cells than the antipsychotic DRD2 antagonist, thioridazine.

[0029] Figure **9**. Optimization of ONC201 inhibition of DRD2 calcium flux. HEK-293T cells were transfected with expression constructs for wild-type DRD2 (**A**) or a control GPCR (**B**). DRD2-specific calcium flux inhibition was investigated at 0.1 and 1 nM dopamine, for ONC201 concentrations between 100 pM and 100 μM. 100 μM ONC201 completely inhibited DRD2 dopamine-induced calcium flux but had no effect on the control GPCR.

[0030] Figure **10**. Comparison of DRD2 inhibitors. DRD2-specific calcium flux inhibition was investigated at 1 nM dopamine, using inhibitors spiperone (squares), domperidone (triangles), and ONC201 (circles) at a range of concentrations. Data for individual assays was normalized using the no-inhibitor value (shown as 10^{-11} M) as 100% activity.

[0031] Figure **11**. Identification of DRD2 residues critical for dopamine-induced calcium flux. (A) Dopamine-induced calcium flux was assayed as before at 1 nM dopamine, across the

entire DRD2 alanine-scan library. The data represent the average of three experiments. Mutant clones were considered to be deficient for calcium flux if they demonstrated flux values less than 2 standard deviations below the average calcium flux value (AV – 2SD) for the entire library. (B) The locations of the 28 mutated residues identified are indicated (green spheres) on the DRD3 crystal structure (PDB id 3PBL; Chien, E.Y. *et al.* (2010) Science **330**:1091-5). The D2R/D3R antagonist eticlopride is shown in cyan.

[0032] Figure **12**. Identification of DRD2 residues critical for ONC201 inhibition of dopamine-induced calcium flux. (A) Dopamine-induced calcium flux was assayed as before at 1 nM dopamine but in the presence of 100 μM ONC201, across the entire DRD2 alanine-scan library. The data represent the average of three experiments normalized to the value for flux value with wild-type DRD2 (%WT). Mutant clones were considered to be critical for ONC201 inhibition if they demonstrated flux values greater than 2 standard deviations above the average calcium flux value (AV + 2SD) for the entire library. (B) The locations of the 8 mutated residues identified are indicated (red spheres) on the DRD3 crystal structure.

[0033] Figure **13**. A reference compound, (+) Butaclamol, and a test compound, ONC201 dihydrochloride, successfully competed for [^{3}H]Methylspiperone, with IC_{50} values of 2.5 nM and 21 μM, respectively.

[0034] Figure **14**. Association kinetic curves for ONC201 dihydrochloride to DRD2S receptor to determine K_{on} and K_{off}.

[0035] Figure **15**. Compound activity with the selected GPCR and Orphan GPCR Biosensor Assays. Compound was tested in antagonist and agonist mode with the desired GPCR and Orphan GPCR Biosensor Assays. For agonist assays, data was normalized to the maximal and minimal response observed in the presence of control ligand and vehicle. For antagonist assays, data was normalized to the maximal and minimal response observed in the presence of EC_{80} ligand and vehicle. The following EC_{80} concentrations were used: CCR4 Arrestin: 0.0078 μM CCL22; CHRM2 Arrestin: 26 μM Acetylcholine; and MC4R Arrestin: 0.0026 μM Melanotan II.

[0036] Figure **16**. ONC206 and ONC212 demonstrated anti-cancer efficacy across various tumor types in the NCI60 cancer cell line panel. ONC203 is an inactive negative control

[0037] Figure **17**. ONC206 is an imipridone with improved DRD2 antagonism. ONC206, an analog of ONC201, exhibits superior antagonism of D2-like dopamine receptor family, and retains highly selective antagonism of D2-like dopamine receptors compared to other antipsychotics, such as a haloperidol.

[0038] Figure **18**. Bone cancer is more responsive to ONC206 than ONC201.

[0039] Figure **19.** Ewing's sarcoma is the most ONC206 responsive bone cancer subtype.

[0040] Figure **20**. ONC206 anti-cancer efficacy is in the nanomolar range in 14 out of 16 Ewing's sarcoma cell lines. ONC206 demonstrated superior efficacy compared to ONC201 in all cell lines

[0041] Figure **21**. The imipridone ONC212 targets an orphan GPCR It is a highly selective agonist of the orphan GPCR tumor suppressor GPR132, and it does not engage DRD2.

[0042] Figure **22**. ONC212 induced cell death in cancer cells (HCT116) but not normal cells (MRC5) at nanomolar concentrations.

[0043] Figure **23**. ONC212 induces the integrated stress response and inhibits Akt/ERK phosphorylation at nanomolar concentrations and at earlier time points compared to ONC201.

[0044] Figure **24**. ONC212 demonstrates oral and IP anti-cancer efficacy in xenograft mouse models of colorectal and breast cancer.

[0045] Figure **25**. Leukemia is more responsive to ONC212 than ONC201.

[0046] Figure **26**. ONC212 demonstrates anti-cancer efficacy (and superior efficacy compared to ONC201) in the nanomolar range in 55 leukemia cell lines regardless of subtype.

[0047] Figure **27**. GPCRs agonized or antagonized (>50%) by 9 imipridones tested. Imipridones selectively target rhodopsin-like Class A GPCRs.

[0048] Figure **28**. Case study of a subject with recurrent glioblastoma (Example 16). (A) Tumor size relative to baseline (%) of total tumor burden in the subject. One cycle is 3 weeks. (B) Contrast MRI scans at baseline, 21, 27 and 36 weeks post-ONC201 initiation of one of 2 malignant lesions.

[0049] Figure **29**. ONC212 demonstrates anti-cancer effects in acute myeloid leukemia (AML) cell lines. (A) Comparison of cell viability of MV411 AML cells treated with ONC212 or cytarabine. (B) Comparison of cell viability of MOLM14, MV411 AML cells, MRC5 lung fibroblasts and Hs27a bone marrow cells treated with ONC212. (C) Cell viability of MOLM14 and MV411 AML cells treated with ONC212 (250nM) for 4, 8, 24, 48, 72 and 96h.

[0050] Figure **30**. ONC212 efficacy in ONC201-resistant AML xenograft model (MV411 AML cells (5×10^6) subcutaneously implanted in the flanks of athymic nude mice). ONC212 and ONC201 were administered orally (PO) as indicated. Tumor volume (A and B) and body weight (C) (n=10) was measured on indicated days. * represents $p < 0.05$ relative to vehicle.

[0051] Figure **31**. ONC206 efficacy in Ewing's sarcoma xenograft model (MHH-ES-1 Ewing's sarcoma cells (5×10^6) subcutaneously implanted in the flanks of athymic nude mice). ONC206 (PO) and methotrexate (IV) were administered on day 1 and day 13 as indicated. Tumor volume (A) and body weight (B) (n=4) was measured on indicated days.

[0052] Figure **32**. ONC213 (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

[0053] Figure **33**. ONC213 demonstrated *in vitro* anti-cancer potency in HCT116/RPMI8226 cancer cells similar to ONC212, but *in vitro* toxicity to normal cells was reduced compared to ONC212.

[0054] Figure **34**. ONC237 (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

[0055] Figure **35**. ONC236 (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

[0056] Figure **36**. ONC234 (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

[0057] Figure **37**. ONC201 linear isomer (TIC-10) (10μM) GPCR profile using a β-arrestin recruitment reporter assay.

[0058] Figure **38**. Number of GPCRs hit for several imipridones.

DETAILED DESCRIPTION OF THE INVENTION

[0059] Scientific and technical terms used here are intended to have the meanings commonly understood by those of ordinary skill in the art. Such terms are found and used in context in various standard references illustratively including J. Sambrook and D. W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; 3rd Ed., 2001; F. M. Ausubel, Ed., Short Protocols in Molecular Biology, Current Protocols; 5th Ed., 2002; B. Alberts et al., Molecular Biology of the Cell, 4th Ed., Garland, 2002; D. L. Nelson and M. M. Cox, Lehninger Principles of Biochemistry, 4th Ed., W.H. Freeman & Company, 2004; Engelke, D. R., RNA Interference (RNAi): Nuts and Bolts of RNAi Technology, DNA Press LLC, Eagleville, PA, 2003; Herdewijn, P. (Ed.), Oligonucleotide Synthesis: Methods and Applications, Methods in Molecular Biology, Humana Press, 2004; A. Nagy, M. Gertsenstein, K. Vintersten, R. Behringer, Manipulating the Mouse Embryo: A Laboratory Manual, 3rd edition, Cold Spring Harbor Laboratory Press; Dec. 15, 2002, ISBN-10: 0879695919; Kursad Turksen (Ed.), Embryonic stem cells: methods and protocols in Methods Mol Biol. 2002;185, Humana Press; Current Protocols in Stem Cell Biology, ISBN: 9780470151808, as well as U.S. Patent No. 8,673,923. The content of each of the references above is hereby incorporated by reference in its entirety.

[0060] The term "substituted" means that any one or more hydrogens on the designated atom is replaced with a selection from the indicated group, provided that the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound. When a substituent is keto (i.e., =O), then 2 hydrogens on the atom are replaced. Keto substituents are

not present on aromatic moieties. Ring double bonds are double bonds that are formed between two adjacent ring atoms (e.g., C=C, C=N, or N=N).

[0061] When a variable (e.g., R^4) occurs more than one time in a constituent or formula for a compound, its definition at each occurrence is independent of its definition at every other occurrence. Thus, for example, if a group is shown to be substituted with 0-3 R^4 moieties, then the group may optionally be substituted with up to three R^4 moieties and R^4 at each occurrence is selected independently from the definition of R^4. Also, combinations of substituents and/or variables are permissible, but only if such combinations result in stable compounds.

[0062] When an atom or chemical moiety is followed by a subscripted numeric range (e.g., $C_{1\text{-}6}$), it will be appreciated that this is meant to encompass each number within the range, as well as all intermediate ranges. For example, "$C_{1\text{-}6}$ alkyl" is meant to include alkyl groups with 1, 2, 3, 4, 5, 6, 1-6, 1-5, 1-4, 1-3, 1-2, 2-6, 2-5, 2-4, 2-3, 3-6, 3-5, 3-4, 4-6, 4-5, and 5-6 carbons.

[0063] The term "alkyl" includes both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. For example, $C_{1\text{-}6}$ alkyl is intended to include C_1, C_2, C_3, C_4, C_5, and C_6 alkyl groups. Examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, isobutyl s-butyl, t-butyl, n-pentyl, s-pentyl, neopentyl and n-hexyl. In certain cases, a straight chain or branched chain alkyl has six or fewer carbon atoms in its backbone (e.g., C_1-C_6 for straight chain, C_3-C_6 for branched chain), and in other cases, a straight chain or branched chain alkyl has four or fewer carbon atoms. Likewise, cycloalkyls have from three to eight carbon atoms in their ring structure, and in other cases, cycloalkyls have five or six carbons in the ring structure. Most preferred is $C_{1\text{-}6}$ alkyl, particularly ethyl, methyl, isopropyl, isobutyl, n-pentyl, n-hexyl and cyclopropylmethyl.

[0064] the term "substituted alkyl" means alkyl as defined above, substituted by one, two or three substituents selected from halogen, -OH, alkoxy, $-NH_2$, $-N(CH_3)_2$, -C(=O)OH, trifluoromethyl, -C≡N, $-C(=O)O(C_1\text{-}C_4)$alkyl, $-C(=O)NH_2$, $-SO_2NH_2$, $-C(=NH)NH_2$, and $-NO_2$, preferably containing one or two substituents selected from halogen, -OH, alkoxy, $-NH_2$, trifluoromethyl, $-N(CH_3)_2$, and -C(=O)OH, more preferably selected from halogen, alkoxy and -OH. Examples of substituted alkyls include, but are not limited to, 2,2-difluoropropyl, 2-carboxycyclopentyl and 3-chloropropyl.

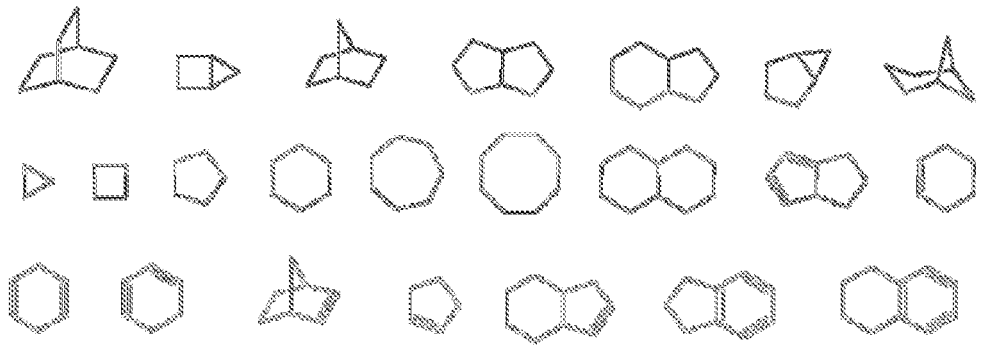
[0065] Unless the number of carbons is otherwise specified, "lower alkyl" is an alkyl group, as defined above, but having from one to six carbon atoms, preferably one to four, in its backbone structure. "Lower alkenyl" and "lower alkynyl" have chain lengths of 2-6 carbon atoms and preferably 2-4 carbon atoms.

[0066] "Alkenyl" includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double bond. For

example, the term "alkenyl" includes straight-chain alkenyl groups (e.g., ethenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl), branched-chain alkenyl groups, cycloalkenyl (e.g., alicyclic) groups (e.g., cyclopropenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl), alkyl or alkenyl substituted cycloalkenyl groups, and cycloalkyl or cycloalkenyl substituted alkenyl groups. In certain cases, a straight chain or branched chain alkenyl group has six or fewer carbon atoms in its backbone (e.g., C_2-C_6 for straight chain, C_3-C_6 for branched chain). Likewise, cycloalkenyl groups may have from three to eight carbon atoms in their ring structure, and in some embodiments, cycloalkenyl groups have five or six carbons in the ring structure. The term "C_2-C_6" includes alkenyl groups containing two to six carbon atoms. The term "C_3-C_6" includes alkenyl groups containing three to six carbon atoms.

[0067] "Alkynyl" includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but which contain at least one triple bond. For example, "alkynyl" includes straight-chain alkynyl groups (e.g., ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl, decynyl), branched-chain alkynyl groups, and cycloalkyl or cycloalkenyl substituted alkynyl groups. In certain embodiments, a straight chain or branched chain alkynyl group has six or fewer carbon atoms in its backbone (e.g., C_2-$C_6$for straight chain, C_3-C_6 for branched chain). The term "C_2-C_6" includes alkynyl groups containing two to six carbon atoms. The term "C_3-C_6" includes alkynyl groups containing three to six carbon atoms.

[0068] The term "cycloalkyl" refers to a mono cyclic or polycyclic non-aromatic radical, where each of the atoms forming the ring (i.e. skeletal atoms) is a carbon atom. In some cases, the cycloalkyl group is saturated or partially unsaturated. In other cases, the cycloalkyl group is fused with an aromatic ring. Cycloalkyl groups include groups with from 3 to 10 ring atoms. Examples of cycloalkyl groups include, but are not limited to, the following moieties:



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[0069] Monocyclic cycloalkyls include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Dicyclic cycloalkyls include, but are not limited to, tetrahydronaphthyl, indanyl, and tetrahydropentalene. Polycyclic cycloalkyls include adamantine and norbornane. The term cycloalkyl includes "unsaturated nonaromatic

carbocyclyl" or "nonaromatic unsaturated carbocyclyl" groups, both of which refer to a nonaromatic carbocycle as defined herein, which contains at least one carbon carbon double bond or one carbon carbon triple bond.

[0070] The term "cycloalkylalkyl" refers to an alkyl group substituted by a cycloalkyl group. Example cycloalkylalkyl groups include cyclopropylalkyl, cyclohexylalkyl.

[0071] The term "heterocycloalkyl" refers to a non-aromatic heterocycle where one or more of the ring-forming atoms is a heteroatom such as an O, N, or S atom. Heterocycloalkyl groups include mono- or polycyclic (e.g., having 2, 3 or 4 fused rings) ring systems, as well as spirocycles. Example heterocycloalkyl groups include morpholino, thiomorpholino, piperazinyl, tetrahydrofuranyl, tetrahydrothienyl, 2,3-dihydrobenzofuryl, 1,3-benzodioxole, benzo-1,4-dioxane, piperidinyl, pyrrolidinyl, isoxazolidinyl, isothiazolidinyl, pyrazolidinyl, oxazolidinyl, thiazolidinyl, and imidazolidinyl. Also included in the definition of heterocycloalkyl can be moieties that have one or more aromatic rings fused (i.e., having a bond in common with) to the nonaromatic heterocyclic ring, for example quinolyl, isoquinolyl, and benzo derivatives of heterocycles. A heterocycloalkyl group having one or more fused aromatic rings are attached though either the aromatic or non-aromatic portion. Also included in the definition of heterocycloalkyl are moieties where one or more ring-forming atoms can be substituted by 1 or 2 oxo or sulfido groups. In some cases, the heterocycloalkyl group has from 1 to about 20 carbon atoms, and in further case from about 3 to about 20 carbon atoms. In some cases, a heterocycloalkyl group contains 3 to about 20, 3 to about 14, 3 to about 7, or 5 to 6 ring-forming atoms. In some cases, a heterocycloalkyl group has 1 to about 4, 1 to about 3, or 1 to 2 heteroatoms. In some cases, a heterocycloalkyl group contains 0 to 3 double bonds. In some cases, a heterocycloalkyl group contains 0 to 2 triple bonds.

[0072] The term "heterocycloalkylalkyl" refers to an alkyl group substituted by a heterocycloalkyl. Example heterocycloalkylalkyls include morpholinoalkyl and piperazinylalkyl.

[0073] The term "aryl" refers to monocyclic or polycyclic (e.g., having 2, 3 or 4 fused rings) aromatic hydrocarbons such as, phenyl, naphthyl, anthracenyl, phenanthrenyl. In some cases, an aryl group has from 6 to about 20 carbon atoms.

[0074] The term "arylalkyl" refers to an alkyl group substituted by an aryl group. Example arylalkyl groups include benzyl and phenylethyl.

[0075] The term "heteroaryl" refers to an aromatic heterocycle having at least one heteroatom ring member such as an O, S, or N atom. Heteroaryl groups include monocyclic and polycyclic (e.g., having 2, 3 or 4 fused rings) systems. A ring-forming N atom in a heteroaryl group can also be oxidized to form an N-oxo moiety. Examples of heteroaryl groups include pyridyl, N-oxopyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, furyl, quinolyl, isoquinolyl,

thienyl, imidazolyl, thiazolyl, indolyl, pyrryl, oxazolyl, benzofuryl, benzothienyl, benzthiazolyl, isoxazolyl, pyrazolyl, triazolyl, tetrazolyl, indazolyl, 1,2,4-thiadiazolyl, isothiazolyl, benzothienyl, purinyl, carbazolyl, benzimidazolyl, indolinyl. In some cases, a heteroaryl group has from 1 to about 20 carbon atoms, and in further cases from about 3 to 20 carbon atoms. In some cases, a heteroaryl group contains 3 to about 14, 3 to about 7, or 5-6 ring-forming atoms. In some cases, a heteroaryl group has 1 to about 4, 1 to about 3, or 1-2 heteroatoms.

[0076] a "heteroarylalkyl" group refers to an alkyl group substituted by a heteroaryl group. An example of a heteroarylalkyl group is pyridylmethyl.

[0077] The terms "halo" or "halogen" refer to a fluorine (F), chlorine (Cl), bromine (Br), or iodine (I) atom; preferably, F, Cl, or Br; more preferably, F or Cl. The term "perhalogenated" refers to a moiety where all hydrogens are replaced by halogens. The term "haloalkyl" refers to alkyl moieties with a halogen replacing a hydrogen on one or more carbons of the hydrocarbon backbone. C_1-C_6 haloalkyl includes a straight chain or branched alkyl with six or fewer backbone carbon atoms and a halogen replacing a hydrogen on one or more backbone carbons.

[0078] The term "alkoxy" or "alkoxyl" includes substituted and unsubstituted alkyl, alkenyl, and alkynyl groups covalently linked to an oxygen atom. C_1-C_6 alkoxy refers to moieties having six or fewer carbon atoms in the hydrocarbon backbone. Examples of alkoxy groups (or alkoxyl radicals) include methoxy, ethoxy, isopropyloxy, propoxy, butoxy, and pentoxy groups. Preferred are (C_1-C_3) alkoxy, particularly ethoxy and methoxy. Examples of substituted alkoxy groups include halogenated alkoxy groups.

[0079] The term "hydroxy" or "hydroxyl" includes groups with an -OH or $-O^-$.

[0080] The term "pharmaceutically acceptable salts" refers to derivatives of compounds where the parent compound is modified by converting an existing acid or base moiety to its salt form. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids. Pharmaceutically acceptable salts include conventional non-toxic salts of a parent compound formed, for example, from non-toxic inorganic or organic acids. Pharmaceutically acceptable salts may be synthesized from a parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting a free acid or base form of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts can be found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, *Journal of Pharmaceutical Science*, 66, 2 (1977), and P. H. Stahl and C. G. Wermuth, editors, *Handbook of Pharmaceutical Salts:*

Properties, Selection and Use, 2nd Revised edition, Weinheim/Zürich:Wiley-VCH/VHCA (2011), each of which is incorporated herein by reference in its entirety.

[0081] Examples of suitable inorganic acids include hydrochloric acid, sulphuric acid, phosphoric acid, or hydrobromic acid, while examples of suitable organic acids include carboxylic acid, sulpho acid, or sulphonic acid, such as acetic acid, tartaric acid, lactic acid, propionic acid, glycolic acid, malonic acid, maleic acid, fumaric acid, tannic acid, succinic acid, alginic acid, benzoic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, cinnamic acid, mandelic acid, citric acid, maleic acid, salicylic acid, trifluoroacetic acid, 3-aminosalicylic acid, ascorbic acid, embonic acid, nicotinic acid, isonicotinic acid, oxalic acid, gluconic acid, amino acids, methanesulphonic acid, ethanesulphonic acid, 2-hydroxyethanesulphonic acid, ethane-1,2-disulphonic acid, benzenesulphonic acid, 4-methylbenzenesulphonic acid or naphthalene-2-sulphonic acid. Examples of suitable inorganic bases include sodium hydroxide, potassium hydroxide and ammonia, while examples of suitable organic bases include amines, e.g., tertiary amines, such as trimethylamine, triethylamine, pyridine, *N,N*-dimethylaniline, quinoline, isoquinoline, α-picoline, β-picoline, γ-picoline, quinaldine, or pyrimidine.

[0082] the term "antibody" encompasses the structure that constitutes the natural biological form of an antibody. In most mammals, including humans, and mice, this form is a tetramer and consists of two identical pairs of two immunoglobulin chains, each pair having one light and one heavy chain, each light chain comprising immunoglobulin domains V_L and C_L, and each heavy chain comprising immunoglobulin domains V_H, Cγ1, Cγ2, and Cγ3. In each pair, the light and heavy chain variable regions (V_L and V_H) are together responsible for binding to an antigen, and the constant regions (C_L, Cγ1, Cγ2, and Cγ3, particularly Cγ2, and Cγ3) are responsible for antibody effector functions. In some mammals, for example in camels and llamas, full-length antibodies may consist of only two heavy chains, each heavy chain comprising immunoglobulin domains V_H, Cγ2, and Cγ3. By "immunoglobulin (Ig)" herein is meant a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulins include but are not limited to antibodies. Immunoglobulins may have a number of structural forms, including full-length antibodies, antibody fragments, and individual immunoglobulin domains including V_H, Cγ1, Cγ2, Cγ3, V_L, and C_L.

[0083] Based on the heavy-chain constant domain amino acid sequence, intact antibodies can be assigned to different "classes." There are five-major classes (isotypes) of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses," *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different antibody classes are called alpha, delta, epsilon, gamma, and mu, respectively. The

subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known to one skilled in the art.

[0084] The terms "antibody" or "antigen-binding fragment," respectively, refer to intact molecules as well as functional fragments thereof, such as Fab, a scFv-Fc bivalent molecule, $F(ab')_2$, and Fv that are capable of specifically interacting with a desired target. In some cases, the antigen-binding fragments comprise:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, which can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule that can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')2 is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains;

(5) Single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule; and

(6) scFv-Fc, is produced by fusing single-chain Fv (scFv) with a hinge region from an immunoglobulin (Ig) such as an IgG, and Fc regions.

[0085] In one embodiment, an antibody provided herein is a monoclonal antibody. In one embodiment, the antigen-binding fragment provided herein is a single chain Fv (scFv), a diabody, a tandem scFv, a scFv-Fc bivalent molecule, an Fab, Fab', Fv, $F(ab')_2$ or an antigen binding scaffold (*e.g.*, affibody, monobody, anticalin, DARPin, Knottin).

[0086] the terms "binds," "binding" or grammatical equivalents, refer to compositions, directly or indirectly, having affinity for each other. "Specific binding" is where the binding is selective between two molecules. A particular example of specific binding occurs between an antibody and an antigen. Typically, specific binding can be distinguished from non-specific when the dissociation constant (K_D) is less than about 1×10^{-5} M or less than about 1×10^{-6} M or 1×10^{-7} M. Specific binding can be detected, for example, by ELISA, immunoprecipitation, coprecipitation, with or without chemical crosslinking, and two-hybrid assays. Appropriate controls can be used to distinguish between "specific" and "non-specific" binding. "Affinity" is the strength of the binding interaction of two molecules, such as an antigen and its antibody, which is defined for

antibodies and other molecules with more than one binding site as the strength of binding of the ligand at one specified binding site. Although the noncovalent attachment of a ligand to antibody or other molecule is typically not as strong as a covalent attachment, "high affinity" is for a ligand that binds to an antibody or other molecule having an affinity constant (K_a) of greater than 10^4 M^{-1}, typically 10^5-10^{11} M^{-1}; as determined by inhibition ELISA or an equivalent affinity determined by comparable techniques, such as Scatchard plots or using K_d/dissociation constant, which is the reciprocal of the K_a.

[0087] The term "selective" with respect to binding, inhibition, stimulation, or modulation means preferential binding, inhibition, stimulation, or modulation, respectively, of a first activity relative to a second activity (e.g., preferential binding of one receptor to another receptor; preferential inhibition relative to other receptors; or preferential inhibition of a mutant to a wild-type or vice versa). In some cases, binding is greater than two times more selective, greater than five times more selective, greater than ten times more selective, greater than fifty times more selective, greater than 100 times more selective, or greater than 1000 times more selective for the desired molecular target or pathway versus an undesired molecular target or pathway. In some cases, a compound will bind a first molecular target or affect a pathway by at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold relative to a second target or pathway under the same conditions. It will be appreciated that in preferred embodiments, binding to the D2-like family of dopamine receptors or a member thereof, will be selective with respect to the D1-like family of dopamine receptors or a member thereof by any of the foregoing amounts. The *in vitro* or *in vivo* activity of a molecular target or pathway may be measured by any suitable reproducible means.

[0088] The term "modulating" refers to "stimulating" or "inhibiting" an activity of a molecular target or pathway. For example, a composition modulates the activity of a molecular target or pathway if it stimulates or inhibits the activity of that target or pathway by at least 10%, by at least about 20%, by at least about 25%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, by at least about 75%, by at least about 80%, by at least about 90%, by at least about 95%, by at least about 98%, or by about 99% or more relative to the activity of that molecular target or pathway under the same conditions but lacking only the presence of the composition. In another example, a composition modulates the activity of a molecular target or pathway if it stimulates or inhibits the activity of that target or pathway by at least 2-fold, at least 5 -fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold relative to the activity of that target or pathway under the same conditions but lacking only the presence of the composition. The activity of a molecular target or pathway may be measured by any reproducible means. For example, the activity of a molecular target or pathway may be

measured *in vitro* or *in vivo* by a suitable assay known in the art for measuring the activity. Control samples (untreated with the composition) can be assigned a relative activity value of 100%.

[0089] In one embodiment, an antibody, antigen-binding fragment, or affinity tag binds its target with a K_D of 0.1 nM - 10 mM, 0.1 nM - 1 mM, or within the 0.1 nM range. In one embodiment, an antibody, antigen-binding fragment, or affinity tag binds its target with a K_D of 0.1-2 nM, 0.1-1 nM, 0.05-1 nM, 0.1-0.5 nM, or 0.1-0.2 nM. In one embodiment, an antibody, antigen-binding fragment, or affinity tag bind its target directly. In one embodiment, an antibody, antigen-binding fragment, or affinity tag bind its target indirectly, for example, binding as a secondary antibody that binds to an antibody bound to the target.

[0090] The word "label" refers to a compound or composition which is conjugated or fused directly or indirectly to a reagent such as a nucleic acid 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 or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition, which is detectable.

[0091] The term "probe" refers to synthetic or biologically produced nucleic acids that contain specific nucleotide sequences which hybridize under stringent conditions to target nucleic acid sequences. The terms "labeled probe," "nucleic acid probe operably linked to a detectable label," or "nucleic acid strand operably linked to a detectable label" refer to a probe which is prepared with a marker moiety or "detectable label" for detection. The marker moiety is attached at either the 5' end, the 3' end, internally, or a combination thereof. That is, one probe may be attached to multiple marker moieties. A preferred moiety is an identifying label such as a fluorophore. A labeled probe may also comprise a plurality of different nucleic acid sequences each labeled with one or more marker moieties. Each marker moiety may be the same or different. It may be beneficial to label different probes (*e.g.*, nucleic acid sequences) each with a different marker moiety. This can be achieved by having a single distinguishable moiety on each probe. For example, probe A is attached to moiety X and probe B is attached to moiety Y. Alternatively, probe A is attached to moieties X and Y while probe B is attached to moiety Z and W. Alternatively, probe A is attached to moieties X and Y, while probe B is attached to moieties Y and Z. All probes "A" and "B" above would be distinguishable and uniquely labeled.

[0092] By "tissue sample" is meant a collection of similar cells obtained from a tissue of a subject or patient, preferably containing nucleated cells with chromosomal material. The four main human tissues are (1) epithelium; (2) connective tissues, including blood vessels, bone and cartilage; (3) muscle tissue; and (4) nerve tissue. The tissue sample source may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or a

blood constituent; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from a time in gestation or development of the subject. A tissue sample may be primary or cultured cells or cell lines. A tissue sample may contain compounds that are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, or antibiotics. By a "section" of a tissue sample is meant a single part or piece of a tissue sample, *e.g.*, a thin slice of tissue or cells cut from a tissue sample. Multiple sections of tissue samples may be taken and subjected to analysis. A "cell line" refers to a permanently established cell culture that will proliferate given appropriate fresh medium and space.

Detection Methods

[0093] In various aspects, provided herein are methods of detecting or measuring a target receptor (*e.g.*, a dopamine receptor or a GPCR) in a biological sample. Targets are detected by contacting the sample with a target detection reagent, *e.g.*, an antibody or fragment thereof, and a labeling reagent. The presence or absence of targets are detected by the presence or absence of the labeling reagent. In some instances, a sample is contacted with the target detection and the labeling reagents concurrently *e.g.*, the detection reagent is a primary antibody and the labeling reagent is a fluorescent dye conjugated to it. Alternatively, the biological sample is contacted with the target detection and labeling reagents sequentially, *e.g.*, the detection reagent is a primary antibody and the labeling reagent includes a secondary antibody. For example, a sample is incubated with a detection reagent, in some cases together with a labeling reagent, under conditions that allow a complex between the detection reagent (and labeling reagent) and target to form. After complex formation the sample is optionally washed one or more times to remove unbound detection reagent (and labeling reagent). When the sample is further contacted with a labeling reagent that specifically binds the detection reagent bound to the target, the sample can optionally be washed one or more times to remove unbound labeling reagent. The presence or absence of the target in the sample is then determined by detecting the labeling reagent.

[0094] The methods described here provide for detection of multiple targets in a sample. Multiple targets are identified by contacting the biological sample with additional detection reagents followed by additional labeling reagent specific for the additional detection reagents using the methods described.

[0095] A detection moiety, i.e., detectable label, is a substance used to facilitate identification and/or quantitation of a target. Detection moieties are directly observed or measured or indirectly observed or measured. Detection moieties include, but are not limited to, radiolabels that can be measured with radiation-counting devices; pigments, dyes or other chromogens that can be visually observed or measured with a spectrophotometer; spin labels that can be measured with a spin label analyzer; and fluorescent moieties, where the output signal is generated by the

excitation of a suitable molecular adduct and that can be visualized by excitation with light that is absorbed by the dye or can be measured with standard fluorometers or imaging systems. The detection moiety can be a luminescent substance such as a phosphor or fluorogen; a bioluminescent substance; a chemiluminescent substance, where the output signal is generated by chemical modification of the signal compound; a metal-containing substance; or an enzyme, where an enzyme-dependent secondary generation of signal occurs, such as the formation of a colored product from a colorless substrate. The detection moiety may also take the form of a chemical or biochemical, or an inert particle, including colloidal gold, microspheres, quantum dots, or inorganic crystals such as nanocrystals or phosphors. The term detection moiety or detectable label can also refer to a "tag" or hapten that can bind selectively to a labeled molecule such that the labeled molecule, when added subsequently, is used to generate a detectable signal. For instance, one can use biotin, iminobiotin or desthiobiotin as a tag and then use an avidin or streptavidin conjugate of horseradish peroxidase (HRP) to bind to the tag, and then use a chromogenic substrate (*e.g.*, tetramethylbenzidine) or a fluorogenic substrate such as Amplex Red or Amplex Gold (Molecular Probes, Inc.) to detect the presence of HRP. Similarly, the tag can be a hapten or antigen (*e.g.*, digoxigenin), and an enzymatically, fluorescently, or radioactively labeled antibody can be used to bind to the tag. Numerous labels are known by those of skill in the art and include, but are not limited to, particles, fluorescent dyes, haptens, enzymes and their chromogenic, fluorogenic, and chemiluminescent substrates.

[0096] A fluorophore is a chemical moiety that exhibits an absorption maximum beyond 280 nm, and when covalently attached in a labeling reagent retains its spectral properties. Fluorophores include a pyrene, an anthracene, a naphthalene, an acridine, a stilbene, an indole or benzindole, an oxazole or benzoxazole, a thiazole or benzothiazole, a porphyrin, a cyanine, a perylene, a 4-amino-7-nitrobenz-2-oxa-1,3-diazole (NBD), a carbocyanine, a carbostyryl, a salicylate, an anthranilate, an azulene, a pyridine, a quinoline, a borapolyazaindacene, a xanthene, an oxazine or a benzoxazine, a carbazine, a phenalenone, a coumarin, a benzofuran and benzphenalenone and derivatives thereof. oxazines include resorufins, aminooxazinones, diaminooxazines, and their benzo-substituted analogs.

[0097] When the fluorophore is a xanthene, the fluorophore may be a fluorescein, a rhodol, or a rhodamine. Fluorescein includes benzo- or dibenzofluoresceins, seminaphthofluoresceins, or naphthofluoresceins. Similarly, rhodol includes seminaphthorhodafluors. Alternatively, the fluorophore is a xanthene that is bound via a single covalent bond at the 9-position of the xanthene. Preferred xanthenes include derivatives of 3H-xanthen-6-ol-3-one, derivatives of 6-amino-3H-xanthen-3-one, or derivatives of 6-amino-3H-xanthen-3-imine. Fluorophores include xanthene (rhodol, rhodamine, fluorescein and derivatives thereof) coumarin, cyanine,

pyrene, oxazine and borapolyazaindacene. In addition, the fluorophore can be sulfonated xanthenes, fluorinated xanthenes, sulfonated coumarins, fluorinated coumarins and sulfonated cyanines. The choice of fluorophore in the labeling reagent will determine the absorption and fluorescence emission properties of the labeling reagent. Physical properties of a fluorophore label include spectral characteristics (absorption, emission and stokes shift), fluorescence intensity, lifetime, polarization and photo-bleaching rate can all be used to distinguish one fluorophore from another.

[0098] Typically, a fluorophore contains one or more aromatic or heteroaromatic rings that are optionally substituted by one or more of a variety of substituents, including halogen, nitro, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, acyl, aryl or heteroaryl ring system, benzo, or other substituents typically present on fluorophores known in the art.

[0099] Preferably, the detection moiety is a fluorescent dye. Fluorescent dyes include, for example, Fluorescein, Rhodamine, Texas Red, Cy2, Cy3, Cy5, Cy0, Cy0.5, Cy1, Cy1.5, Cy3.5, Cy7, VECTOR Red, ELF™ (Enzyme-Labeled Fluorescence), FluorX, Calcein, Calcein-AM, CRYPTOFLUOR™'S, Orange (42 kDa), Tangerine (35 kDa), Gold (31 kDa), Red (42 kDa), Crimson (40 kDa), BHMP, BHDMAP, Br-Oregon, Lucifer Yellow, Alexa dye family, N-(6-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)caproyl) (NBD), BODIPY™, boron dipyrromethene difluoride, Oregon Green, MITOTRACKER™ Red, DiOC7 (3), DiIC18, Phycoerythrin, Phycobiliproteins BPE (240 kDa) RPE (240 kDa) CPC (264 kDa) APC (104 kDa), Spectrum Blue, Spectrum Aqua, Spectrum Green, Spectrum Gold, Spectrum Orange, Spectrum Red, NADH, NADPH, FAD, Infra-Red (IR) Dyes, Cyclic GDP-Ribose (cGDPR), Calcofluor White, Tyrosine and Tryptophan. Many fluorophores can also function as chromophores and thus they are also preferred chromophores.

[00100] In addition to fluorophores, enzymes also find use as detectable moieties. Enzymes are desirable detectable moieties because amplification of a detectable signal can be achieved resulting in increased assay sensitivity. The enzyme itself does not produce a detectable response but breaks down a substrate when it is contacted by an appropriate substrate such that the converted substrate produces a fluorescent, colorimetric or luminescent signal. Enzymes amplify a detectable signal because one enzyme on a labeling reagent can result in multiple substrates being converted to a detectable signal. This is advantageous where there is a low quantity of target present in the sample or a fluorophore does not exist that will give comparable or stronger signal than the enzyme. However, fluorophores are preferred because they do not require additional assay steps, and thus reduce the overall time to complete an assay. The enzyme substrate is selected to yield the preferred measurable product, *e.g.* colorimetric, fluorescent or chemiluminescence. Such substrates are extensively used in the art.

[00101] A preferred colorimetric or fluorogenic substrate and enzyme combination uses oxidoreductases such as horseradish peroxidase and a substrate such as 3,3′-diaminobenzidine (DAB) and 3-amino-9-ethylcarbazol-e (AEC), which yield a distinguishing color (brown and red, respectively). Other colorimetric oxidoreductase substrates that yield detectable products include, but are not limited to: 2,2-azino-bis(3-ethylbenzothiaz-oline-6-sulfonic acid) (ABTS), o-phenylenediamine (OPD), 3,3′,5,5′-tetramethylbenzidine (TMB), o-dianisidine, 5-aminosalicylic acid, 4-chloro-1-naphthol. Fluorogenic substrates include, but are not limited to, homovanillic acid or 4-hydroxy-3-methoxyphenylacetic acid, reduced phenoxazines and reduced benzothiazines, including Amplexe Red reagent and its variants and reduced dihydroxanthenes, including dihydrofluoresceins and dihydrorhodamines including dihydrorhodamine 123. Peroxidase substrates that are tyramides represent a unique class of peroxidase substrates in that they can be intrinsically detectable before action of the enzyme but are "fixed in place" by the action of a peroxidase in the process described as tyramide signal amplification (TSA). These substrates are extensively utilized to label targets in samples that are cells, tissues or arrays for their subsequent detection by microscopy, flow cytometry, optical scanning and fluorometry.

[00102] Additional colorimetric (and in some cases fluorogenic) substrate and enzyme combination use a phosphatase enzyme such as an acid phosphatase, an alkaline phosphatase or a recombinant version of such a phosphatase in combination with a colorimetric substrate such as 5-bromo-6-chloro-3-indolyl phosphate (BCIP), 6-chloro-3-indolyl phosphate, 5-bromo-6-chloro-3-indolyl phosphate, p-nitrophenyl phosphate, or o-nitrophenyl phosphate or with a fluorogenic substrate such as 4-methylumbelliferyl phosphate, 6,8-difluoro-7-hydroxy4-methylcoumarinyl phosphate (DiFMUP) fluorescein diphosphate, 3-0-methylfluorescein phosphate, resorufin phosphate, 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate (DDAO phosphate), or ELF 97, ELF 39 or related phosphates.

[00103] Glycosidases, in particular β-galactosidase, β-glucuronidase and β-glucosidase, are additional suitable enzymes. Appropriate colorimetric substrates include, but are not limited to, 5-bromo4-chloro-3-indolyl β-D-galactopyranoside (X-gal) and similar indolyl galactosides, glucosides, and glucuronides, o-nitrophenyl β-D-galactopyranoside (ONPG) and p-nitrophenyl β-D-galactopyranoside. Preferred fluorogenic substrates include resorufin β-D-galactopyranoside, fluorescein digalactoside (FDG), fluorescein diglucuronide and their structural variants, 4-methylumbelliferyl β-D-galactopyranoside, carboxyumbelliferyl β-D-galactopyranoside and fluorinated coumarin β-D-galactopyranosides. Additional enzymes includehydrolases such as cholinesterases and peptidases, oxidases such as glucose oxidase and cytochrome oxidases, and reductases for which suitable substrates are known.

[00104] Enzymes and their appropriate substrates that produce chemiluminescence are preferred for some assays. These include, but are not limited to, natural and recombinant forms of luciferases and aequorins. Chemiluminescence-producing substrates for phosphatases, glycosidases and oxidases such as those containing stable dioxetanes, luminol, isoluminol and acridinium esters are additionally useful. For example, the enzyme is luciferase or aequorin. The substrates are luciferine, ATP, Ca^{++} and coelenterazine.

[00105] In addition to enzymes, haptens such as biotin are useful detectable moieties. Biotin is useful as it is in an enzyme system that can further amplify a detectable signal, and it can serve as a tag in affinity chromatography for isolation purposes. For detection, an enzyme conjugate that has affinity for biotin is used, such as avidin-HRP. Subsequently, a peroxidase substrate is added to produce a detectable signal. Haptens also include hormones, naturally occurring and synthetic drugs, pollutants, allergens, affector molecules, growth factors, chemokines, cytokines, lymphokines, amino acids, peptides, chemical intermediates, or nucleotides.

[00106] In some cases, a detectable moiety is a fluorescent protein. Exemplary fluorescent proteins include green fluorescent protein (GFP), phycobiliproteins and their derivatives, luciferase or aequorin. Fluorescent proteins, especially phycobiliprotein, are particularly useful for creating tandem dye labeled labeling reagents. These tandem dyes comprise a fluorescent protein and a fluorophore to obtain a larger stokes shift where the emission spectra is farther shifted from the fluorescent protein's absorption spectra. This is particularly advantageous to detect a low amount of target in a sample where the emitted fluorescent light is maximally optimized, in other words the fluorescent protein reabsorbs little to none of the emitted light. The fluorescent protein and fluorophore function as an energy transfer pair where the fluorescent protein emits at a wavelength the fluorophore absorbs, and the fluorphore then emits at a wavelength farther from the fluorescent protein than could be obtained with only the fluorescent protein. A particularly useful combination is phycobiliproteins and sulforhodamine fluorophores, or sulfonated cyanine fluorophores; or sulfonated xanthene derivatives. Alternatively, the fluorophore is an energy donor and the fluorescent protein is an energy acceptor.

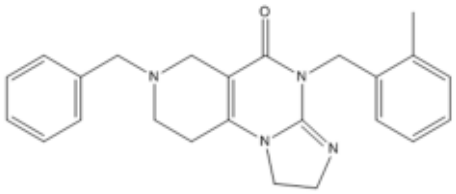
Methods of visualizing the detection moiety depend on the label.

[00107] In some cases, a sample is illuminated with a light wavelength selected to give a detectable optical response, and observed with a means for detecting that response. Equipment useful for illuminating fluorescent compounds include hand-held ultraviolet lamps, mercury arc lamps, xenon lamps, lasers and laser diodes. These illumination sources are optically integrated into laser scanners, fluorescent microplate readers or standard or microfluorometers. The degree or location of signal, compared to a standard or expected response, indicates whether and to what degree the sample possesses a given characteristic or desired target.

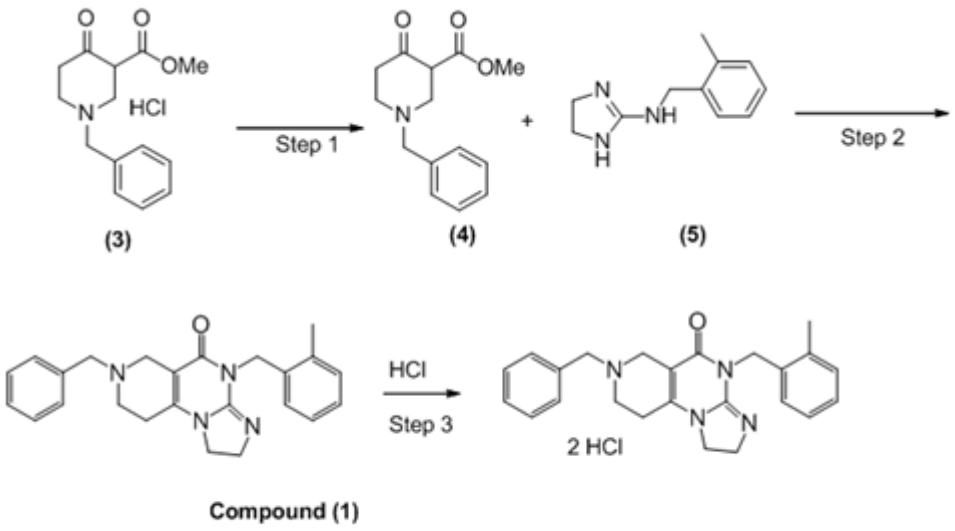
[00108] An optical response is detected by visual inspection, or by using one of the following devices: CCD camera, video camera, photographic film, laser-scanning devices, fluorometers, photodiodes, quantum counters, epifluorescence microscopes, scanning microscopes, flow cytometers, fluorescence microplate readers, or by means for amplifying the signal such as photomultiplier tubes. When a sample is examined using a flow cytometer, examination of it optionally includes sorting portions of it according to their fluorescence response.

[00109] When an indirectly detectable label is used, then illuminating typically includes adding a reagent to produce a detectable signal such as a colorimetric enzyme substrate. Radioisotopes are also considered indirectly detectable where an additional reagent is not needed, rather the radioisotope is exposed to X-ray film or other mechanism to record and measure the signal. This is true for some chemiluminescent signals that are observed after exposure to film.

I. ONC201 (COMPOUND (1)), SALTS THEREOF AND SYNTHESES THEREOF



[00110] ONC201 (compound (**1**)) and its analogs, and their pharmaceutically acceptable salts, as well as syntheses for them, are provided herein. In *in vitro* models, animal models, and human clinical trials, ONC201 has broad anti-cancer activity, low toxicity including few, if any, adverse effects, low genotoxicity, and high bioavailability including orally. These features allow ONC 201 and various analogs to be well suited for a variety of applications. ONC201 can be made by the synthesis shown in Scheme **1**.

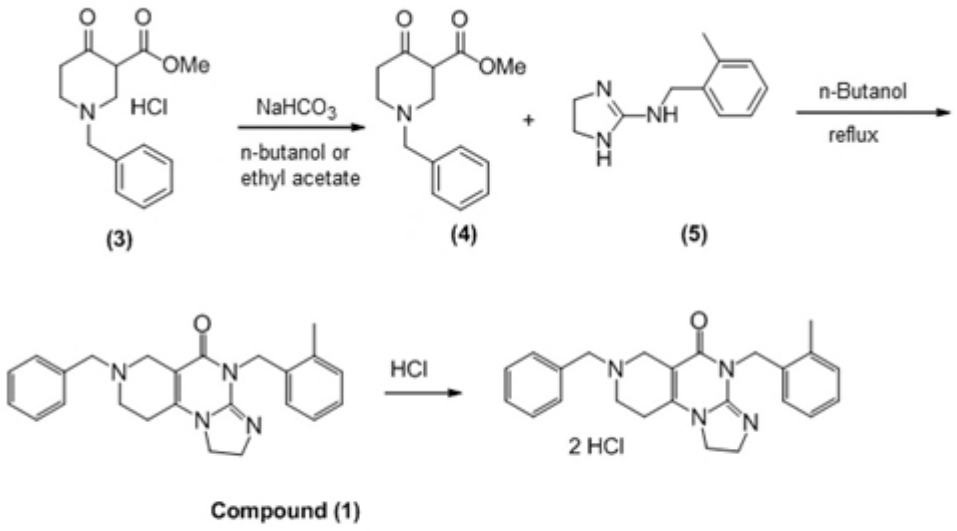


Scheme 1

[00111] Synthesis of an ONC201 dihydrochloride salt starts with commercially available intermediary N-Benzyl-3-carbomethoxy-4-piperidone hydrochloride, compound (**3**). In one embodiment, the synthesis includes neutralizing compound (**3**) with a base (Step **1**) to produce compound (**4**), a free base. In one embodiment, compound (**3**) is neutralized with an inorganic base to produce compound (**4**). In one embodiment, compound (**3**) is neutralized with an organic base to produce compound (**4**). In one embodiment, compound (**3**) is neutralized in the presence of an alcohol, for example, n-butanol. In one embodiment, compound (**3**) is neutralized in the presence of at least one organic solvent, for example, n-butanol and/or ethyl acetate. In one embodiment, compound (**3**) is neutralized in the presence of a base and at least one organic solvent, for example, $NaHCO_3$ and n-butanol. In one embodiment, compound (**3**) is neutralized in the presence of n-butanol and triethyl amine (Et_3N).

[00112] In one embodiment, the synthesis includes reacting compound (**4**) with compound (**5**) (Step **2**) to produce intermediary compound (**1**). In one embodiment, the reaction in Step **2** includes heating compound (**4**) with compound (**5**). In one embodiment, the reaction in Step **2** includes refluxing heating compound (**4**) and compound (**5**) in the presence of a solvent. In one embodiment, the reaction in Step **2** includes use of Dean-stark trap to remove water and/or methanol (MeOH) formed in the reaction.

[00113] In one embodiment, an ONC201 dihydrochloride salt is synthesized (Step **3**). In one embodiment, this reaction (Step **3**) includes treating ONC201 with HCl in dioxane. In one embodiment, Step **3** includes treating ONC201with 4N HCl in dioxane. In one embodiment, the synthesis optionally includes recrystallizing the ONC201 di-salt. In a preferred embodiment, the ONC201 di-hydrochloride salt is synthesized as shown in Scheme **2**.



Scheme 2

II. TNF-RELATED APOPTOSIS-INDUCING LIGAND ("TRAIL")

[00114] TRAIL protein can be assayed in a sample obtained from a subject to detect TRAIL expression induced by compounds and their salts described herein. Immunoassays can be used to assay TRAIL in a sample, including enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunofiltration assay (ELIFA), flow cytometry, immunoblot, immunoprecipitation, immunohistochemistry, immunocytochemistry, luminescent immunoassay (LIA), fluorescent immunoassay (FIA), and radioimmunoassay. Assays may be used to obtain qualitative and/or quantitative results. Specific details of suitable methods for both qualitative and quantitative sample assays are described in standard references, including E. Harlow & D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988; F. Breitling & S. Diibel, Recombinant Antibodies, John Wiley & Sons, New York, 1999; H. Zola, Monoclonal Antibodies: Preparation and Use of Monoclonal Antibodies and Engineered Antibody Derivatives, Basics: From Background to Bench, BIOS Scientific Publishers, 2000; B.K.C. Lo, Antibody Engineering: Methods and Protocols, Methods in Molecular Biology, Humana Press, 2003; F.M. Ausubel et al., Eds., Short Protocols in Molecular Biology, Current Protocols, Wiley, 2002; S. Klussman, Ed., The Aptamer Handbook: Functional Oligonucleotides and Their Applications, Wiley, 2006; Ormerod, M.G., Flow Cytometry: a practical approach, Oxford University Press, 2000; Givan, A.L., Flow Cytometry: first principles, Wiley, New York, 2001; Gorczyca, W., Flow Cytometry in Neoplastic Hematology: morphologic-immunophenotypic correlation, Taylor & Francis, 2006; Crowther, J.R., The ELISA Guidebook (Methods in Molecular Biology), Humana Press, 2000; Wild, D., The Immunoassay Handbook, 3rd Edition, Elsevier Science, 2005, and J. Sambrook and D.W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 3rd ed., 2001.

[00115] Protocols to assay and analyze a sample for TRAIL to detect an effect of a pharmaceutical composition are described in U.S. Patent 8,673,923 to Wafik S. El-deiry *et al.*, which is incorporated by reference herein in its entirety.

[00116] In one embodiment, TRAIL assays are used to monitor a subject. For example, a sample is obtained from a subject before treatment with a pharmaceutical composition and at one or more times during and/or following treatment to assess the treatment's effectiveness. In another example, a sample is obtained from a subject at various times to assess the course or progress of disease or healing. In one embodiment, death receptors from circulating tumor cells are assayed to see if a treatment described here increases the amount or type of death receptors.

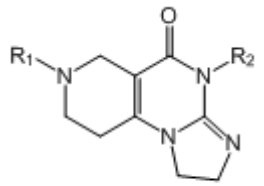
[00117] Cancers treated using methods and compositions described herein are characterized by abnormal cell proliferation including pre-neoplastic hyperproliferation, cancer *in-situ*,

neoplasms and metastasis. Methods and compositions described herein can be used for prophylaxis, as well as amelioration of cancer signs or symptoms. “Treatment” of a cancer in a subject includes: preventing, inhibiting or ameliorating cancer in the subject, such as slowing cancer progression or reducing or ameliorating a cancer sign or symptom. Examples of cancers treated using methods and compositions described herein include breast cancer, CNS cancers, colon cancer, ovarian cancer, prostate cancer, leukemia, lung cancer, and lymphoma.

III. COMPOUNDS OF FORMULA (10) AND SALTS THEREOF

[00118] In one aspect, provided herein are compounds and salts of formula (**10**) and methods of making them. Persons skilled in the art will understand that the general principles and concepts described here in conjunction with ONC201 (compound (**1**)) and its salts, including principles and concepts related to methods and pharmaceutical compositions, apply with equal force to compounds of formula (**10**) and salts thereof.

[00119] In one embodiment, provided herein are compounds of formula (**10**):



(10)

, wherein R_1 and R_2 are independently selected from H, alkyl, aryl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heterocycloalkylalkyl, heteroaryl, arylalkyl, heteroarylalkyl, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, and acyl radicals. In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2-CH_3-Ph) (i.e., ONC201). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2,4-di F-Ph) (i.e., ONC206). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(4-CF_3-Ph) (i.e., ONC212). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(3,4-di F-Ph) (i.e., ONC213). In one embodiment, R_1 is CH_2 (3,4-di-Cl-Ph and R_2 is CH_2-(4-CF_3-Ph) (i.e., ONC234). In one embodiment, R_1 is CH_2-3-thienyl and R_2 is CH_2-(4-CF_3-Ph) (i.e., ONC236).

[00120] In one embodiment, R_1 and R_2 are independently selected from the group consisting of H, $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, $C_{1\text{-}4}$alkylthienyl, $C_{1\text{-}4}$alkylpyridinyl, $C_{1\text{-}4}$alkylisoxazolidinyl, $C_{1\text{-}4}$alkylmorpholinyl, $C_{1\text{-}4}$alkylthiazolyl, and $C_{1\text{-}4}$alkylpyrazinyl wherein $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, $C_{1\text{-}4}$alkylthienyl, $C_{1\text{-}4}$alkylpyridinyl, $C_{1\text{-}4}$alkylisoxazolidinyl, $C_{1\text{-}4}$alkylmorpholinyl, $C_{1\text{-}4}$alkylthiazolyl, and $C_{1\text{-}4}$alkylpyrazinyl are optionally substituted with $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkoxyl, hydroxyl, perhalogenated $C_{1\text{-}4}$alkyl, or halo. In one embodiment, R_1 and/or R_2 is a substituted or unsubstituted, arylalkyl or heteroarylalkyl. In one embodiment, the heteroarylalkyl is selected from $C_{1\text{-}4}$alkylpyrrolyl, $C_{1\text{-}4}$alkylfuryl,

C_{1-4}alkylpyridyl, C_{1-4}alkyl-1,2,4-thiadiazolyl, C_{1-4}alkylpyrimidyl, C_{1-4}alkylthienyl, C_{1-4}alkylisothiazolyl, C_{1-4}alkylimidazolyl, C_{1-4}alkyltetrazolyl, C_{1-4}alkylpyrazinyl, C_{1-4}alkylpyrimidyl, C_{1-4}alkylquinolyl, C_{1-4}alkylisoquinolyl, C_{1-4}alkylthiophenyl, C_{1-4}alkylbenzothienyl, C_{1-4}alkylisobenzofuryl, C_{1-4}alkylpyrazolyl, C_{1-4}alkylindolyl, C_{1-4}alkylpurinyl, C_{1-4}alkylcarbazolyl, C_{1-4}alkylbenzimidazolyl, and C_{1-4}alkylisoxazolyl.

[00121] In one embodiment, R_1 and/or R_2 is a benzyl optionally substituted with one or more of the following substituents on the benzyl ring: X, $-CH_3$, $-NO_2$, $-OCH_3$, -CN, $-CXH_2$, $-CX_2H$, C_2-C_4 alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_1-C_4 alkyl; and where p is an integer from 2 to 20 and X is a halogen, including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl.

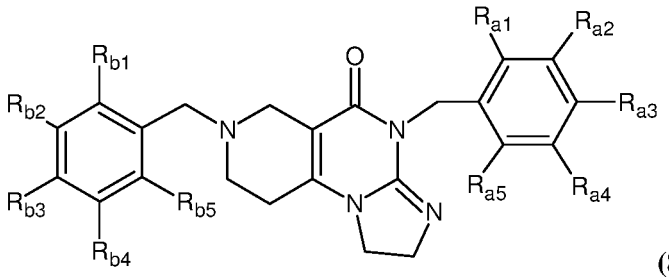
[00122] In one embodiment, R_1 is selected from H, CH_3, CH_2Ph, CH_2-(4-CF_3-Ph), CH_2-(4-F-Ph), CH_2-(4-Cl-Ph), CH_2-(OCH_3-Ph), CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2-(3-thienyl), CH_2-2-pyridinyl, CH_2-4-methyl-2-thiazolyl, CH_2-2-pyrazinyl, CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-(3,4-di Cl-Ph), CH_2-(3,4-di F-Ph), CH_2-(3,5-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CH(OH)Ph$, (4-F-Ph)-4-oxobutyl, $CH_2CH_2NHCOOC(CH_3)_3$, $CH_2CH_2CH_2NH_2$, and $CD_2C_6D_5$. In one embodiment, R_2 is selected from H, CH_3, CH_2Ph, CH_2-(4-CF_3-Ph), CH_2-((2-Cl)-Ph), CH_2-((2-F)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-(2,4-di Cl-Ph), CH_2-(3,4-di Cl-Ph), CH_2-(3,4-di F-Ph), CH_2-(3,5-di F-Ph), CH_2-((2-CH_3)-Ph), CH_2(2-CH_3, 4-F-Ph), CH_2-((4-OCH_3)-Ph), CH_2-(3-pyridinyl), CH_2-(3-isoxazolidinyl), CH_2CH_2-(4-morpholinyl), CH_2-(2-F, 4-CF_3-Ph), $CH_2CH(OH)Ph$, $(CH_2)_3CO$-4F-Ph, , (4-F-Ph)-4-oxobutyl, $CH_2CH_2NHCOOC(CH_3)_3$, $CH_2CH_2CH_2NH_2$, and $CD_2C_6D_5$.

[00123] In one embodiment, R_1 is H. In one embodiment, R_1 is a substituted or unsubstituted arylalkyl, *e.g.*, a benzyl (CH_2Ph) or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[00124] In one embodiment, R_2 is a substituted or an unsubstituted arylalkyl, *e.g.*, benzyl or phenylethyl. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the arylalkyl is substituted with one or more substituents selected from halo, CH_3, CF_3 or OCH_3. In one embodiment, R_2 is a substituted or an unsubstituted heterocycloalkylalkyl, *e.g.*, piperazinylalkyl or morpholinoalkyl. In one embodiment, R_2 is a substituted or an unsubstituted heteroarylalkyl, *e.g.*, pyridylmethyl or isoxazolidinylmethyl. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl,

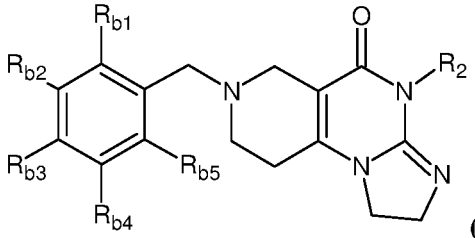
or halo. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with at least one substituent selected from halo, CH_3, CF_3 or OCH_3.

[00125] In one embodiment, compound (**10**) has the structure of formula (**80**):



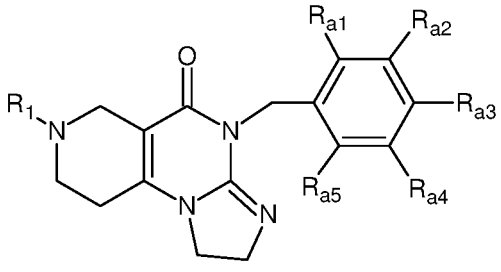
(**80**), wherein R_{a1}, R_{a2}, R_{a3}, R_{a4}, R_{a5}, R_{b1}, R_{b2}, R_{b3}, R_{b4}, and R_{b5} are each independently selected from the group consisting of H, X, $-CH_3$, $-NO_2$, $-OCH_3$, $-CN$, $-CXH_2$, $-CX_2H$, C_2-C_4 alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_1-C_4 alkyl; and where p is an integer from 2 to 20 and X is a halogen.

[00126] In one embodiment, compound (**10**) has the structure of formula (**90**)



(**90**), wherein R_2 is as defined above, and wherein R_{b1}, R_{b2}, R_{b3}, R_{b4}, and R_{b5} are each independently selected from the group consisting of H, X, $-CH_3$, $-NO_2$, $-OCH_3$, $-CN$, $-CXH_2$, $-CX_2H$, C_{2-4} alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_{1-4}alkyl; and where p is an integer from 2 to 20 and X is a halogen.

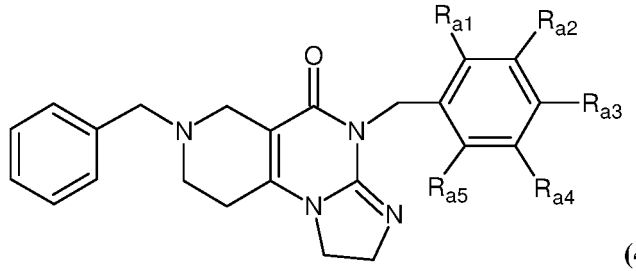
[00127] In one embodiment, compound (**10**) has the structure of formula (**40**)



(**40**), where R_1 is as defined above, and where R_{a1}, R_{a2}, R_{a3}, R_{a4}, and R_{a5} are each independently selected from the group consisting of H, X, $-CH_3$, $-NO_2$, $-OCH_3$, $-CN$, $-CXH_2$, $-CX_2H$, C_{2-4}alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m,

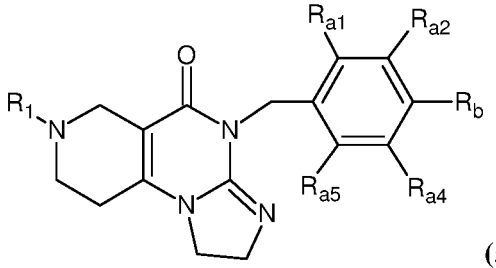
$C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_{1-4} alkyl; p is an integer from 2 to 20; and X is a halogen. In one embodiment, R_1 is H. In one embodiment, R_1 is a substituted or unsubstituted arylalkyl, such as benzyl or phenylethyl. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the benzyl is substituted with one or more halo. In one embodiment, the benzyl is substituted with one or more substituents selected from halo, CH_3, CF_3, and OCH_3. In one embodiment, the benzyl is substituted with one halo, *e.g.*, F at an ortho or para position. In one embodiment, the benzyl is substituted with two halogen, *e.g.*, F at both meta positions.

[00128] In one embodiment, compound (**40**) has the structure of compound (**45**):



(**45**), where R_{a1}, R_{a2}, R_{a3}, R_{a4}, and R_{a5} are as defined above. In one embodiment, the benzyl is substituted with one or more halogens. In one embodiment, the benzyl is substituted with one or more substituents selected from halo, CH_3, CF_3, and OCH_3. In one embodiment, R_{a1} or R_{a5} is a halo, *e.g.*, F. In one embodiment, both R_{a2} and R_{a3} are halo, *e.g.*, F.

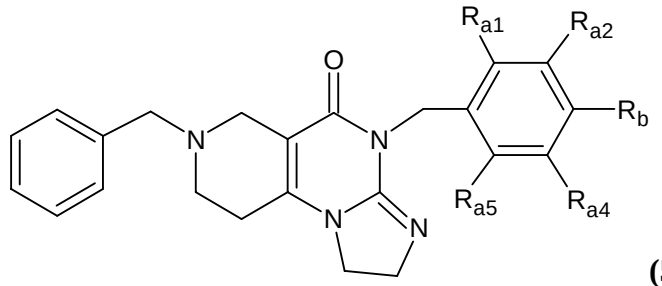
[00129] In one embodiment, compound (**10**) has the structure of compound (**50**)



(**50**), wherein R_1 is as defined above, and wherein R_b is selected from the group consisting of H, X, $-CH_3$, $-NO_2$, $-OCH_3$, -CN, $-CXH_2$, $-CX_2H$, C_{2-4}alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or C_{1-4}alkyl; and where p is an integer from 2 to 20 and X is a halogen, and wherein R_{a1}, R_{a2}, R_{a4}, and R_{a5} are each independently selected from the group consisting of H, X, $-CH_3$, $-NO_2$, $-OCH_3$, -CN, $-CXH_2$, $-CX_2H$, C_{2-4}alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$, OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or C_{1-4} alkyl; and where p is an integer from 2 to 20 and X is a halogen. In one embodiment, R_1 is H. In one embodiment, R_1 is a substituted or unsubstituted arylalkyl, such as

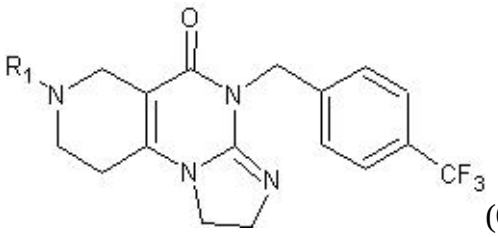
a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, R_b is selected from halo, CH_3, CF_3, and OCH_3. In one embodiment, one or more of R_{a1}, R_{a2}, R_{a4}, and R_{a5} is selected from halo, CH_3, CF_3, and OCH_3. In one embodiment, R_{a1}, R_{a2}, R_{a4}, and R_{a5} are H, and R_b is selected from halo, CH_3, CF_3, and OCH_3. In one embodiment, R_b is a halogen, *e.g.*, F, and R_{a1} is CH_3. In one embodiment, R_b is F or Cl, and R_{a2} is F or Cl. In one embodiment, R_b is CF_3. In one embodiment, R_b is OCH_3. In one embodiment, R_b and R_{a1} are Cl.

[00130] In one embodiment, compound (**50**) has the structure of compound (**55**):



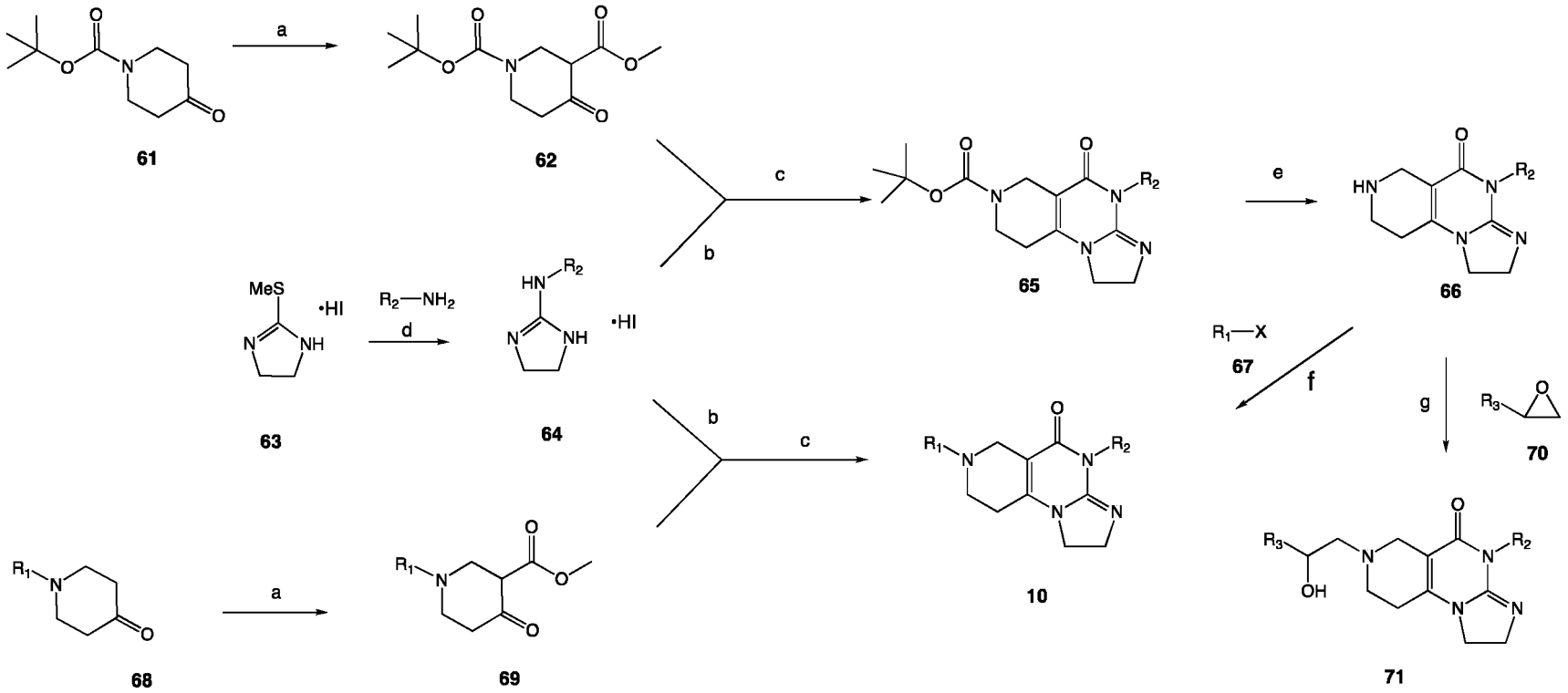
(55), where R_{a1}, R_{a2}, R_{a4}, R_{a5}, and R_b are as defined above. In one embodiment, R_b is selected from halo, CH_3, CF_3, and OCH_3. In one embodiment, one or more of R_{a1}, R_{a2}, R_{a4}, and R_{a5} is selected from halo, CH_3, CF_3, and OCH_3. In one embodiment, R_{a1}, R_{a2}, R_{a4}, and R_{a5} are H, and R_b is selected from halo, CH_3, CF_3, and OCH_3. In one embodiment, R_b is halo, *e.g.*, F, and R_{a1} is CH_3. In one embodiment, R_b is F or Cl, and R_{a2} is F or Cl. In one embodiment, R_b is CF_3. In one embodiment, R_b is OCH_3. In one embodiment, R_b and R_{a1} are Cl.

[00131] In one embodiment, compound (**10**) has the structure of compound (**60**)



(**60**). In one embodiment, R_1 is H. In one embodiment, R_1 is a substituted or unsubstituted arylalkyl, such as benzyl or phenylethyl. In one embodiment, R_1 is a substituted or unsubstituted heterocycloalkylalkyl or a substituted or unsubstituted heteroarylalkyl, such as CH_2-(2-thienyl), CH_2-(3-thienyl), CH_2-4-methyl-2-thiazolyl, CH_2-2-pyrazinyl, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(3-isoxazolidinyl), CH_2-2-pyridinyl, CH_2-3-pyridinyl, and CH_2CH_2-(4-morpholinyl). In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the benzyl is substituted with one or more halogens. In one embodiment, the benzyl is substituted with one or more substituents selected from halo (*e.g.*, F), CH_3, CF_3, and OCH_3. In one embodiment, the benzyl is substituted at the para position with a halo, CH_3, CF_3, or OCH_3 substituent. In one embodiment, R_1 is fluorophenyloxobutyl or hydroxyphenylethyl

Scheme **3** illustrates the synthesis of compounds of formula (**10**):



Methods: a. NaH, dimethyl carbonate, toluene, 80°C 4 h; b. 1N $NaOH/CH_2Cl_2$ to convert to free base, then heat in dioxane 70 °C; c. 1-butanol/reflux 3-6 h (Dean-Stark trap) PPTS; d. dioxane 70 °C; e. HCl in dioxane -25 °C - RT to give HCl salt ; f. Na_2CO_3, DIEA 80 °C; g. $NaOH/CH_2Cl_2$ to make free base, then MeOH reflux, 3.5 h

Scheme 3

[00132] Compounds of formula (**10**) (i.e., imipridones) are synthesized starting from a substituted piperidone, which is converted by reaction with a substitued aminoimidazoline to give the core compound (**10**). There are two routes, one in which the R_1 substituent is present in the piperidone (*e.g.*, **68**). In that route, (**68**) is acylated with dimethyl carbonate using sodium hydride in toluene at 80 °C to form piperidone ester (**69**). Commercially available methylthioimidazoline HI salt (**63**) is reacted with an amine in dioxane at 70 °C to afford the R_2-substituted aminoimidazoline (**64**) as its HI salt. Direct reaction of (**64**) with piperidone ester (**69**) in 1-butanol at reflux with removal of water via a Dean-Stark trap over 3-6 h gives the tricylic compound (**10**). In a variant of this scheme, N-BOC protected piperidone (**61**) is converted by the same methods to BOC protected compound (**65**), which is treated with HCl in dioxane to remove the BOC group and then converted to the free base of (**66**) with 1N NaOH with extraction with methylene chloride. Subsequent treatment of (**66**) with a halide (**67**) or epoxide (**70**) affords desired compound (**10**).

[00133] Crude products may be purified by column chromatography eluting with methylene chloride:methanol or by HPLC using acetonitrile:TFA:H_2O to produce final products as either free bases or as TFA salts. Treatment of free bases with HCl in dioxane or lyophilization of TFA salts generates products (**10**) as HCl or TFA salts. Alternatively, the free base may be treated with another inorganic or organic acid to form other salts, generally selected from those known to be pharmaceutically acceptable. Salts of compound (**10**) are usually solids and examples have been crystallized from ethanol or other solvents to give high quality crystals. The tricyclic structure has been definitively confirmed in the case of compound (**1**) by an X-ray crystal structure and NMR.

[00134] Compounds described herein can be used, with or without an aminoalkyl linker (*e.g.*, compound (**33**)), to identify molecules (*e.g.*, proteins) that interact with them in a cellular context. Expression of these binding targets may be used to predict response to imipridones or analogs thereof (i.e. serve as biomarkers). These compounds can also be used to screen for structurally unrelated molecules using competition assays known in the art to identify drugs able to outcompete the target interaction with a higher affinity. In addition, these molecules may have improved drug properties or allow additional applications by altering drug properties including safety, potency, pharmacokinetics, biodistribution, or metabolism.

TABLE 1:EXAMPLES OF COMPOUNDS OF FORMULA (10)

No.	ONC Number	R_1	R_2
1	ONC201	CH_2Ph	CH_2-((2-CH_3)-Ph)
13		CH_2Ph	CH_3
14	ONC202	CH_2Ph	CH_2-((2-Cl)-Ph)
15	ONC203	CH_2Ph	CH_2-(2-thienyl)
16	ONC204	CH_2Ph	CH_2CH_2Ph
17	ONC205	CH_2Ph	CH_2CH_2(4-N-benzyl-piperazine)
18	ONC206	CH_2Ph	CH_2-(2,4-di F-Ph)
19	ONC207	H	CH_2-((2-CH_3)-Ph)
20	ONC208	CH_3	CH_2-((2-CH_3)-Ph)
21	ONC209	CH_2CH_2Ph	CH_2-((2-CH_3)-Ph)
22		CH_2CH_2-(4-N-benzyl-piperizine)	CH_2-((2-CH_3)-Ph)
23		$CH_2CHOHPh$	CH_2-((2-CH_3)-Ph)
24		$(CH_2)_3CO$-4F-Ph	CH_2-((2-CH_3)-Ph)
32	ONC215	$CH_2CH_2NHCOOC(CH_3)_3$	CH_2-((2-CH_3)-Ph)
33	ONC216	$CH_2CH_2CH_2NH_2$	CH_2-((2-CH_3)-Ph)
41	ONC210	CH_2Ph	CH_2-(3,5-di F-Ph)
51	ONC211	CH_2Ph	CH_2-(3,4-di Cl-Ph)
52	ONC212	CH_2Ph	CH_2-(4-CF_3-Ph)
53	ONC213	CH_2Ph	CH_2-(3,4-di F-Ph)
54	ONC214	$CD_2C_6D_5$	CH_2-((2-CH_3)-Ph)
43	ONC217	CH_2Ph	CH_2-(2-F-Ph)
55	ONC218	CH_2Ph	CH_2(2-CH_3, 4-F-Ph)
56	ONC219	CH_2Ph	CH_2-(2,4-di Cl-Ph)
57	ONC220	CH_2Ph	CH_2-((4-OCH_3)-Ph)
34	ONC226	CH_2Ph	CH_2-(3-pyridinyl)
35	ONC222	CH_2Ph	CH_2-(3-isoxazolidinyl)
36	ONC224	CH_2Ph	CH_2CH_2-(4-morpholinyl)
37	ONC223	CH_2Ph	CH_2-(4-CH_3-Ph)
38	ONC221	H	CH_2-(4-CF_3-Ph)
73	ONC227	CH_2-(4-CF_3-Ph)	CH_2-(4-CF_3-Ph)
72	ONC225	CH_2Ph	CH_2-(2-F, 4-CF_3-Ph)
74	ONC228	CH_2-(4-F-Ph)	CH_2-(4-CF_3-Ph)
75	ONC229	CH_2-(OCH_3-Ph)	CH_2-(4-CF_3-Ph)

No.	ONC Number	R_1	R_2
76	ONC230	(4-F-Ph)-4-oxobutyl	CH_2-(4-CF_3-Ph)
77	ONC231	CH_2-3-pyridyl	CH_2-(4-CF_3-Ph)
78	ONC232	CH_2-4-methyl-2-thiazolyl	CH_2-(4-CF_3-Ph)
79	ONC233	CH_2-2-pyrazinyl	CH_2-(4-CF_3-Ph)
81	ONC234	CH_2-(3,4-di Cl-Ph)	CH_2-(4-CF_3-Ph)
82	ONC235	CH_2-(4-Cl-Ph)	CH_2-(4-CF_3-Ph)
83	ONC236	CH_2-3-thienyl	CH_2-(4-CF_3-Ph)
84	ONC237	$CH_2CH(OH)Ph$	CH_2-(4-CF_3-Ph)

IV. ASSESSING SENSITIVITY AND EFFICACY OF TREATMENT REGIMENS

[00135] Measuring expression, gene mutation, or gene copy number of a dopamine receptor or another G protein–coupled receptor (GPCR) may be used to predict response or sensitivity to a method of treatment described herein and to identify subjects likely to be responsive to a method of treatment described herein, such as treatment with a compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In one aspect, provided herein are methods of identifying whether a subject having a condition is likely to be responsive to a treatment regimen described herein. In one embodiment, the methods comprises (i) obtaining a biological sample from the subject; (ii) measuring expression levels of at least one dopamine receptor or G protein–coupled receptor (GPCR) in the sample; (iii) comparing the levels measured in the sample to those for a pre-determined standard; and (iv) determining whether the subject is likely to be responsive to the treatment regimen, based on the levels measured in the sample to those for the pre-determined standard. In one embodiment, the step of measuring an expression level of a dopamine receptor or GPCR in the sample include the steps of (i) contacting the sample with an antibody or antigen-binding fragment that specifically binds to the receptor to form a complex of the antibody or antigen-binding fragment with the receptor; and (ii) measuring the amount of the complex. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the cancer is a neuro-oncology disease. In one embodiment, the cancer is a neuroendocrine tumor. In one embodiment, the cancer is selected from the group consisting of meningioma, ependymoma, glioma, neuroblastoma, and diffuse intrinsic pontine glioma. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the psychiatric disorder is selected from the group consisting of psychosis, bipolar disorder, and major depressive disorder. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the infection is a bacterial infection. In one embodiment, the infection is a gram-negative bacterial infection. In

one embodiment, the infection is a gram-positive bacterial infection. In one embodiment, the bacterial infection is an infection of a bacteria selected from the group consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. In one embodiment, the gram-positive bacterial infection is a *Staphylococcus* infection. In one embodiment, the *Staphylococcus* infection is an *S. aureus* infection (*e.g.*, a methicillin-resistant *S. aureus* (MRSA) infection). In one embodiment, the treatment regimen comprises administering an effective amount of a therapeutic, such as a compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In one embodiment, the dopamine receptor is from the D2-like family of dopamine receptorsIn one embodiment, the dopamine receptor is DRD2. In one embodiment, the dopamine receptor is DRD3. In one embodiment, the dopamine receptor is DRD4. In one embodiment, the dopamine receptor is DRD2, DRD3, or both. In one embodiment, the GPCR is a Class A GPCR. In one embodiment, the GPCR is GPR132. In one embodiment, the GPCR is selected from the group consisting of GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7, and combinations thereof. In one embodiment, the dopamine receptor is DRD5, the treatment regimen comprises administering an effective amount of a therapeutic, such as a compound of formula (**10**) or a pharmaceutically acceptable salt thereof, and an increased level of expression of DRD5 measured in the sample relative to the pre-determined standard indicates that the subject is or is not likely to be responsive to the treatment regimen.

[00136] In another aspect, provided herein are methods of assessing the effectiveness of a treatment regimen described herein, monitoring, or providing a prognosis for a subject with a condition. In one embodiment, the methods comprises (i) obtaining a biological sample from the subject; (ii) measuring expression levels of at least one dopamine receptor or G protein–coupled receptor (GPCR) in the sample; (iii) comparing the levels measured in the sample to those for a pre-determined standard; and (iv) determining a prognosis or determining whether the subject is responsive to the treatment regimen, based on the levels measured in the sample to those for the pre-determined standard. In one embodiment, the step of measuring an expression level of a dopamine receptor or GPCR in the sample include the steps of (i) contacting the sample with an antibody or antigen-binding fragment that specifically binds to the receptor to form a complex of the antibody or antigen-binding fragment with the receptor; and (ii) measuring the amount of the complex. In one embodiment, the methods comprise (i) obtaining a biological sample from the subject; (ii) measuring gene copy number or mutations in at least one dopamine receptor in the sample; (iii) comparing the copy number measured or mutations found in the sample to those for a pre-determined standard; and (iv) determining

whether the subject is responsive to the treatment regimen, based on the copy number measured or mutations found in the sample to those for the pre-determined standard. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the cancer is a neuro-oncology disease. In one embodiment, the cancer is a neuroendocrine tumor. In one embodiment, the cancer is selected from the group consisting of meningioma, ependymoma, glioma, neuroblastoma, and diffuse intrinsic pontine glioma. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the psychiatric disorder is selected from the group consisting of psychosis, bipolar disorder, and major depressive disorder. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the infection is a bacterial infection. In one embodiment, the infection is a gram-negative bacterial infection. In one embodiment, the infection is a gram-positive bacterial infection. In one embodiment, the bacterial infection is an infection of a bacteria selected from the group consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. In one embodiment, the gram-positive bacterial infection is a *Staphylococcus* infection. In one embodiment, the *Staphylococcus* infection is an *S. aureus* infection (*e.g.*, a methicillin-resistant *S. aureus* (MRSA) infection). In one embodiment, the treatment regimen comprises administering an effective amount of a therapeutic, such as a compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In one embodiment, the dopamine receptor is selected from DRD2, DRD2S, DRD2L, and DRD3. In one embodiment, the dopamine receptor is from the D2-like family of dopamine receptors. In one embodiment, the dopamine receptor is from the D1-like family of dopamine receptors. In one embodiment, the dopamine receptor is DRD1. In one embodiment, the dopamine receptor is DRD2. In one embodiment, the dopamine receptor is DRD3. In one embodiment, the dopamine receptor is DRD4. In one embodiment, the dopamine receptor is DRD5. In one embodiment, the dopamine receptor is DRD2, DRD3, or both. In one embodiment, the GPCR is a Class A GPCR. In one embodiment, the GPCR is GPR132. In one embodiment, the GPCR is selected from the group consisting of GPR132, GPR91, MTNR1A, GPR162, GPR137, BAI3, LGR4, PTGIR, CXCR7, and combinations thereof.

[00137] In one embodiment, the dopamine receptor is DRD5, the treatment regimen comprises administering an effective amount of a compound of formula (**10**) or a pharmaceutically acceptable salt thereof, and an increased level of expression of DRD5 measured in the sample relative to the pre-determined standard indicates that the treatment regimen is or is not effective. In one embodiment, the dopamine receptor is DRD5, the treatment regimen comprises administering an effective amount of a therapeutic, such as a

compound of formula (**10**) or a pharmaceutically acceptable salt thereof, and mutation in the DRD5 gene measured in the sample indicates that the treatment regimen is or is not effective. In one embodiment, the dopamine receptor is DRD5, the treatment regimen comprises administering an effective amount of a therapeutic, such as a compound of formula (**10**) or a pharmaceutically acceptable salt thereof, and the misense mutation Q366R in the DRD5 gene measured in the sample indicates that the treatment regimen is or is not effective.

[00138] In another aspect, provided herein are methods of identifying whether a subject having a condition is likely to be responsive to a treatment regimen described herein. In one embodiment, the methods comprises (i) obtaining a biological sample from the subject; (ii) measuring gene copy number or mutations in at least one dopamine receptor in the sample; (iii) comparing the copy number measured or mutations found in the sample to those for a pre-determined standard; and (iv) determining whether the subject is likely to be responsive to the treatment regimen, based on the copy number measured or mutations found in the sample to those for the pre-determined standard. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the cancer is a neuro-oncology disease. In one embodiment, the cancer is a neuroendocrine tumor. In one embodiment, the cancer is selected from the group consisting of meningioma, ependymoma, glioma, neuroblastoma, and diffuse intrinsic pontine glioma. In one embodiment, the subject has, or is at risk of having, a psychiatric disorder. In one embodiment, the psychiatric disorder is selected from the group consisting of psychosis, schizophrenia, bipolar disorder, and major depressive disorder. In one embodiment, the subject has, or is at risk of having, an infection. In one embodiment, the infection is a bacterial infection. In one embodiment, the infection is a gram-negative bacterial infection. In one embodiment, the infection is a gram-positive bacterial infection. In one embodiment, the bacterial infection is an infection of a bacteria selected from the group consisting of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. In one embodiment, the gram-positive bacterial infection is a *Staphylococcus* infection. In one embodiment, the *Staphylococcus* infection is an *S. aureus* infection (*e.g.*, a methicillin-resistant *S. aureus* (MRSA) infection). In one embodiment, the treatment regimen comprises administering an effective amount of a therapeutic, such as a compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In one embodiment, the dopamine receptor is from the D2-like family of dopamine receptors. In one embodiment, the dopamine receptor is DRD1. In one embodiment, the dopamine receptor is DRD2. In one embodiment, the dopamine receptor is DRD3. In one embodiment, the dopamine receptor is DRD4. In one embodiment, the dopamine receptor is DRD5. In one embodiment, the

dopamine receptor is DRD2, DRD3, or both. In one embodiment, the dopamine receptor is DRD5, the treatment regimen comprises administering an effective amount of a therapeutic, such as a compound of formula (**10**) or a pharmaceutically acceptable salt thereof, and mutation in the DRD5 gene measured in the sample indicates that the subject is or is not likely to be responsive to the treatment regimen. In one embodiment, the dopamine receptor is DRD5, the treatment regimen comprises administering an effective amount of a therapeutic, such as a compound of formula (**10**) or a pharmaceutically acceptable salt thereof, and the misense mutation Q366R in the DRD5 gene measured in the sample indicates that the subject is or is not likely to be responsive to the treatment regimen.

[00139] In addition, measuring expression, post-translational modifications, or activity levels of or mutations in eIF2-α, ATF4, CHOP, DR5, or cleaved or total cytokeratin 18 may be used to predict response or sensitivity to a method of treatment described herein and to identify subjects likely to be responsive to a method of treatment described herein, such as treatment with a compound of formula (**10**), a pharmaceutically acceptable salt thereof, or an analog thereof. In addition, measuring expression, post-translational modifications, or activity levels of or mutations in eIF2-α, ATF4, CHOP, DR5, or cleaved or total cytokeratin 18 can be used to assess the effectiveness of or monitor a method of treatment described herein. Furthermore, measuring expression, post-translational modifications, or activity levels of or mutations in eIF2-α, ATF4, CHOP, DR5, or cleaved or total cytokeratin 18 can be used to screen *in vivo*, *in vitro*, or *in silico* for structurally unrelated anti-cancer molecules. For example, competition and other assays known in the art may be used to identify drugs able to outcompete the target interaction with a higher affinity to compare changes in those levels to the respective changes produced by a compound of formula (**10**) or an analog thereof. Assays can also be performed on living mammalian cells, which more closely approximate the effects of a particular serum level of drug in the body, or on microsomal extracts prepared from cultured cell lines.

[00140] In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the treatment regimen comprises administering an effective amount of an imipridone, such as ONC201, or an analog thereof. In one embodiment, the treatment regimen comprises administering an effective amount of ONC201. In one embodiment, the treatment regimen comprises administering an effective amount of a compound of formula (**10**). In one embodiment, the compound of formula (**10**) is a compound of formula (**40**), *e.g.*, a compound of formula (**45**). In one embodiment, a compound of formula (**10**) is a compound of formula (**50**), *e.g.*, a compound formula (**55**). In one embodiment, the compound of formula (**10**) is a compound of formula (**80**). In one embodiment, the compound of formula (**10**) is a compound

of formula (**90**). In one embodiment, the compound of formula (**10**) is a compound of formula (**60**). In one embodiment, analogs of compound (**1**) have a structure selected from the structures of formula (**25**), formula (**26**), formula (**27**), formula (**28**), formula (**29**), formula (**30**), or formula (**31**).

[00141] Levels for a pre-determined standard can be, e.g., the average or median levels measured in samples from subjects. The levels for a pre-determined standard can be measured under the same or substantially similar experimental conditions as in measuring a sample from a subject. The levels for the pre-determined standard may be obtained from subjects who are responsive to treatment with an imipridone, such as ONC201, or an analog thereof. In one embodiment, the pre-determined standard is obtained from subjects who are responsive to treatment with the compound, and if the levels in a sample from a subject are similar to those in the standard, then the subject can be classified as likely to be responsive to treatment. The levels for the pre-determined standard may be obtained from subjects who are not responsive to treatment with the compound. In one embodiment, the pre-determined standard is obtained from subjects who are not responsive to treatment with the compound, and if the levels in a sample from a subject are different (*e.g.*, up- or down-regulated) than in the pre-determined standard, then the subject can be classified as likely to be responsive to treatment. The levels for the pre-determined standard may be obtained from normal healthy subjects.

[00142] Immunoassays can be used to assay protein or methylation levels in a sample, including enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunofiltration assay (ELIFA), flow cytometry, immunoblot, immunoprecipitation, immunohistochemistry, immunocytochemistry, luminescent immunoassay (LIA), fluorescent immunoassay (FIA), and radioimmunoassay. m^6A mRNA methylation levels can be obtained by methylated RNA immunoprecipitation (Me-RIP)) or other quantitative biochemical assays known in the art.

[00143] Nucleic acid mutations can be determined by any of a number of known procedures. For example, a biologic sample from an individual can first be obtained. Such biological samples include, but are not limited to, a bodily fluid (such as urine, saliva, plasma, or serum) or a tissue sample (such as a buccal tissue sample or buccal cell). The biologic sample can then be sequenced or scanned using known methods. For example, DNA arrays can be used to analyze at least a portion of the subject's genomic sequence. Furthermore, whole or partial genome sequence information can be used. Such sequences can be determined using standard sequencing methods including chain-termination (Sanger dideoxynucleotide), dye-terminator sequencing, and SOLID™ sequencing (Applied Biosystems). Whole genome sequences can be cut by restriction enzymes or sheared (mechanically) into shorter fragments for sequencing. DNA sequences can also be amplified using known methods such as PCR and vector-based

cloning methods (*e.g., Escherichia coli*). In one embodiment, at least a portion of a subject's genetic material (*e.g.*, DNA, RNA, mRNA, cDNA, other nucleotide bases or derivatives of these) is scanned or sequenced using, *e.g.*, conventional DNA sequencers or chip-based technologies, to identify the presence or absence of mutations or copy number variations.

[00144] In one aspect, provided herein are methods of identifying and treating a subject having a condition and who is likely to be responsive to a treatment regimen described herein. In one embodiment, the method comprises (i) identifying whether a subject having a condition is likely to be responsive to a treatment regimen described herein; and (ii) treating with the treatment regimen a subject determined likely to be responsive to that treatment regimen. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the treatment regimen comprises administering an effective amount an imipridone, *e.g.*, ONC201 or an analog thereof. In one embodiment, the treatment regimen comprises administering an effective amount of compound (**1**). In one embodiment, the treatment regimen comprises administering an effective amount of a compound of formula (**10**). In one embodiment, the compound of formula (**10**) is a compound of formula (**40**), *e.g.*, a compound of formula (**45**). In one embodiment, a compound of formula (**10**) is a compound of formula (**50**), *e.g.*, a compound formula (**55**). In one embodiment, the compound of formula (**10**) is a compound of formula (**80**). In one embodiment, the compound of formula (**10**) is a compound of formula (**90**). In one embodiment, the compound of formula (**10**) is a compound of formula (**60**). In one embodiment, analogs of compound (**1**) have a structure selected from the structures of formula (**25**), formula (**26**), formula (**27**), formula (**28**), formula (**29**), formula (**30**), or formula (**31**).

[00145] Levels for a pre-determined standard can be, e.g., the average or median levels measured in samples from subjects. The levels for a pre-determined standard can be measured under the same or substantially similar experimental conditions as in measuring a sample from a subject. The levels for the pre-determined standard may be obtained from subjects who are responsive to treatment with an imipridone, such as ONC201 or an analog thereof. In one embodiment, the pre-determined standard is obtained from subjects who are responsive to treatment with the compound, and if the levels in a sample from a subject are similar to those in the standard, then the subject can be classified as likely to be responsive to treatment. The levels for the pre-determined standard may be obtained from subjects who are not responsive to treatment with the compound. In one embodiment, the pre-determined standard is obtained from subjects who are not responsive to treatment with the compound, and if the levels in a sample from a subject are different (e.g., up- or down-regulated) than those in the pre-determined standard, then the subject can be classified as likely to be responsive to

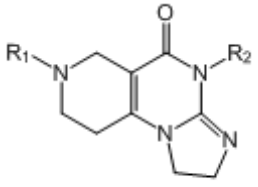
treatment. The levels for the pre-determined standard may be obtained from normal healthy subjects. Immunoassays can be used to assay protein levels in a sample.

[00146] In one aspect, provided herein are methods of treating and assessing the effectiveness of a treatment in a subject having a condition. In one embodiment, the method comprises (i) treating the subject according to a method of treatment described herein (ii) assessing as decribed herein the effectiveness of the treatment. In one embodiment, the subject has, or is at risk of having, cancer. In one embodiment, the treatment regimen comprises administering an effective amount of an imipridone, such as ONC201 or an analog thereof. In one embodiment, the treatment regimen comprises administering an effective amount of compound (**1**). In one embodiment, the treatment regimen comprises administering an effective amount of a compound of formula (**10**). In one embodiment, the compound of formula (**10**) is a compound of formula (**40**), *e.g.*, a compound of formula (**45**). In one embodiment, a compound of formula (**10**) is a compound of formula (**50**), *e.g.*, a compound formula (**55**). In one embodiment, the compound of formula (**10**) is a compound of formula (**80**). In one embodiment, the compound of formula (**10**) is a compound of formula (**90**). In one embodiment, the compound of formula (**10**) is a compound of formula (**60**). In one embodiment, analogs of compound (**1**) have a structure selected from the structures of formula (**25**), formula (**26**), formula (**27**), formula (**28**), formula (**29**), formula (**30**), or formula (**31**).

[00147] Other conditions that may be suitable for the methods described herein include Attention Deficit Disorder; Addiction; Epilepsy; Viral infection; Inflammation; Neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis; Cardiovascular diseases such as coronary artery disease, cardiomyopathy, hypertensive heart disease, heart failure, pulmonary heart disease, cardiac dysrhythmias, inflammatory heart disease, endocarditis, inflammatory cardiomegaly, myocarditis, valvular heart disease, cerebrovascular disease, peripheral arterial disease, congenital heart disease, rheumatic heart disease; Diabetes; and light chain amyloidosis.

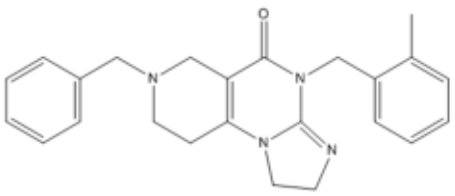
V. COMPOSITIONS

[00148] In one aspect, pharmaceutical compositions are provided, comprising compounds of formula (**10**):



(10)

or of formula (**1**):



and their pharmaceutically acceptable saltsIn one embodiment, the salt is a pharmaceutically

acceptable mono-salt of the compound. In one embodiment, the salt is a pharmaceutically acceptable di-salt of the compound. In one embodiment, the salt is a pharmaceutically acceptable mono- or multi-salt (*e.g.*, a di-salt or tri-salt) thereof selected from the group consisting of hydrochloride, hydrobromide, hydrogensulphate, sulfates, phosphates, fumarates, succinates, oxalates and lactates, bisulfates, hydroxyl, tartrate, nitrate, citrate, bitartrate, carbonate, malate, maleate, fumarate sulfonate, methylsulfonate, formate, acetate, and carboxylate. In one embodiment, the salt is a salt selected from the group consisting of p-toluene-sulfonate, benzenesulfonate, citrate, methanesulfonate, oxalate, succinate, tartrate, fumarate and maleate. In one embodiment, the salt is a salt selected from the group consisting of ammonium, sodium, potassium, calcium, magnesium, zinc, lithium, and/or with counter-ions such as methylamino, dimethylamino, diethylamino and triethylamino counter-ions. In one embodiment, the salt is a. di-hydrochloride salt or a di-hydrobromide salt.

[00149] Compound **(1)** (ONC201) has the same chemical structure that would be revealed by structural analysis (*e.g.*, NMR, X-ray diffraction) of compound NSC 350625, available from the National Cancer Institute's Developmental Therapeutics Program Repository.

[00150] In one embodiment, the pharmaceutical composition includes a di-salt (*e.g.*, a di-hydrochloride salt) of ONC201 or an analog thereof (*e.g.*, an imipridone). Salts (*e.g.*, di-salts or tri-salts) of an ONC201 analog can be prepared from an ONC201 analog, which can be synthesized as described herein, or using standard chemical synthetic methodology known to one of ordinary skill in the art.

[00151] In one embodiment, the pharmaceutical composition includes at least one pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers, include, but are not limited to, those in Handbook of Pharmaceutical Excipients, 7th ed., edited by Raymond C. Rowe *et al.*, American Pharmaceutical Association, Washington, USA and Pharmaceutical Press, London; and earlier editions. Exemplary pharmaceutically acceptable carriers, methods for making pharmaceutical compositions and various dosage forms, as well as administration modes are well-known in the art, for example as detailed in Pharmaceutical Dosage Forms: Tablets, edited by Larry L. Augsburger & Stephen W. Hoag., London: Informa Healthcare, 2008; and in L.V. Allen, Jr. et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, 8th ed., Philadelphia, Pa.: Lippincott, Williams & Wilkins, 2004; A.R. Gennaro, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins, 21st ed., 2005, particularly chapter 89; and J.G. Hardman et al., Goodman & Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill Professional, 10th ed., 2001.

[00152] In one embodiment, pharmacuetical compositions are formulated for ocular administration. In one embodiment, pharmaceutical compositions are formulated for topical

administration. In one embodiment, pharmaceutical compositions are formulated as drops, ointments, or liquids. In one embodiment, pharmaceutical compositions include conventional pharmaceutical carriers such as aqueous, powdery or oily bases, thickeners.

[00153] In one embodiment, a pharmaceutical composition is a formulation for intravenous administration. In one embodiment, the intravenous formulation comprises a compound of formula (**10**) or a pharmaceutically acceptable salt thereof dissolved in a solvent. In one embodiment, the solvent comprises water. In one embodiment, the intravenous formulation includes the compound or its salt in a concentration of about 0.05, about 0.25, about 0.5, about 2.5, about 5, about 25, or about 50 mg/mL. In one embodiment, the intravenous formulation includes the compound or its salt in a concentration of from about 0.05, 0.5, or 5 mg/mL to about 1, 10, or 100 mg/mL. In one embodiment, the intravenous formulation includes from about 0.005% 0.05%, or 0.5% to about 0.1%, 1%, or 10% of the compound or its salt. In one embodiment, the intravenous formulation includes about 0.05%, 0.5%, or 5% of the compound or its salt. In one embodiment, the intravenous formulation includes a higher or a lower concentration of the compound or its salt.

[00154] In one embodiment, the intravenous formulation has a pH of about 3. In one embodiment, the formulation is adjusted to pH 3 with a phosphate buffer. In one embodiment, the intravenous formulation includes dextrose or sodium chloride. In one embodiment, the intravenous formulation includes the compound or its salt in a concentration of about 5 mg/mL and pH 3 and forms a stable solution. In one embodiment, the intravenous formulation includes the compound or its salt in a concentration of about 5 mg/mL and pH < 5 and forms a stable solution. In one embodiment, the intravenous formulation includes the compound or its salt and one or more antioxidants. In one embodiment, the intravenous formulation includes a mixture of mono- and di-hydrochloride salts of the compound. In one embodiment, the intravenous formulation includes the compound or its salt as a 1 % solution in a concentration of about 10 mg/mL. For example, the intravenous formulation is a solution with a pH of about 3.3. In one embodiment, the pH is less than 4.0.

[00155] In one embodiment, the pharmaceutical composition further includes a pharmaceutically acceptable carrier. In one embodiment, a suitable pharmaceutically acceptable carrier includes an aqueous carrier. In one embodiment, the aqueous carrier includes sterile water. In one embodiment, the formulation includes dextrose and/or sodium. In one embodiment, the pharmaceutically acceptable carrier includes an oil.

[00156] In one embodiment, an intravenous formulation comprises ONC201 or an analog thereof or a di-hydrochloride salt thereof dissolved in water at 25 mg/mL. In one embodiment, the formulation is adjusted to pH 3 with phosphate buffer. In one embodiment, the formulation

includes dextrose, sodium chloride or both. In one embodiment, the formulation includes a higher or a lower concentration of the di-hydrochloride salt of ONC201 or an analog thereof. In one embodiment, the formulation includes ONC201 or an analog thereof or a di-hydrochloride salt thereof in a concentration of about 5 mg/mL. In one embodiment, the formulation of about 5 mg/mL forms a stable solution and pH 3. In one embodiment, the formulation of about 5 mg/mL has a pH < 5 and forms a stable solution. In one embodiment, the intravenous formulation includes ONC201 or an analog thereof or a di-hydrochloride salt thereof and one or more antioxidants. In one embodiment, the intravenous formulation includes a mixture of mono- and di-hydrochloride salts of ONC201 or an analog thereof. In one embodiment, the intravenous formulation includes ONC201 or an analog thereof or a di-hydrochloride salt thereof as a 1 % solution in a concentration of about 10 mg/mL. For example, the intravenous formulation is a solution having a pH of about 3.3. In one embodiment, the pH is less than 4.0.

[00157] In one embodiment, the intravenous formulation includes from about 0.5% to about 10% (or from about 5 mg/mL to about 100 mg/mL) of ONC201 or an analog thereof or a di-salt thereof. In one embodiment, the formulation includes from about 5 % (or about 50 mg/mL) of ONC201 or an analog thereof or a di-salt thereof. In one embodiment, the intravenous infusion rate may be slowed to decrease side effects of ONC201 or an analog thereof or a di-salt thereof.

[00158] In one embodiment, the pharmaceutical composition comprises about 0.1-99% of an ONC201 salt or an analog thereof; and a pharmaceutically acceptable carrier, e.g., an oil or sterile water or other aqueous carrier. In one embodiment, the composition comprises a mono or di-salt of ONC201 or an analog thereof in a range of from about 5% to about 50% for oral dosage forms.

[00159] In one embodiment, a pharmaceutical composition includes an antioxidant. Suitable antioxidants include: ascorbic acid derivatives such as ascorbic acid, erythorbic acid, sodium ascorbate, thiol derivatives such as thioglycerol, cysteine, acetylcysteine, cystine, dithioerythreitol, dithiothreitol, glutathione, tocopherols, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), sulfurous acid salts such as sodium sulfate, sodium bisulfite, acetone sodium bisulfite, sodium metabisulfite, sodium sulfite, sodium formaldehyde sulfoxylate, and sodium thiosulfate, nordihydroguaiaretic acid. It should be noted that antioxidants used for aqueous formulations typically include: sodium sulphite, sodium metabisulphite, sodium formaldehyde sulphoxylate and ascorbic acid and combinations thereof, whereas antioxidants used in oil-based solutions, organic solvents, include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate and combinations thereof. In yet other embodiments, an antioxidant can be one or more of a flavanoid, an isoflavone, monothioglycerol, L-cysteine, thioglycolic acid, α-tocopherol, ascorbic acid

6-palmitate, dihydrolipoic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), vitamin E, propyl gallate, β-carotene, ascorbic acid. Antioxidants can typically be used in about 0.1% to 1.0% by weight, more typically about 0.2%.

[00160] In one embodiment, the pharmaceutical composition includes an imipridone, such as ONC201 or an analog thereof, or a pharmaceutically acceptable salt thereof and at least one other therapeutic agent. For example, the other therapeutic agent is selected from the group consisting of hormone analogs and antihormones, aromatase inhibitors, LHRH agonists and antagonists, inhibitors of growth factors, growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors; antimetabolites; antitumour antibiotics; platinum derivatives; alkylation agents; antimitotic agents; tubuline inhibitors; PARP inhibitors, topoisomerase inhibitors, serine/threonine kinase inhibitors, tyrosine kinase inhibitors, protein protein interaction inhibitors, RAF inhibitors, MEK inhibitors, ERK inhibitors, IGF-1R inhibitors, ErbB receptor inhibitors, rapamycin analogs, BTK inhibitors, CRM1 inhibitors (e.g., KPT185), P53 modulators (e.g., Nutlins), antiangiogenics (e.g., axitinib, aflibercept, sorafenib, and regorafenib), amifostin, anagrelid, clodronat, filgrastin, interferon, interferon α, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer, 2-chlorodesoxyadenosine, 2-fluorodesoxy-cytidine, 2-methoxyoestradiol, 2C4,3-alethine, 131-1-TM-601, 3CPA, 7-ethyl-10-hydroxycamptothecin, 16-aza-epothilone B, A 105972, A 204197, abiraterone, aldesleukin, alitretinoin, allovectin-7, altretamine, alvocidib, amonafide, anthrapyrazole, AG-2037, AP-5280, apaziquone, apomine, aranose, arglabin, arzoxifene, atamestane, atrasentan, auristatin PE, ABT-199 (Venetoclax), ABT-263 (Navitoclax), AVLB, AZ10992, ABX-EGF, AMG-479 (ganitumab), ARRY 162, ARRY 438162, ARRY-300, ARRY-142886/AZD-6244 (selumetinib), ARRY-704/AZD-8330, AR-12, AR-42, AS-703988, AXL-1717, AZD-8055, AZD-5363, AZD-6244, ARQ-736, ARQ 680, AS-703026 (primasertib), avastin, AZD-2014, azacytidine, azaepothilone B, azonafide, BAY-43-9006, BAY 80-6946, BBR-3464, BBR-3576, bevacizumab, BEZ-235, biricodar dicitrate, BCX-1777, BKM-120, bleocin, BLP-25, BMS-184476, BMS-247550, BMS-188797, BMS-275291, BMS-663513, BMS-754807, BNP-1350, BNP-7787, BIBW 2992 (afatinib, tomtovok), BIBF 1120 (vargatef), BI 836845, BI 2536, BI 6727, BI 836845, BI 847325, BI 853520, BUB-022, bleomycinic acid, bleomycin A, bleomycin B, brivanib, bryostatin-1, bortezomib, brostallicin, busulphan, BYL-719, CA-4 prodrug, CA-4, CapCell, calcitriol, canertinib, canfosfamide, capecitabine, carboxyphthalatoplatin, CCl-779, CC-115, CC-223, CEP-701, CEP-751, CBT-1 cefixime, ceflatonin, ceftriaxone, celecoxib, celmoleukin, cemadotin, CH4987655/RO-4987655, chlorotrianisene, cilengitide, ciclosporin, CDA-II, CDC-394, CKD-602, CKI-27, clofarabin, colchicin, combretastatin A4, COT

inhibitors, CHS-828, CH-5132799, CLL-Thera, CMT-3 cryptophycin 52, CTP-37, CTLA-4 monoclonal antibodies, CP-461, CV-247, cyanomorpholinodoxorubicin, cytarabine, D 24851, decitabine, deoxorubicin, deoxyrubicin, deoxycoformycin, depsipeptide, desoxyepothilone B, dexamethasone, dexrazoxanet, diethylstilbestrol, diflomotecan, didox, DMDC, dolastatin 10, doranidazole, DS-7423, E7010, E-6201, edatrexat, edotreotide, efaproxiral, eflornithine, EGFR inhibitors, EKB-569, EKB-509, enzastaurin, enzalutamide, elsamitrucin, epothilone B, epratuzumab, ER-86526, erlotinib, ET-18-0CH3, ethynylcytidine, ethynyloestradiol, exatecan, exatecan mesylate, exemestane, exisulind, fenretinide, figitumumab, floxuridine, folic acid, FOLFOX, FOLFOX4, FOLFIRI, formestane, fotemustine, galarubicin, gallium maltolate, gefinitib, gemtuzumab, gimatecan, glufosfamide, GCS-100, GDC-0623, GDC-0941 (pictrelisib), GDC-0980, GDC-0032, GDC-0068, GDC-0349, GDC-0879, G17DT immunogen, GMK, GPX-100, gp100-peptide vaccines, GSK-5126766, GSK-690693, GSK-1120212 (trametinib), GSK-2118436 (dabrafenib), GSK-2126458, GSK-2132231A, GSK-2334470, GSK-2110183, GSK-2141795, GW2016, granisetron, herceptine, hexamethylmelamine, histamine, homoharringtonine, hyaluronic acid, hydroxyurea, hydroxyprogesterone caproate, ibandronate, ibrutinib, ibritumomab, idatrexate, idenestrol, IDN-5109, IGF-1R inhibitors, IMC-1C11, IMC-A12 (cixutumumab), immunol, indisulam, interferon α-2a, interferon α-2b, pegylated interferon α-2b, interleukin-2, INK-1117, INK-128, INSM-18, ionafarnib, ipilimumab, iproplatin, irofulven, isohomohalichondrin-B, isoflavone, isotretinoin, ixabepilone, JRX-2, JSF-154, J-107088, conjugated oestrogens, kahalid F, ketoconazole, KW-2170, KW-2450, lobaplatin, leflunomide, lenograstim, leuprolide, leuporelin, lexidronam, LGD-1550, linezolid, lutetium texaphyrin, lometrexol, losoxantrone, LU 223651, lurtotecan, LY-S6AKT1, LY-2780301, mafosfamide, marimastat, mechloroethamine, MEK inhibitors, MEK-162, methyltestosteron, methylprednisolone, MEDI-573, MEN-10755, MDX-H210, MDX-447, MDX-1379, MGV, midostaurin, minodronic acid, mitomycin, mivobulin, MK-2206, MK-0646 (dalotuzumab), MLN518, motexaf in gadolinium, MS-209, MS-275, MX6, neridronate, neratinib, Nexavar, neovastat, nilotinib, nimesulide, nitroglycerin, nolatrexed, norelin, N-acetylcysteine, 06-benzylguanine, oblimersen, omeprazole, oncophage, oncoVEXGM-CSF, ormiplatin, ortataxel, OX44 antibodies, OSI-027, OSI-906 (linsitinib), 4-1BB antibodies, oxantrazole, oestrogen, panitumumab, patupilone, pegfilgrastim, PCK-3145, pegfilgrastim, PBI-1402, PBI-05204, PDO325901, PD-1 antibodies, PEG-paclitaxel, albumin-stabilized paclitaxel, PEP-005, PF-05197281, PF-05212384, PF-04691502, PHT-427, P-04, PKC412, P54, PI-88, pelitinib, pemetrexed, pentrix, perifosine, perillylalcohol, pertuzumab, PI3K inhibitors, PI3K/mTOR inhibitors, PG-TXL, PG2, PLX-4032/RO-5185426 (vemurafenib), PLX-3603/RO-5212054,

PT-100, PWT-33597, PX-866, picoplatin, pivaloyloxymethylbutyrate, pixantrone, phenoxodiol O, PKI166, plevitrexed, plicamycin, polyprenic acid, porfiromycin, prednisone, prednisolone, quinamed, quinupristin, R115777, RAF-265, ramosetron, ranpirnase, RDEA-119/BAY 869766, RDEA-436, rebeccamycin analogs, receptor tyrosine kinase (RTK) inhibitors, regorafenib, revimid, RG-7167, RG-7304, RG-7421, RG-7321, RG 7440, rhizoxin, rhu-MAb, rinfabate, risedronate,rituximab, robatumumab, rofecoxib, RO-31-7453, RO-5126766, RO-5068760, RPR 109881A, rubidazone, rubitecan, R-flurbiprofen, RX-0201, S-9788, sabarubicin, SAHA, sargramostim, satraplatin, SB 408075, Se-015/Ve-015, SU5416, SU6668, SDX-101, semustin, seocalcitol, SM-11355, SN-38, SN-4071, SR-27897, SR-31747, SR-13668, SRL-172, sorafenib, spiroplatin, squalamine, suberanilohydroxamic acid, sutent, T 900607, T 138067, TAK-733, TAS-103, tacedinaline, talaporf in, Tarceva, tariquitar, tasisulam, taxotere, taxoprexin, tazarotene, tegafur, temozolamide, tesmilifene, testosterone, testosterone propionate, tesmilifene, tetraplatin, tetrodotoxin, tezacitabine, thalidomide, theralux, therarubicin, thymalfasin, thymectacin, tiazofurin, tipifarnib, tirapazamine, tocladesine, tomudex, toremofin, trabectedin, TransMID-107, transretinic acid, traszutumab, tremelimumab, tretinoin, triacetyluridine, triapine, triciribine, trimetrexate, TLK-286TXD 258, tykerb/tyverb, urocidin, valrubicin, vatalanib, vincristine, vinflunine, virulizin, WX-UK1, WX-554, vectibix, xeloda, XELOX, XL-147, XL-228, XL-281, XL-518/R-7420/GDC-0973, XL-765, YM-511, YM-598, ZD-4190, ZD-6474, ZD-4054, ZD-0473, ZD-6126, ZD-9331, ZD1839, ZSTK-474, zoledronat, zosuquidar, and combinations thereof.

[00161] In one embodiment, the other therapeutic agent comprises a hormone analog, an antihormone or both selected from tamoxifen, toremifene, raloxifene, fulvestrant, megestrol acetate, flutamide, nilutamide, bicalutamide, aminoglutethimide, cyproterone acetate, finasteride, buserelin acetate, fludrocortisone, fluoxymesterone, medroxy-progesterone, octreotide, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more LHRH agonists and/or antagonists selected from goserelin acetate, luprolide acetate, triptorelin pamoate and combinations thereof and wherein the LHRH antagonists are selected from Degarelix, Cetrorelix, Abarelix, Ozarelix, Degarelix combinations thereof. In one embodiment, the other therapeutic agent comprises one or more growth factor inhibitors selected from inhibitors of: platelet derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insuline-like growth factors (IGF), human epidermal growth factor (HER) and hepatocyte growth factor (HGF). In one embodiment, the other therapeutic agent comprises one or more inhibitors of the human epidermal growth factor selected from HER2, HER3, and HER4. In one embodiment, the other therapeutic agent comprises one or more tyrosine kinase

inhibitors selected from cetuximab, gefitinib, imatinib, lapatinib and trastuzumab, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more aromatase inhibitors selected from anastrozole, letrozole, liarozole, vorozole, exemestane, atamestane, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more antimetabolites which are antifolates selected from methotrexate, raltitrexed, and pyrimidine analogs. In one embodiment, the other therapeutic agent comprises one or more antimetabolites which are pyrimidine analogs selected from 5-fluorouracil, capecitabin and gemcitabin. In one embodiment, the other therapeutic agent comprises one or more antimetabolites which are purine and/or adenosine analogs selected from mercaptopurine, thioguanine, cladribine and pentostatin, cytarabine, fludarabine, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more antitumour antibiotics selected from anthracyclins, doxorubicin, daunorubicin, epirubicin and idarubicin, mitomycin-C, bleomycin, dactinomycin, plicamycin, streptozocin and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more platinum derivatives selected from cisplatin, oxaliplatin, carboplatin and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more alkylation agents selected from estramustin, meclorethamine, melphalan, chlorambucil, busulphan, dacarbazin, cyclophosphamide, ifosfamide, temozolomide, nitrosoureas, and combinations thereof. In one embodiment, the other therapeutic agent comprises nitrosoureas selected from carmustin, lomustin, thiotepa, and combinations thereof. In one embodiment, the other therapeutic agent comprises antimitotic agents selected from Vinca alkaloids and taxanes. In one embodiment, the other therapeutic agent comprises one or more taxanes selected from paclitaxel, docetaxel, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more Vinca alkaloids selected from vinblastine, vindesin, vinorelbin, vincristine, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more topoisomerase inhibitors which are epipodophyllotoxins. In one embodiment, the other therapeutic agent comprises one or more epipodophyllotoxins selected from etoposide and etopophos, teniposide, amsacrin, topotecan, irinotecan, mitoxantron, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more serine/threonine kinase inhibitors selected from PDK 1 inhibitors, B-Raf inhibitors, mTOR inhibitors, mTORC1 inhibitors, PI3K inhibitors, dual mTOR/PI3K inhibitors, STK 33 inhibitors, AKT inhibitors, PLK 1 inhibitors, inhibitors of CDKs, Aurora kinase inhibitors, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more tyrosine kinase inhibitors which are PTK2/FAK inhibitors. In one embodiment, the other therapeutic agent comprises one or more protein protein interaction inhibitors selected from

IAP, Mcl-1, MDM2/MDMX and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more rapamycin analogs selected from everolimus, temsirolimus, ridaforolimus, sirolimus, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more therapeutic agents selected from amifostin, anagrelid, clodronat, filgrastin, interferon, interferon α, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer, and combinations thereof. In one embodiment, the other therapeutic agent comprises one or more therapeutic agents selected from 2-chlorodesoxyadenosine, 2-fluorodesoxy-cytidine, 2-methoxyoestradiol, 2C4,3-alethine, 131-1-TM-601, 3CPA, 7-ethyl-10-hydroxycamptothecin, 16-aza-epothilone B, A 105972, A 204197, abiraterone, aldesleukin, alitretinoin, allovectin-7, altretamine, alvocidib, amonafide, anthrapyrazole, AG-2037, AP-5280, apaziquone, apomine, aranose, arglabin, arzoxifene, atamestane, atrasentan, auristatin PE, ABT-199 (Venetoclax), ABT-263 (Navitoclax), AVLB, AZ10992, ABX-EGF, AMG-479 (ganitumab), ARRY 162, ARRY 438162, ARRY-300, ARRY-142886/AZD-6244 (selumetinib), ARRY-704/AZD-8330, AR-12, AR-42, AS-703988, AXL-1717, AZD-8055, AZD-5363, AZD-6244, ARQ-736, ARQ 680, AS-703026 (primasertib), avastin, AZD-2014, azacytidine, azaepothilone B, azonafide, BAY-43-9006, BAY 80-6946, BBR-3464, BBR-3576, bevacizumab, BEZ-235, biricodar dicitrate, BCX-1777, BKM-120, bleocin, BLP-25, BMS-184476, BMS-247550, BMS-188797, BMS-275291, BMS-663513, BMS-754807, BNP-1350, BNP-7787, BIBW 2992 (afatinib, tomtovok), BIBF 1120 (vargatef), BI 836845, BI 2536, BI 6727, BI 836845, BI 847325, BI 853520, BUB-022, bleomycinic acid, bleomycin A, bleomycin B, brivanib, bryostatin-1, bortezomib, brostallicin, busulphan, BYL-719, CA-4 prodrug, CA-4, CapCell, calcitriol, canertinib, canfosfamide, capecitabine, carboxyphthalatoplatin, CCl-779, CC-115, CC-223, CEP-701, CEP-751, CBT-1 cefixime, ceflatonin, ceftriaxone, celecoxib, celmoleukin, cemadotin, CH4987655/RO-4987655, chlorotrianisene, cilengitide, ciclosporin, CDA-II, CDC-394, CKD-602, CKI-27, clofarabin, colchicin, combretastatin A4, COT inhibitors, CHS-828, CH-5132799, CLL-Thera, CMT-3 cryptophycin 52, CTP-37, CTLA-4 monoclonal antibodies, CP-461, CV-247, cyanomorpholinodoxorubicin, cytarabine, D 24851, decitabine, deoxorubicin, deoxyrubicin, deoxycoformycin, depsipeptide, desoxyepothilone B, dexamethasone, dexrazoxanet, diethylstilbestrol, diflomotecan, didox, DMDC, dolastatin 10, doranidazole, DS-7423, E7010, E-6201, edatrexat, edotreotide, efaproxiral, eflornithine, EGFR inhibitors, EKB-569, EKB-509, enzastaurin, enzalutamide, elsamitrucin, epothilone B, epratuzumab, ER-86526, erlotinib, ET-18-0CH3, ethynylcytidine, ethynyloestradiol, exatecan, exatecan mesylate, exemestane, exisulind, fenretinide, figitumumab, floxuridine, folic acid, FOLFOX, FOLFOX4, FOLFIRI, formestane, fotemustine, galarubicin, gallium maltolate,

gefinitib, gemtuzumab, gimatecan, glufosfamide, GCS-100, GDC-0623, GDC-0941 (pictrelisib), GDC-0980, GDC-0032, GDC-0068, GDC-0349, GDC-0879, G17DT immunogen, GMK, GPX-100, gp100-peptide vaccines, GSK-5126766, GSK-690693, GSK-1120212 (trametinib), GSK-2118436 (dabrafenib), GSK-2126458, GSK-2132231A, GSK-2334470, GSK-2110183, GSK-2141795, GW2016, granisetron, herceptine, hexamethylmelamine, histamine, homoharringtonine, hyaluronic acid, hydroxyurea, hydroxyprogesterone caproate, ibandronate, ibrutinib, ibritumomab, idatrexate, idenestrol, IDN-5109, IGF-1R inhibitors, IMC-1C11, IMC-A12 (cixutumumab), immunol, indisulam, interferon α-2a, interferon α-2b, pegylated interferon α-2b, interleukin-2, INK-1117, INK-128, INSM-18, ionafarnib, ipilimumab, iproplatin, irofulven, isohomohalichondrin-B, isoflavone, isotretinoin, ixabepilone, JRX-2, JSF-154, J-107088, conjugated oestrogens, kahalid F, ketoconazole, KW-2170, KW-2450, lobaplatin, leflunomide, lenograstim, leuprolide, leuporelin, lexidronam, LGD-1550, linezolid, lutetium texaphyrin, lometrexol, losoxantrone, LU 223651, lurtotecan, LY-S6AKT1, LY-2780301, mafosfamide, marimastat, mechloroethamine, MEK inhibitors, MEK-162, methyltestosteron, methylprednisolone, MEDI-573, MEN-10755, MDX-H210, MDX-447, MDX-1379, MGV, midostaurin, minodronic acid, mitomycin, mivobulin, MK-2206, MK-0646 (dalotuzumab), MLN518, motexaf in gadolinium, MS-209, MS-275, MX6, neridronate, neratinib, Nexavar, neovastat, nilotinib, nimesulide, nitroglycerin, nolatrexed, norelin, N-acetylcysteine, 06-benzylguanine, oblimersen, omeprazole, oncophage, oncoVEXGM-CSF, ormiplatin, ortataxel, OX44 antibodies, OSI-027, OSI-906 (linsitinib), 4-1BB antibodies, oxantrazole, oestrogen, panitumumab, patupilone, pegfilgrastim, PCK-3145, pegfilgrastim, PBI-1402, PBI-05204, PDO325901, PD-1 antibodies, PEG-paclitaxel, albumin-stabilized paclitaxel, PEP-005, PF-05197281, PF-05212384, PF-04691502, PHT-427, P-04, PKC412, P54, PI-88, pelitinib, pemetrexed, pentrix, perifosine, perillylalcohol, pertuzumab, PI3K inhibitors, PI3K/mTOR inhibitors, PG-TXL, PG2, PLX-4032/RO-5185426 (vemurafenib), PLX-3603/RO-5212054, PT-100, PWT-33597, PX-866, picoplatin, pivaloyloxymethylbutyrate, pixantrone, phenoxodiol O, PKI166, plevitrexed, plicamycin, polyprenic acid, porfiromycin, prednisone, prednisolone, quinamed, quinupristin, R115777, RAF-265, ramosetron, ranpirnase, RDEA-119/BAY 869766, RDEA-436, rebeccamycin analogs, receptor tyrosine kinase (RTK) inhibitors, revimid, RG-7167, RG-7304, RG-7421, RG-7321, RG 7440, rhizoxin, rhu-MAb, rinfabate, risedronate,rituximab, robatumumab, rofecoxib, RO-31-7453, RO-5126766, RO-5068760, RPR 109881A, rubidazone, rubitecan, R-flurbiprofen, RX-0201, S-9788, sabarubicin, SAHA, sargramostim, satraplatin, SB 408075, Se-015/Ve-015, SU5416, SU6668, SDX-101, semustin, seocalcitol, SM-11355, SN-38, SN-4071, SR-27897, SR-31747,

SR-13668, SRL-172, sorafenib, spiroplatin, squalamine, suberanilohydroxamic acid, sutent, T 900607, T 138067, TAK-733, TAS-103, tacedinaline, talaporf in, Tarceva, tariquitar, tasisulam, taxotere, taxoprexin, tazarotene, tegafur, temozolamide, tesmilifene, testosterone, testosterone propionate, tesmilifene, tetraplatin, tetrodotoxin, tezacitabine, thalidomide, theralux, therarubicin, thymalfasin, thymectacin, tiazofurin, tipifarnib, tirapazamine, tocladesine, tomudex, toremofin, trabectedin, TransMID-107, transretinic acid, traszutumab, tremelimumab, tretinoin, triacetyluridine, triapine, triciribine, trimetrexate, TLK-286TXD 258, tykerb/tyverb, urocidin, valrubicin, vatalanib, vincristine, vinflunine, virulizin, WX-UK1, WX-554, vectibix, xeloda, XELOX, XL-147, XL-228, XL-281, XL-518/R-7420/GDC-0973, XL-765, YM-511, YM-598, ZD-4190, ZD-6474, ZD-4054, ZD-0473, ZD-6126, ZD-9331, ZD1839, ZSTK-474, zoledronat, zosuquidar, and combinations thereof.

[00162] In one embodiment, the other therapeutic agent comprises a steroid, including dexamethasone, prednisolone, methyl prednisolone, prednisone, hydrocortisone, triamcinolone, betamethasone, and cortivazol. In one embodiment, the other therapeutic agent comprises an anti-emetic, Anti-emetics include, but are not limited to, 5-HT3 receptor agonists (*e.g.*, dolasetron, granisetron, ondansetron, tropisetron, palonosetron, and mirtazapine), dopamine agonists (*e.g.*, domperidone, olanzapine, droperidol, haloperidol, chlorpromazine, prochlorperazine, alizapride, prochlorperazine, and metoclopramide), NK1 receptor antagonists (*e.g.*, aprepitant and casopitant), antihistamines (such as cyclizine, diphenhydramine, dimenhydrinate, doxylamine, meclizine, promethazine, hydroxyzine), cannabinoids (*e.g.*, cannabis, dronabinol, nabilone, and sativex), benzodiazepines (*e.g.*, midazolam and lorazepam), anticholinergics (*e.g.*, hyoscine), trimethobenzamide, ginger, emetrol, propofol, peppermint, muscimol, and ajwain.

[00163] In one embodiment, the other therapeutic agent comprises an anti-cancer agent, which includes a mitotic inhibitor. In one embodiment, the mitotic inhibitor includes a taxane. In one embodiment, the mitotic inhibitor includes a taxane selected from paclitaxel and docetaxel.

[00164] In one embodiment, the pharmaceutical composition includes an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof; and at least one anti-cancer agent, which includes one or more of acivicin, aclarubicin, acodazole, acronine, adozelesin, aldesleukin, alitretinoin, allopurinol, altretamine, ambomycin, ametantrone, amifostine, aminoglutethimide, amsacrine, anastrozole, anthramycin, arsenic trioxide, asparaginase, asperlin, azacitidine, azetepa, azotomycin, batimastat, benzodepa, bevacizumab, bicalutamide, bisantrene, bisnafide dimesylate, bizelesin, bleomycin, brequinar, bropirimine, busulfan, cactinomycin, calusterone, capecitabine, caracemide, carbetimer, carboplatin,

carmustine, carubicin, carzelesin, cedefingol, celecoxib, chlorambucil, cirolemycin, cisplatin, cladribine, crisnatol mesylate, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, decitabine, dexormaplatin, dezaguanine, dezaguanine mesylate, diaziquone, docetaxel, doxorubicin, droloxifene, dromostanolone, duazomycin, edatrexate, eflomithine, elsamitrucin, enloplatin, enpromate, epipropidine, epirubicin, erbulozole, esorubicin, estramustine, etanidazole, etoposide, etoprine, fadrozole, fazarabine, fenretinide, floxuridine, fludarabine, fluorouracil, flurocitabine, fosquidone, fostriecin, fulvestrant, gemcitabine, hydroxyurea, idarubicin, ifosfamide, ilmofosine, interleukin II (IL-2, including recombinant interleukin II or rIL2), interferon α-2a, interferon α-2b, interferon α-n1, interferon α-n3, interferon β-Ia, interferon gamma-Ib, iproplatin, irinotecan, lanreotide, letrozole, leuprolide, liarozole, lometrexol, lomustine, losoxantrone, masoprocol, maytansine, mechlorethamine hydrochlride, megestrol, melengestrol acetate, melphalan, menogaril, mercaptopurine, methotrexate, metoprine, meturedepa, mitindomide, mitocarcin, mitocromin, mitogillin, mitomalcin, mitomycin, mitosper, mitotane, mitoxantrone, mycophenolic acid, nelarabine, nocodazole, nogalamycin, ormnaplatin, oxisuran, paclitaxel, pegaspargase, peliomycin, pentamustine, peplomycin, perfosfamide, pipobroman, piposulfan, piroxantrone hydrochloride, plicamycin, plomestane, porfimer, porfiromycin, prednimustine, procarbazine, puromycin, pyrazofurin, riboprine, rogletimide, safingol, semustine, simtrazene, sparfosate, sparsomycin, spirogermanium, spiromustine, spiroplatin, streptonigrin, streptozocin, sulofenur, talisomycin, tamoxifen, tecogalan, tegafur, teloxantrone, temoporfin, teniposide, teroxirone, testolactone, thiamiprine, thioguanine, thiotepa, tiazofurin, tirapazamine, topotecan, toremifene, trestolone, triciribine, trimetrexate, triptorelin, tubulozole, uracil mustard, uredepa, vapreotide, verteporfin, vinblastine, vincristine sulfate, vindesine, vinepidine, vinglycinate, vinleurosine, vinorelbine, vinrosidine, vinzolidine, vorozole, zeniplatin, zinostatin, zoledronate, zorubicin and combinations thereof.

[00165] Examples of suitable anti-cancer agents include those described Goodman and Gilman's The Pharmacological Basis of Therapeutics, 12th Ed., edited by Laurence Brunton, Bruce Chabner, Bjorn Knollman, McGraw Hill Professional, 2010.

[00166] In some exemplary embodiments, the pharmaceutical composition includes a salt (e.g., a mono-or di- salt) of an imipridone, *e.g.*, ONC201, or an analog thereof and at least one other therapeutic agent, where the other therapeutic agent comprises an anti-angiogenic agent, for example, bevacizumab. In one embodiment, the anti-angiogenic agent is selected from aflibercept, axitinib, angiostatin, endostatin, l6kDa prolactin fragment, laminin peptides, fibronectin peptides, tissue metalloproteinase inhibitors (TIMP 1, 2, 3, 4), plasminogen activator inhibitors (PAI-1, -2), tumor necrosis factor α, (high dose, invitro), TGF-β1,

interferons (IFN-α, -β, γ), ELR-CXC chemokines, IL-12; SDF-1; MIG; platelet factor 4 (PF-4); IP-10, thrombospondin (TSP), SPARC, 2-methoxyoestradiol, proliferin-related protein, suramin, sorafenib, regorafenib, thalidomide, cortisone, linomide, fumagillin (AGM-1470; TNP-470), tamoxifen, retinoids, CM101, dexamethasone, leukemia inhibitory factor (LIF), hedgehog inhibitor and combinations thereof.

[00167] A pharmaceutical combination can include first and second therapeutic agents in any desired proportions provided that the synergistic or cooperative effect still occurs. A synergistic pharmaceutical combination preferably contains the first and second therapeutic agents in a ratio of from about 1:9 to about 9:1. In one embodiment, a synergistic combination contains the first and second therapeutic agents in a ratio of from about 1:8 to about 8:1, from about 1:7 to about 7:1, from about 1:6 to about 6:1, from about 1:5 to about 5:1, from about 1:4 to about 4:1, from about 1:3 to about 3:1, or from about 1:2 to about 2:1. In one embodiment, the synergistic combination contains the therapeutic agents in a ratio of approximately 1:1.

[00168] In one embodiment, the second therapeutic agent is selected from Allopurinol, Arsenic Trioxide, Azacitidine, Bortezomib, Bevacizumab, Capecitabine, Carboplatin, Celecoxib, Chlorambucil, Clofarabine, Cytarabine, Dacarbazine, Daunorubicin HCl, Docetaxel, Doxorubicin HCl, Floxuridine, Gemcitabine HCl, Hydroxyurea, Ifosfamide, Imatinib Mesylate, Ixabepilone, Lenalidomide, Megestrol acetate, Methotrexate, Mitotane, Mitoxantrone HCl, Oxaliplatin, Paclitaxel, Pralatrexate, Romidepsin, Sorafenib, Streptozocin, Tamoxifen Citrate, Topotecan HCl, Tretinoin, Vandetanib, Vismodegib, Vorinostat, and combinations thereof.

[00169] In one embodiment, the second therapeutic agent comprises a small molecule multi-kinase inhibitor, *e.g.*, sorafenib or regorafenib. In one embodiment, the second therapeutic agent comprises a Hedgehog Pathway Inhibitor, *e.g.*, vismodegib. In one embodiment, the second therapeutic agent includes a drug selected from Table 2 below.

Table 2: Classes Of Drugs

Classes of drugs	Examples
Purine analogs	allopurinol, oxypurinol, clofarabine, and tisopurine
Pyrimidine analogs	5-fluorouracil, Floxuridine (FUDR), capecitabine, cytarabine, 6-azauracil (6-AU), and gemcitabine (Gemzar)
Proteasome inhibitors	bortezomib, carfilzomib, cediranib, disulfiram, epigallocatechin-3-gallate, salinosporamide A, ONCX 0912, CEP-18770, MLN9708, epoxomicin, and MG132.
Anti-angiogenic	bevacizumab, aflibercept, sunitinib, sorafenib, pazopanib,

Classes of drugs	Examples
	vandetanib, cabozantinib, axitinib, ponatinib, regorafenib, ranibizumab, lapatinib, and vandetanib.
Platinum-based antineoplastic drugs	cisplatin, carboplatin, oxaliplatin, satraplatin, picoplatin, nedaplatin, and triplatin.
COX-2 inhibitors	celecoxib, valdecoxib (Bextra), parecoxib (Dynastat), lumiracoxib, etoricoxib, and rofecoxib.
Nitrogen mustards	cyclophosphamide, chlorambucil, uramustine, ifosfamide, melphalan, bendamustine, and mustine.
Alkylating agents	cyclophosphamide, mechlorethamine or mustine (HN2) (trade name Mustardgen), uramustine or uracil mustard, melphalan, chlorambucil, ifosfamide, bendamustine, carmustine, lomustine, streptozocin, and busulfan.
Anthracyclines	Daunorubicin (Daunomycin), Daunorubicin (liposomal), Doxorubicin (Adriamycin), Doxorubicin (liposomal), Epirubicin, Idarubicin, Valrubicin, and Mitoxantrone.
Taxanes	Paclitaxel (Taxol), Docetaxel (Taxotere), and albumin-bound paclitaxel (Abraxane).
Nucleotide synthesis inhibitor	methotrexate, pralatrexate, hydroxyurea, and 5-fluorodeoxyuridine, 3,4-dihydroxybenzylamine.
Bcr-abl inhibitors	imatinib, nilotinib, dasatinib, bosutinib and ponatinib.
Other	arsenic trioxide, thalidomide, revlimid, and mitotane.
Topoisomerase inhibitor	amsacrine, etoposide, etoposide phosphate, teniposide, doxorubicin, Topotecan (Hycamtin), Irinotecan (CPT-11, Camptosar), Exatecan, Lurtotecan, ST 1481, CKD 602, ICRF-193, and genistein.
HDAC inhibitors	Vorinostat (SAHA), Romidepsin (Istodax), Panobinostat (LBH589), Valproic acid (as Mg valproate), Belinostat (PXD101), Mocetinostat (MGCD0103), Abexinostat (PCI-24781), Entinostat (MS-275), SB939, Resminostat (4SC-201), Givinostat, Quisinostat (JNJ-26481585), CUDC-101, AR-42, CHR-2845, CHR-3996, 4SC-202, CG200745, ACY-1215, ME-344, sulforaphane, Kevetrin, and ATRA.

Classes of drugs	Examples
Multi-kinase inhibitors	sorafenib, regorafenib, and vandetanib.
Hormone therapies	tamoxifen, toremifene, Arimidex (anastrozole), Aromasin (exemestane), Femara (letrozole), and Fulvestrant (Faslodex).
Hedgehog signaling Inhibitors	vismodegib, BMS-833923, IPI-926, LDE-225, PF-04449913, LEQ 506, and TAK-441.
Checkpoint Inhibitors	Opdivo (nivolumab), Durvalumab (Medi4736), Keytruda (pembrolizumab, MK3475), BGB-A317, AMP-224, PDR001, REGN 281, Atezolizumab (MPDL3280A), Pidilizumab (BMS-936559, CT-011, ONO-4538), Avelumab (MSB0010718 C), Yervoy (ipilimumab), tremelimumab
BCL2 Inhibitors	AT-101, Bcl-2/xL inhibitor, Navitoclax (ABT-263), Venetoclax (ABT-199), Apogossypol, PTN1258, obatoclax, G3139

[00170] In one embodiment, the second therapeutic agent includes drugs that target tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors. In one embodiment, the second therapeutic agent includes a recombinant TRAIL or an agonistic antibody that activates one or more TRAIL receptors. In one embodiment, the second therapeutic agent includes one or more antibodies or recombinant TRAIL that activate signaling by DR4, DR5 or both. In one embodiment, the second therapeutic agent includes one or more of AMG-655, LBY-135, mapatumumab, lexatumumab, Apomab, and rhApo2L/TRAIL. In one embodiment, the second therapeutic agent includes an active agent selected from Camptothecin, 5-FU, capecitabine, cisplatin, doxorubicin, irinotecan, paclitaxel, cisplatin, bortezomib, BH3I-2, rituximab, radiation, triterpenoids, sorafenib, gemcitabine, HDAC inhibitors, carboplatin, T-101 (a gossypol derivate), ABT-263, ABT-737, and GX-15-070 (obatoclax), vorinostat, cetuximab, panitumumab, bevacizumab, ganitumab, interferon gamma, sorafenib, XIAP antagonists, Bcl-2 antagonists, and Smac mimetics.

VI. DOSE

[00171] In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose ranging from about 40, 50, 60, or 100 mg to about 2000 mg; from about 4, 5, 6, or 10 mg to about 200 mg; or from about 0.4, 0.5, 0.6, or 1 mg to about 20 mg where the weight can be based on the

compound in its free base form. In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 50 mg to about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 5 mg to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, and 200 mg; or from about 0.5 mg to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 mg. In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 40 mg to about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 4 mg to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 0.4 mg to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 mg. In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 60 mg to about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 6 mg to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 0.6 mg to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg. In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 100 mg to about 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900 mg, or 2000 mg; from about 10 mg to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 1 mg to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg. In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 200 mg to about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 20 mg to about 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 2 mg to about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg, based on the compound in its free base form. In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 400 mg to about 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, or 2000 mg; from about 40 mg to about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 mg; or from about 4 mg to about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,

15, 16, 17, 18, 19, or 20 mg based on the compound in its free base form. In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof thereof in a dose level ranging from about 50 mg to about 60, 70, 80, 90, or 100 mg; from about 60 mg to about 70, 80, 90, or 100 mg; from about 70 mg to about 80, 90 or 100 mg, from about 80 mg to about 90 or 100 mg; from about 90 mg to about 100 mg; from about 5 mg to about 6, 7, 8, 9, or 10 mg; from about 6 mg to about 7, 8, 9, or 10 mg; from about 7 mg to about 8, 9 or 10 mg, from about 8 mg to about 9 or 10 mg; from about 9 mg to about 10 mg; from about 0.5 mg to about 0.6, 0.7, 0. 8, 0.9, or 1 mg; from about 0.6 mg to about 0.7, 0.8, 0.9, or 1 mg; from about 0.7 mg to about 0.8, 0.9 or 1 mg, from about 0.8 mg to about 0.9 or 1 mg; or from about 0.9 mg to about 1 mg.

[00172] In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose ranging from about 1 mg/kg to about 40 mg/kg; 0.1 mg/kg to about 4 mg/kg; or 0.01 mg/kg to about 0.40 mg/kg. In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose level ranging from about 1, 2, 3, 4, 5, 6, 7, 8, or 9 mg/kg to about 10, 20, 30, or 40 mg/kg; from about 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 mg/kg to about 20, 30, or 40 mg/kg; from about 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29 mg/kg to about 30 or 40 mg/kg; from about 30, 31, 32, 33, 34, 35, 36, 37, 38, or 39 mg/kg to about 40 mg/kg; from about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, or 0.9 mg/kg to about 1, 2, 3, or 4 mg/kg; from about 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, or 1.9 mg/kg to about 2, 3, or 4 mg/kg; from about 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, or 2.9 mg/kg to about 3 or 4 mg/kg; or from about 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, or 3.9 mg/kg to about 4 mg/kg; from about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 mg/kg to about 0.10, 0.20, 0.30, or 0.40 mg/kg; from about 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, or 0.19 mg/kg to about 0.20, 0.30, or 0.40 mg/kg; from about 0.20, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, or 0.29 mg/kg to about 0.30 or 0.40 0.mg/kg; or from about 0.30, 0.31, 0.32, 0.33, 0.34, 0.35, 0.36, 0.37, 0.38, or 0.39 mg/kg to about 0.40 mg/kg.

[00173] In one embodiment, a pharmaceutical composition comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose ranging from about 37.5 mg/m^2 to about 1500 mg/m^2; from about 3.75 mg/m^2 to about 150 mg/m^2; or from about 0.4 mg/m^2 to about 15 mg/m^2 In one embodiment, a pharmaceutical composition comprises comprises an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in a dose ranging from about 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265,

270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 505, 510, 515, 520, 525, 530, 535, 540, 545, 550, 555, 560, 565, 570, 575, 580, 585, 590, 595, 600, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895, 900, 905, 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995, 1000, 1005, 1010, 1015, 1020, 1025, 1030, 1035, 1040, 1045, 1050, 1055, 1060, 1065, 1070, 1075, 1080, 1085, 1090, 1095, 1100, 1105, 1110, 1115, 1120, 1125, 1130, 1135, 1140, 1145, 1150, 1155, 1160, 1165, 1170, 1175, 1180, 1185, 1190, 1195, 1200, 1205, 1210, 1215, 1220, 1225, 1230, 1235, 1240, 1245, 1250, 1255, 1260, 1265, 1270, 1275, 1280, 1285, 1290, 1295, 1300, 1305, 1310, 1315, 1320, 1325, 1330, 1335, 1340, 1345, 1350, 1355, 1360, 1365, 1370, 1375, 1380, 1385, 1390, 1395, 1400, 1405, 1410, 1415, 1420, 1425, 1430, 1435, 1440, 1445, 1450, 1455, 1460, 1465, 1470, 1475, 1480, 1485, 1490, 1495 mg/m^2 to about 1500 mg/m^2; from about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 mg/m^2 to about 150 mg/m^2;or from about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 111, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 mg/m^2 to about 15 mg/m^2.

VII. DOSAGE FORMS

[00174] Suitable pharmaceutical compositions for use in the methods described herein can be formulated into a dosage form that can be administered to a patient. In one embodiment, a pharmaceutical composition is in the form of an oral dosage unit or parenteral dosage unit. In one embodiment, a pharmaceutical composition is in the form of an oral dosage unit. In one embodiment, an oral dosage unit is fractionated into several, smaller doses, which are administered to a subject over a predetermined period of time in order to reduce toxicity of a therapeutic agent being administered. In one embodiment, an oral dosage unit is administered by a tablet or capsule comprising a controlled release formulation that can include a plurality of particles, granules, pellets, minitablets or tablets. In one embodiment, the pharmaceutical

composition is in the form of a parenteral dosage unit. In one embodiment, the parenteral dosage unit is selected from the group consisting of intravenous (IV), subcutaneous (SC), and intramuscular (M), rectal (PR) and transdermal dosage units. In one embodiment, the composition is in a dosage form selected from the group consisting of sterile solutions, suspensions, suppositories, tablets and capsules. In one embodiment, the composition is an oral dosage form selected from the group consisting of a tablet, caplet, capsule, lozenge, syrup, liquid, suspension and elixir. In one embodiment, the composition is in an oral dosage form selected from the group consisting of tablets, hard shell capsules, soft gelatin capsules, beads, granules, aggregates, powders, gels, solids and semi-solids.

[00175] In one embodiment, suitable forms of pharmaceutical compositions for use in the methods described herein include dermatological compositions adapted for cutaneous topical administration. For example, dermatological compositions include a cosmetically or pharmaceutically acceptable medium. Dermatological compositions for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. In one embodiment, conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners, skin enhancers can be necessary or desirable and therefore used. Examples of suitable enhancers include ethers such as diethylene glycol monoethyl ether (available commercially as TRANSCUTOL®) and diethylene glycol monomethyl ether; surfactants such as sodium laurate, sodium lauryl sulfate, cetyltrimethylammonium bromide, benzalkonium chloride, Poloxamer (231, 182, 184), Tween (20, 40, 60, 80), and lecithin (US Pat. 4,783,450); alcohols such as ethanol, propanol, octanol, benzyl alcohol; polyethylene glycol and esters thereof such as polyethylene glycol monolaurate; amides and other nitrogenous compounds such as urea, dimethylacetamide (DMA), dimethylformamide (DMF), 2-pyrrolidone, l-methyl-2-pyrrolidone, ethanolamine, diethanolamine and triethanolamine; terpenes; alkanones; and organic acids, particularly citric acid and succinic acid. AZONE® and sulfoxides such as DMSO and C10MSO may also be used, but are less preferred.

[00176] In one embodiment, the pharmaceutical composition is in a dosage form selected from the group consisting of sustained release forms, controlled release forms, delayed release forms and response release forms.

VIII. METHODS OF USE

[00177] The compositions and methods described herein have utility in treating many disease conditions, including cancer (e.g., colorectal, brain, and glioblastoma). In one embodiment, the compositions and methods described herein are used to treat diseases such as ocular melanoma, desmoplastic round cell tumor, chondrosarcoma, leptomengial disease, diffuse large B-cell

lymphoma, Acute Lymphoblastic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, AIDS-Related Cancers, AIDS-Related Lymphoma, Anal or Rectal Cancer, Appendix Cancer, Astrocytomas, and Atypical Teratoid/Rhabdoid Tumor. In one embodiment, the compositions and methods described herein are used to treat diseases such as Basal Cell Carcinoma, Basal Cell Nevus Syndrome, Gorlin-Nevus Syndrome, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma, Brain Tumor, Breast Cancer, Bronchial Tumors, Burkitt Lymphoma, and Spinal Cord Tumors. In one embodiment, the compositions and methods described herein are used to treat diseases such as Carcinoid Tumor, Carcinoma of Unknown Primary, Central Nervous System Atypical Teratoid/Rhabdoid Tumor, Leptomeningeal Disease, Central Nervous System Embryonal Tumors, Central Nervous System Lymphoma, Cervical Cancer, Chordoma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Chronic Myeloproliferative Disorders, Colon Cancer, Colorectal Cancer, Craniopharyngioma, and Cutaneous T-Cell Lymphoma (including Sezary syndrome and mycosis fungoides (MF)). In one embodiment, the compositions and methods described herein are used to treat cdiseases such as Embryonal Tumors of Central Nervous System, Endometrial Cancer, Ependymoblastoma, Ependymoma, Esophageal Cancer, Ewing Sarcoma Family of Tumors, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, and Eye Cancer, including Intraocular Melanoma and Retinoblastoma. In one embodiment, the compositions and methods described herein are used to treat diseases such as Gallbladder Cancer, Gastric (Stomach) Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumor (GIST), Germ Cell Tumor, Gestational Trophoblastic Tumor, and Glioma. In one embodiment, the compositions and methods described herein are used to treat a cancer selected from the group consisting of Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular (Liver) Cancer, Histiocytosis, Hodgkin Lymphoma, and Hypopharyngeal Cancer. In one embodiment, the compositions and methods described herein are used to treat diseases such as Kaposi Sarcoma and Kidney (Renal Cell) Cancer. In one embodiment, the compositions and methods described herein are used to treat diseases such as Langerhans Cell Histiocytosis, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, including Non-Small Cell Lung Cancer, and Small Cell Lung Cancer, Non-Hodgkin Lymphoma, and Primary Central Nervous System Lymphoma. In one embodiment, the compositions and methods described herein are used to treat diseases such as Waldenström's macroglobulinemia (lymphoplasmacytic lymphoma), Malignant Fibrous Histiocytoma of Bone and Osteosarcoma, Medulloblastoma, Medulloepithelioma, Melanoma, Merkel Cell Carcinoma, Mesothelioma, Metastatic Squamous Neck Cancer with Occult Primary, Multiple Endocrine Neoplasia Syndrome, Mouth Cancer, Multiple

Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndromes, complex karyotype, blastic phase leukemia, Myelodysplastic/Myeloproliferative Neoplasms, Multiple Myeloma, and Myeloproliferative Disorders. In one embodiment, the compositions and methods described herein are used to treat cancer. In one embodiment, the compositions and methods described herein are used to treat diseases such as Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, and Neuroblastoma. In one embodiment, the compositions and methods described herein are used to treat diseases such as Oral Cancer,Lip and Oral Cavity Cancer, Oropharyngeal Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma of Bone, Ovarian Cancer, Ovarian Germ Cell Tumor, Ovarian Epithelial Cancer, and Ovarian Low Malignant Potential Tumor. In one embodiment, the compositions and methods described herein are used to treat diseases such as Pancreatic Cancer, Papillomatosis, Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer, Pineal Parenchymal Tumors of Intermediate Differentiation, Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors, Pituitary Tumor, Pleuropulmonary Blastoma, Pregnancy and Breast Cancer, Primary Central Nervous System Lymphoma, and Prostate Cancer. In one embodiment, the compositions and methods described herein are used to treat a cancer selected from the group consisting of Rectal Cancer, Renal Cell (Kidney) Cancer, Renal Pelvis and Ureter, Respiratory Tract Carcinoma Involving the NUT Gene on Chromosome 15, Retinoblastoma, and Rhabdomyosarcoma. In one embodiment, the compositions and methods described herein are used to treat high grade prostate cancer. In one embodiment, the compositions and methods described herein are used to treat medium grade prostate cancer. In one embodiment, the compositions and methods described herein are used to treat low grade prostate cancer. In one embodiment, the compositions and methods described herein are used to treat castration-resistant prostate cancer. In one embodiment, the compositions and methods described herein are used to treat a nervous system tumor. In one embodiment, the compositions and methods described herein are used to treat a central nervous system tumor. In one embodiment, the compositions and methods described herein are used to treat a peripheral nervous system tumor. In one embodiment, the compositions and methods described herein are used to treat a paraganglioma. In one embodiment, the compositions and methods described herein are used to treat a pheochromocytoma.

[00178] In *in vitro* models, in animal models, and in human clinical trials compound (**1**) (ONC201) has broad anti-cancer activity, low toxicity including few, if any, adverse effects, low genotoxicity, and high bioavailability including oral bioavailability. These features allow ONC 201 and various analogs to be particularly well suited for pediatric patients. These features also make ONC 201 and various analogs particularly well suited for chronic therapy,

for high risk patients, and to ensure long-lasting responses or stable disease or to prevent disease recurrence.

[00179] In one embodiment, the compositions and methods described herein are used to treat a pediatric cancer (*e.g.*, pediatric solid tumors, pediatric sarcomas, pediatric Ewing's sarcomas, pediatric gliomas, pediatric central nervous system cancers, pediatric neuroblastoma, pediatric leukemia and pediatric lymphoma).

[00180] In one embodiment, the compositions and methods described herein are used to treat a proliferative skin disorder such as psoriasis. In one embodiment, the compositions and methods described herein are used to treat a cancer selected from the group consisting of Salivary Gland Cancer, Sarcoma, Sézary Syndrome, Skin Cancer, Ocular Cancer, Skin Carcinoma, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma, Squamous Neck Cancer with Occult Primary, and Supratentorial Primitive Neuroectodermal Tumors. In one embodiment, the compositions and methods described herein are used to treat a cancer selected from the group consisting of T-Cell Lymphoma, Testicular Cancer, Throat Cancer, Thymoma and Thymic Carcinoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, and Gestational Trophoblastic Tumor. In one embodiment, the compositions and methods described herein are used to treat a cancer selected from the group consisting of Carcinoma of Unknown Primary Site, Cancer of Unknown Primary Site, Unusual Cancers of Childhood, Transitional Cell Cancer of the Renal Pelvis and Ureter, Urethral Cancer, and Uterine Sarcoma. In one embodiment, the compositions and methods described herein are used to treat cancer selected from the group consisting of Vaginal Cancer and Vulvar Cancer. In one embodiment, the compositions and methods described herein are used to treat a cancer selected from the group consisting of Wilms Tumor and Women's Cancers.

[00181] In one embodiment, the compositions and methods described herein are used as a first-line therapy (sometimes called primary therapy). In one embodiment, the compositions and methods described herein are used as a second-line therapy. In one embodiment, the compositions and methods described herein are used as a third-line therapy. In one embodiment, the compositions and methods described herein are used as a salvage therapy. The term "salvage therapy" means a therapeutic agent that can be taken with any regimen after a subject's initial treatment regimen has failed or after the subject's condition has not responded to an initial treatment. In one embodiment, the compositions and methods described herein are used as a rescue therapy. In one embodiment of the rescue therapy, the compositions are used as a rescue agent to counteract the action of an initial treatment. In one embodiment of the rescue therapy, the compositions are used as rescue agent which is administered to a subject who has developed resistance to a standard or an initial treatment. In one embodiment, the

compositions and methods described herein are used as a neoadjuvant therapy. In one embodiment, the neoadjuvant therapy comprises administration of one or more of the therapeutic agents described herein to a subject before a main or first line treatment. In one embodiment, the neoadjuvant therapy reduces the size or extent of the cancer being treated before a main or first line treatment is administered to the subject undergoing treament. In one embodiment, the compositions and methods described herein are used as an adjuvant therapy. In one embodiment, the adjuvant therapy comprises administration of one or more therapeutic agents described herein to a subject, wherein the one or more therapeutic agent that modify the effect of other therapeutic agents that are already administered to the subject or are concurrently administered to the subject or subsequently administered to the subject.

[00182] In one embodiment, the compositions and methods described herein exhibit reduced chance of drug-drug interactions. In one embodiment, an imipridone, such as ONC201, or an analog thereof are eliminated from the patient's body before it can interact with another pharmaceutically active agent.

[00183] In one embodiment, the compositions and methods of described herein exhibit toxicity levels that facilitates combinations with other pharamaceutical agents.

[00184] The methods and compositions described herein are not limited to a particular animal species. In one embodiment, a subject treated according to methods and using compositions described herein, can be mammalian or non-mammalian. In one embodiment, a mammalian subject mammal includes, but is not limited to, a human; a non-human primate; a rodent such as a mouse, rat, or guinea pig; a domesticated pet such as a cat or dog; a horse, cow, pig, sheep, goat, or rabbit. In one embodiment, a non-mammalian subject includes, but is not limited to, a bird such as a duck, goose, chicken, or turkey. In one embodiment, the subject is a human. In one embodiment, subjects can be either gender and any age. The composition and methods can also be used to prevent cancer. The composition and methods can also be used to stimulate the immune system.

[00185] The methods and compositions described herein are not limited to a particular age of the subject. In one embodiment, a subject treated according to methods and using compositions described herein is over 50 years old, over 55 years old, over 60 years old, or over 65 years old. In one embodiment, a subject treated according to methods and using compositions described herein is under 50 years old, under 55 years old, under 60 years old, or under 65 years old.

[00186] . In one embodiment, a subject treated according to methods and using compositions described herein is a pediatric patient. In one embodiment, the pediatric patient is younger than 18 years old, younger than 17 years old, younger than 16 years old, younger than 15 years old, younger than 14 years old, is younger than 13 years old, younger than 12 years old, younger

than 11 years old, younger than 10 years old, younger than 9 years old, younger than 8 years old, younger than 7 years old, younger than 6 years old, younger than 5 years old, younger than 4 years old, younger than 3 years old, younger than 2 years old, younger than 1 year old. In one embodiment, the pediatric patient is younger than 12 months old, younger than 11 months old, younger than 10 months old, younger than 9 months old, younger than 8 months old, younger than 7 months old, younger than 6 months old, is younger than 5 months old, younger than 4 months old, younger than 3 months old, younger than 2 months old, younger than 1 month old. In one embodiment, the pediatric patient younger than 4 weeks old, younger than 3 weeks old, younger than 2 weeks old, younger than 1 weeks old. In one embodiment, the pediatric patient is younger than 7 days old, younger than 6 days old, younger than 5 days old, younger than 4 days old, younger than 3 days old, younger than 2 days old, or younger than 1 day old. In one embodiment, the pediatric patient is a neonate. In one embodiment, the pediatric patient is prematurely born.

[00187] In one embodiment, the patient is less than 45 kg in weight, less than 40 kg in weight, less than 35 kg in weight, less than 30 kg in weight, less than 25 kg in weight, less than 20 kg in weight, less than 15 kg in weight, less than 14 kg in weight, less than 10 kg in weight, less than 5 kg in weight, less than 4 kg in weight, less than 3 kg in weight, less than 2 kg in weight, or less than 1 kg in weight.

[00188] In one embodiment, the subject has received at least one prior therapeutic agent. In one embodiment the subject has received at least two, at least three, or at least four prior therapeutic agents. In one embodiment the prior therapeutic agent is ibrutinib, bortezomib, carfilzomib, temozolomide, bevacizumab, cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone, cytarabine, cisplatin, rituximab, 5-fluorouracil, oxaliplatin, leucovorin, or lenalidomide.

[00189] In one embodiment, the subject has been treated with radiation. In one embodiment, the subject has been treated with surgery. In one embodiment, the subject has been treated with adoptive T-cell therapy.

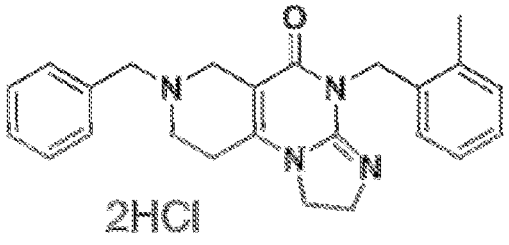
[00190] In one embodiment, the cancer no longer responds to treatment with ibrutinib, bortezomib, carfilzomib, temozolomide, bevacizumab, cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone, cytarabine, cisplatin, rituximab, 5-fluorouracil, oxaliplatin, leucovorin, lenalidomide, radiation, surgery, or a combination thereof.

[00191] In one embodiment, the compositions and methods described herein have a dose response relation in cancer cells that is different from the dose response relation of the same compositions and methods in normal cells. The dose response relation of ONC201 on proliferation and cell death in normal and tumor cells was determined by measuring cell

viability following treatment with ONC201 at various concentrations for 72 hours. The tumors tested included a human colon cancer cell line (HCT116), breast tumor cell line (MDA-MB-231), and a human primary glioblastoma cell line (U87). And the normal cells tested included human foreskin fibroblasts (HFF), human fetal lung fibroblast (MRC-5) cells, and a human lung fibroblast cell line (WI-38). Doxorubicin was used as a positive control at 1 μg/mL in normal fibroblasts. Cell viability of normal cells tested was at least about 75% at about 1-5 mg/mL of ONC201, whereas viability of tumor cells was significantly lower (*e.g.*, at or below 50%) at the same ONC201 concentration. Moreover, as ONC201 concentration increased beyond about 5 mg/mL viability of tumor cells fell to below 25%, whereas viability of normal cells remained at about 75%. Cell viability assays in human fetal lung fibroblast (MRC-5) cells were performed following 72 hour treatment with compound (**1**) (5 μM) or DMSO and a recovery period in complete drug-free media after treatment. Cell recovery was seen with ONC201, but not with DMSO.

[00192] In one embodiment, the compositions and methods described herein have utility in treating cancer in a subject. In one embodiment, the compositions and methods described herein have utility in treating cancer in a human subject. In one embodiment, the treatment method comprises administering to a subject in need of such treatment, a pharmaceutically effective amount of an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

[00193] In one embodiment, the treatment method comprises administering to a subject in need of such treatment: (i) a first therapeutic agent including an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in combination with (ii) a second therapeutic agent, wherein the first and the second therapeutic agents are administered either simultaneously or sequentially. The second therapeutic agent can be any suitable therapeutic agent, including any pharmaceutically active agent disclosed herein. A pharmaceutically acceptable ONC201 salt includes the di-hydrochloride salt below:



[00194] It is understood that a di-hydrochloride salt of ONC201 or an analog thereof (including a compound of formula (**10**)), or an alternative di-salt thereof apparent from the

teaching of this disclosure, can be substitued for ONC201 or an analog thereof in a composition or dosing regimen described hererin.

[00195] In one embodiment, the treatment method comprises administering a synergistic pharmaceutical combination, either simultaneously or sequentially, to a subject in need of such treatment, wherein the synergistic pharmaceutical combination comprises (i) a first therapeutic agent comprising an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof; and (ii) a second therapeutic agent. In one embodiment, the treatment method comprises administering to a subject in need of such treatment, either simultaneously or sequentially, therapeutically synergistic effective amounts of the first therapeutic agent in combination with the second therapeutic agent. In one embodiment, the treatment method comprises administering to a subject in need of such treatment, an effective amount of the first therapeutic agent in combination with an effective amount of the second therapeutic agent, wherein the combination provides a synergistic effect in the *in vivo* treatment of a cancer sensitive to the combination, and wherein the first and the second therapeutic agents are administered either simultaneously or sequentially. In one embodiment, the treatment method comprises administering to a subject in need of such treatment, an effective amount of the first therapeutic agent in combination with an effective amount of a second therapeutic agent, wherein the combination provides a synergistic effect in the *in vivo* treatment of a minimal residual disease sensitive to the combination, and wherein the first and second therapeutic agents are administered either simultaneously or sequentially.In one embodiment, the second therapeutic agent is given before or prior to the first therapeutic agent.

[00196] In one embodiment, the treatment method targets a cancer selected from the group consisting of solid tumors, liquid tumors, lymphomas, leukemias, or myelomas.

[00197] In one embodiment, the treatment method targets a solid tumor, wherein the solid tumor is selected from the group consisting of: Cervical Cancer, Endometrial Cancer, Extracranial Germ Cell Tumor; Extragonadal Germ Cell Tumor; Germ Cell Tumor; Gestational Trophoblastic Tumor; Ovarian Cancer, Ovarian Germ Cell Tumor, Ovarian Epithelial Cancer, and Ovarian Low Malignant Potential Tumor; Penile Cancer, Prostate Cancer; Pregnancy and Breast Cancer; high grade prostate cancer; medium grade prostate cancer; low grade prostate cancer; castration-resistant prostate cancer; Breast Cancer; Bile Duct Cancer; Extrahepatic Bile Duct Cancer; Gallbladder Cancer; Hepatocellular (Liver) Cancer; Kidney (Renal Cell) Cancer; Liver Cancer, Renal Cell (Kidney) Cancer, Renal Pelvis and Ureter; Basal Cell Carcinoma; Basal Cell Nevus Syndrome, Gorlin-Nevus Syndrome, Melanoma, Merkel Cell Carcinoma, Papillomatosis, Multiple Endocrine Neoplasia Syndrome; Pancreatic Cancer, Parathyroid Cancer, ocular melanoma; Eye Cancer; Retinoblastoma;

Malignant Fibrous Histiocytoma; Ewing Sarcoma Family of Tumors; desmoplastic round cell tumor; chondrosarcoma, Kaposi Sarcoma, Rhabdomyosarcoma; Spinal Cord Tumors, Leptomeningeal Disease, Central Nervous System Embryonal Tumors, Chordoma, Embryonal Tumors of Central Nervous System, Ependymoblastoma, Ependymoma, Neuroblastoma; Pineal Parenchymal Tumors of Intermediate Differentiation, Pineoblastoma; Adrenocortical Carcinoma; Bone Cancer, Osteosarcoma; Malignant Fibrous Histiocytoma of Bone and Osteosarcoma; Osteosarcoma and Malignant Fibrous Histiocytoma of Bone; Carcinoid Tumor, Carcinoma of Unknown Primary, Bronchial Tumors, Lung Cancer, Pleuropulmonary Blastoma; Respiratory Tract Carcinoma Involving the NUT Gene on Chromosome 15, Astrocytomas, Atypical Teratoid/Rhabdoid Tumor; Central Nervous System Atypical Teratoid/Rhabdoid Tumor, Craniopharyngioma, Glioma, Brain cancer, Medulloblastoma, Medulloepithelioma, Supratentorial Primitive Neuroectodermal Tumors; Pituitary Tumor; Gastric (Stomach) Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumor (GIST), Bladder Cancer, Anal or Rectal Cancer, Appendix Cancer, Esophageal Cancer, Hypopharyngeal Cancer; Laryngeal Cancer, Lip and Oral Cavity Cancer, Metastatic Squamous Neck Cancer with Occult Primary, Mouth Cancer, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Oral Cancer, Lip and Oral Cavity Cancer, Oropharyngeal Cancer, Paranasal Sinus and Nasal Cavity Cancer, Pharyngeal Cancer; Head and Neck Cancer, and Mesothelioma.

[00198] In one embodiment, the treatment method targets a lymphoma selected from the group consisting of: diffuse large B-cell lymphoma, AIDS-Related Lymphoma, Cutaneous T-Cell Lymphoma, Sezary syndrome, mycosis fungoides (MF); Histiocytosis; Burkitt Lymphoma, and Central Nervous System Lymphoma; Non-Hodgkin Lymphoma, and Primary Central Nervous System Lymphoma, Hodgkin Lymphoma, Waldenström's macroglobulinemia; Mycosis Fungoides; Primary Central Nervous System Lymphoma; lymphoplasmacytic lymphoma, and Primary Central Nervous System Lymphoma.

[00199] In one embodiment, the treatment method targets a Non-Hodgkin's lymphoma (NHL) selected from the group consisting of: mantle cell lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, marginal zone lymphoma, small lymphocytic lymphoma, lyphoplasmacytic NHL, Waldenstrom's macroglobulinaemia, and skin lymphomas.

[00200] In one embodiment, the treatment method targets a leukemia selected from the group consisting of: Acute Lymphoblastic Leukemia (ALL), Chronic Lymphocytic Leukemia (CLL), Chronic Myeloproliferative Disorders; Hairy Cell Leukemia; Acute Myeloid Leukemia (AML); Chronic Myelogenous Leukemia (CML); and Langerhans Cell Histiocytosis.

[00201] In one embodiment, the treatment method targets an acute leukemia selected from the group consisting of: acute lymphotyte leukemia, acute myeloid leukemia, chronic lymphoblasitc leukemia, chronic myeloid leukemia, myelodysplastic syndrome, and myeloproliferative disease.

[00202] In one embodiment, the treatment method targets a myeloma selected from the group consisting of: IgA myeloma; IgG myeloma; IgM myeloma; IgD myeloma; IgE myeloma; light chain myeloma; non secretory myeloma; complex karyotype, blastic phase leukemia; Multiple Myeloma/Plasma Cell Neoplasm, Multiple Myeloma, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Neoplasms, and Myeloproliferative Disorders.

[00203] In one embodiment, the treatment method targets a peripheral nervous system tumor. In one embodiment, the treatment method targets a paraganglioma. In one embodiment, the treatment method targets a pheochromocytoma.

[00204] In one embodiment, treatment of cancer comprises prevention of tumor growth in a cancer subject. In one embodiment, treatment of cancer comprises prevention of formation of cancer metastases in a cancer subject. In one embodiment, treatment of cancer comprises targeted treatment of minimal residual disease in a cancer subject known to have the minimal residual disease in a cancer or a subject at risk for having minimal residual disease.

[00205] This might be indicated after treatment of the primary tumor by surgery and/or after chemotherapy (radiotherapy) has been initiated or determined to be efficaceous. Disseminated tumor cells may be in their dormant state and often cannot be attacked by chemotherapy (radiotherapy). A thus treated patient seemingly is in a healed state, and refered to as "minimal residual disease." Nevertheless, the dormant tumor cells have a potential to form metastases if they become metastasising cells due to a growth stimulus after a longer dormant state.

[00206] The term "minimal residual disease" denotes a small number of cancer cells that remain in a subject during or after treatment when the subject is in remission (exhibiting no symptoms or signs of the disease). The methods described herein are preferably applied to a form of the diseases listed herein, including adult and childhood forms of these diseases.

[00207] In one embodiment, the treatment method is useful for treating an autoimmune disease. Autoimmune diseases include, but are not limited to alopecia areata, antiphospholipid, autoimmune hepatits, celiac disease, diabetes type 1, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hemolytic anemia, idiopathic thrombocytopenic purpura, inflammatory bowel disease, inflammatory myopathies, multiple sclerosis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erythematosus, and vitiligo.

[00208] In one embodiment, the treatment method is useful for treating autoimmune and inflammatory disorders of the peripheral nerve system such as amyotrophic lateral sclerosis (Lou Gehrig's disease), based on various causes such as metabolic disorders that include diabetes, B12 and folate vitamin deficiencies, chemotherapy medications and medicines used to treat HIV, poisons that cause peripheral nerve damage, cancers that develop peripheral neuropathies as well as paraneoplastic syndromes, alcohol abuse, chronic kidney disease, injuries that cause compression on nerves and other lesions, infections such as Lyme disease, Guillain Barre syndrome, connective tissue disease, rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus, certain inflammatory conditions such as sarcoidosis, coeliac disease, hereditary diseases such as charcot marie tooth syndrome, Friedreich's ataxia, and/or idiopathic where no specific cause is found but inflammatory and/or autoimmune mechanisms are the cause of onset.

[00209] In one embodiment, the treatment method is useful for treating autoimmune and inflammatory disorders with ocular manifestations. Such ocular manifestations include, but are not limited to, ocular cicatricial pemphigoid, Mooren's corneal ulcer, various forms of uveitis, rheumatoid arthritis, systemic lupus erythematosus, polyarteritis nodosa, relapsing polychondritis, Wegener's granulomatosis, scleroderma, Behcet's disease, Reiter's disease, inflammatory bowel disease (ulcerative colitis and Crohn's disease) and ankylosing spondylitis, retinitis pigmentosa, macular degeneration, keratoconjunctivitis sicca, scleritis, episcleritis, keratitis, peripheral corneal ulceration, and less common entities such as choroiditis, retinal vasculitis, episcleral nodules, retinal detachments, and/or macular edema.

[00210] In one embodiment, the treatment method is useful for treating acute allograft rejection in transplant patients. In one embodiment, the treatment method is useful for treating ischemic stroke. In one embodiment, the treatment method is useful for treating inflammatory diseases including arthritis, psoriasis, asthma, and colitis.

[00211] In one embodiment, a therapeutic agent includes a pharmaceutically acceptable mono-salt of ONC201 or an analog thereof (*e.g.*, a compound of formula (**10**)). In one embodiment, a therapeutic agent includes a pharmaceutically acceptable ONC201 di-salt or an analog thereof (*e.g.*, a compound of formula (**10**)). As described herein, some of the analogs can be tri-salts In one embodiment, a therapeutic agent includes ONC201 or an analog thereof (*e.g.*, a compound of formula (**10**)) in the form of a pharmaceutically acceptable mono- or di-salt selected from the group consisting of hydrochloride, hydrobromide, hydrogensulphate, sulfates, phosphates, fumarates, succinates, oxalates and lactates, bisulfates, hydroxyl, tartrate, nitrate, citrate, bitartrate, carbonate, malate, maleate, fumarate sulfonate, methylsulfonate, formate, acetate, and carboxylate. In one embodiment, a therapeutic agent includes ONC201 or

an analog thereof in the form of a pharmaceutically acceptable mono- or di-salt selected from p-toluene-sulfonate, benzenesulfonate, methanesulfonate, oxalate, succinate, tartrate, citrate, fumarate and maleate. In one embodiment, a therapeutic agent includes ONC201 or an analog thereof in the form of a pharmaceutically acceptable mono- or di-salt having a counter ion selected from the group consisting of ammonium, sodium, potassium, calcium, magnesium, zinc, lithium, and/or with counter-ions such as methylamino, dimethylamino, diethylamino, triethylamino counter-ions, and combinations thereof. In one embodiment, a therapeutic agent includes a compound described herein in the form of a halide di-salt, such as a di-hydrochloride salt or a di-hydrobromide salt.

[00212] In one embodiment of the treatment method, the second therapeutic agent includes an anti-cancer agent. In one embodiment of the treatment method, the second therapeutic agent is selected from acivicin, aclarubicin, acodazole, acronine, adozelesin, aldesleukin, alitretinoin, allopurinol, altretamine, ambomycin, ametantrone, amifostine, aminoglutethimide, amsacrine, anastrozole, anthramycin, arsenic trioxide, asparaginase, asperlin, azacitidine, azetepa, azotomycin, batimastat, benzodepa, bevacizumab, bicalutamide, bisantrene, bisnafide dimesylate, bizelesin, bleomycin, brequinar, bropirimine, busulfan, cactinomycin, calusterone, capecitabine, caracemide, carbetimer, carboplatin, carmustine, carubicin, carzelesin, cedefingol, celecoxib, chlorambucil, cirolemycin, cisplatin, cladribine, crisnatol mesylate, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, decitabine, dexormaplatin, dezaguanine, dezaguanine mesylate, diaziquone, docetaxel, doxorubicin, droloxifene, dromostanolone, duazomycin, edatrexate, eflomithine, elsamitrucin, enloplatin, enpromate, epipropidine, epirubicin, erbulozole, esorubicin, estramustine, etanidazole, etoposide, etoprine, fadrozole, fazarabine, fenretinide, floxuridine, fludarabine, fluorouracil, flurocitabine, fosquidone, fostriecin, fulvestrant, gemcitabine, hydroxyurea, idarubicin, ifosfamide, ilmofosine, interleukin II (IL-2, including recombinant interleukin II or rIL2), interferon α-2a, interferon α-2b, interferon α-n1, interferon α-n3, interferon β-Ia, interferon gamma-Ib, iproplatin, irinotecan, lanreotide, letrozole, leuprolide, liarozole, lometrexol, lomustine, losoxantrone, masoprocol, maytansine, mechlorethamine hydrochlride, megestrol, melengestrol acetate, melphalan, menogaril, mercaptopurine, methotrexate, metoprine, meturedepa, mitindomide, mitocarcin, mitocromin, mitogillin, mitomalcin, mitomycin, mitosper, mitotane, mitoxantrone, mycophenolic acid, nelarabine, nocodazole, nogalamycin, ormnaplatin, oxisuran, paclitaxel, pegaspargase, peliomycin, pentamustine, peplomycin, perfosfamide, pipobroman, piposulfan, piroxantrone hydrochloride, plicamycin, plomestane, porfimer, porfiromycin, prednimustine, procarbazine, puromycin, pyrazofurin, riboprine, rogletimide, safingol, semustine, simtrazene, sparfosate, sparsomycin, spirogermanium,

spiromustine, spiroplatin, streptonigrin, streptozocin, sulofenur, talisomycin, tamoxifen, tecogalan, tegafur, teloxantrone, temoporfin, teniposide, teroxirone, testolactone, thiamiprine, thioguanine, thiotepa, tiazofurin, tirapazamine, topotecan, toremifene, trestolone, triciribine, trimetrexate, triptorelin, tubulozole, uracil mustard, uredepa, vapreotide, verteporfin, vinblastine, vincristine sulfate, vindesine, vinepidine, vinglycinate, vinleurosine, vinorelbine, vinrosidine, vinzolidine, vorozole, zeniplatin, zinostatin, zoledronate, zorubicin and combinations thereof.

[00213] In one embodiment of the treatment method, the second therapeutic agent is selected, from hormone analogs and antihormones, aromatase inhibitors, LHRH agonists and antagonists, inhibitors of growth factors, growth factor antibodies, growth factor receptor antibodies, tyrosine kinase inhibitors; antimetabolites; antitumour antibiotics; platinum derivatives; alkylation agents; antimitotic agents; tubuline inhibitors; PARP inhibitors, topoisomerase inhibitors, serine/threonine kinase inhibitors, tyrosine kinase inhibitors, protein protein interaction inhibitors, MEK inhibitors, ERK inhibitors, IGF-1R inhibitors, ErbB receptor inhibitors, rapamycin analogs, amifostin, anagrelid, clodronat, filgrastin, interferon, interferon α, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer, 2-chlorodesoxyadenosine, 2-fluorodesoxy-cytidine, 2-methoxyoestradiol, 2C4,3-alethine, 131-1-TM-601, 3CPA, 7-ethyl-10-hydroxycamptothecin, 16-aza-epothilone B, A 105972, A 204197, abiraterone, aldesleukin, alitretinoin, allovectin-7, altretamine, alvocidib, amonafide, anthrapyrazole, AG-2037, AP-5280, apaziquone, apomine, aranose, arglabin, arzoxifene, atamestane, atrasentan, auristatin PE, ABT-199 (Venetoclax), ABT-263 (Navitoclax), AVLB, AZ10992, ABX-EGF, AMG-479 (ganitumab), ARRY 162, ARRY 438162, ARRY-300, ARRY-142886/AZD-6244 (selumetinib), ARRY-704/AZD-8330, AR-12, AR-42, AS-703988, AXL-1717, AZD-8055, AZD-5363, AZD-6244, ARQ-736, ARQ 680, AS-703026 (primasertib), avastin, AZD-2014, azacytidine, azaepothilone B, azonafide, BAY-43-9006, BAY 80-6946, BBR-3464, BBR-3576, bevacizumab, BEZ-235, biricodar dicitrate, BCX-1777, BKM-120, bleocin, BLP-25, BMS-184476, BMS-247550, BMS-188797, BMS-275291, BMS-663513, BMS-754807, BNP-1350, BNP-7787, BIBW 2992 (afatinib, tomtovok), BIBF 1120 (vargatef), BI 836845, BI 2536, BI 6727, BI 836845, BI 847325, BI 853520, BUB-022, bleomycinic acid, bleomycin A, bleomycin B, brivanib, bryostatin-1, bortezomib, brostallicin, busulphan, BYL-719, CA-4 prodrug, CA-4, CapCell, calcitriol, canertinib, canfosfamide, capecitabine, carboxyphthalatoplatin, CCl-779, CC-115, CC-223, CEP-701, CEP-751, CBT-1 cefixime, ceflatonin, ceftriaxone, celecoxib, celmoleukin, cemadotin, CH4987655/RO-4987655, chlorotrianisene, cilengitide, ciclosporin, CDA-II, CDC-394, CKD-602, CKI-27, clofarabin, colchicin, combretastatin A4, COT

inhibitors, CHS-828, CH-5132799, CLL-Thera, CMT-3 cryptophycin 52, CTP-37, CTLA-4 monoclonal antibodies, CP-461, CV-247, cyanomorpholinodoxorubicin, cytarabine, D 24851, decitabine, deoxorubicin, deoxyrubicin, deoxycoformycin, depsipeptide, desoxyepothilone B, dexamethasone, dexrazoxanet, diethylstilbestrol, diflomotecan, didox, DMDC, dolastatin 10, doranidazole, DS-7423, E7010, E-6201, edatrexat, edotreotide, efaproxiral, eflornithine, EGFR inhibitors, EKB-569, EKB-509, enzastaurin, enzalutamide, elsamitrucin, epothilone B, epratuzumab, ER-86526, erlotinib, ET-18-0CH3, ethynylcytidine, ethynyloestradiol, exatecan, exatecan mesylate, exemestane, exisulind, fenretinide, figitumumab, floxuridine, folic acid, FOLFOX, FOLFOX4, FOLFIRI, formestane, fotemustine, galarubicin, gallium maltolate, gefinitib, gemtuzumab, gimatecan, glufosfamide, GCS-100, GDC-0623, GDC-0941 (pictrelisib), GDC-0980, GDC-0032, GDC-0068, GDC-0349, GDC-0879, G17DT immunogen, GMK, GPX-100, gp100-peptide vaccines, GSK-5126766, GSK-690693, GSK-1120212 (trametinib), GSK-2118436 (dabrafenib), GSK-2126458, GSK-2132231A, GSK-2334470, GSK-2110183, GSK-2141795, GW2016, granisetron, herceptin, hexamethylmelamine, histamine, homoharringtonine, hyaluronic acid, hydroxyurea, hydroxyprogesterone caproate, ibandronate, ibritumomab, idatrexate, idenestrol, IDN-5109, IGF-1R inhibitors, IMC-1C11, IMC-A12 (cixutumumab), immunol, indisulam, interferon α-2a, interferon α-2b, pegylated interferon α-2b, interleukin-2, INK-1117, INK-128, INSM-18, ionafarnib, ipilimumab, iproplatin, irofulven, isohomohalichondrin-B, isoflavone, isotretinoin, ixabepilone, JRX-2, JSF-154, J-107088, conjugated oestrogens, kahalid F, ketoconazole, KW-2170, KW-2450, lobaplatin, leflunomide, lenograstim, leuprolide, leuporelin, lexidronam, LGD-1550, linezolid, lutetium texaphyrin, lometrexol, losoxantrone, LU 223651, lurtotecan, LY-S6AKT1, LY-2780301, mafosfamide, marimastat, mechloroethamine, MEK inhibitors, MEK-162, methyltestosteron, methylprednisolone, MEDI-573, MEN-10755, MDX-H210, MDX-447, MDX-1379, MGV, midostaurin, minodronic acid, mitomycin, mivobulin, MK-2206, MK-0646 (dalotuzumab), MLN518, motexaf in gadolinium, MS-209, MS-275, MX6, neridronate, neratinib, Nexavar, neovastat, nilotinib, nimesulide, nitroglycerin, nolatrexed, norelin, N-acetylcysteine, 06-benzylguanine, oblimersen, omeprazole, oncophage, oncoVEXGM-CSF, ormiplatin, ortataxel, OX44 antibodies, OSI-027, OSI-906 (linsitinib), 4-1BB antibodies, oxantrazole, oestrogen, panitumumab, patupilone, pegfilgrastim, PCK-3145, pegfilgrastim, PBI-1402, PBI-05204, PDO325901, PD-1 antibodies, PEG-paclitaxel, albumin-stabilized paclitaxel, PEP-005, PF-05197281, PF-05212384, PF-04691502, PHT-427, P-04, PKC412, P54, PI-88, pelitinib, pemetrexed, pentrix, perifosine, perillylalcohol, pertuzumab, PI3K inhibitors, PI3K/mTOR inhibitors, PG-TXL, PG2, PLX-4032/RO-5185426 (vemurafenib), PLX-3603/RO-5212054,

PT-100, PWT-33597, PX-866, picoplatin, pivaloyloxymethylbutyrate, pixantrone, phenoxodiol O, PKI166, plevitrexed, plicamycin, polyprenic acid, porfiromycin, prednisone, prednisolone, quinamed, quinupristin, R115777, RAF-265, ramosetron, ranpirnase, RDEA-119/BAY 869766, RDEA-436, rebeccamycin analogs, receptor tyrosine kinase (RTK) inhibitors, revimid, RG-7167, RG-7304, RG-7421, RG-7321, RG 7440, rhizoxin, rhu-MAb, rinfabate, risedronate,rituximab, robatumumab, rofecoxib, RO-31-7453, RO-5126766, RO-5068760, RPR 109881A, rubidazone, rubitecan, R-flurbiprofen, RX-0201, S-9788, sabarubicin, SAHA, sargramostim, satraplatin, SB 408075, Se-015/Ve-015, SU5416, SU6668, SDX-101, semustin, seocalcitol, SM-11355, SN-38, SN-4071, SR-27897, SR-31747, SR-13668, SRL-172, sorafenib, spiroplatin, squalamine, suberanilohydroxamic acid, sutent, T 900607, T 138067, TAK-733, TAS-103, tacedinaline, talaporf in, Tarceva, tariquitar, tasisulam, taxotere, taxoprexin, tazarotene, tegafur, temozolamide, tesmilifene, testosterone, testosterone propionate, tesmilifene, tetraplatin, tetrodotoxin, tezacitabine, thalidomide, theralux, therarubicin, thymalfasin, thymectacin, tiazofurin, tipifarnib, tirapazamine, tocladesine, tomudex, toremofin, trabectedin, TransMID-107, transretinic acid, traszutumab, tremelimumab, tretinoin, triacetyluridine, triapine, triciribine, trimetrexate, TLK-286TXD 258, tykerb/tyverb, urocidin, valrubicin, vatalanib, vincristine, vinflunine, virulizin, WX-UK1, WX-554, vectibix, xeloda, XELOX, XL-147, XL-228, XL-281, XL-518/R-7420/GDC-0973, XL-765, YM-511, YM-598, ZD-4190, ZD-6474, ZD-4054, ZD-0473, ZD-6126, ZD-9331, ZD1839, ZSTK-474, zoledronat, zosuquidar, and combinations thereof.

[00214] In one embodiment of the treatment method, the second therapeutic agent is selected from tamoxifen, toremifene, raloxifene, fulvestrant, megestrol acetate, flutamide, nilutamide, bicalutamide, aminoglutethimide, cyproterone acetate, finasteride, buserelin acetate, fludrocortisone, fluoxymesterone, medroxy-progesterone, octreotide, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent is selected from LHRH agonists and LHRH antagonists. In one embodiment, a LHRH agonist is selected from goserelin acetate, luprolide acetate, triptorelin pamoate and combinations thereof. In one embodiment, the second therapeutic agent includes a LHRH antagonist is selected from Degarelix, Cetrorelix, Abarelix, Ozarelix, Degarelix combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes an inhibitor of a growth factor. In one embodiment, the inhibitor of a growth factor is selected from inhibitors of: platelet derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insuline-like growth factors (IGF), human epidermal growth factor (HER), hepatocyte growth factor (HGF), and combinations thereof. In

one embodiment, the human epidermal growth factor (HER) is selected from HER2, HER3, and HER4.

[00215] In one embodiment of the treatment method, the second therapeutic agent includes a tyrosine kinase inhibitor. In one embodiment of the treatment method, the tyrosine kinase inhibitor is selected from cetuximab, gefitinib, imatinib, lapatinib and trastuzumab, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes an aromatase inhibitor. In one embodiment of the treatment method, the aromatase inhibitor is selected from anastrozole, letrozole, liarozole, vorozole, exemestane, atamestane, and combinations thereof.

[00216] In one embodiment of the treatment method, the second therapeutic agent includes an antimetabolite. In one embodiment of the treatment method, the antimetabolite comprises an antifolate. In one embodiment of the treatment method, the antifolate is selected from methotrexate, raltitrexed, pyrimidine analogs, and combinations thereof. In one embodiment of the treatment method, the antimetabolite is a pyrimidine analog. In one embodiment of the treatment method, the pyrimidine analog is selected from 5-fluorouracil, capecitabin, gemcitabin, and combination thereof. In one embodiment of the treatment method, the antimetabolite is a purine analog or an adenosine analog. In one embodiment of the treatment method, the purine analog or adenosine analog is selected from mercaptopurine, thioguanine, cladribine and pentostatin, cytarabine, fludarabine, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes an antitumour antibiotic. In one embodiment of the treatment method, the antitumor antibiotic is selected from anthracyclins, doxorubicin, daunorubicin, epirubicin and idarubicin, mitomycin-C, bleomycin, dactinomycin, plicamycin, streptozocin and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes a platinum derivative. In one embodiment of the treatment method, the platinum derivative is selected from cisplatin, oxaliplatin, carboplatin and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes an alkylation agent. In one embodiment of the treatment method, the alkylation agent is selected from estramustin, meclorethamine, melphalan, chlorambucil, busulphan, dacarbazin, cyclophosphamide, ifosfamide, temozolomide, nitrosoureas, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes a nitrosourea. In one embodiment of the treatment method, the nitrosourea is selected from carmustin, lomustin, thiotepa, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes an antimitotic agent. In one embodiment of the treatment method, the antimitotic agent is selected from Vinca alkaloids and taxanes. In one embodiment of the

treatment method, the taxane is selected from paclitaxel, docetaxel, and combinations thereof. In one embodiment of the treatment method, the Vinca alkaloids are selected from vinblastine, vindesin, vinorelbin, vincristine, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes a topoisomerase inhibitor. In one embodiment of the treatment method, the topoisomerase inhibitor is an epipodophyllotoxin. In one embodiment of the treatment method, the topoisomerase inhibitor, which is an epipodophyllotoxin selected from etoposide, etopophos, teniposide, amsacrin, topotecan, irinotecan, mitoxantron, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes a serine/threonine kinase inhibitor. In one embodiment of the treatment method, the serine/threonine kinase inhibitor is selected from PDK 1 inhibitors, B-Raf inhibitors, mTOR inhibitors, mTORC1 inhibitors, PI3K inhibitors, dual mTOR/PI3K inhibitors, STK 33 inhibitors, AKT inhibitors, PLK 1 inhibitors, inhibitors of CDKs, Aurora kinase inhibitors, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes a tyrosine kinase inhibitor. In one embodiment of the treatment method, the second therapeutic agent includes a PTK2/FAK inhibitor. In one embodiment of the treatment method, the second therapeutic agent includes a protein protein interaction inhibitor. In one embodiment of the treatment method, the protein protein interaction inhibitor is selected from IAP, Mcl-1, MDM2/MDMX and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent includes a rapamycin analog. In one embodiment of the treatment method, the rapamycin analog is selected from everolimus, temsirolimus, ridaforolimus, sirolimus, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent is selected from amifostin, anagrelid, clodronat, filgrastin, interferon, interferon α, leucovorin, rituximab, procarbazine, levamisole, mesna, mitotane, pamidronate and porfimer, and combinations thereof. In one embodiment of the treatment method, the second therapeutic agent is selected from 2-chlorodesoxyadenosine, 2-fluorodesoxy-cytidine, 2-methoxyoestradiol, 2C4,3-alethine, 131-1-TM-601, 3CPA, 7-ethyl-10-hydroxycamptothecin, 16-aza-epothilone B, A 105972, A 204197, abiraterone, aldesleukin, alitretinoin, allovectin-7, altretamine, alvocidib, amonafide, anthrapyrazole, AG-2037, AP-5280, apaziquone, apomine, aranose, arglabin, arzoxifene, atamestane, atrasentan, auristatin PE, ABT-199 (Venetoclax), ABT-263 (Navitoclax), AVLB, AZ10992, ABX-EGF, AMG-479 (ganitumab), ARRY 162, ARRY 438162, ARRY-300, ARRY-142886/AZD-6244 (selumetinib), ARRY-704/AZD-8330, AR-12, AR-42, AS-703988, AXL-1717, AZD-8055, AZD-5363, AZD-6244, ARQ-736, ARQ 680, AS-703026 (primasertib), avastin, AZD-2014, azacytidine, azaepothilone B, azonafide, BAY-43-9006, BAY 80-6946, BBR-3464, BBR-3576, bevacizumab, BEZ-235, biricodar

dicitrate, BCX-1777, BKM-120, bleocin, BLP-25, BMS-184476, BMS-247550, BMS-188797, BMS-275291, BMS-663513, BMS-754807, BNP-1350, BNP-7787, BIBW 2992 (afatinib, tomtovok), BIBF 1120 (vargatef), BI 836845, BI 2536, BI 6727, BI 836845, BI 847325, BI 853520, BUB-022, bleomycinic acid, bleomycin A, bleomycin B, brivanib, bryostatin-1, bortezomib, brostallicin, busulphan, BYL-719, CA-4 prodrug, CA-4, CapCell, calcitriol, canertinib, canfosfamide, capecitabine, carboxyphthalatoplatin, CCl-779, CC-115, CC-223, CEP-701, CEP-751, CBT-1 cefixime, ceflatonin, ceftriaxone, celecoxib, celmoleukin, cemadotin, CH4987655/RO-4987655, chlorotrianisene, cilengitide, ciclosporin, CDA-II, CDC-394, CKD-602, CKI-27, clofarabin, colchicin, combretastatin A4, COT inhibitors, CHS-828, CH-5132799, CLL-Thera, CMT-3 cryptophycin 52, CTP-37, CTLA-4 monoclonal antibodies, CP-461, CV-247, cyanomorpholinodoxorubicin, cytarabine, D 24851, decitabine, deoxorubicin, deoxyrubicin, deoxycoformycin, depsipeptide, desoxyepothilone B, dexamethasone, dexrazoxanet, diethylstilbestrol, diflomotecan, didox, DMDC, dolastatin 10, doranidazole, DS-7423, E7010, E-6201, edatrexat, edotreotide, efaproxiral, eflornithine, EGFR inhibitors, EKB-569, EKB-509, enzastaurin, enzalutamide, elsamitrucin, epothilone B, epratuzumab, ER-86526, erlotinib, ET-18-0CH3, ethynylcytidine, ethynyloestradiol, exatecan, exatecan mesylate, exemestane, exisulind, fenretinide, figitumumab, floxuridine, folic acid, FOLFOX, FOLFOX4, FOLFIRI, formestane, fotemustine, galarubicin, gallium maltolate, gefinitib, gemtuzumab, gimatecan, glufosfamide, GCS-100, GDC-0623, GDC-0941 (pictrelisib), GDC-0980, GDC-0032, GDC-0068, GDC-0349, GDC-0879, G17DT immunogen, GMK, GPX-100, gp100-peptide vaccines, GSK-5126766, GSK-690693, GSK-1120212 (trametinib), GSK-2118436 (dabrafenib), GSK-2126458, GSK-2132231A, GSK-2334470, GSK-2110183, GSK-2141795, GW2016, granisetron, herceptine, hexamethylmelamine, histamine, homoharringtonine, hyaluronic acid, hydroxyurea, hydroxyprogesterone caproate, ibandronate, ibritumomab, idatrexate, idenestrol, IDN-5109, IGF-1R inhibitors, IMC-1C11, IMC-A12 (cixutumumab), immunol, indisulam, interferon α-2a, interferon α-2b, pegylated interferon α-2b, interleukin-2, INK-1117, INK-128, INSM-18, ionafarnib, ipilimumab, iproplatin, irofulven, isohomohalichondrin-B, isoflavone, isotretinoin, ixabepilone, JRX-2, JSF-154, J-107088, conjugated oestrogens, kahalid F, ketoconazole, KW-2170, KW-2450, lobaplatin, leflunomide, lenograstim, leuprolide, leuporelin, lexidronam, LGD-1550, linezolid, lutetium texaphyrin, lometrexol, losoxantrone, LU 223651, lurtotecan, LY-S6AKT1, LY-2780301, mafosfamide, marimastat, mechloroethamine, MEK inhibitors, MEK-162, methyltestosteron, methylprednisolone, MEDI-573, MEN-10755, MDX-H210, MDX-447, MDX-1379, MGV, midostaurin, minodronic acid, mitomycin, mivobulin, MK-2206, MK-0646 (dalotuzumab), MLN518,

motexaf in gadolinium, MS-209, MS-275, MX6, neridronate, neratinib, Nexavar, neovastat, nilotinib, nimesulide, nitroglycerin, nolatrexed, norelin, N-acetylcysteine, 06-benzylguanine, oblimersen, omeprazole, oncophage, oncoVEXGM-CSF, ormiplatin, ortataxel, OX44 antibodies, OSI-027, OSI-906 (linsitinib), 4-1BB antibodies, oxantrazole, oestrogen, panitumumab, patupilone, pegfilgrastim, PCK-3145, pegfilgrastim, PBI-1402, PBI-05204, PDO325901, PD-1 antibodies, PEG-paclitaxel, albumin-stabilized paclitaxel, PEP-005, PF-05197281, PF-05212384, PF-04691502, PHT-427, P-04, PKC412, P54, PI-88, pelitinib, pemetrexed, pentrix, perifosine, perillylalcohol, pertuzumab, PI3K inhibitors, PI3K/mTOR inhibitors, PG-TXL, PG2, PLX-4032/RO-5185426 (vemurafenib), PLX-3603/RO-5212054, PT-100, PWT-33597, PX-866, picoplatin, pivaloyloxymethylbutyrate, pixantrone, phenoxodiol O, PKI166, plevitrexed, plicamycin, polyprenic acid, porfiromycin, prednisone, prednisolone, quinamed, quinupristin, R115777, RAF-265, ramosetron, ranpirnase, RDEA-119/BAY 869766, RDEA-436, rebeccamycin analogs, receptor tyrosine kinase (RTK) inhibitors, revimid, RG-7167, RG-7304, RG-7421, RG-7321, RG 7440, rhizoxin, rhu-MAb, rinfabate, risedronate,rituximab, robatumumab, rofecoxib, RO-31-7453, RO-5126766, RO-5068760, RPR 109881A, rubidazone, rubitecan, R-flurbiprofen, RX-0201, S-9788, sabarubicin, SAHA, sargramostim, satraplatin, SB 408075, Se-015/Ve-015, SU5416, SU6668, SDX-101, semustin, seocalcitol, SM-11355, SN-38, SN-4071, SR-27897, SR-31747, SR-13668, SRL-172, sorafenib, spiroplatin, squalamine, suberanilohydroxamic acid, sutent, T 900607, T 138067, TAK-733, TAS-103, tacedinaline, talaporf in, Tarceva, tariquitar, tasisulam, taxotere, taxoprexin, tazarotene, tegafur, temozolamide, tesmilifene, testosterone, testosterone propionate, tesmilifene, tetraplatin, tetrodotoxin, tezacitabine, thalidomide, theralux, therarubicin, thymalfasin, thymectacin, tiazofurin, tipifarnib, tirapazamine, tocladesine, tomudex, toremofin, trabectedin, TransMID-107, transretinic acid, traszutumab, tremelimumab, tretinoin, triacetyluridine, triapine, triciribine, trimetrexate, TLK-286TXD 258, tykerb/tyverb, urocidin, valrubicin, vatalanib, vincristine, vinflunine, virulizin, WX-UK1, WX-554, vectibix, xeloda, XELOX, XL-147, XL-228, XL-281, XL-518/R-7420/GDC-0973, XL-765, YM-511, YM-598, ZD-4190, ZD-6474, ZD-4054, ZD-0473, ZD-6126, ZD-9331, ZD1839, ZSTK-474, zoledronat, zosuquidar, and combinations thereof.

[00217] In one embodiment, the other therapeutic agent comprises a steroid, including dexamethasone, prednisolone, methyl prednisolone, prednisone, hydrocortisone, triamcinolone, betamethasone, and cortivazol. In one embodiment, the other therapeutic agent comprises an anti-emetic. Anti-emetics include, but are not limited to, 5-HT3 receptor agonists (such as dolasetron, granisetron, ondansetron, tropisetron, palonosetron, and mirtazapine), dopamine agonists (such as domperidone, olanzapine, droperidol, haloperidol,

chlorpromazine, prochlorperazine, alizapride, prochlorperazine, and metoclopramide), NK1 receptor antagonists (such as aprepitant and casopitant), antihistamines (such as cyclizine, diphenhydramine, dimenhydrinate, doxylamine, meclizine, promethazine, hydroxyzine), cannabinoids (such as cannabis, dronabinol, nabilone, and sativex), benzodiazepines (such as midazolam and lorazepam), anticholinergics (such as hyoscine), trimethobenzamide, ginger, emetrol, propofol, peppermint, muscimol, and ajwain.

[00218] Pharmaceutical compositions may be administered to a subject via any suitable route of administration. In one embodiment, the pharmaceutical composition is administered to a subject orally, parenterally, transdermally or transmucosally. In one embodiment, the pharmaceutical composition is administered to a subject parenterally. In one embodiment, the pharmaceutical composition is administered to a subject via a parenteral route of administration selected from the group consisting of intravenous (IV), subcutaneous (SC), and intramuscular (IM). In one embodiment, the pharmaceutical composition is administered to a subject via a route of administration selected from rectal and transdermal. In one embodiment, the pharmaceutical composition is administered to a subject in a dosage form selected from the group consisting of sterile solutions, suspensions, suppositories, tablets and capsules. In one embodiment, the pharmaceutical composition is administered to a subject in an oral dosage form selected from the group consisting of a tablet, caplet, capsule, lozenge, syrup, liquid, suspension and elixir. In one embodiment, the pharmaceutical composition is administered to a subject in an oral dosage form selected from the group consisting of tablets, hard shell capsules, soft gelatin capsules, beads, granules, aggregates, powders, gels, solids and semi-solids.

[00219] In one embodiment, the pharmaceutical composition is administered to a subject as a dosage form selected from the group consisting of sustained release forms, controlled release forms, delayed release forms and response release forms.

[00220] In one embodiment, the pharmaceutical composition is administered to a subject once daily. In one embodiment, the pharmaceutical composition is administered to a subject accoridng to an infrequent dosing regimen (e.g., administered once per week or less frequently). In one embodiment, the pharmaceutical composition is administered to a subject accoridng to a frequent dosing regimen (e.g., administered more than once per week). In one embodiment, the pharmaceutical composition is administered to a subject once weekly. In one embodiment, the pharmaceutical composition is administered to a subject once every four weeks. In one embodiment, the pharmaceutical composition is administered to a subject twice a week. In one embodiment, the pharmaceutical composition is administered to a subject once every two weeks. In one embodiment, the pharmaceutical composition is administered to a subject once every three weeks. In one embodiment, the pharmaceutical composition is

administered to a subject in a repeated cycle of once weekly, once every two weeks, once every three weeks, once every four weeks or combinations thereof.

[00221] In one embodiment, the treatment method comprises administering to a subject in need of such treatment: (i) a first therapeutic agent including a compound comprising an imipridone, such as ONC201, or an analog thereof, or a pharmaceutically acceptable salt thereof in combination with (ii) a second therapeutic agent, wherein the first therapeutic agent and the second therapeutic agent are administered either simultaneously or sequentially; and further comprises assaying the expression of an endoplasmic reticulum (ER) stress response gene in a biological sample. In one embodiment, the endoplasmic reticulum stress response gene is selected from the group that includes, but is not limited to, C/EBP-Homologous Protein (CHOP), Activating Transcription Factor 3 (ATF3) and both CHOP and ATF3. In one embodiment, the endoplasmic reticulum stress response gene is selected from the group that includes, but is not limited to, ATF3, Activating Transcription Factor 4 (ATF4) CHOP, IRE1, Binding immunoglobulin protein (BiP), Eukaryotic translation initiation factor 2A (eIF2a), X-box binding protein 1 (XBP1). The biological sample may be tumor, peripheral blood mononuclear cells, or skin biopsy. The biological sample may be obtained before, during, or after drug administration. In one embodiment, the treatment method further comprises adjusting a dose of the first therapeutic agent to achieve induction of about 50%, 75%, 100%, 125%, 150%, 175%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 425%, 450%, 475%, 500%, 525%, 550%, 575%, 600%, or greater than 600% of one or more ER stress gene. In one embodiment, the treatment method further comprises adjusting a dose of the first therapeutic agent to achieve induction of about 50% to about 100%, about 100% to about 150%, about 150% to about 200%, about 200% to about 250%, about 250% to about 300%, about 300% to about 350%, about 350% to about 400%, about 400% to about 450%, about 450% to about 500%, about 500% to about 550%, about 550% to about 600%, or greater than 600% of ER stress genes. In one embodiment, the treatment method further comprises adjusting a dose of the first therapeutic agent to achieve induction of about 50% to about 100%, about 100% to about 200%, about 200% to about 300%, about 300% to about 400%, about 400% to about 500%, about 500% to about 600%, or greater than 600% of ER stress genes.

[00222] In one embodiment, the treatment method comprises administering to a subject in need of such treatment: (i) a first therapeutic agent including a compound comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in combination with (ii) a second therapeutic agent, wherein the first therapeutic agent and the second therapeutic agent are administered either simultaneously or sequentially; and further comprises assaying the expression of proteasomal activity in a biological sample. In one

embodiment the proteasomal activity may be chymotrysin-like, trypsin-like, and/or caspase-like activity. In one embodiment, the biological sample may be tumor, peripheral blood mononuclear cells, or skin cells. The biological sample may be obtained before, during, or after drug administration. In one embodiment, the treatment method further comprises adjusting the dose to achieve inhibition of about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% of the proteasomal activity. In one embodiment, the treatment method further comprises adjusting the dose to achieve inhibition of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the proteasomal activity. In one embodiment, the treatment method further comprises adjusting the dose to achieve inhibition of about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, or greater than 90% of the proteasomal activity.

[00223] In an aspect, provided herein are treatment methods, which comprise administering to a subject in need of such treatment a combination of a first therapeutic agent including an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof (*e.g.*, a di-salt or tri-salt) and a second therapeutic agent, the method comprising:

(i) administering to the subject the first therapeutic agent;

(ii) waiting until a predetermined waiting time has elapsed after the time of administration of the first therapeutic agent to the subject; and/or until adverse events are resolved or resolving; and

(iii) administering the second therapeutic agent to the subject, wherein the predetermined waiting time is chosen so as to obtain a delayed therapeutic effect of the first therapeutic agent without an increased risk of possible combined toxic effects of the first and second therapeutic agents. In one embodiment, the predetermined waiting time is determined based on the clearance rate of the compound of the first therapeutic agent or a metabolite thereof. In one embodiment, the predetermined waiting time is determined by a quantitative assessment of renal function and parameters of renal. In one embodiment, the predetermined waiting time is determined by an assay for the determination of renal function, wherein the assay is selected from the group consisting of serum level the compound of the first therapeutic agent or a metabolite thereof; clearance rate of the compound of the first therapeutic agent or a metabolite thereof; 24-hour urinary clearance of the compound of the first therapeutic agent or a metabolite thereof.

[00224] In one embodiment of the treatment method, the predetermined waiting time substantially equals the time required for systemic clearance of the compound of the first therapeutic agent or a metabolite thereof from the subject's body. In one embodiment of the treatment method, the predetermined waiting time substantially equals the time required for renal clearance of the compound of the first therapeutic agent or a metabolite thereof from the subject's body. In one embodiment of the treatment method, the predetermined waiting time substantially equals the time required for hepatic clearance of the compound of the first therapeutic agent or a metabolite thereof from the subject's body. In one embodiment of the treatment method, the predetermined waiting time substantially equals the time required for total clearance of the compound of the first therapeutic agent or a metabolite thereof from the subject's body. In one embodiment of the treatment method, the predetermined waiting time is about 4 hours. In other embodimens the waiting time is 1 day. In one embodiment, the waiting time is until C_{max} of the compound of the first therapeutic agent has passed. In other embodiments, the waiting time is after most of the adverse events are resolved or are resolving. In one embodiment of the treatment method, the predetermined waiting time is about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days. In one embodiment of the treatment method, the predetermined waiting time is a range of about 1-7 days, about 1-6 days, about 1-5 days, about 1-4 days, about 1-3 days, or about 1 to 2 days. In one embodiment, the waiting time is up to 3 weeks. The preceeding are considered "therapeutic time periods."

[00225] When the order of administration is reversed, timing for the administration of the first therapeutic agent can be after the C_{max} of the second therapeutic agent (i.e., the first administered drug) has passed. In one embodiment, administration of the first therapeutic agent can be after most or substantially all of the first administered drug has been eliminated from the body or the toxicity effects for the first administered drug are resolved or are resolving.

[00226] In one embodiment, the treatment method further comprises monitoring levels of the compound of the first therapeutic agent or a metabolite thereof in the subject using pharmacokinetic profiling. In some such embodiments, monitoring levels of the compound of the first therapeutic agent or a metabolite thereof in the subject using pharmacokinetic profiling comprises constructing a pharmacokinetic profile of the compound of the first therapeutic agent or a metabolite thereof for the subject using concentrations of the compound of the first therapeutic agent or a metabolite thereof in at least two samples obtained from the subject at time points suitable to construct a pharmacokinetic profile. In one embodiment, which include monitoring levels of the compound of the first therapeutic agent or a metabolite thereof in the subject using pharmacokinetic profiling, samples are collected from the subject at point-of-care or point of use by sampling or self-sampling on point-of-care devices or point of use devices or

on matrices suitable for storage of the samples prior to quantitation in a laboratory. In one embodiment, each of the point-of-care devices or point of use devices is capable of quantitating the compound of the first therapeutic agent or a metabolite thereof. In one embodiment, which include monitoring levels of the compound of the first therapeutic agent or a metabolite thereof in the subject, one or more samples are collected from the subject at point-of-care or point of use by biopsy device for analysis at the point-of-care or point of use devices or for storage prior to analysis by a laboratory. In one embodiment, a biopsy is taken after a time interval of 3-8 hours following administration the first therapeutic agent to the subject. In one embodiment, a biopsy is taken after a time interval of 3-24 hours following administration of the first therapeutic agent to the subject. In one embodiment, a biopsy is taken after a time interval of 8-24 hours following administration of the first therapeutic agent thereof to the subject. In one embodiment, a biopsy is taken after a time interval of 2 days following administration of the first therapeutic agent to the subject. In one embodiment, a biopsy is taken after a time interval of 3 days following administration of the first therapeutic agent to the subject. In one embodiment, a biopsy is taken after a time interval of 4 days following administration of the first therapeutic agent to the subject. In one embodiment, a biopsy is taken after a time interval of 1-7 days following administration of the first therapeutic agent.

[00227] In one embodiment, the pharmacokinetic profile includes pharmacokinetic parameters suitable for guiding dosing of the first therapeutic agent for the subject being treated. In one embodiment of the treatment method, the C_{max} of the first therapeutic agent following its administration to the subject ranges from about 1000 ng/dL to 1500 ng/dL for a therapeutic time period. In one embodiment, C_{max} is less than 1500 ng/dL and greater than 85 ng/dL for a therapeutic time period. In one embodiment, the C_{max} of the first therapeutic following its administration to the subject ranges from about 1000 ng/mL to 1500 ng/mL for a therapeutic time period. In one embodiment, C_{max} is less than 1500 ng/mL and greater than 85 ng/mL for a therapeutic time period.

[00228] In one embodiment, maximum concentration of the first therapeutic agent in blood (whole blood, plasma, or serum) ("C_{max}") of a subject after administering it to the subject is a C_{max} of from about 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, or 1490 ng/dL to about 1500 ng/dL; from about 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137,

138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/dL to about 150 ng/dL; or from about 10, 10.5, 11, 11.5, 120, 12.5, 13, 13.5, 14, or 14.5 ng/dL to about 15 ng/dL.

[00229] In one embodiment, maximum concentration of the first therapeutic agent in blood (whole blood, plasma, or serum) ("C_{max}") of the subject following its administration is a C_{max} of from about 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, or 1490 ng/mL to about 1500 ng/mL; from about 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/mL to about 150 ng/mL; or from about 10, 10.5, 11, 11.5, 120, 12.5, 13, 13.5, 14, or 14.5 ng/mL to about 15 ng/mL.

[00230] In one embodiment, maximum concentration of the first therapeutic agent in blood (whole blood, plasma, or serum) ("C_{max}") of a subject following its administration is selected from about 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, or 1490 ng/dL. In one embodiment, the C_{max} of the first therapeutic agent in blood (whole blood, plasma, or serum) ("C_{max}") of a subject following its administration is selected from about 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/dL. In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 10, 10.5, 11, 11.5, 120, 12.5, 13, 13.5, 14, or 14.5 ng/dL.

[00231] In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1110, 1120, 1130, 1140, 1150, 1160, 1170, 1180, 1190, 1200, 1210, 1220, 1230, 1240, 1250, 1260, 1270, 1280, 1290, 1300, 1310, 1320, 1330, 1340, 1350, 1360, 1370, 1380, 1390, 1400, 1410, 1420, 1430, 1440, 1450, 1460, 1470, 1480, or 1490 ng/mL. In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/mL. In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 10, 10.5, 11, 11.5, 120, 12.5, 13, 13.5, 14, or 14.5 ng/mL.

[00232] In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 85, 95, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225, 235, 245, 255, 265, 275, 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395, 405, 415, 425, 435, 445, 455, 465, 475, 485, 495, 505, 515, 525, 535, 545, 555, 565, 575, 585, 595, 605, 615, 625, 635, 645, 655, 665, 675, 685, 695, 705, 715, 725, 735, 745, 755, 765, 775, 785, 795, 805, 815, 825, 835, 845, 855, 865, 875, 885, 895, 905, 915, 925, 935, 945, 955, 965, 975, 985, 995, 1005, 1015, 1025, 1035, 1045, 1055, 1065, 1075, 1085, 1095, 1105, 1115, 1125, 1135, 1145, 1155, 1165, 1175, 1185, 1195, 1205, 1215, 1225, 1235, 1245, 1255, 1265, 1275, 1285, 1295, 1305, 1315, 1325, 1335, 1345, 1355, 1365, 1375, 1385, 1395, 1405, 1415, 1425, 1435, 1445, 1455, 1465, 1475, 1485, 1495, or 1500 ng/dL. In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/dL. In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 ng/dL.

[00233] In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 85, 95, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225, 235, 245, 255, 265, 275, 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395, 405, 415, 425, 435, 445, 455, 465, 475, 485, 495, 505, 515, 525, 535, 545, 555, 565, 575, 585, 595, 605, 615, 625, 635, 645, 655, 665, 675, 685, 695, 705, 715, 725, 735, 745, 755, 765, 775, 785, 795, 805, 815, 825, 835, 845, 855, 865, 875, 885, 895, 905, 915, 925, 935, 945, 955, 965, 975, 985, 995, 1005, 1015, 1025, 1035, 1045, 1055, 1065, 1075, 1085, 1095, 1105, 1115, 1125, 1135, 1145, 1155, 1165, 1175, 1185, 1195, 1205, 1215, 1225, 1235, 1245, 1255, 1265, 1275, 1285, 1295, 1305, 1315, 1325, 1335, 1345, 1355, 1365, 1375, 1385, 1395, 1405, 1415, 1425, 1435, 1445, 1455, 1465, 1475, 1485, 1495, or 1500 ng/mL. In one embodiment, the C_{max} of the first therapeutic following its administration is selected from about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112,

113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/mL. In one embodiment, the C_{max} of the first therapeutic agent following its administration is selected from about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 ng/mL.

[00234] In one embodiment, the C_{max} of the first therapeutic agent after administering it to the subject ranges from about 85 ng/dL to 1500 ng/dL; from about 8.5 ng/dL to 150 ng/dL; or from about 0.85 ng/dL to 15 ng/dL. In one embodiment, the C_{max} of the first therapeutic agent in a subject's blood (whole blood, plasma, or serum) after its administration is selected from about 85, 95, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225, 235, 245, 255, 265, 275, 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395, 405, 415, 425, 435, 445, 455, 465, 475, 485, 495, 505, 515, 525, 535, 545, 555, 565, 575, 585, 595, 605, 615, 625, 635, 645, 655, 665, 675, 685, 695, 705, 715, 725, 735, 745, 755, 765, 775, 785, 795, 805, 815, 825, 835, 845, 855, 865, 875, 885, 895, 905, 915, 925, 935, 945, 955, 965, 975, 985, 995, 1005, 1015, 1025, 1035, 1045, 1055, 1065, 1075, 1085, 1095, 1105, 1115, 1125, 1135, 1145, 1155, 1165, 1175, 1185, 1195, 1205, 1215, 1225, 1235, 1245, 1255, 1265, 1275, 1285, 1295, 1305, 1315, 1325, 1335, 1345, 1355, 1365, 1375, 1385, 1395, 1405, 1415, 1425, 1435, 1445, 1455, 1465, 1475, 1485, or 1495 ng/dL to about 1500 ng/dL; from about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/dL to about 150 ng/dL; or from about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 ng/dL to about 15 ng/dL.

[00235] In one embodiment, the C_{max} of the first therapeutic agent following its administration ranges from about 85 ng/mL to 1500 ng/mL; from about 8.5 ng/mL to 150 ng/mL; or from about 0.85 ng/mL to 15 ng/mL. In one embodiment, the C_{max} of the first therapeutic following its administration is selected from about 85, 95, 105, 115, 125, 135, 145, 155, 165, 175, 185, 195, 205, 215, 225, 235, 245, 255, 265, 275, 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395, 405, 415, 425, 435, 445, 455, 465, 475, 485, 495, 505, 515, 525, 535, 545, 555, 565, 575, 585, 595, 605, 615, 625, 635, 645, 655, 665, 675, 685, 695, 705, 715, 725, 735, 745, 755, 765, 775, 785, 795, 805, 815, 825, 835, 845, 855, 865, 875, 885, 895, 905, 915, 925, 935, 945, 955, 965, 975, 985, 995, 1005, 1015, 1025, 1035, 1045, 1055, 1065, 1075,

1085, 1095, 1105, 1115, 1125, 1135, 1145, 1155, 1165, 1175, 1185, 1195, 1205, 1215, 1225, 1235, 1245, 1255, 1265, 1275, 1285, 1295, 1305, 1315, 1325, 1335, 1345, 1355, 1365, 1375, 1385, 1395, 1405, 1415, 1425, 1435, 1445, 1455, 1465, 1475, 1485, or 1495 ng/mL to about 1500 ng/mL; from about 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 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, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, or 149 ng/mL to about 150 ng/mL; or from about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, or 14.5 ng/mL to about 15 ng/mL.

[00236] In one embodiment, the total drug exposure over time, measured as the area under the curve ("AUC") of a plot of the concentration of the drug in blood (whole blood, plasma, or serum) of a subject following administration of the drug against time after administration of the drug ranges from about 150 ng hr/mL to about 8000 ng hr/mL; from about 15 ng hr/mL to about 800 ng hr/mL; or from about 1.5 ng hr/mL to about 80 ng hr/mL. In one embodiment, AUC is less than 8000 ng hr/mL and is greater than or equal to 150 ng hr/mL. In one embodiment, AUC is less than 800 ng hr/mL and is greater than or equal to 15 ng hr/mL. In one embodiment, AUC is less than 80 ng hr/mL and is greater than or equal to 1.5 ng hr/mL.

[00237] In one embodiment, the total drug exposure over time is an AUC of from about 100 ng hr/mL to about 8000 ng hr/mL; from about 10 ng hr/mL to about 800 ng hr/mL; or from about 1 ng hr/mL to about 80 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC of from about from about 150, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 2600, 2800, 3000, 3200, 3400, 3600, 3800, 4000, 4200, 4400, 4600, 4800, 5000, 5200, 5400, 5600, 5800, 6000, 6200, 6400, 6600, 6800, 7000, 7200, 7400, 7600, or 7800 ng hr/mL to about 8000 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC of from about 15, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, or 780 ng hr/mL to about 800 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC of from about from about 1.5, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, or 78 ng hr/mL to about 80 ng hr/mL.

[00238] In one embodiment, the total drug exposure over time is an AUC of from about 100 ng hr/mL to about 8000 ng hr/mL, from about 10 ng hr/mL to about 800 ng hr/mL; or from

about 1 ng hr/mL to about 80 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC of from about from about 150 ng hr/mL to about 7800, 7600, 7400, 7200, 7000, 6800, 6600, 6400, 6200, 6000, 5800, 5600, 5400, 5200, 5000, 4800, 4600, 4400, 4200, 4000, 3800, 3600, 3400, 3200, 3000, 2800, 2600, 2400, 2200, 2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, or 200 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC of from about from about 15 ng hr/mL to about 780, 760, 740, 720, 700, 680, 660, 640, 620, 600, 580, 560, 540, 520, 500, 480, 460, 440, 420, 400, 380, 360, 340, 320, 300, 280, 260, 240, 220, 200, 180, 160, 140, 120, 100, 80, 60, 40, or 20 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC of from about from about 1.5 ng hr/mL to about 78, 76, 74, 72, 70, 68, 66, 64, 62, 60, 58, 56, 54, 52, 50, 48, 46, 44, 42, 40, 38, 36, 34, 32, 30, 28, 26, 24, 22, 20, 18, 16, 14, 12, 10, 8, 6, 4, or 2 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC of from about 100 ng hr/mL to about 200 ng hr/mL; from about 10 ng hr/mL to about 20 ng hr/mL; or from about 1 ng hr/mL to about 2 ng hr/mL.

[00239] In one embodiment, the total drug exposure over time is an AUC selected from about 100, 150, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 2600, 2800, 3000, 3200, 3400, 3600, 3800, 4000, 4200, 4400, 46000, 4800, 5000, 5200, 5400, 5600, 5800, 6000, 6200, 6400, 6600, 6800, 7000, 7200, 7400, 7600, 7800, and 8000 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC selected from about 10, 15, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 4600, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, 780, and 800 ng hr/mL. In one embodiment, the total drug exposure over time is an AUC selected from about 1, 15, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 460, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, and 80 ng hr/mL.

[00240] In another aspect, provided herein are methods of treatment, or use of a composition to treat a disease state, which comprises administering to a subject in need of such treatment a combination of a first therapeutic agent and a second therapeutic agent, the method comprising:

(i) administering to the subject the first therapeutic agent including an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof;

(ii) monitoring levels of the compound of the first therapeutic agent or a metabolite thereof in the subject using pharmacokinetic profiling; and

(iii) administering the second therapeutic agent conditional on the level of the first therapeutic agent in the subject. In one embodiment, the monitoring step includes constructing a pharmacokinetic profile of the compound of the first therapeutic agent or a metabolite thereof for the subject using concentrations of the compound of the first therapeutic agent or a metabolite thereof in a plurality of samples obtained from the subject at time points suitable to

construct a pharmacokinetic profile. In one embodiment, at least two samples are collected at point-of-care or point of use by sampling or self-sampling on point-of-care devices or point of use devices or on matrices suitable for storage of the samples prior to quantitation of the compound or a metabolite thereof by a laboratory. In one embodiment, each point-of-care devices or point of use devices is capable of quantitating the compound or a metabolite thereof. In one embodiment, the pharmacokinetic profile includes pharmacokinetic parameters suitable for guiding dosing of the compound or a salt thereof for the subject. In one embodiment, the samples include from 2-12 samples. In one embodiment, the samples are collected over a time period of up to 8 hours, up to 24 hours, up to 48 hours, or up to 72 hours. In one embodiment, the pharmacokinetic parameters include at least one parameter selected from the group consisting of AUC, AUC_{inf}, T_{max}, C_{max}, time above threshold, steady state concentration, absorption rate, clearance rate, distribution rate, terminal T-1/2 or parameters drawn from noncompartmental pharmacokinetic (PK) or compartmental PK analysis, including physiological model-based compartmental PK analysis. In one embodiment, the treatment method further comprises generating a report including the pharmacokinetic profile of the subject. In one embodiment, the report includes a recommendation regarding dosing based on the pharmacokinetic profile of the subject. In one embodiment, a reduction in dosage of ONC201, the analog thereof, or the pharmaceutically acceptable salt thereof is indicated to reduce risk of toxicity based on one or more pharmacokinetic parameters. In one embodiment, the reduction in dosage of the compound or salt thereof is indicated based on time above threshold, wherein the threshold is the drug concentration above which toxicity occurs, or one or more of AUC, AUC_{inf}, mean residence time (MRT), exponentials defining the pharmacokinetic profile, volume of distribution at steady state (Vss), volume of distribution during the terminal phase (Vz) or combination of a group of pharmacokinetic variable to adequately describe the pharmacokinetic profile. In one embodiment, a dose adjustment of the compound or salt thereof is indicated to increase efficacy based on one or more pharmacokinetic parameters. In one embodiment, an increase in dosage of the compound or salt thereof is indicated based on one or more of AUC, AUC_{inf}, MRT, exponentials defining the pharmacokinetic profile, steady state volume (Vss) of distribution, volume of distribution during the terminal phase (Vz) or combination of a group of pharmacokinetic variables to adequately describe the pharmacokinetic profile. In one embodiment, the dose of the compound or salt thereof is adjusted to within 5% to 25% of a desired target value. In one embodiment, each of the samples is applied to the point-of-care device or the point of use device for determining the concentration of the compound or a metabolite thereof, wherein the point-of-care device or the point of use device comprises a lateral flow strip having a

construction and composition such that an application of one or more of the samples to the lateral flow strip causes a fraction of the drug in the sample to bind to with a component of the lateral flow strip such that a detectable signal proportional to the concentration of the drug in the applied sample is produced. In one embodiment, the samples are applied to matrices suitable for storage of the samples prior to quantitation by a laboratory. In one embodiment, the samples are stored as dried blood spots. In one embodiment, drug concentrations are measured by ELISA, LC MS MS, LC UV or LCMS. In one embodiment, the pharmacokinetic parameters include at least one of steady state concentration, absorption, and terminal $T_{1/2}$. In one embodiment, at least one of the samples is whole blood.

IX. MULTIMODAL THERAPEUTIC METHODS

[00241] In one aspect, provided herein are multimodal therapeutic methods in which administration of an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof to a subject in need of such treatment is supplemented by administration of other therapeutic modalities. In one embodiment, the multimodal therapeutic method comprises administering to a subject a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in conjunction with radiation therapy or after radiation is determined to not have been efficacious. In one embodiment, the multimodal therapeutic method comprises administering to a subject a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in conjunction with radiation therapy, wherein the pharmaceutical composition comprising theimipridone, such as ONC201, the analog thereof, or pharmaceutically acceptable salt thereof and the radiation therapy are administered concurrently or sequentially in any order. In one embodiment, the multimodal therapeutic method comprises administering to a subject a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in conjunction with radiation therapy in a sequential arrangement. In one embodiment, the multimodal therapeutic method comprises administering to a subject in need of such treatment a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof thereof concurrently with radiation therapy. In one embodiment, the multimodal therapeutic method is used for the treatment of cancer. In one embodiment, the multimodal therapeutic method includes administering to a cancer subject in need of such treatment a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof and irradiating cancer cells with a radiation beam. In one embodiment, the multimodal therapeutic method

uses the technique of conformal radiotherapy (CRT) to deliver a dose volume histogram (DVH) prescribed to a cancer subject. In one embodiment, the multimodal therapeutic method uses the technique of intensity modulated radiation therapy (IMRT) to deliver radiation to cancer cells. In one embodiment, the multimodal therapeutic method uses techniques that compensate for motion of tumors in the subject during treatment (e.g., where doses of radiation must be administered to a thoracic tumor which moves as the patient breathes). For example, the multimodal therapeutic method use Four Dimensional Computed Tomography (4D CT) scanning techniques to adjust the delivered radiation field to compensate for tumor motion over the breathing cycle.

[00242] Any suitable type of radiation, including gamma radiation which is given fractionated, IMRT (intensity modulated radiation therapy), gamma knife, proton therapy and brachytherapy can be used with the multimodal therapeutic method. Radiation therapy and administering an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof can be used to treat brain tumors such as glioblastoma or disease that has metastasized to the brain from lung cancer. The multimodal therapeutic method can be used to treat lung cancer, pancreatic cancer, rectal cancer, breast cancer, sarcoma, prostate cancer, gynecological malignancies, and lymphoma. The gamma knife is used frequently to treat brain metastases. In one embodiment, the multimodal therapeutic method includes use of proton therapy to treat cancer, including brain tumors, prostate cancer and any tumor proximate vital organs where it is very important to minimize toxicity to nearby normal tissue.

[00243] In one embodiment, a multimodal therapeutic method includes administering to a cancer subject in need of such treatment a pharmaceutical composition comprising an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof in combination with adoptive cell therapy (*e.g.*, CAR-T (JCAR 14, 15, 16, 17, KTE-C19, or CTL019); other T Cell (AFM13); or NK (CDNO-109 or NK-92)) either simultaneously or in combination.

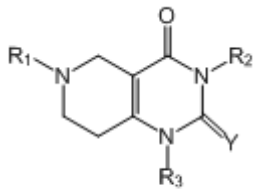
[00244] In one embodiment, the multimodal therapeutic method eliminates minimal residual disease without adding to toxicity resulting from treatment by an imipridone, such as ONC201, an analog thereof, or a pharmaceutically acceptable salt thereof. In one embodiment, the multimodal therapeutic method improves prognosis and/or reduces adverse side-effects associated with a disease state or condition in a subject undergoing treatment.

X. ADDITIONAL IMIPRIDONE DERIVATIVES, ANALOGS, AND SALTS

[00245] In one aspect, provided herein are compounds that are analogs of the compounds of formula (**10**) and methods of making them. Persons skilled in the art will understand that the

general principles and concepts described above in conjunction with ONC201 and compounds of formula (**10)** and their salts, including principles and concepts related to methods and pharmaceutical compositions, apply with equal force to the following analogs and salts thereof.

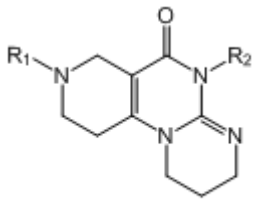
[00246] In one embodiment, the analogs have the structure of compound (**25**):



(25)

, wherein Y is NR_4 or O, and wherein R_1, R_2, R_3, and R_4 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. In one embodiment, R_1, R_2, R_3, and R_4 are optionally substituted. In one embodiment, some or all hydrogens in R_1, R_2, R_3, and R_4 are substituted by deuterium. In other embodiments, R_1, R_2, R_3, and R_4 are independently selected from the group consisting of H, $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, and $C_{1\text{-}4}$alkylthienyl, wherein $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, and $C_{1\text{-}4}$benzyl-piperazine are optionally substituted with $C_{1\text{-}4}$alkyl, hydroxyl, or halo. In still other embodiments, R_1, R_2, R_3, and R_4 are independently selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph.

[00247] In one embodiment, the analogs have the structure of compound (**26**):



(26)

, wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. In one embodiment, R_1 and R_2 are independently selected from the group consisting of H, $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, and $C_{1\text{-}4}$alkylthienyl, wherein $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl,

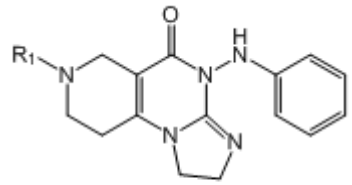
C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, R_1 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. In one embodiment, R_2 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph.

[00248] In one embodiment, R_1 is a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or –OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. In one embodiment, R_2 is a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or –OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen.

[00249] In one embodiment, R_1 is a H. In one embodiment, R_1 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[00250] In one embodiment, R_2 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the arylalkyl is substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3. In one embodiment, R_2 is a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. In one embodiment, R_2 is a substituted or an unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3.

[00251] In one embodiment, the analogs have the structure of compound (**27**):

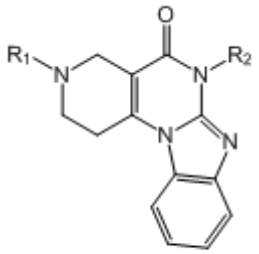


(27)

, wherein R_1 is H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. In one embodiment, R_1 is selected from the group consisting of H, $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, and $C_{1\text{-}4}$alkylthienyl, wherein $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, and $C_{1\text{-}4}$benzyl-piperazine are optionally substituted with $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkoxyl, hydroxyl, perhalogenated $C_{1\text{-}4}$alkyl, or halo. In one embodiment, R_1 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph.

[00252] In one embodiment, R_1 is a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or –OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. In one embodiment, R_1 is a H. In one embodiment, R_1 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkoxyl, hydroxyl, perhalogenated $C_{1\text{-}4}$alkyl, or halo.

[00253] In one embodiment, the analogs have the structure of compound (**28**):



(28)

, wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl,

acyl, and heterocycle radicals. In one embodiment, R_1 and R_2 are independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzyl-piperazine, and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, R_1 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, CH_2CH_2(4-N-benzyl-piperazine), and $(CH_2)_3CO$-4F-Ph. In one embodiment, R_2 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. In one embodiment, when R_1 is CH_2Ph, R_2 is not CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2,4-di F-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(4-CF_3-Ph).

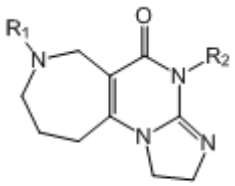
[00254] In one embodiment, R_1 is a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or –OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. In one embodiment, R_2 is a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or –OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen.

[00255] In one embodiment, R_1 is a H. In one embodiment, R_1 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[00256] In one embodiment, R_2 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the arylalkyl is substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3. In one embodiment, R_2 is a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. In one embodiment, R_2 is a substituted or an unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl,

or halo. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$.

[00257] In one embodiment, the analogs have the structure of compound (**29**):



(29)

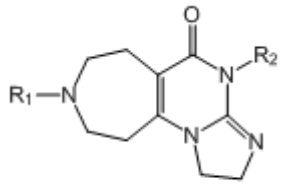
, wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. In one embodiment, R_1 and R_2 are independently selected from the group consisting of H, $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, and $C_{1\text{-}4}$alkylthienyl, wherein $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, and $C_{1\text{-}4}$benzyl-piperazine are optionally substituted with $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkoxyl, hydroxyl, perhalogenated $C_{1\text{-}4}$alkyl, or halo. In one embodiment, R_1 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3$CO-4F-Ph. In one embodiment, R_2 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3$CO-4F-Ph. In one embodiment, when R_1 is CH_2Ph, R_2 is not CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2,4-di F-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(4-CF_3-Ph).

[00258] In one embodiment, R_1 is a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: $-CH_3$, $-NO_2$, $-OCH_3$, $-CXH_2$, $-CX_2H$, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, or $-OC_pX_{2p+1}$, where p is an integer from 2 to 20 and where X is a halogen including refers to F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. In one embodiment, R_2 is a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: $-CH_3$, $-NO_2$, $-OCH_3$, $-CXH_2$, $-CX_2H$, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, or $-OC_pX_{2p+1}$, where p is an integer from 2 to 20 and where X is a halogen.

[00259] In one embodiment, R_1 is a H. In one embodiment, R_1 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

[00260] In one embodiment, R_2 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the arylalkyl is substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$. In one embodiment, R_2 is a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. In one embodiment, R_2 is a substituted or an unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$.

[00261] In one embodiment, the analogs have the structure of compound (**30**):



(30)

, wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl, acyl, and heterocycle radicals. In one embodiment, R_1 and R_2 are independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzyl-piperazine, and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, R_1 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. In one embodiment, R_2 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. In one embodiment, when R_1 is CH_2Ph, R_2 is not CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph

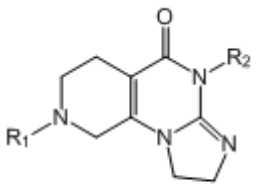
and R_2 is CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2,4-di F-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(4-CF_3-Ph).

[00262] In one embodiment, R_1 is a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: $-CH_3$, $-NO_2$, $-OCH_3$, $-CXH_2$, $-CX_2H$, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, or $-OC_pX_{2p+1}$, where p is an integer from 2 to 20 and where X is a halogen including refers to F, Cl, Br, or I, preferably, F, Cl, or Br, more preferably, F or Cl. In one embodiment, R_2 is a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: $-CH_3$, $-NO_2$, $-OCH_3$, $-CXH_2$, $-CX_2H$, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, or $-OC_pX_{2p+1}$, where p is an integer from 2 to 20 and where X is a halogen.

[00263] In one embodiment, R_1 is a H. In one embodiment, R_1 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkoxyl, hydroxyl, perhalogenated $C_{1\text{-}4}$alkyl, or halo.

[00264] In one embodiment, R_2 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkoxyl, hydroxyl, perhalogenated $C_{1\text{-}4}$alkyl, or halo. In one embodiment, the arylalkyl is substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$. In one embodiment, R_2 is a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. In one embodiment, R_2 is a substituted or unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkoxyl, hydroxyl, perhalogenated $C_{1\text{-}4}$alkyl, or halo. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$.

[00265] In one embodiment, the analogs have the structure of compound (**31**):



(31)

, wherein R_1 and R_2 independently represent H, alkyl, cycloalkyl, cycloalkylalkyl, carboxyl, haloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, aralkyl, hydroxyalkyl, alkoxy, aryloxy, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, alkanoyl, mercapto, alkylthio, arylthio, alkylsulfinyl, arylsulfinyl, alkylsulfonyl, arylsulfonyl, heteroaryl,

acyl, and heterocycle radicals. In one embodiment, R_1 and R_2 are independently selected from the group consisting of H, C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, C_{1-4}benzyl-piperazine, and C_{1-4}alkylthienyl, wherein C_{1-4}alkyl, C_{1-4}alkylphenyl, C_{1-4}alkylphenylketone, and C_{1-4}benzyl-piperazine are optionally substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, R_1 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. In one embodiment, R_2 is selected from the group consisting of H, CH_3, CH_2Ph, CH_2-((2-Cl)-Ph), CH_2-(2-thienyl), CH_2CH_2Ph, CH_2CH_2(4-N-benzyl-piperazine), CH_2-(2,4-di F-Ph), CH_2-((2-CH_3)-Ph), $CH_2CHOHPh$, and $(CH_2)_3CO$-4F-Ph. In one embodiment, when R_1 is CH_2Ph, R_2 is not CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2-CH_3-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2,4-di F-Ph). In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(4-CF_3-Ph).

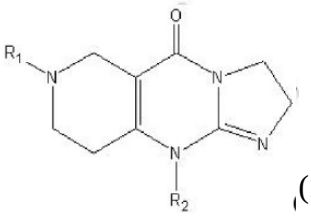
[00266] In one embodiment, R_1 is a benzyl optionally substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or –OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl. In one embodiment, R_2 is a benzyl substituted with one or more of the following substituents alone or in combination in the ortho, meta, and/or para positions of the benzyl ring: -CH_3, -NO_2, -OCH_3, -CXH_2, -CX_2H, -CX_3, -$CH_2(CX_3)$, -$CH(CX_3)_2$, -$C(CX_3)_3$, -C_pX_{2p+1}, -OCX_3, or –OC_pX_{2p+1}, where p is an integer from 2 to 20 and where X is a halogen.

[00267] In one embodiment, R_1 is a H. In one embodiment, R_1 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo.

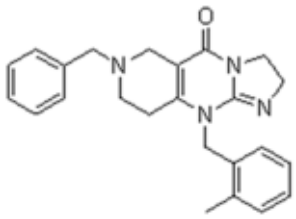
[00268] In one embodiment, R_2 is a substituted or an unsubstituted arylalkyl, such as a benzyl or phenylethyl group. In one embodiment, the arylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl, or halo. In one embodiment, the arylalkyl is substituted with one or more substituents selected from the group consisting of halo, -CH_3, -CF_3, and -OCH_3. In one embodiment, R_2 is a substituted or an unsubstituted heterocycloalkylalkyl, such as a morpholinoalkyl or piperazinylalkyl group. In one embodiment, R_2 is a substituted or an unsubstituted heteroarylalkyl, such as an isoxazolidinylmethyl or pyridylmethyl group. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with C_{1-4}alkyl, C_{1-4}alkoxyl, hydroxyl, perhalogenated C_{1-4}alkyl,

or halo. In one embodiment, the heterocycloalkylalkyl or heteroarylalkyl is substituted with one or more substituents selected from the group consisting of halo, $-CH_3$, $-CF_3$, and $-OCH_3$.

[00269] In one embodiment, provided herein are compounds of formula (**100**):



(**100**), wherein R_1 and R_2 are independently selected from H, alkyl, cycloalkyl, cycloalkylalkyl, heterocycloalkyl, heterocycloalkylalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl, alkoxyalkyl, alkoxycarbonyl, aralkoxy, aralkylthio, and acyl radicals. In one embodiment, R_1 is CH_2Ph and R_2 is CH_2-(2-CH_3-Ph), which is an ONC201 linear isomer (i.e., TIC-10)



TIC-10, which lacks anti-cancer activity (Jacob *et al.*, *Angew. Chem. Int. Ed.*, (2014) **53**:6628; Wagner *et al.*, *Oncotarget* (2015) **5**(24):12728). But as shown in the Examples TIC-10 is a CXCR7 agonist. CXCR7 agonists can be used for liver regeneration and preventing or treating liver fibrosis (*Nature* (2014) **505**:97).

[00270] In one embodiment, R_1 and R_2 are independently selected from the group consisting of H, $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, $C_{1\text{-}4}$alkylthienyl, $C_{1\text{-}4}$alkylpyridinyl, $C_{1\text{-}4}$alkylisoxazolidinyl, $C_{1\text{-}4}$alkylmorpholinyl, $C_{1\text{-}4}$alkylthiazolyl, and $C_{1\text{-}4}$alkylpyrazinyl wherein $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkylphenyl, $C_{1\text{-}4}$alkylphenylketone, $C_{1\text{-}4}$benzyl-piperazine, $C_{1\text{-}4}$alkylthienyl, $C_{1\text{-}4}$alkylpyridinyl, $C_{1\text{-}4}$alkylisoxazolidinyl, $C_{1\text{-}4}$alkylmorpholinyl, $C_{1\text{-}4}$alkylthiazolyl, and $C_{1\text{-}4}$alkylpyrazinyl are optionally substituted with $C_{1\text{-}4}$alkyl, $C_{1\text{-}4}$alkoxyl, hydroxyl, perhalogenated $C_{1\text{-}4}$alkyl, or halo. In one embodiment, R_1 and/or R_2 is a substituted or unsubstituted, arylalkyl or heteroarylalkyl. In one embodiment, the heteroarylalkyl is selected from $C_{1\text{-}4}$alkylpyrrolyl, $C_{1\text{-}4}$alkylfuryl, $C_{1\text{-}4}$alkylpyridyl, $C_{1\text{-}4}$alkyl-1,2,4-thiadiazolyl, $C_{1\text{-}4}$alkylpyrimidyl, $C_{1\text{-}4}$alkylthienyl, $C_{1\text{-}4}$alkylisothiazolyl, $C_{1\text{-}4}$alkylimidazolyl, $C_{1\text{-}4}$alkyltetrazolyl, $C_{1\text{-}4}$alkylpyrazinyl, $C_{1\text{-}4}$alkylpyrimidyl, $C_{1\text{-}4}$alkylquinolyl, $C_{1\text{-}4}$alkylisoquinolyl, $C_{1\text{-}4}$alkylthiophenyl, $C_{1\text{-}4}$alkylbenzothienyl, $C_{1\text{-}4}$alkylisobenzofuryl, $C_{1\text{-}4}$alkylpyrazolyl, $C_{1\text{-}4}$alkylindolyl, $C_{1\text{-}4}$alkylpurinyl, $C_{1\text{-}4}$alkylcarbazolyl, $C_{1\text{-}4}$alkylbenzimidazolyl, and $C_{1\text{-}4}$alkylisoxazolyl.

[00271] In one embodiment, R_1 and/or R_2 is a benzyl optionally substituted with one or more of the following substituents on the benzyl ring: X, $-CH_3$, $-NO_2$, $-OCH_3$, $-CN$, $-CXH_2$, $-CX_2H$, C_2-C_4 alkyl, $-CX_3$, $-CH_2(CX_3)$, $-CH(CX_3)_2$, $-C(CX_3)_3$, $-C_pX_{2p+1}$, $-OCX_3$, $-OC_pH_{2p+1}$, $-OC_pX_{2p+1}$,

OR^m, SR^m, NR^mR^n, $NR^mC(O)R^n$, SOR^m, SO_2R^m, $C(O)R^m$, and $C(O)OR^m$; R^m and R^n are independently selected from H or a C_1-C_4 alkyl; and where p is an integer from 2 to 20 and X is a halogen, including F, Cl, Br, or I; preferably, F, Cl, or Br; more preferably, F or Cl.

XI. EXAMPLES

[00272] It should be understood that the description and examples below are meant for purposes of illustration only and are not meant to limit the scope of this disclosure. The examples below are meant to illustrate the embodiments disclosed and are not to be construed as being limitations to them. Additional compounds, other than those described below, may be prepared by the following reaction schemes or appropriate variations or modifications thereof.

Example 1. Synthesis of 2-Chlorobenzylamino-2-imidazoline hydriodide

[00273] To a stirred solution of 2-methylthio-2-imidazoline hydriodide (244 mg, 1.00 mMol) in dry dioxane (2.0 mL) was added 2-chlorobenzylamine (141 mg, 1.0 mMol). The reaction mixture was stirred for 90 min at 70 °C under argon. The solution was cooled to room temperature, filtered on a sintered funnel, washed with cold dioxane (2 mL) and dried under vacuum. The white solid compound 4•HI (R_2=2-chlorobenzyl) was obtained (242 mg, 72%) and used without further purification.

Example 2. Synthesis of 2-Chlorobenzylamino-2-imidazoline

[00274] To a stirred solution of 2-chlorobenzylamino-2-imidazoline hydriodide (242 mg, 0.72 mMol) in water (3 mL), was added 1.0 N sodium hydroxide (2 mL) at 7 °C. The reaction mixture was stirred for 30 min at 7 °C under argon. After that methylene chloride (5 mL) was added and the mixture stirred for another 5 min. The reaction mixture was extracted with methylene chloride (2× 2.5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The resulting free base (150 mg, 100%) was obtained as a viscous liquid and was used for the next reaction without any further purification. MS(ESI) 210(M+H).

Example 3. Synthesis of Methyl-1-benzyl 4-oxo-3-piperidine carboxylate (Compound (**6**)).

[00275] To a stirred methyl-1-benzyl 4-oxo-3-piperidine carboxylate hydrochloride (5.7 g, 20 mMol) in ethyl acetate (50 mL), was added triethylamine (6 mL) at 7 °C. The reaction mixture was stirred for 30 min at 7 °C under an argon atmosphere. The reaction mixture was extracted with ethyl acetate (2× 50 mL) and washed with water (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The resulting free base residue (5, R_1=benzyl) as a viscous oil was used in the next reaction without any further purification MS(ESI) 248(M+H)

Example 4. Synthesis of ONC202 (Compound (**14**))

[00276] To a solution of 2-chlorobenzylamino-2- imidazoline (150 mg, 0.72 mMol), methyl 1-benzyl 4- oxo-3-piperidine carboxylate (5, R_1=benzyl) (195 mg, 0.79 mMol) in 1-butanol (2 mL) was added PPTS (10 mg) and the mixture was stirred at room temperature for 48 h. After that the reaction mixture was refluxed at 125 °C to 130 °C for 2h. The solvents were removed under vacuum, extracted with ethyl acetate (10 mL), and washed with saturated sodium bicarbonate solution (2×10 mL) and water (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude free base was purified by RP HPLC (10%-40% acetonitrile / water) to give ONC902 TFA salt as a white solid (228 mg, 50% yield) MS(ESI) 407 (M+H).

[00277] The same process was used starting with different benzylamines to prepare various analogs, *e.g.*, ONC203, 204, 205, 206, 912, 210, 211, 212, 213, 214, 217, 218, 219, 220, 221, 222, 223, 224, 225, and 226.

Example 5. Synthesis of ONC207 (Compound (**19**))

[00278] To a suspension of 60% sodium hydride (3.5 g, 88 mMol) in dry toluene (50 mL), dimethyl carbonate (4.32 g, 48.0 mMol) was added dropwise in 0.5 h at room temperature under nitrogen. After addition of a few drops of methanol, 1-tert-butoxycarbonyl-4-piperidone (4.8 g, 24 mMol) dissolved in dry toluene (20 mL) was added dropwise to the reaction mixture while stirring at 80 °C over 1h. The reaction mixture was stirred for 3 h at the same temperature and then cooled to 0 °C (ice bath) and adjusted to pH 6-6.5 with acetic acid. The resulting cold mixture was diluted with water (10mL) and adjusted to pH 8 with 5% sodium hydroxide solution. The toluene layer was separated and the aqueous layer was extracted with toluene (20 mL). The combined organic layer was dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The compound was dried in vacuum to give methyl-1-tert-butoxycarbonyl- 4-oxo-3-piperidine carboxylate (5.0 g, 80%). The compound obtained was carried to the next reaction without any further purification.

[00279] 2-methybenzylamino-2-imidazoline (190 mg, 1 mMol), methyl 1-tert-butoxycarbonyl- 4-oxo-3-piperidine carboxylate (315 mg, 1.1 mMol) in 1-butanol (2 mL) was added PPTS (10.0 mg) and the mixture was stirred at room temperature for 48 h. After that the reaction mixture was refluxed at 125 °C to 130 °C for 2h. The solvents were removed under vacuum, extracted with ethyl acetate (10 mL), washed with saturated sodium bicarbonate solution (2×10 mL) and water (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude free base was cleaved with 10% trifluoroacetic acid in

dichloromethane, purified by RP HPLC (10%-40% acetonitrile/water) to give ONC907 (262 mg, 50%) TFA salt as a white solid MS(ESI) 297 (M+H).

Example 6. Synthesis of ONC209 (Compound (**21**))

[00280] A mixture of ONC907 (100 mg, 0.2 mMol), phenylethyl bromide (55.0 mg, 0.28 mMol) and potassium carbonate (150 mg, 1.0 mMol) in N,N-dimethylformamide (3 mL) was heated to 70 °C for 12 h. The solvents were removed under vacuum, extracted with ethyl acetate (10 mL), and washed with water (5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude free base was purified by RP HPLC (10%-40% acetonitrile/water) to give ONC209 (62 mg, 50%) TFA salt as a white solid MS(ESI) 401 (M+H).

[00281] The same process was used starting with different halides to give ONC215 and 214. Compounds 227, 228, 229, 230, 231, 232, 233, 234, 235, and 236 were prepared using an analogous process from Examples 1 and 5 starting with different benzylamines. Then treating the intermediate compound where R_1 is H with different halides as above.

[00282] Compound ONC216 was prepared from ONC215 by treatment with TFA.

[00283] Compound (**72**) was prepared by reacting the precursor NH compound prepared in analogy to Example 5 and treating it with styrene oxide.

Example 7. Synthesis of ONC208 (Compound (**20**))

[00284] To a solution of 2-methylbenzylamino-2-imidazoline (190.0 mg, 1.0 mmol), methyl 1-methyl 4-oxo-3-piperidine carboxylate (185.0 mg, 1.0 mMol) in 1-butanol (2.0 mL) was added PPTS (10.0 mg) and the mixture was stirred at room temperature for 48 h. After that the reaction mixture was refluxed at 125 °C to 130 °C for 2h. The solvents were removed under vacuum, extracted with ethyl acetate (10 mL), washed with saturated sodium bicarbonate solution (2×10 mL) and water (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated. The crude free base was purified by HPLC 10%-40% acetonitrile and water to give ONC908 (270.0 mg, 50%) TFA salt as a white solid MS(ESI) 311 (M+H).

Example 8. Synthesis of ONC201 (Compound (**1**))

[00285] To a stirred 800 mL saturated $NaHCO_3$ in a 2 L round bottom flask, compound (**3**) (239.7 g, 0.845 mol, 1.6 equiv) was added in portions. n-Butanol (500 mL) was added to the resulting mixture, which was stirred for 30 min and then transferred to a separating funnel. The organic phase, containing compound (**4**), was separated and transferred to a 2 L three-neck round bottom flask equipped with mechanical stirring, N_2 inlet, a thermocouple, a condenser and a Dean-Stark trap.To the contents of the flask, Compound (**5**) (100 g, 0.528 mol, 1 equiv)

and pyridinium p-toluenesulfonate (PPTS) (6.63 gm 0.026 mol, 5 mol%) were added. The resulting mixture was heated to reflux for 6 hours. Water in the reaction mixture was separated into the Dean-Stark trap as necessary. Refluxing temperature increased from 93 °C to 118 °C. Reaction progress was monitored by HPLC. When the peak area of compound (**1**) on HPLC remained constant with the reaction time, the reaction was stopped.

Example 9. Synthesis of Di-Salt of ONC201 (Compound (**1**)•2HCl)

[00286] Without isolation of the compound (**1**), the reaction mixture from Example 8 was washed with water (500 mL) and diluted with methyl tert-butyl ether (MTBE) (800 mL). The organic phase was washed with water (500 mL × 2) and transferred to a 3 L three-neck round bottom flask equipped with mechanical stirring, N_2 inlet, a thermocouple, a condenser and a Dean-Stark trap. While agitating the reaction mixture, 1 N HCl in dioxane-MTBE solution was added dropwise (4 N HCl in dioxane: 300 mL, 1.2 mol, 2.27 equiv; MTBE: 1200 mL) until no more solid precipitated out of the reaction mixture upon addition of HCl. The reaction mixture was heated to reflux at 60-65 °C for 2 hours. Water was separated into the Dean-Stark trap as necessary. Upon cooling to room temperature, the solid precipitate was filtered through a sintered glass funnel and washed with n-butanol-MTBE (1: 2, 600 mL) and MTBE (600 mL) respectively. The solid was dried in a vacuum oven at 65°C overnight (16 hours) to afford 200 g yellow solid.

[00287] To a 2 L three-neck round bottom flask equipped with mechanical stirring, N_2 inlet, a thermocouple and a condenser, the above solid (200 g) was added, followed by ethanol (1000 mL). The mixture was heated to reflux at 78°C for 2 hours. Upon cooling to room temperature, the solid was filtered through a sintered glass funnel and washed with ethanol (200 mL × 3). The wet solid was dried in the vacuum oven at 85°C for 3 days until the residual solvent met specification. 120 g of compound (2) was obtained as a white solid in a yield of 49%, with HPLC purity 99.7%.

Example 10. Activity of Imipridones

[00288] A number of imipridones were prepared based on the syntheses above. For each compound, viability of human cancer cells at 72 hours post-treatment with the compound was measured. The change in potency (relative to ONC201) was determined and shown in Table 3.

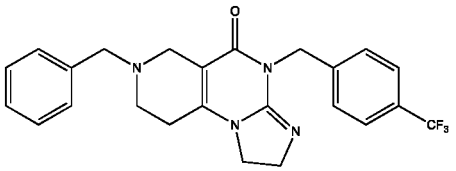
TABLE 3: RELATIVE POTENCY OF ONC201 ANALOGS

No.	Identifier	R_1	R_2	Relative Potency*
1	**ONC201**	CH_2Ph	CH_2-((2-CH_3)-Ph)	**N/A**
14	**ONC202**	CH_2Ph	CH_2(2-Cl-Ph)	**B**

No.	Identifier	R_1	R_2	Relative Potency*
15	ONC203	CH_2Ph	CH_2-(2-thienyl)	C
16	ONC204	CH_2Ph	CH_2CH_2Ph	C
17	ONC205	CH_2Ph	CH_2CH_2(4-N-benzyl-piperazine)	C
18	ONC206	CH_2Ph	CH_2-(2,4-di F-Ph)	A
19	ONC207	H	CH_2-((2-CH_3)-Ph)	B
20	ONC208	CH_3	CH_2-((2-CH_3)-Ph)	B
21	ONC209	CH_2CH_2Ph	CH_2-((2-CH_3)-Ph)	B
32	ONC215	$(CH_2)_3$-NH-BOC	CH_2-((2-CH_3)-Ph)	B
33	ONC216	$(CH_2)_3$-NH_2	CH_2-((2-CH_3)-Ph)	B
41	ONC210	CH_2Ph	CH_2-(3,5-di F-Ph)	B
51	ONC211	CH_2Ph	CH_2-(3,4-di Cl-Ph)	B
52	ONC212	CH_2Ph	CH_2-(4-CF_3-Ph)	A
53	ONC213	CH_2Ph	CH_2-(3,4-di F-Ph)	A
54	ONC214	$CD_2C_6D_5$	CH_2-((2-CH_3)-Ph)	B
43	ONC217	CH_2Ph	CH_2(2-F-Ph)	C
55	ONC218	CH_2Ph	CH_2(2-CH_3, 4-F-Ph)	A
56	ONC219	CH_2Ph	CH_2-(2,4-di Cl-Ph)	A
57	ONC220	CH_2Ph	CH_2-((4-OCH_3)-Ph)	A
35	ONC222	CH_2Ph	CH_2-(3-isoxazolidinyl)	C
36	ONC224	CH_2Ph	CH_2CH_2-(4-morpholinyl)	A
38	ONC221	H	CH_2-(4-CF_3-Ph)	A
72	ONC225	CH_2Ph	CH_2-(2-F, 4-CF_3-Ph)	A
37	ONC223	CH_2Ph	CH_2-(4-CH_3-Ph)	A
34	ONC226	CH_2Ph	CH_2-(3-pyridinyl)	A
77	ONC231	CH_2-3-pyridyl	CH_2-(4-CF_3-Ph)	A
78	ONC232	CH_2-4-methyl-2-thiazolyl	CH_2-(4-CF_3-Ph)	B
79	ONC233	CH_2-2-pyrazinyl	CH_2-(4-CF_3-Ph)	B
81	ONC234	CH_2-(3,4-di Cl-Ph)	CH_2-(4-CF_3-Ph)	A
83	ONC236	CH_2-3-thienyl	CH_2-(4-CF_3-Ph)	A
84	ONC237	$CH_2CH(OH)Ph$	CH_2-(4-CF_3-Ph)	C
73	ONC227	CH_2-(4-CF_3-Ph)	CH_2-(4-CF_3-Ph)	B
74	ONC228	CH_2-(4-F-Ph)	CH_2-(4-CF_3-Ph)	A
75	ONC229	CH_2-(4-OCH_3-Ph)	CH_2-(4-CF_3-Ph)	B
76	ONC230	4-F-Ph-4-oxobutyl	CH_2-(4-CF_3-Ph)	A

* Relative to the potency of ONC201; **A** Indicates a potency increase of >2-fold of ONC201; **B** Indicates potency that is within 2-fold of ONC201; and **C** Indicates a potency decrease of >2-fold of ONC201.

ONC212



[00289] The IC_{50} of ONC201 and ONC212 (5nM - 5μM, 72h) upon treatment of several acute myeloid leukemia (AML) cell lines (n=3) were determined and shown below in Table 11.

TABLE 11

AML cell line	ONC201 IC_{50} (μM)	ONC212 IC_{50} (μM)
MV411	3.25	0.01
HL60	>5	0.21
MOLM14	3.92	0.01

[00290] Cell viability of MV411 AML cells treated with ONC212 and cytarabine (5nM – 5μM, 24h) (n=3) was measured (Figure 29A). Furthermore, cell viability MOLM14, MV411 AML cells, MRC5 lung fibroblasts and Hs27a bone marrow cells treated with ONC212 (5nM – 5μM, 72h) (n=3) was measured (Figure 29B). Cell viability of MOLM14 and MV411 AML cells treated with ONC212 (250 nM) for 4, 8, 24, 48, 72 and 96h was measured. ONC212 medium was replaced by fresh medium at these time points and cell viability was determine at 96h for all samples. (n=2) (Figure 29C).

[00291] In addition, a single dose of compound (**52**) (ONC212) by oral or intraperitoneal administration to human colon cancer xenograft-bearing mice resulted in significant reduction of tumor volume compared to vehicle-treated control cohorts (Figure 24). Compound (**52**) has a wide therapeutic window, as it is well tolerated at doses at least up to 225 mg/kg in mice.

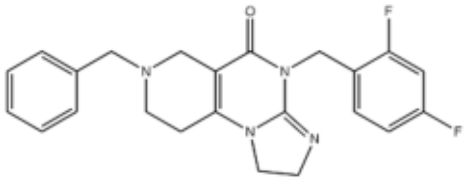
[00292] Furthermore, ONC212 demonstrated efficacy in ONC201-resistant AML xenograft model (Figure 30). MV411 AML cells (5×10^6) were subcutaneously implanted in the flanks of athymic nude. ONC212 and ONC201 were administered orally (PO) as indicated. Tumor volume (A and B) and body weight (C) (n=10) was measured on indicated days. * represents $p < 0.05$ relative to vehicle.

[00293] ONC212 efficacy in AML was evaluated *in vitro* and was upto 400 fold more potent compared to ONC201 (Table 11). ONC212 was also efficacious in AML cells resistant to standard of care cytarabine (Fig 29A). Despite robust improvement in efficacy ONC212 maintains a wide therapeutic window *in vitro* and is non-toxic to normal cells at efficacious concentrations (Fig 29B). An 8 hr exposure of ONC212 at 250nM was sufficient to cause robust reduction in cell viability in MOLM14 and MV411 AML cells (Fig 29C). At least 24-48h exposure was required with ONC201 for efficacy.

[00294] ONC212 efficacy was determined in a leukemia xenograft model with MV411 AML cells resistant to standard-of-care cytarabine (Fig 30). ONC212 50 mg/kg significantly reduced leukemia xenograft tumor growth with oral weekly administration while ONC201 was not efficacious in this model at similar doses (Fig 30A). Interesting, biweekly ONC212 dosing with 25 mg/kg and weekly/biweekly dosing with 5 mg/kg was not efficacious (Fig 30B). None of these ONC212 administration regimens were associated with body weight loss (Fig 30C) or gross observations.

[00295] ONC212 25 mg/kg represents NOAEL in mouse and rat non-GLP oral single dose studies which is also the efficacious dose in mouse xenograft studies. ONC212 is approximately 10 fold more toxic compared to ONC201 (NOAEL 225 mg/kg in rat non-GLP oral single dose study).

ONC206



[00296] ONC206 demonstrated efficacy in a Ewing's sarcoma xenograft model (Fig. 31). MHH-ES-1 Ewing's sarcoma cells (5×10^6) were subcutaneously implanted in the flanks of athymic nude mice. ONC206 (PO) and methotrexate (IV) were administered on day 1 and day 13 as indicated. Tumor volume (Fig. 31A) and body weight (Fig. 31B) (n=4) was measured on indicated days.

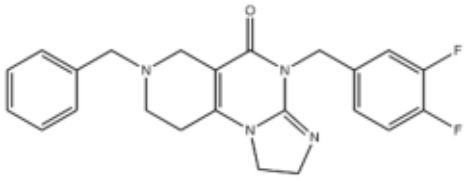
[00297] In addition, the IC_{50} of ONC201 and ONC206 (5nM - 5μM, 72h) upon treatment of several cell lines (n=3) were determined and shown below in Table 11.

TABLE 12

Cell line	ONC201 IC_{50} (μM)	ONC206 IC_{50} (μM)
MV411 (AML)	3.25	0.2
K562 (CML)	>5	0.22
MOLM14 (AML)	3.92	0.27
MHH-ES-1 (Ewing's sarcoma)	5.65	0.61
HFF (Normal Fibroblast)		>5

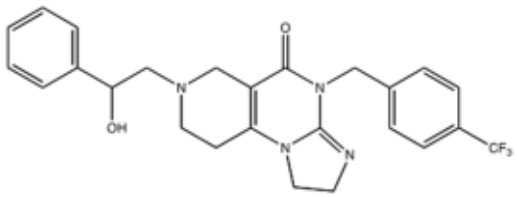
[00298] ONC206 showed up to 20 fold improvement compared to ONC201 in *in vitro* potency with no in vitro toxicity to normal cells at therapeutic doses (Table 12). With ONC206, only 2-fold increased toxicity (NOAEL 125 mg/kg) was noted overall relative to ONC201 (NOAEL 225 mg/kg) in rat non-GLP oral single dose study. *In vivo* efficacy in Ewing's sarcoma model with no toxicity (Fig 31). ONC206 efficacy was comparable to chemotherapy methotrexate, but chemotherapy was associated with body weight loss.

ONC213



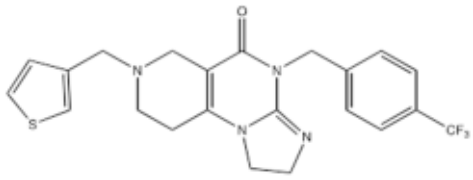
[00299] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC213 selectively targets DRD2/3 and GPR132/91 (Figure 32). Dual targeting of DRD2/3 and GPR132/91 represents a novel strategy for anti-cancer efficacy without toxicity. ONC213 is a DRD2/3 inhibitor and a GPR132/91 agonist. DRD2/3 potency of ONC213 is more than ONC201 but less than ONC206. GPR132 potency of ONC213 is less than ONC212. Specifically, ONC213 demonstrated *in vitro* anti-cancer potency in HCT116/RPMI8226 cancer cells similar to ONC212, but *in vitro* toxicity to normal cells was reduced compared to ONC212 (Figure 33). The safety profile of ONC213 confirmed in mouse MTD study with NOAEL 75 mg/kg three times that of ONC212 (25mg/kg). The GPR91 agonist activity of ONC213 provides an opportunity for immunology, immune-oncology and hematopoietic applications (*Nature Immunology* **9**:1261 (2008); *J Leukoc Biol.* **85**(5):837 (May 2009)).

ONC237



[00300] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC237 selectively targets DRD5 and GPR132 (Figure 34). ONC237 is a GPR132 agonist and DRD5 antagonist and has reduced anticancer efficacy (IC_{50} 31.2 µM) compared to ONC201. This data show that combining GPR132 agonist activity with DRD5 (D1-like dopamine receptor) antagonist activity results in poor anti-cancer effects compared to ONC213 which combines GPR132 agonist and DRD2/3 antagonist activity.

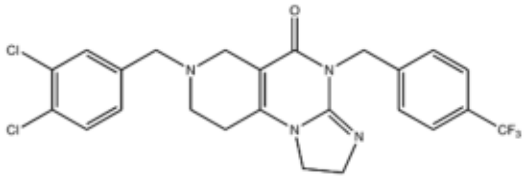
ONC236



[00301] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC236 is a highly selective

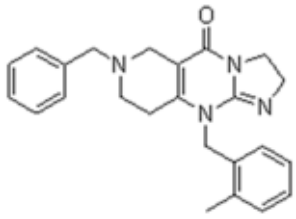
GPR132 agonist (Figure 35). ONC236 has anticancer efficacy (IC_{50} 88nM) comparable to ONC212 (10nM) better than ONC206/ONC201, completeness of response is better than ONC201 but not ONC212 in HCT116 cells.

ONC234



[00302] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC234 is a broad spectrum and potent GPCR targeting small molecule (Figures 36 and 38). ONC234 hits several GPCRs including activity as an antagonist activity for adrenergic, histamine, serotonin, CHRM, CCR, DRD2/5 receptors, as well as CXCR7 agonist activity.ONC236 has anticancer efficacy (IC_{50} 234nM) similar to ONC206, completeness of response same as ONC212, and better than ONC201 in HCT116 cells.

ONC201 LINEAR ISOMER (TIC-10)



(TIC-10)

[00303] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that the ONC201 linear isomer (TIC-10) is a CXCR7 agonist (Figure 37). CXCR7 agonists can be used for liver regeneration and preventing/treating fibrosis, such as liver fibrosis (*Nature* **505**:97 (2014)). Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue, including as a result of wound healing. Examples of fibrosis includes, pulmonary fibrosis, including cystic fibrosis and idiopathic pulmonary fibrosis; radiation-induced lung injury following treatment for cancer; liver fibrosis (cirrhosis); heart fibrosis, including atrial fibrosis, endomyocardial fibrosis, and old myocardial infarction; glial scar; arthrofibrosis; Crohn's Disease; dupuytren's contracture; keloids; mediastinal fibrosis; myelofibrosis; Peyronie’s disease; nephrogenic systemic fibrosis; progressive massive fibrosis; retroperitoneal fibrosis; scleroderma/systemic sclerosis; and adhesive capsulitis.

Example 11. GPCR Antagonism of ONC201

[00304] ONC201 was evaluated in a whole cell, functional assay of β-Arrestin G protein–coupled receptor (GPCR) activity that directly measures dopamine receptor activity by detecting the interaction of β-Arrestin with the activated GPCR that serves as a reporter. For each dopamine receptor (DRD1, DRD2S, DRD2L, DRD3, DRD4, and DRD5), cell lines overexpressing reporter constructs were expanded from freezer stocks. Cells were seeded in a total volume of 20 μL into white walled, 384-well microplates and incubated at 37°C prior to testing, with antagonist followed by agonist challenge at the EC_{80} concentration. Intermediate dilution of sample stocks was performed to generate 5× sample in assay buffer. 3.5 μL of 5× sample was added to cells and incubated at 37°C or room temperature for 30 minutes. Vehicle concentration was 1%. 5 μL of 6× EC_{80} agonist in assay buffer was added to cells and incubated at 37°C or room temperature for 90 or 180 minutes prior to assay readout. % Antagonism was calculated using the following formula %: Antagonism =100% × (1 — (mean RLU of test sample — mean RLU of vehicle control) / (mean RLU of EC_{80} control — mean RLU of vehicle control).

Example 12: Selective Antagonism of DRD2 by ONC201.

[00305] ONC201 is a first-in-class small molecule discovered in a phenotypic screen for p53-independent inducers of tumor selective proapoptotic pathways. Oral ONC201 is being evaluated as a new therapeutic agent in five early phase clinical trials for select advanced cancers based on pronounced efficacy in aggressive and refractory tumors and excellent safety.

[00306] In this Example, the prediction and validation of selective direct molecular interactions between ONC201 and specific dopamine receptor family members are reported. Experimental GPCR profiling indicated that ONC201 selectively antagonizes the D2-like, but not D1-like, dopamine receptor subfamily. Reporter assays in a heterologous expression system revealed that ONC201 selectively antagonizes both short and long isoforms of DRD2 and DRD3, with weaker potency for DRD4 and no antagonism of DRD1 or DRD5. Increased secretion of prolactin is a clinical hallmark of DRD2 antagonism by several psychiatric medications that potently target this receptor. ELISA measurements in peripheral blood of patients treated with ONC201 in the first-in-human trial with advanced solid tumors determined that 10/11 patients evaluated exhibited induction of prolactin (mean of 2-fold).

[00307] Using the TCGA database, the D2-like dopamine receptor subfamily, particularly DRD2, was found to be prevalent and selectively overexpressed in several malignancies. Preclinical reports show that DRD2 inhibition imparts antitumor efficacy, without killing

normal cells, via induction of ATF4/CHOP and inhibition of Akt and ERK signaling that are all attributes of ONC201.

Methods

[00308] ONC201 dihydrochloride was obtained from Oncoceutics. Kinase inhibition assays for the kinome were performed as described (*see* Anastassiadis *et al.*, *Nat Biotech* **29**:1039 (2011)). GPCR arrestin recruitment and cAMP modulation reporter assays were performed as described (*see* McGuinness *et al.*, *Journal of Biomolecular Screening* **14**:49 (2009)). PathHunterTM (DiscoveRx) β-arrestin cells expressing one of several GPCR targets were plated onto 384-well white solid bottom assay plates (Corning 3570) at 5000 cells per well in a 20 µL volume in an appropriate cell plating reagent. Cells were incubated at 37 °C, 5% CO_2 for 18-24 h. Samples were prepared in buffer containing 0.05% fatty-acid free BSA (Sigma). For agonist mode tests, samples (5 µL) were added to pre-plated cells and incubated for 90 minutes at 37 °C, 5% CO_2. For antagonist mode tests, samples (5 µL) were added to pre-plated cells and incubated for 30 minutes at 37 °C, 5% CO_2 followed by addition of EC_{80} agonist (5 µL) for 90 minutes at 37 °C, 5% CO_2. For Schild analysis, samples (5 µL) were added to pre-plated cells and incubated for 30 minutes at 37 °C, 5% CO_2 followed by addition of serially dliuted agonist (5 µL) for 90 minutes at 37 °C, 5% CO_2. Control wells defining the maximal and minimal response for each assay mode were tested in parallel. Arrestin recruitment was measured by addition of 15 µL PathHunter Detection reagent and incubated for 1-2 h at room temperature and read on a Perkin Elmer Envision Plate Reader. For agonist and antagonist tests, data was normalized for percent efficacy using the appropriate controls and fitted to a sigmoidal dose-response (variable slope), Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*HillSlope)), where X is the log concentration of compound. For Schild analysis, data was normalized for percent efficacy using the appropriate controls and fitted to a Gaddum/Schild EC_{50} shift using global fitting, where Y=Bottom + (Top-Bottom)/(1+10^((LogEC-X)*HillSlope)), Antag=1+(B/(10^(-1*pA2)))^SchildSlope and LogEC=Log(EC_{50}*Antag). EC_{50} / IC_{50} analysis was performed in CBIS data analysis suite (Cheminnovation) and Schild analysis performed in GraphPad Prism 6.0.5.

Results

[00309] ONC201 is a small molecule in phase II clinical trials for select advanced cancers. It was discovered in a phenotypic screen for p53-independent inducers of the pro-apoptotic TRAIL pathway. Although the contribution of ONC201-induced ATF4/CHOP upregulation and inactivation of Akt/ERK signaling (Allen *et al.*, *Science translational medicine* 5, 171ra117-171ra117 (2013)) to its anti-cancer activity has been characterized, its molecular binding target has remained elusive.

[00310] *In vitro* profiling of GPCR activity using a hetereologous reporter assay for arrestin recruitment, a hallmark of GPCR activation, indicated that ONC201 selectively antagonizes the D2-like (DRD2/3/4), but not D1-like (DRD1/5), dopamine receptor subfamily (Figures 4B and 5A). Antagonism of adrenoceptor alpha receptors or other GPCRs was not observed under the evaluated conditions. Among the DRD2 family, ONC201 antagonized both short and long isoforms of DRD2 and DRD3, with weaker potency for DRD4. Further characterization of ONC201-mediated antagonism of arrestin recruitment to DRD2L was assessed by a Gaddum/Schild EC_{50} shift analysis, which determined a dissociation constant of 2.9 μM for ONC201 that is equivalent to its effective dose in many human cancer cells (Figure 4C). Confirmatory results were obtained for cAMP modulation in response to ONC201, which is another measure of DRD2L activation (Figure 4D). The ability of dopamine to reverse the dose-dependent antagonism of up to 100 μM ONC201 suggests direct, competitive antagonism of DRD2L (Figures 5B and 5C). In agreement with the ONC201 specificity predicted by BANDIT, no significant interactions were identified between ONC201 and nuclear hormone receptors, the kinome, or other drug targets of FDA-approved cancer therapies (Figures 5D and 5E). Interestingly, a biologically inactive constitutional isomer of ONC201 (Wagner *et al.*, *Oncotarget* **5**:12728 (2014)) did not inhibit DRD2L, suggesting that antagonism of this receptor could be linked to its biological activity (Figure 5F). In summary, these studies establish that ONC201 selectively antagonizes the D2-like dopamine receptor subfamily, which appears to be a promising therapeutic target in oncology, and ONC201 is the first compound to exploit this treatment paradigm in several ongoing Phase II clinical studies.

Example 13: Shotgun Mutagenesis Epitope Mapping of DRD2.

[00311] Shotgun Mutagenesis uses a high-throughput cellular expression technology to express and analyze large libraries of mutated target proteins within eukaryotic cells. Every residue in a protein is individually mutated to an alanine, or other specified residue, to assay changes in function. Proteins are expressed within standard mammalian cell lines, therefore even difficult proteins that require eukaryotic translational or post-translational processing can be mapped.

[00312] First, conditions were evaluate and identified for screening the DRD2 antagonist ONC201 with wild-type DRD2 using the Shotgun Mutagenesis screening assay. Then, a DRD2 Ala-scan library was prepared and the residues critical for ONC201 binding were mapped at single amino acid resolution using Shotgun Mutagenesis technology.

DRD2 Shotgun Mutagenesis Library:

Parental plasmid: DRD2

Library size: 442 mutant clones (complete protein)

Mutation Strategy: Alanine scan mutagenesis

Cell type: HEK-293T

Screening Assay: Calcium flux

Epitope Tag: C-terminal V5/HIS6

[00313] Parental Construct: DNA encoding the full-length human DRD2 (Accession No: NP_000786.1; MDPLNLSWYD DDLERQNWSR PFNGSDGKAD RPHYNYYATL LTLLIAVIVF GNVLVCMAVS REKALQTTTN YLIVSLAVAD LLVATLVMPW VVYLEVVGEW KFSRIHCDIF VTLDVMMCTA SILNLCAISI DRYTAVAMPM LYNTRYSSKR RVTVMISIVW VLSFTISCPL LFGLNNADQN ECIIANPAFV VYSSIVSFYV PFIVTLLVYI KIYIVLRRRR KRVNTKRSSR AFRAHLRAPL KGNCTHPEDM KLCTVIMKSN GSFPVNRRRV EAARRAQELE MEMLSSTSPP ERTRYSPIPP SHHQLTLPDP SHHGLHSTPD SPAKPEKNGH AKDHPKIAKI FEIQTMPNGK TRTSLKTMSR RKLSQQKEKK ATQMLAIVLG VFIICWLPFF ITHILNIHCD CNIPPVLYSA FTWLGYVNSA VNPIIYTTFN IEFRKAFLKI LHC (SEQ ID NO: 1) was subcloned into a mammalian high-expression vector. This parental construct was sequence-verified and then validated for mammalian cell expression by detection of calcium flux in response to dopamine. DNA yields from plasmid preparations have been validated for high-throughput processing.

[00314] Assay Set-up: A DRD2-specific calcium flux assay was successfully optimized for DRD2 expressed in human cells. An agonist dose-response assay was used to identify a suitable dopamine concentration for use in optimizing the inhibition of DRD2-specific calcium flux by antagonist ONC201. Subsequent dose-response inhibition assays identified a concentration of ONC201 that inhibited the DRD2 dopamine response by >95%.

Calcium Flux Assay Optimization:

[00315] **Receptor Activity Assay.** DRD2 activity was assessed using a published GPCR assay (Greene, T.A. *et al.*, (2011) PLoS One 6, e20123). Briefly, HEK-293T cells were transfected with expression constructs for wild-type DRD2 or a negative control GPCR, in 384-well format. After 22 hr, calcium flux experiments were performed over a range of dopamine concentrations (300 pM – 100 nM), using a Flexstation II- 384 fluorescence reader (Molecular Devices). Data sets were analyzed and represented as percentage over baseline signal using Prism 5.0 software (GraphPad Software, Inc).

[00316] For cells expressing DRD2, but not a control GPCR, addition of dopamine resulted in increases in cellular calcium flux, measured as increased fluorescence. A dose response plot of the fluorescence peak height versus dopamine concentration demonstrated the strong dopamine-induced calcium flux (EC_{50} = 0.45 nM) in cells expressing DRD2, but not the

control GPCR. This suggested that the calcium flux assay could be used to test for ONC201 inhibition.

DRD2 Calcium Flux Inhibition Assay Optimization

[00317] Following identification of the EC_{50} for dopamine in the calcium flux assay, ONC201 inhibition of DRD2-specific calcium flux was investigated at several dopamine concentrations. Using 1 nM dopamine (> 2-fold higher than the dopamine EC_{50}) with a range of ONC201 concentrations (1 nM to 100 μM), ONC201 inhibition of dopamine-induced DRD2 calcium flux was observed at the highest concentrations tested (Figure 9A), with complete inhibition by 100 μM ONC201 (IC_{50} = 21.5 μM). Inhibition of calcium flux by 100 μM ONC201 was not the result of a broad inhibition of GPCRs or of a non-specific effect on cells since ONC201 had no effect on the calcium flux activity of cells expressing a control GPCR (Figure 9B).

[00318] Analysis of a number of replicate values obtained for inhibition of DRD2 calcium flux by 100 μM ONC201 indicated a robust assay, with a Z' value of 0.61. The Z' value is a measurement of assay quality, calculated from the means and standard deviations obtained for replicate determinations of calcium flux obtained with or without ONC201.

Comparison of DRD2 inhibitors.

[00319] The ONC201 inhibition of DRD2 was compared to that by the DRD2 antagonists spiperone and domperidone (Figure 10), which have been described as inhibiting DRD2 at concentrations lower than the 100 μM required for inhibition by ONC201. These antagonists were screened at concentrations between 100 pM and 1 μM, and both showed complete inhibition of dopamine-induced calcium flux, with spiperone having an IC_{50} = 19 nM, and domperidone an IC_{50} = 47 nM. These values were consistent with previous characterizations and demonstrate that the relatively high IC_{50} obtained for ONC201 (21.5 μM) does not result from the use of a calcium flux assay to measure DRD2 activity.

[00320] Optimal screening conditions were determined for ONC201 inhibition of DRD2-specific calcium flux in response to dopamine. These conditions give a robust response to dopamine, this response is reduced by >95% by addition of ONC201 to 100 μM, and the assay demonstrated low variability between replicates. These data indicate that the selected conditions are suitable for successful high-throughput screening. Further screening of the DRD2 mutation library was at a dopamine concentration of 1 nM and an ONC201 concentration of 100 μM.

Screening the DRD2 alanine-scan library for response to dopamine.

[00321] The DRD2 alanine-scan mutation library (and with alanines changed to serines) comprised 442 clones, covering residues 2 - 443 of the DRD2 protein, 100% of target residues.

The DRD2 mutation library was first screened by calcium flux assay with dopamine (1 nM) in the absence of ONC201 to identify residues whose mutation diminished dopamine-induced calcium flux. We identified 28 amino acid residues that were critical for dopamine-induced DRD2 flux (**Figure 11**).

[00322] Residues were identified from the analysis are listed in Table 4 and shown in Figure 11. Clones were considered to be deficient for calcium flux if they demonstrated flux values less than 2 standard deviations below the average calcium flux value (AV – 2SD) for the entire library.

TABLE 4: DRD2 RESIDUES CRITICAL FOR DOPAMINE-INDUCED CALCIUM FLUX

Mutation	Calcium Flux % WT
C182A	0
I184A	0
S197A	0
T119A	1
S193A	1
D80A	3
R132A	3
D114A	4
H393A	4
F198A	10
V83A	10
I377A	11
Y416A	12
C118A	14

Mutation	Calcium Flux % WT
S7A	15
W386A	15
S121A	16
I394A	16
E248A	19
V190A	20
Y199A	20
C107A	20
S419A	20
F189A	22
I122A	23
T205A	24
N23A	25
L125A	25
I128A	27

Screening the DRD2 alanine-scan library for ONC 201 inhibition of dopamine-induced signaling identified residues required for inhibition by ONC201.

[00323] To identify residues important for the inhibition of DRD2 by ONC201, the DRD2 alanine-scan mutation library was screened by the calcium flux assay for the ability to respond to dopamine in the presence of an inhibiting concentration of ONC201, using dopamine at 1 nM and ONC201 at 100 μM. Eight residues critical for ONC201 inhibitory activity were identified (Figure 12). All residues identified by this screen showed high calcium flux with dopamine alone (Table 5). Clones were considered to be critical for inhibition by ONC201 at 100 μM if they demonstrated flux values greater than 2 standard deviations above the average calcium flux value (AV + 2SD) for the entire library. Also shown in Table 5 for these critical clones are the calcium flux values obtained from similar experiments performed with 250 μM ONC201 or without ONC201 (dopamine 1 nM), and in addition the % conservation of the critical residues across the 5 DRD receptors, with the residues found in each receptor.

TABLE 5: DRD2 RESIDUES CRITICAL FOR ONC201 INHIBITION OF DOPAMINE-INDUCED CALCIUM FLUX

Calcium Flux as a % of flux with WT DRD2 (100)				DRD % Conservation	DRD				
Mutation	ONC201 100 μM	ONC201 250 μM	Dopamine 1nM		1	2	3	4	5
I397A	122	89	105	20	P	**I**	T	A	P
E95A	97	39	123	100	E	**E**	E	E	E
V91A	94	58	119	40	K	**V**	V	F	K
Y192A	85	11	64	60	S	**Y**	Y	Y	S
V196A	79	22	119	40	I	**V**	V	C	I
A177S	77	26	85	40	A	**A**	T	V	D
T165A	67	28	92	20	L	**T**	A	A	L
L81A	63	20	83	100	L	**L**	L	L	L

[00324] Since the average inhibition by 100 μM ONC201 across the library was approximately 75%, we also conducted a screen at 250 μM ONC201 to determine if critical residues would be the same at higher levels of inhibition. Under this condition dopamine-induced calcium flux was inhibited by approximately 93%, and the previously identified residues **V91**, **E95**, and **I397** were also critical for inhibition at 250 μM ONC201 (Table 5), using the same criteria of flux values greater than 2 standard deviations above the average calcium flux value (AV + 2SD) for the library.

Conclusions:

[00325] In initial screens of the DRD2 alanine-scan mutation library by dopamine–induced calcium flux assay, 28 mutations greatly decreased calcium flux, identifying resides critical for DRD2 function. As found in a similar analysis of the GPCR CXCR4, the critical residues were distributed throughout the protein, in the predicted dopamine binding pocket, the transmembrane regions and in the cytoplasmic exposed portion of DRD2. These 28 residues are critical for either dopamine binding, signal transduction through the transmembrane domains, or G protein coupling. A detailed analysis comparable to that performed for CXCR4, as well as the structural analysis of the DRD3-eticlopride structure (Chien *et al.*, 2010), can be used to assign specific function to each DRD2 critical residue.

[00326] To identify residues important for the inhibition of DRD2 by ONC201, the DRD2 alanine-scan mutation library was screened by calcium flux assay with dopamine and 100 μM ONC201. These screens identified 8 residues as critical for ONC201 inhibition of DRD2-dependent dopamine-induced calcium flux - **L81**, **V91**, **E95**, **T165**, **A177**, **Y192**, **V196**, and **I397**. Residues **V91**, **E95**, and **I397** were also identified as critical for resistance to DRD2 inhibition by 250 μM ONC201, suggesting that they are key ONC201-interacting residues. These residues define a relatively large ligand binding site, which is not unexpected due to the larger size of ONC201 compared to dopamine and eticlopride. The locations of these residues

are generally consistent with a role in mediating ONC201 inhibition of DRD2-dependent dopamine-induced calcium flux. Residues critical for inhibition of a GPCR taste receptor by probenecid were previously identified (Greene et al., 2011), with the location of the residues consistent with a non-competitive mechanism of inhibition. In contrast, the residues identified here for DRD2 are consistent with competitive inhibition by ONC201 at the dopamine binding site. When modeled on the structure of homologous receptor DRD3, the majority of the residues identified surround the binding pocket containing a co-crystallized antagonist eticlopride, with 5 of the 8 identified residues conserved between DRD2 and DRD3. Two of the residues appear to more distal from the putative binding site (A177 and L81) and may affect ONC201 binding in a more allosteric fashion. Additional residues that contribute to ONC201 inhibition may be identified using DRD2 agonists with structures distinct from dopamine.

Example 14: Determination of the Association & Dissociation Rate Constants of unlabelled ONC201 Dihydrochloride on the Human D2S Receptor.

[00327] In this Example, the *k*on / *k*off rates of unlabeled ONC201 dihydrochloride on the D2S receptor was determined. The *k*on / *k*off rate estimation was performed by competitive ligand binding according to the method described in: M.R. Dowling & S.J. Charlton (2006) *Brit. J. Pharmacol.* **148**:927-937 and H.J. Motulsky & L.C. Mahan (1984) *Mol. Pharmacol.* **25**:1-9. Referring to this method, the *k*on / *k*off rates of the unlabeled test compounds were calculated from its *K*i value (competition binding) and its effect on the binding kinetics of the radioligand (competition kinetics).

[00328] First, the IC_{50} and *K*i values of ONC201 dihydrochloride, and selection of the adequate compound concentrations for the competition kinetics experiment, were determined. Then, the *k*on and *k*off rate constants of the radioligand ([^{3}H]Methylspiperone) was determined. Finally, the *k*on and *k*off rate constants of the unlabeled ONC201 dihydrochloride was determined. ONC201 dihydrochloride was tested at 8 concentrations in duplicate (n = 2) in the competition binding assay, and the IC_{50} and *K*i values were determined.

[00329] The reference compound, (+) Butaclamol, and the test compound, ONC201-2HCL, successfully competed for [^{3}H]Methylspiperone, with IC_{50} values of 2.5 nM and 21 μM, respectively. Previously, the compound ONC201-2HCL yielded a similar IC_{50} value of 16 μM. For the competition binding assay, the following 6 concentrations of ONC201-2HCL were selected: 5 / 10 / 20 / 40 / 60 / 80 μM.

[00330] The the binding kinetics of [^{3}H]Methylspiperone on the D2S receptor was determined. For this, [^{3}H]Methylspiperone (at one concentration of 0.3 nM) was incubated with the D2S receptor membranes for 12 different incubation times to measure the association rate. The non-specific binding was measured with Butaclamol (10 μM) for each incubation

time. The dissociation was initiated by addition of an excess of Butaclamol (10 µM) after 60 minutes incubation of [^{3}H]Methylspiperone (0.3 nM) with the D2S receptor membranes, and the signal decrease was measured after 12 different incubation times. The experiment was performed in triplicate (n = 3) with incubation times adjusted to 0 / 30 / 60 / 80 / 120 / 180/ 240 / 300 / 360 / 420 / 480 minutes and 24 hours for the association and 2 / 5 / 8 / 10 / 15 / 20 / 25 / 30 / 40 / 60 / 120 / 180 minutes for the dissociation kinetics.

[00331] [3H]Methylspiperone displayed a k_{on} value of 2.3 × 10^8 $M^{-1}min^{-1}$ and a k_{off} value of 0.009506 min^{-1} (and thus a $t_{1/2}$ value of 73 minutes) on the D2S receptor. The K_d calculated from the results of the association / dissociation experiment (0.04 nM) is in the same range as compared to the K_d observed in the saturation experiment (0.15 nM), thereby validating the experiment.

[00332] The effect of the unlabeled ONC201-2HCl at six concentrations on the association kinetics of [^{3}H]Methylspiperone (0.3 nM) was tested. The non-specific binding was measured with Butaclamol (10 µM). The same 12 incubation times as above were used: 2 / 5 / 8 / 10 / 15 / 20 / 25 / 30 / 40 / 60 / 120 / 180 minutes. A measurement in the absence of compound was performed as negative control.

[00333] ONC201-2HCl displayed a k_{on} value of 4.1 × 10^5 $M^{-1}min^{-1}$ and a k_{off} value of 1.32 min^{-1} (and thus a $t_{1/2}$ value of 0.53 minutes) on the D2S receptor. The K_i calculated from the results of the association / dissociation experiment (3.2 µM) is in the same range as compared to the K_i observed in the saturation experiment (7 µM), thereby validating the experiment. In conclusion, **ONC201-2HCl displays a much slower association and a much faster dissociation as compared to [^{3}H]Methylspiperone**.

Example 15: Bactericidal Activity of Imipridones.

Materials and Methods

[00334] Test material: ONC201 dihydrochloride; Control: Microcrystalline Cellulose.

[00335] Method: Harmonized EP/USP Microbial Examination of Nonsterile Products (Current USP <61>/<62>).

Results

TABLE 6: VERIFICATION OF THE INOCULUM RECOVERY CONTROL AND MICROBIAL ENUMERATION TEST

1:300 with TSB Mod Dilution	Indicator Organisms Count							
	Ec	Sa	Pa	Bs	Ca (TSA)	Ab (TSA)	CA (SDA)	Ab (SDA)
Inoculum	27	31	28	52	48	21	52	20
434019	N/A	0	24	48	51	18	46	19

TABLE 7: THE VALIDATION FOR SPECIFIED MICROORGANISMS

Sample	BTGN	Ec	Pa	Sa	Ca
1:300 with TSB Mod Dilution	P	P	P	F	P

P = Pass F = Fail NA = Not Applicable; Ec = Escherichia coli ATCC# 8739; Pa = Pseudomonas aeruginosa ATCC# 9027; Sa = Staphylococcus aureus ATCC# 6538; Bs = Bacillus subtilis ATCC# 6633; Ca = Candida albicans ATCC# 10231; Ab =Aspergillus brasiliensis ATCC# 16404; BTGN = Bile Tolerant Gram Negative bacteria; Cs = Clostridium species; TSA = Trypticase Soy Agar; SDA = Sabouraud Dextrose Agar.

[00336] ONC201 dihydrochloride when tested at the 1:300 dilution with TSB Mod, did not meet the requirements of the USP <61>/<62>

[00337] Microbial Limit Suitability Test. Inhibition was observed for Staphylococcus aureus for USP<61>/<62>. Therefore, it can be assumed that the failure to isolate the inoculated microorganism is attributable to the bactericidal activity of ONC201 dihydrochloride and thus it is not likely to be contaminated with the inhibited species of microorganism.

[00338] Next, the Minimal Inhibitory Concentration (MIC) for six imipridones was determined against wild type and methicillin-resistant *Staphylococcus aureus*.

Materials and Methods

Compounds

[00339] ONC201 and ONC206 were previously solubilized at 40 mM in DMSO. ONC212, ONC207 and ONC213 were solubilized at 20 mg/mL in DMSO and an ONC201 linear isomer (TIC-10) was solubilized at 10 mg/mL in DMSO. Methicillin and/or vancomycin were evaluated in parallel as positive control antibiotics and were purchased from Sigma-Aldrich and solubilized in deionized H_2O at a concentration of 10 mg/mL.

Bacteria

[00340] The bacterial strains employed in these assays were obtained from the American Type Culture Collection (ATCC). All bacterial strains were propagated as recommended by the ATCC. Each strain was stored as a frozen glycerol stock at -80°C and a 10 µL loop of the frozen stock was used to inoculate each culture for these assays. The strains with their classification and properties are listed in Table 8 below.

TABLE 8: STRAINS OF *STAPHYLOCOCCUS AUREUS* AND CHARACTERISTICS

ATCC #	Classification	Properties	Assay Media
29213	Gram Positive Cocci	QC Wild Type Strain	Trypticase Soy Broth (TSB)
33591		Hospital Acquired Methicillin Resistant	Nutrient Broth
700699		Hospital Acquired, MDR, Reduced Susceptibility to Vancomycin	Brain Heart Infusion Broth + 0.004 g/L Vancomycin

Minimal Inhibitory Concentration (MIC) Determination

[00341] The susceptibility of the bacterial organisms to the test compounds was evaluated by determining the MIC of each compound using a micro-broth dilution analysis according to the methods recommended by the Clinical and Laboratory Standards Institute CLSI. All microbial strains were obtained from American Type Culture Collections (ATCC) and cultured according to the supplier's recommendations. Evaluation of the susceptibility of each organism against the test compounds included a positive control antibiotic(s). For each organism, a standardized inoculum was prepared by direct suspension of freshly plated colonies in the appropriate media as indicated in Table 8 to an optical density 625 nm (OD_{625}) of 0.1 (equivalent to a 0.5 McFarland standard). The suspended inoculum was diluted to a concentration of approximately 1×10^6 colony forming units per milliliter (CFU/mL) and 100 µL placed into triplicate wells of a 96-well plate containing 100 µL of test compound serially diluted 2-fold in the appropriate broth. One hundred microliters (100 µL) of the inoculum was also added to triplicate wells containing 100 µL of two-fold serial dilutions of a positive control antibiotic and to wells containing 100 µL of media only. This dilution scheme yielded final concentrations for each microbial organism estimated to be 5×10^5 CFU/mL. Test compound concentrations ranged from a high-test of 100 to a low test of 0.2 µM using a two-fold dilution scheme. The plates were incubated for 24 or 48 hours (*Staphylococcus aureus* 700699) at 37°C and the microbial growth at each concentration of compound was determined by measuring the optical density at 625 nm on a Molecular Devices SpectraMax Plus-384 plate reader and visually by scoring the plates +/- for bacterial growth. The MIC for each compound was determined as the lowest compound dilution that completely inhibited microbial growth.

Results

[00342] Six (6) imipridones were evaluated for their ability to inhibit the growth of three strains of *Staphylococcus aureus*. ONC201, ONC207, and an ONC201 linear isomer (TIC-10) were inactive against all three strains up to a concentration of 100 µg/mL. Against wild type *Staphylococcus aureus* (ATCC 29213) the MIC of ONC206, ONC212 and ONC213 was 6.25 µg/mL, 3.13 µg/mL and 25 µg/mL, respectively. Against *Staphylococcus aureus* (ATCC

33591) the MIC of ONC206, ONC212 and ONC213 was 12.5 µg/mL, 3.13 µg/mL and 3.13 µg/mL, respectively. The activity was similar against the MDR *Staphylococcus aureus* (ATCC 700699) with all three compounds having a MIC of 12.5 µg/mL. Vancomycin, the positive control compound, was active at the expected concentration and methicillin was found to be inactive up to a concentration of 100 µg/mL against the two methicillin resistant strains of bacteria. Data are presented in Table 9.

TABLE 9: MIC DETERMINATION OF 6 IMIPRIDONES FOR 3 *STAPHYLOCOCCUS AUREUS* STRAINS

Compound (µg/mL)	*Staphylococcus aureus* ATCC 29213				*Staphylococcus aureus* ATCC 33591				*Staphylococcus aureus* ATCC 700699 (48 hours)			
	MIC_{90}	MIC_{95}	MIC_{99}	Visual	MIC_{90}	MIC_{95}	MIC_{99}	Visual	MIC_{90}	MIC_{95}	MIC_{99}	Visual
ONC201	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
ONC206	6.25	6.25	6.25	6.25	12.5	25	>100	12.5	12.5	12.5	25	12.5
ONC207	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
ONC212	3.13	3.13	3.13	3.125	3.13	6.25	100	3.125	6.25	12.5	12.5	12.5
ONC213	12.5	12.5	25	25	3.13	6.25	100	3.125	6.25	12.5	12.5	12.5
TIC-10	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Methicillin	---	---	---	---	>100	>100	>100	>100	>100	>100	>100	>100
Vancomycin	3.13	3.13	6.25	3.125	0.39	0.39	0.78	0.391	12.5	25	25	25

Discussion

[00343] Six (6) imipridones were evaluated for activity against 3 strains of *Staphylococcus aureus*. ONC201, ONC207, and TIC-10 were inactive against all three strains. ONC206, ONC212 and ONC213 had varying activity ranging from 3.13 µg/mL to 25 µg/mL against all three bacterial strains. Relative to vancomycin the activity of these three imipridones was equivalent or 2 to 8-fold less against strain 29213. All three of these imipridones had 10 to 30-fold less activity compared to vancomycin against strain 33591 and the activity for all three compounds was 2-fold higher than vancomycin against strain 700699.

[00344] These experiments are repeated with additional impiridones and for additional bacteria, including both Gram-positive and Gram-negative bacteria, such as those in Table 10.

TABLE 10

Organism	Condition	Gram +/Gram -
Enterococcus faecium	Noscomial bacteremia, wound infections, endocarditis, UTIs	+
Staphylococcus aureus	Bacteremia, endocarditis	+
Klebsiella pneumonia	Pneumonia, UTIs, Upper respiratory tract infections	-
Acinetobacter baumannii	Infections in ICU and burn patients; also being seen in general hospital and nursing homes	-
Pseudomonas aeruginosa	Pneumoniae, CF	-
Enterobacter cloacae	UTIs, respiratory infections	-

Example 16: Case study of ONC201 treatment in a subject with recurrent glioblastoma

[00345] This Example provides a case study of a 22 year old female with recurrent glioblastoma (unmethylated MGMT, H3.3 K27M mutant) treated with 625mg of ONC201 once every three weeks. Figure 28 (A) Tumor size relative to baseline (%) of total tumor burden in the subject. One cycle is 3 weeks. (B) Contrast MRI scans at baseline, 21, 27 and 36 weeks post-ONC201 initiation of one of 2 malignant lesions in the subject with 625mg q3w ONC201.

[00346] It will be appreciated by one skilled in the art that changes could be made to the exemplary embodiments shown and described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the exemplary embodiments shown and described, but it is intended to cover modifications within the spirit and scope of this invention as defined by the claims. For example, specific features of the exemplary embodiments may or may not be part of the claimed invention and features of the disclosed embodiments may be combined. Unless specifically set forth here, the terms "a", "an" and "the" are not limited to one element but instead should be read to mean "at least one."

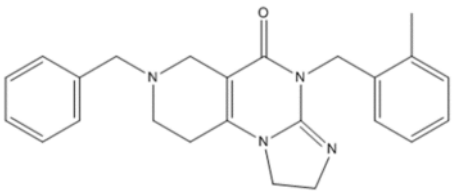
[00347] It is to be understood that the figures and descriptions may have been simplified to focus on elements that are relevant for a clear understanding, while eliminating, for purposes of clarity, other elements that those of ordinary skill in the art will appreciate may also comprise a portion of the invention. However, because such elements are well known in the art, and because they do not necessarily facilitate a better understanding of the invention, a description of such elements is not provided herein.

[00348] Further, to the extent that a method does not rely on the particular order of steps set forth, the particular order should not be construed as limitation on the claims. Claims directed to a method should not be limited to performance of the steps in the order written, and one skilled in the art can readily appreciate that they can be varied and still remain within the spirit and scope of this invention.

[00349] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety here.

CLAIMS

1. Use of a compound of formula (**1**)



(**1**),

or a pharmaceutically acceptable salt thereof,
in the manufacture of a pharmaceutical composition for treating a central nervous system cancer in a subject in need thereof, wherein the central nervous system cancer has a histone H3 mutation.

2. The use according to claim 1, wherein the cancer is meningioma, ependymoma, glioma, neuroblastoma or diffuse intrinsic pontine glioma.

3. The use according to claim 1 or 2, wherein the cancer has an epigentically silenced unmethylated O(6)-methylguanine-DNA methyltransferase (MGMT) gene.

4. The use according to any one of claims 1 to 3, wherein the subject is a human.

5. The use according to any one of claims 1 to 4, wherein the pharmaceutically acceptable salt is a di-hydrochloride salt.