

1,3,4-OXADIAZOLE AND 1,3,4-THIADIAZOLE DERIVATIVES AS IMMUNOMODULATORS

This application claims the benefit of Indian provisional application number 4012/CHE/2013, filed on September 06, 2013; which hereby incorporated by reference.

TECHNICAL FIELD

The present invention relates to 1,3,4-oxadiazole and 1,3,4-thiadiazole
5 compounds therapeutically useful as immune modulators. The invention also relates to pharmaceutical compositions comprising the said 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds as therapeutic agents.

BACKGROUND OF THE INVENTION

Programmed cell death-1 (PD-1) is a member of the CD28 superfamily that
10 delivers negative signals upon interaction with its two ligands, PD-L1 or PD-L2. PD-1 and its ligands are broadly expressed and exert a wider range of immunoregulatory roles in T cells activation and tolerance compared with other CD28 members. PD-1 and its ligands are involved in attenuating infectious immunity and tumor immunity, and facilitating chronic infection and tumor progression. The biological significance of PD-1
15 and its ligand suggests the therapeutic potential of manipulation of PD-1 pathway against various human diseases (Ariel Pedoeem et al., Curr Top Microbiol Immunol. (2011); 350:17-37).

T-cell activation and dysfunction relies on direct and modulated receptors. Based on their functional outcome, co-signaling molecules can be divided as co-stimulators and
20 co-inhibitors, which positively and negatively control the priming, growth, differentiation and functional maturation of a T-cell response (Li Shi, et al., Journal of Hematology & Oncology 2013, 6:74).

Therapeutic antibodies that block the programmed cell death protein-1 (PD-1) immune checkpoint pathway prevent T-cell down regulation and promote immune
25 responses against cancer. Several PD-1 pathway inhibitors have shown robust activity in various phases of clinical trials (RD Harvey, Clinical Pharmacology & Therapeutics (2014); 96 2, 214–223).

Programmed death-1 (PD-1) is a co-receptor that is expressed predominantly by T cells. The binding of PD-1 to its ligands, PD-L1 or PD-L2, is vital for the
30 physiological regulation of the immune system. A major functional role of the PD-1 signaling pathway is the inhibition of self-reactive T cells, which serve to protect against

autoimmune diseases. Elimination of the PD-1 pathway can therefore result in the breakdown of immune tolerance that can ultimately lead to the development of pathogenic autoimmunity. Conversely, tumor cells can at times co-opt the PD-1 pathway to escape from immunosurveillance mechanisms. Therefore, blockade of the PD-1 pathway has become an attractive target in cancer therapy. Current approaches include six agents that are either PD-1 and PD-L1 targeted neutralizing antibodies or fusion proteins. More than forty clinical trials are underway to better define the role of PD-1 blockade in variety of tumor types. (Hyun-Tak Jin et al., Clinical Immunology (Amsterdam, Netherlands) (2014), 153(1), 145-152).

International applications WO 01/14557, WO 02/079499, WO 2002/086083, WO 03/042402, WO 2004/004771, WO 2004/056875, WO2006121168, WO2008156712, WO2010077634, WO2011066389, WO2014055897, WO2014059173, WO2014100079 and US patent US08735553 report PD-1 or PD-L1 inhibitory antibodies or fusion proteins.

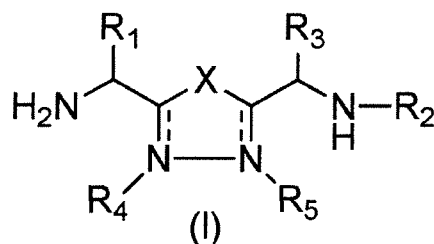
Further, International applications, WO2011161699, WO2012/168944, WO2013144704 and WO2013132317 report peptides or peptidomimetic compounds which are capable of suppressing and/or inhibiting the programmed cell death 1 (PD1) signaling pathway.

Still there is a need for more potent, better and/or selective immune modulators of PD-1 pathway. The present invention provides 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds which are capable of suppressing and/or inhibiting the programmed cell death 1 (PD1) signalling pathway.

SUMMARY OF INVENTION

In accordance with the present invention, 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds or a pharmaceutically acceptable salt or a stereoisomer thereof, provided which are capable of suppressing and/or inhibiting the programmed cell death 1 (PD1) signalling pathway.

In one aspect, the present invention provides a 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds of formula (I):



wherein,

R₁ is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;

X is S or O;

5 R₂ is hydrogen or -CO-Aaa;

Aaa is an amino acid residue selected from Ser, Asn or Thr; wherein a C-terminus thereof is a free terminus, is amidated or is esterified;

R₃ is side chain of an amino acid selected from Ser, Ala, Glu, Gln, Asn or Asp;

---- is an optional bond;

10 R₄ and R₅ independently are hydrogen or absent;

or a pharmaceutically acceptable salt or a stereoisomer thereof.

In a further aspect of the present invention, it relates to the pharmaceutical composition comprising a compound of formula (I) or a pharmaceutically acceptable salt or a stereoisomer and processes for preparing thereof.

15 In yet another aspect of the present invention, it provides use of 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds of formula (I) or a pharmaceutically acceptable salt or a stereoisomer thereof, which are capable of suppressing and/or inhibiting the programmed cell death 1 (PD1) signaling pathway.

DETAILED DESCRIPTION OF THE INVENTION

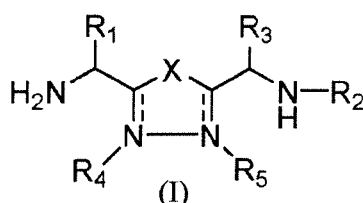
20 The present invention provides 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds as therapeutic agents useful for treatment of disorders via immunopotentialization comprising inhibition of immunosuppressive signal induced due to PD-1, PD-L1, or PD-L2 and therapies using them.

Each embodiment is provided by way of explanation of the invention, and not by way of limitation of the invention. In fact, it will be apparent to those skilled in the art that various modification and variations can be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment can be used on another embodiment to yield a still further embodiment. Thus it is intended that the present invention cover such

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modifications and variations as come within the scope of the appended claims and their equivalents. Other objects, features, and aspects of the present invention are disclosed in, or are obvious from, the following detailed description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary
 5 embodiments only, and is not to be construed as limiting the broader aspects of the present invention.

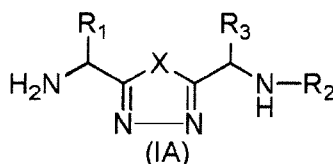
In one embodiment, the present invention relates to compounds of formula (I)



wherein,

- 10 R_1 is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;
 X is S or O;
 R_2 is hydrogen or -CO-Aaa;
 Aaa is an amino acid residue selected from Ser, Asn or Thr; wherein a C-terminus thereof is a free terminus, is amidated or is esterified;
 15 R_3 is side chain of an amino acid selected from Ser, Ala, Glu, Gln, Asn or Asp;
 ---- is an optional bond;
 R_4 and R_5 independently are hydrogen or absent;
 or a pharmaceutically acceptable salt or a stereoisomer thereof.
 In yet another embodiment, the present invention provides compounds of formula

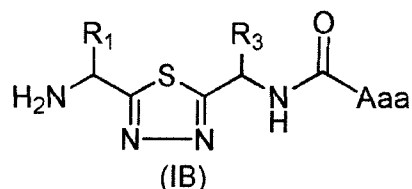
20 (IA)



- or a pharmaceutically acceptable salt or a stereoisomer thereof; wherein,
 R_1 is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;
 X is S or O;
 25 R_2 is hydrogen or -CO-Aaa;
 R_3 is side chain of an amino acid selected from Ser, Ala, Glu, Gln, Asn or Asp;

Aaa is an amino acid residue selected from Ser, Asn or Thr; wherein a C-terminus thereof is a free terminus, is amidated or is esterified.

In yet another further embodiment, the present invention provides compounds of formula (IB)



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or a pharmaceutically acceptable salt or a stereoisomer thereof; wherein,

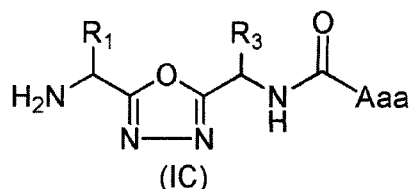
R₁ is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;

R₃ is side chain of an amino acid selected from Ser, Ala, Glu, Gln, Asn or Asp;

Aaa is an amino acid residue selected from Ser, Asn or Thr; wherein a C-terminus thereof is a free terminus, is amidated or is esterified.

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In yet another further embodiment, the present invention provides compounds of formula (IC)



or a pharmaceutically acceptable salt or a stereoisomer thereof; wherein,

R₁ is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;

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R₃ is side chain of an amino acid selected from Ser, Ala, Glu, Gln, Asn or Asp;

Aaa is an amino acid residue selected from Ser, Asn or Thr; wherein a C-terminus thereof is a free terminus, is amidated or is esterified.

In yet another further embodiment, the present invention provides compounds of formula (I), wherein,

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R₁ is side chain of Ser or Thr;

R₂ is -CO-Aaa;

Aaa is an amino acid residue Ser or Thr; wherein the C-terminus is free;

R₃ is side chain of Asn, Gln, Glu or Asp.

The embodiment below are illustrative of the present invention and are not intended to limit the claims to the specific embodiments exemplified.

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According to one embodiment, specifically provided are compounds of the formula (I) and (IA), in which X is O.

According to another embodiment, specifically provided are compounds of the formula (I) and (IA) in which X is S.

5 According to yet another embodiment, specifically provided are compounds of the formula (I) and (IA) in which R₂ is hydrogen.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which R₄ and R₅ are hydrogen.

10 According to yet another embodiment, specifically provided are compounds of the formula (I) in which R₄ and R₅ are absent.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which R₂ is -CO-Ser.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which R₂ is -CO-Thr.

15 According to yet another embodiment, specifically provided are compounds of the formula (I), (IA), (IB) and (IC) in which R₁ is side chain of Ser.

According to yet another embodiment, specifically provided are compounds of the formula (I), (IA), (IB) and (IC) in which R₁ is side chain of Thr.

20 According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IC) in which R₁ is side chain of Phe, Ala or Asn.

According to yet another embodiment, specifically provided are compounds of the formula (I), (IA), (IB) and (IC) in which R₃ is side chain of Asn.

According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IB) in which R₃ is side chain of Ser.

25 According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IC) in which R₃ is side chain of Gln.

According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IC) in which R₃ is side chain of Glu.

30 According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IC) in which R₃ is side chain of Ala or Asp.

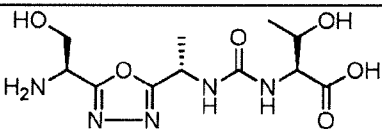
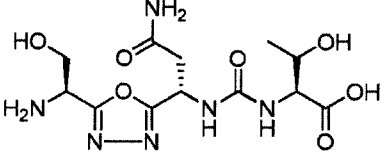
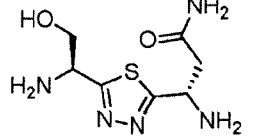
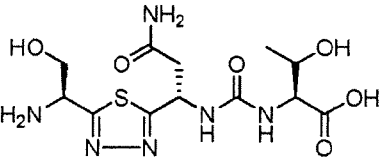
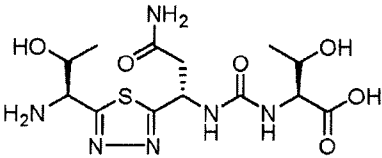
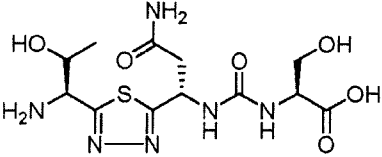
According to yet another embodiment, specifically provided are compounds of the formula (IB) and (IC) in which Aaa is Ser.

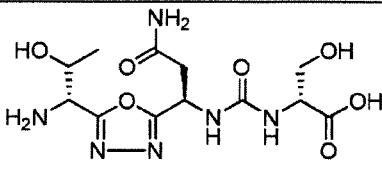
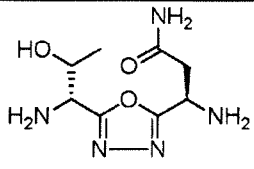
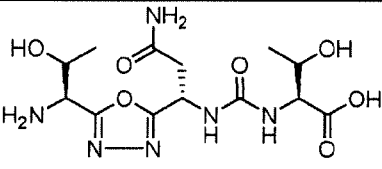
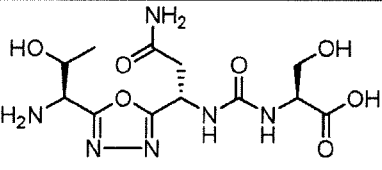
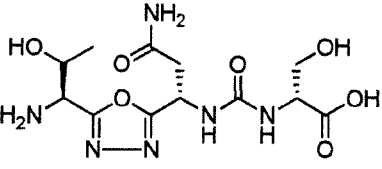
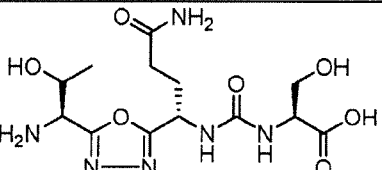
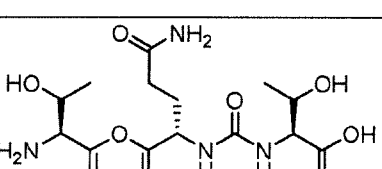
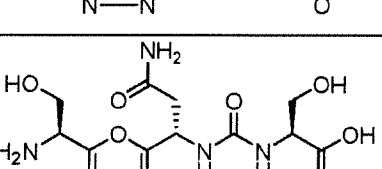
According to yet another embodiment, specifically provided are compounds of the formula (IC) in which Aaa is Thr.

According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IB) in which one, more or all amino acid/s is/are D amino acid/s.

In an embodiment, specific compounds of formula (I) without any limitation are enumerated in Table (1):

Table 1

Compound No.	Structure
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or a pharmaceutically acceptable salt thereof or a stereoisomer thereof.

The compounds as disclosed in the present invention are formulated for pharmaceutical administration.

In one embodiment, the present invention provides a pharmaceutical composition comprising the compound as disclosed, and a pharmaceutically acceptable carrier or a diluent.

In another embodiment, the said pharmaceutical composition further comprising at least one of an anticancer agent, chemotherapy agent, or antiproliferative compound.

In one embodiment, the present invention provides the compounds as disclosed in the present invention for use as a medicament.

In another embodiment, the present invention provides the compounds as disclosed in the present invention for use as a medicament for the treatment of cancer or
5 infectious disease.

In another embodiment, the present invention provides the compounds as disclosed in the present invention for use as a medicament for the treatment bone cancer, cancer of the head or neck, pancreatic cancer, skin cancer, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal
10 region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of
15 soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumours of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumour
20 angiogenesis, spinal axis tumour, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

In another embodiment, the present invention provides the compounds as disclosed in the present invention for use in the treatment of cancer.

25 In another embodiment, the present invention provides the compounds as disclosed in the present invention for use in the treatment of infectious disease.

In one embodiment, the present invention provides the compounds as disclosed in the present invention for use as a medicament for the treatment of bacterial infectious disease, a viral infectious disease or a fungal infectious disease.

30 In one embodiment, the present invention provides a method of treatment of cancer, wherein the method comprises administration of an effective amount of the compound of the present invention to the subject in need thereof.

In another embodiment the present invention provides a method of modulating an immune response mediated by PD-1 signaling pathway in a subject, comprising administering to the subject therapeutically effective amount of the compound of the present invention such that the immune response in the subject is modulated.

5 In yet another embodiment the present invention provides a method of inhibiting growth of tumour cells and/or metastasis in a subject, comprising administering to the subject a therapeutically effective amount of compound of the present invention capable of inhibiting the programmed cell death 1 (PD1) signaling pathway.

The said tumour cells include cancer such as but not limited to bone cancer,
10 cancer of the head or neck, pancreatic cancer, skin cancer, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the
15 esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumours of childhood, lymphocytic
20 lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumour angiogenesis, spinal axis tumour, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

25 In yet another further embodiment the present invention provides a method of treating an infectious disease in a subject comprising administering to the subject a therapeutically effective amount of the compound of the present invention capable of inhibiting the programmed cell death 1 (PD1) signaling pathway such that the subject is treated for the infectious disease.

30 Still yet another embodiment of the present invention provides a method of treating bacterial, viral and fungal infections in a subject comprising administering to the subject a therapeutically effective amount of the compound of the present invention

capable of inhibiting the programmed cell death 1 (PD1) signalling pathway such that the subject is treated for the bacterial, viral and fungal infections.

The infectious disease includes but not limited to HIV, Influenza, Herpes, Giardia, Malaria, Leishmania, the pathogenic infection by the virus Hepatitis (A, B, & C), herpes virus (e.g., VZV, HSV-I, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus, pathogenic infection by the bacteria chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, E. coli, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme's disease bacteria, pathogenic infection by the fungi Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger, etc.), Genus Mucorales (mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum, and pathogenic infection by the parasites Entamoeba histolytica, Balantidium coli, Naegleria fowleri, Acanthamoeba sp., Giardia lamblia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, Nippostrongylus brasiliensis.

The compounds of the present invention may be used as single drugs or as a pharmaceutical composition in which the compound is mixed with various pharmacologically acceptable materials.

The pharmaceutical composition is usually administered by oral or inhalation routes, but can be administered by parenteral administration route. In the practice of this invention, compositions can be administered, for example, by orally, intravenous infusion, topically, intraperitoneally, intravesically or intrathecally. Examples of the parenteral administration includes but not limited to intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that

can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Oral administration, parenteral administration, subcutaneous administration and intravenous administration are the preferred methods of administration.

5 The dosage of the compounds of the present invention varies depending on age, weight, symptom, therapeutic efficacy, dosing regimen and/or treatment time. Generally, they may be administered by oral or inhalation routes, in an amount of 1 mg to 100 mg per time, from once a couple of days, once 3 days, once 2 days, once a day to a couple of
10 times a day, in the case of an adult, or continuously administered by oral or inhalation routes from 1 to 24 hours a day. Since the dosage is affected by various conditions, an amount less than the above dosage may sometimes work well enough, or higher dosage may be required in some cases.

 The compounds of the present invention may be administered in combination with other drugs for (1) complementation and/or enhancement of prevention and/or
15 therapeutic efficacy of the preventive and/or therapeutic drug of the present invention, (2) dynamics, absorption improvement, dosage reduction of the preventive and/or therapeutic drug of the present invention, and/or (3) reduction of the side effects of the preventive and/or therapeutic drug of the present invention.

 A concomitant medicine comprising the compounds of the present invention and
20 other drug may be administered as a combination preparation in which both components are contained in a single formulation, or administered as separate formulations. The administration by separate formulations includes simultaneous administration and administration with some time intervals. In the case of the administration with some time intervals, the compound of the present invention can be administered first, followed by
25 another drug or another drug can be administered first, followed by the compound of the present invention. The administration method of the respective drugs may be the same or different.

 The dosage of the other drug can be properly selected, based on a dosage that has been clinically used. The compounding ratio of the compound of the present invention
30 and the other drug can be properly selected according to age and weight of a subject to be administered, administration method, administration time, disorder to be treated, symptom and combination thereof. For example, the other drug may be used in an amount of 0.01 to 100 parts by mass, based on 1 part by mass of the compound of the

present invention. The other drug may be a combination of two or more kind of arbitrary drugs in a proper proportion. The other drug that complements and/or enhances the preventive and/or therapeutic efficacy of the compound of the present invention includes not only those that have already been discovered, but those that will be discovered in future, based on the above mechanism.

Diseases on which this concomitant use exerts a preventive and/or therapeutic effect are not particularly limited. The concomitant medicine can be used for any diseases, as long as it complements and/or enhances the preventive and/or therapeutic efficacy of the compound of the present invention.

The compound(s) of the present invention can be used with an existing chemotherapeutic concomitantly or in a mixture form. Examples of the chemotherapeutic include an alkylation agent, nitrosourea agent, antimetabolite, anticancer antibiotics, vegetable-origin alkaloid, topoisomerase inhibitor, hormone drug, hormone antagonist, aromatase inhibitor, P-glycoprotein inhibitor, platinum complex derivative, other immunotherapeutic drugs and other anticancer drugs. Further, it can be used with a cancer treatment adjunct, such as a leucopenia (neutropenia) treatment drug, thrombocytopenia treatment drug, antiemetic and cancer pain intervention drug, concomitantly or in a mixture form.

In one embodiment, the compound(s) of the present invention can be used with other immunomodulators and/or a potentiating agent concomitantly or in a mixture form. Examples of the immunomodulator include various cytokines, vaccines and adjuvants. Examples of these cytokines, vaccines and adjuvants that stimulates immune responses include but not limited to GM-CSF, M-CSF, G-CSF, interferon- α , β , or γ , IL-1, IL-2, IL-3, IL-12, Poly (I:C) and C_pG.

In another embodiment, the potentiating agents includes cyclophosphamide and analogs of cyclophosphamide, anti-TGF β and Imatinib (Gleevac), a mitosis inhibitor, such as paclitaxel, Sunitinib (Sutent) or other antiangiogenic agents, an aromatase inhibitor, such as letrozole, an A2a adenosine receptor (A2AR) antagonist, an angiogenesis inhibitor, anthracyclines, oxaliplatin, doxorubicin, TLR4 antagonists, and IL-18 antagonists.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in art to which the subject

matter herein belongs. As used herein, the following definitions are supplied in order to facilitate the understanding of the present invention.

As used herein, the term 'compound(s)' refers to the compounds disclosed in the present invention.

5 As used herein, the term "comprise" or "comprising" is generally used in the sense of include, that is to say permitting the presence of one or more features or components.

As used herein, the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

10 As used herein, the term "amino" refers to -NH₂ group. Unless set forth or recited to the contrary, all amino groups described or claimed herein may be substituted or unsubstituted.

As used herein, the term "amino acid" refers to amino acids having L or D stereochemistry at the alpha carbon.

15 "Pharmaceutically acceptable salt" is taken to mean an active ingredient, which comprises a compound of the formula (I) in the form of one of its salts, in particular if this salt form imparts improved pharmacokinetic properties on the active ingredient compared with the free form of the active ingredient or any other salt form of the active ingredient used earlier. The pharmaceutically acceptable salt form of the active
20 ingredient can also provide this active ingredient for the first time with a desired pharmacokinetic property which it did not have earlier and can even have a positive influence on the pharmacodynamics of this active ingredient with respect to its therapeutic efficacy in the body.

"Pharmaceutically acceptable" means that which is useful in preparing a
25 pharmaceutical composition that is generally safe, non-toxic, and neither biologically nor otherwise undesirable and includes that which is acceptable for veterinary as well as human pharmaceutical use.

The term "stereoisomer" refers to any enantiomers, diastereoisomers, or geometrical isomers of the compounds of formula (I), wherever they are chiral or when
30 they bear one or more double bond. When the compounds of the formula (I) and related formulae are chiral, they can exist in racemic or in optically active form. Since the pharmaceutical activity of the racemates or stereoisomers of the compounds according to the invention may differ, it may be desirable to use the enantiomers. In these cases, the

end product or even the intermediates can be separated into enantiomeric compounds by chemical or physical measures known to the person skilled in the art or even employed as such in the synthesis. In the case of racemic amines, diastereomers are formed from the mixture by reaction with an optically active resolving agent. Examples of suitable
5 resolving agents are optically active acids such as the R and S forms of tartaric acid, diacetyltartaric acid, dibenzoyltartaric acid, mandelic acid, malic acid, lactic acid, suitable N-protected amino acids (for example N-benzoylproline or N-benzenesulfonylproline), or the various optically active camphorsulfonic acids. Also advantageous is chromatographic enantiomer resolution with the aid of an optically
10 active resolving agent (for example dinitrobenzoylphenylglycine, cellulose triacetate or other derivatives of carbohydrates or chirally derivatised methacrylate polymers immobilised on silica gel).

The term "subject" includes mammals (especially humans) and other animals, such as domestic animals (e.g., household pets including cats and dogs) and non-
15 domestic animals (such as wildlife).

"Therapeutically effective amount" or "efficient amount" refers to sufficient amount of the compound(s) of the present invention that (i) treats or prevents the particular disease, disorder or syndrome (ii) attenuates, ameliorates or eliminates one or more symptoms of the particular disease, disorder or syndrome or (iii) prevents or delays
20 the onset of one or more symptoms of the particular disease, disorder or syndrome described herein. In the case of cancer, the therapeutically effective amount of the drug may decrease the number of cancer cells; decrease the cancer size; inhibit (i.e., slow to some extent and alternatively stop) cancer cell infiltration into peripheral organs; suppress (i.e., slow to some extent and alternatively stop) tumor metastasis; inhibit, to
25 some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. In the case of infectious disease states, the therapeutic effective amount is an amount sufficient to decrease or alleviate an infectious diseases, the symptoms of an infections caused by bacterial, viral and fungal.

Naturally-occurring amino acids are identified throughout by the conventional
30 three-letter abbreviations indicated in the below table 2.

Table 2 (Amino acid codes)

Name	3-letter code	Name	3-letter code
Asparagine	Asn	Glutamine	Gln

Aspartic acid	Asp	Phenylalanine	Phe
Alanine	Ala	Serine	Ser
Glutamic acid	Glu	Threonine	Thr

The abbreviations used in the entire specification may be summarized hereinbelow with their particular meaning.

°C (degree Celsius); δ (delta); % (percentage); brine (NaCl solution);
 5 CH₂Cl₂/DCM (Dichloromethane); br s (Broad singlet); Cs₂CO₃ (Caesium carbonate); d (Doublet); DMF (Dimethyl formamide); DMSO (Dimethyl sulphoxide); DMSO-d₆ (Deuterated DMSO); EDC.HCl/EDCI (1-(3-Dimethyl aminopropyl)-3-carbodiimide hydrochloride); Et₂NH (Diethylamine); Fmoc (Fluorenylmethyloxycarbonyl chloride); g or gr (gram); H or H₂ (Hydrogen); H₂O (Water); HOBT/HOBT (1-Hydroxy benzotriazole); HCl (Hydrochloric acid); h or hr (Hours); Hz (Hertz); HPLC (High-
 10 performance liquid chromatography); I₂ (Iodine); K₂CO₃ (Potassium carbonate); LCMS (Liquid chromatography mass spectroscopy); MeOH (Methanol); mmol (Millimoles); M (Molar); μ l (Microlitre); mL (Millilitre); mg (Milligram); m (Multiplet); MHz (Megahertz); MS (ES) (Mass spectroscopy-electro spray); min. (Minutes); Na (Sodium);
 15 NaHCO₃ (Sodium bicarbonate); NH₂NH₂.H₂O (Hydrazine hydrate); NMM (N-methyl morpholine); Na₂SO₄ (Sodium sulphate); N₂ (Nitrogen); NMR (Nuclear magnetic resonance spectroscopy); PD-L1 (Programmed death-ligand 1); PD-L2 (Programmed cell death 1 ligand 2); prep-HPLC/preparative HPLC (Preparative High-performance liquid chromatography); S (Singlet); ^tBu (tertiary butyl); TEA/Et₃N (Triethyl amine);
 20 TLC (Thin Layer Chromatography); THF (Tetrahydrofuran); TIPS (Triisopropylsilane); TFA/CF₃COOH (Trifluoroacetic acid); t (Triplet); t_R = (Retention time); TPP (Triphenylphosphine); etc.

EXPERIMENTAL

An embodiment of the present invention provides the preparation of compounds
 25 of formula (I) according to the procedures of the following examples, using appropriate materials. Those skilled in the art will understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare these compounds. Moreover, by utilizing the procedures described in detail, one of ordinary skill in the art can prepare additional compounds of the present invention.

The starting materials are generally available from commercial sources such as Sigma-Aldrich, USA or Germany; Chem-Impex USA; G.L. Biochem, China and Spectrochem, India.

Purification and characterization of compounds

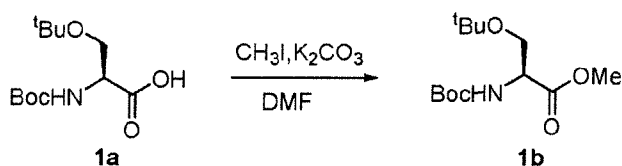
Analytical HPLC method: Analytical HPLC was performed using on ZIC HILIC 200 A° column (4.6 mm × 250 mm, 5 µm), Flow rate: 1.0 mL / min. The elution conditions used are: Buffer A: 5 mmol ammonium acetate, Buffer B: Acetonitrile, Equilibration of the column with 90 % buffer B and elution by a gradient of 90 % to 40 % buffer B during 30 min.

Preparative HPLC Method: Preparative HPLC was performed using on SeQuant ZIC HILIC 200 A^o column (10 mm × 250 mm, 5 μm), Flow rate: 5.0 ml / min. The elution conditions used are: Buffer A: 5 mmol ammonium acetate (adjust to pH-4 with Acetic Acid), Buffer B: Acetonitrile, Equilibration of the column with 90 % buffer B and elution by a gradient of 90 % to 40 % buffer B during 20 min.

LCMS was performed on API 2000 LC/MS/MS triple quad (Applied bio systems) with Agilent 1100 series HPLC with G1315 B DAD, using Mercury MS column or using Agilent LC/MSD VL single quad with Agilent 1100 series HPLC with G1315 B DAD, using Mercury MS column or using Shimadzu LCMS 2020 single quad with Prominence UFLC system with SPD-20 A DAD.

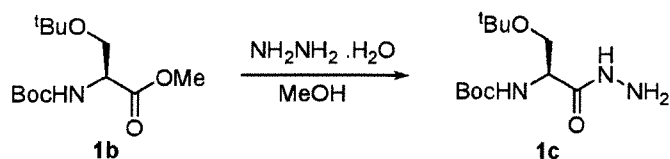
20 **Example 1: Synthesis of compound 1**

Step 1a:



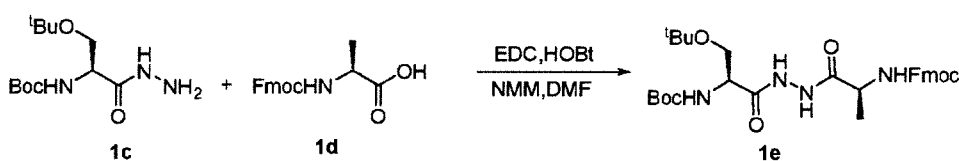
Potassium carbonate (7.9 g, 57.39 mmol) and Methyl iodide (1.3 mL, 21.04 mmol) were added to a solution of compound **1a** (5.0 g, 19.13 mmol) in DMF (35 mL) and stirred at room temperature for 2 h. The completeness of the reaction was confirmed by TLC analysis. The reaction mixture was partitioned between water and ethyl acetate. Organic layer was washed with water, brine, dried over Na₂SO₄ and evaporated under reduced pressure to get 5.0 g of compound **1b** (Yield: 96.1%). LCMS: 176.1 (M-Boc)⁺.

Step 1b:



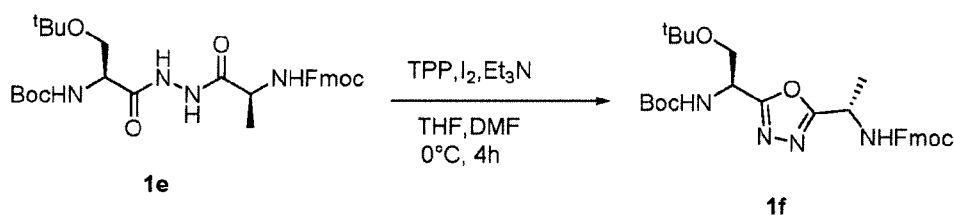
Hydrazine hydrate (7.2 mL) was added to a solution of compound **1b** (5.0 g, 18.16 mmol) in methanol (30 mL) and stirred at room temperature for 2 h. The completeness of the reaction was confirmed by TLC analysis. The reaction mixture was evaporated under reduced pressure, the residue obtained was partitioned between water and ethyl acetate. Organic layer was washed with water, brine, dried over Na₂SO₄ and evaporated under reduced pressure to get 4.0 g of compound **1c** (Yield: 80.0%). LCMS: 276.3 (M+H)⁺.

Step 1c:



NMM (0.67 mL, 6.52 mmol) was slowly added to a stirred solution of **1c** (1.2 g, 4.35 mmol), **1d** (1.43 g, 4.35 mmol), HOBT (0.7 g, 5.22 mmol) and EDC.HCl (0.99 g, 5.22 mmol) in DMF (15 mL) at 0°C. The reaction mixture was stirred at room temperature for 12 h. The completeness of the reaction was confirmed by TLC analysis. The reaction was quenched with ice and the solid precipitated was filtered and dried under vacuum to obtain 2.0 g of pure product **1e** (Yield: 83.3%). LCMS: 591.5 (M+Na)⁺.

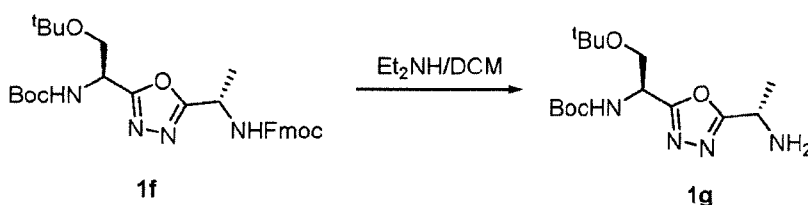
Step 1d:



To a stirred solution of **1e** (1.5 g, 2.63 mmol) in dry THF (15.0 mL) and DMF (5.0 mL) triphenylphosphine (1.38 g, 5.27 mmol) and iodine (1.33 g, 5.27 mmol) were added at 0°C. After the iodine was completely dissolved, Et₃N (1.52 mL, 10.54 mmol) was added to this reaction mixture at ice cold temperature. Reaction mixture was allowed to attain room temperature and stirred for 4 h. The completeness of the reaction was confirmed by TLC analysis. The reaction was quenched with ice water and extracted with ethyl acetate. Organic layer was washed with saturated sodium thiosulphate and brine solution.

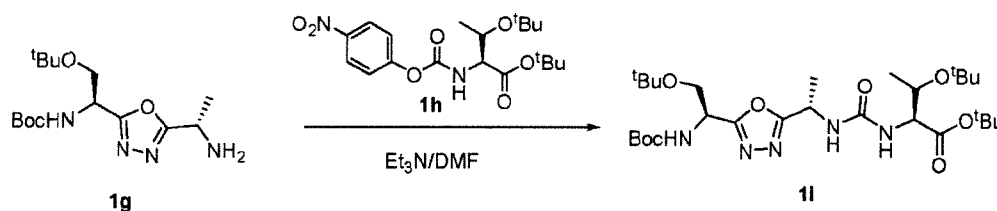
The separated Organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to get residue, which was further purified by silica gel column chromatography (eluent: 30% ethyl acetate in hexane) to afford 0.8 g of compound **1f** (Yield: 55%). LCMS: 551.3 ($\text{M}+\text{H}$)⁺.

5 **Step 1e:**



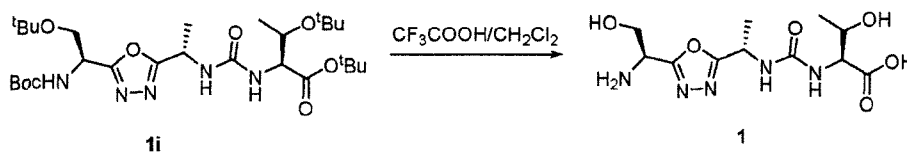
Fmoc group was deprotected by the addition of diethylamine (20.0 mL) to a solution of compound **1f** (0.8 g, 1.45 mmol) in CH_2Cl_2 (20.0 mL) at 0°C . The reaction was stirred at room temperature for 2 h. The resulting solution was concentrated in vacuum to get a thick gummy residue. The crude compound was purified by neutral alumina column chromatography (eluent: 2% methanol in chloroform) to afford 0.38 g of compound **1g** (Yield: 80.0%); LCMS: 329.4 ($\text{M}+\text{H}$)⁺.

Step 1f:



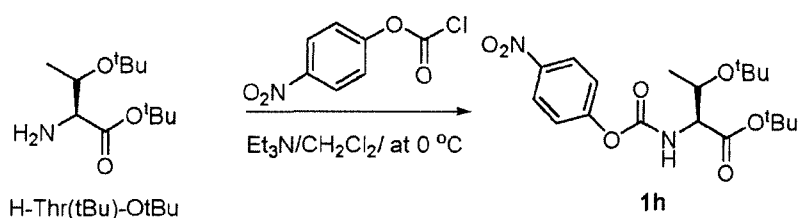
Compound **1g** (0.38 g, 1.16 mmol), TEA (0.33 mL, 2.32 mmol) dissolved in DMF (10 mL) were added drop wise to a solution of **1h** (0.55 g, 1.39 mmol) at 0°C for urea bond formation and the mixture was stirred at room temperature for 2 h. The completeness of the reaction was confirmed by TLC analysis. The reaction was quenched with ice water, the solid precipitated was filtered and dried under vacuum to get crude compound, which was further purified by silica gel column chromatography (eluent: 0-35% ethyl acetate in hexane) to get 0.4 g of product **1i** (Yield: 59.7%). LCMS: 586.4 ($\text{M}+\text{H}$)⁺.

Step 1g:



To a solution of compound **1i** (0.4 g, 0.68 mmol) in CH₂Cl₂ (5 mL), trifluoroacetic acid (5 mL) and catalytic amount of triisopropylsilane were added and stirred at room temperature for 3 h to remove the acid sensitive protecting groups. The resulting solution was concentrated under nitrogen and the solid material was purified by preparative HPLC method as described under experimental conditions (Yield: 0.05 g). LCMS: 318.0 (M+H)⁺; HPLC: t_R = 10.96 min.

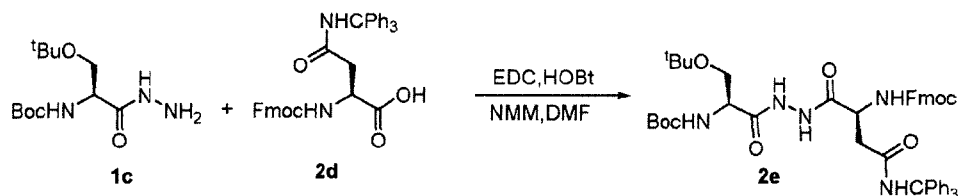
Synthesis of compound 1h (NO₂-C₆H₄-OCO-Thr(tBu)-O^tBu):



To a solution of 4-nitrophenylchloroformate (4.79 g, 23.77 mmol) in DCM (25.0 mL) was added a solution of H-Thr(tBu)-OtBu (5.0 g, 21.61 mmol) TEA (6.2 mL, 43.22 mmol) in CH₂Cl₂ (25 mL) slowly at 0°C and allowed to stir for 30 min. The completion of the reaction was confirmed by TLC analysis. After completion of reaction it was diluted with DCM and washed with 1.0 M of citric acid followed by 1.0 M sodium carbonate solution. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure to afford crude compound **1h**, which was further purified by silica gel column chromatography (eluent: 0-5% ethyl acetate in hexane) to get 3.0 g of product **1h**. ¹H NMR (CDCl₃, 400 MHz): δ 1.17 (s, 9H), 1.28 (d, 3H), 1.50 (s, 9H), 4.11 (m, 1H), 4.28 (m, 1H), 5.89 (d, 1H), 7.37 (d, 2H), 8.26 (d, 2H).

Example 2: Synthesis of compound 2

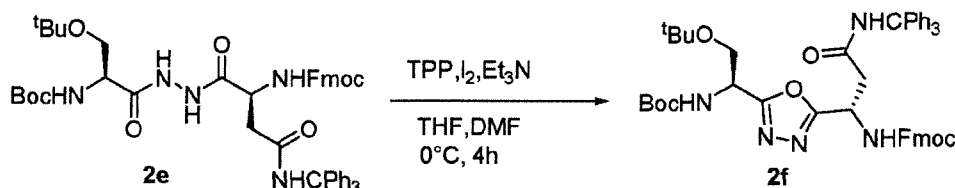
Step 2a:



NMM (1.8 mL, 18.15 mmol) was slowly added to a stirred solution of **1c** (2.0 g, 7.26 mmol), **2d** (4.3 g, 7.26 mmol), HOBT (1.17 g, 8.7 mmol) and EDC.HCl (1.66 g, 8.7 mmol) in DMF (15 mL) at 0°C. The reaction mixture was stirred at room temperature for 12 h. The completeness of the reaction was confirmed by TLC analysis. The reaction

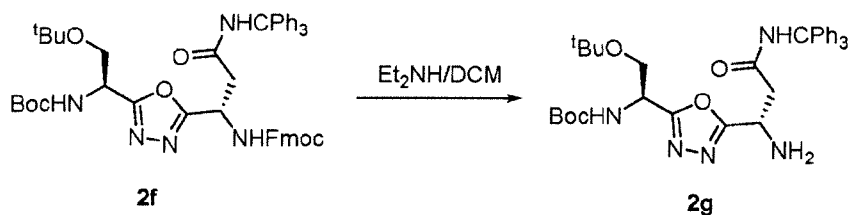
was quenched with ice, the solid precipitated was filtered and dried under vacuum to afford 3.7 g of pure product **2e** (Yield: 59.6%). LCMS: 854.4 (M+H)⁺.

Step 2b:



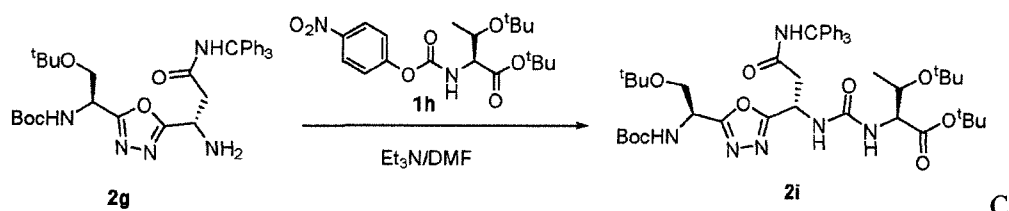
To a stirred solution of **2e** (3.7 g, 4.33 mmol) dissolved in dry THF (25.0 mL) and DMF (10.0 mL), triphenylphosphine (2.28 g, 8.66 mmol) and iodine (2.2 g, 8.66 mmol) were added at 0°C. After the iodine was completely dissolved, Et₃N (2.5 mL, 17.32 mmol) was added at same temperature. The reaction mixture was stirred at room temperature for 4 h. The completeness of the reaction was confirmed by TLC analysis. The reaction was quenched with ice water and extracted with ethyl acetate. The organic layer was washed with saturated sodium thiosulphate and brine solution. The separated organic layer was dried over Na₂SO₄ and evaporated under reduced pressure, which was further purified by silica gel column chromatography (eluent: 30% ethyl acetate in hexane) to get 2.0 g of compound **2f** (Yield: 55%). LCMS: 858.4 (M+Na)⁺.

15 **Step 2c:**



Diethylamine (30.0 mL) was added to a solution of compound **2f** (2.0 g, 1.17 mmol) in CH₂Cl₂ (30.0 mL) at 0°C. The reaction mixture was stirred at room temperature for 1 h. The resulting solution was concentrated in vacuum to get a thick gummy residue. The crude compound was purified by neutral alumina column chromatography (eluent: 2% methanol in chloroform) to afford 1.0 g of compound **2g** (Yield: 71.4%). LCMS: 614.5 (M+H)⁺.

Step 2d:

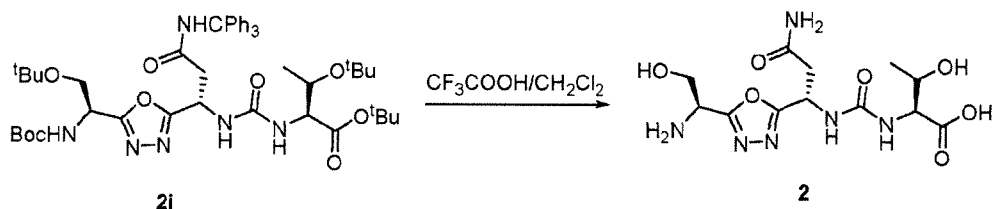


C

Compound **2g** (1.0 g, 1.63 mmol) and TEA (0.47 mL, 3.2 mmol) dissolved in DMF (10 mL) were added drop wise to a solution of **1h** (0.7 g, 1.79 mmol) at 0 °C. The reaction mixture was then allowed to reach room temperature and continued the stirring for 2 h.

- 5 The completeness of the reaction was confirmed by TLC analysis. The reaction was quenched with ice water, the solid precipitated was filtered and dried under vacuum. The crude compound obtained was further purified by silica gel column chromatography (eluent: 0-30% ethyl acetate in hexane) to get 0.8 g of product **2i** (Yield: 57.1%). LCMS: 871.6 (M+H)⁺.

10 Step 2e:



To a solution of compound **2i** (0.8 g, 0.92 mmol) in CH₂Cl₂ (6 mL), trifluoroacetic acid (6 mL) and catalytic amount of triisopropylsilane were added and stirred at room temperature for 3 h. The resulting solution was concentrated under nitrogen and the solid material was purified by preparative HPLC method described under experimental conditions (Yield: 0.065 g). HPLC: t_R = 12.01 min.; LCMS: 361.34 (M+H)⁺.

- 15

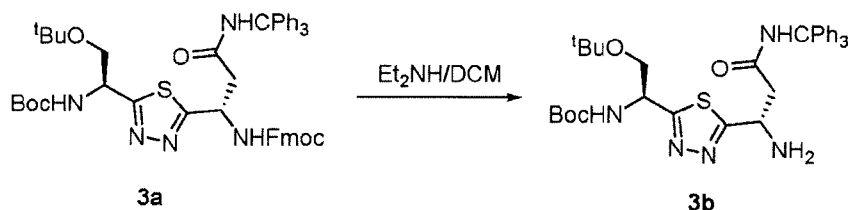
Example 3: Synthesis of compound 3

Step 3a:

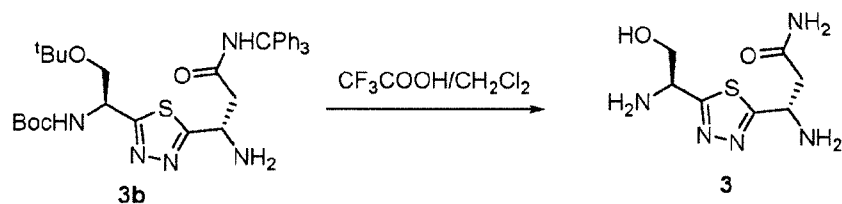


- 20 Lawesson's reagent (2.85 g, 7.03 mmol) was added to a solution of compound **2e** (4 g, 4.68 mmol) in THF (40 mL) and stirred at 75°C for 4 h. The completeness of the reaction was confirmed by TLC analysis. The reaction mixture was evaporated under reduced

pressure and the obtained residue was partitioned between ice water and ethyl acetate. The organic layer was washed with NaHCO_3 solution followed brine solution. The organic layer was dried over Na_2SO_4 , filtered and evaporated under reduced pressure to get residue which was further purified by silica gel column chromatography (eluent: 0-5% ethyl acetate in hexane) to afford 2.7 g of compound **3a** (Yield: 67.66%). LCMS: 852.3 ($\text{M}+\text{H}$)⁺,

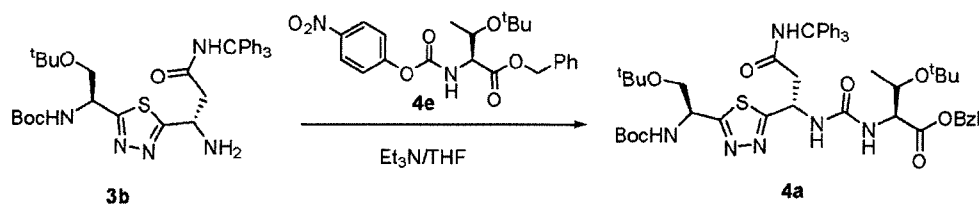
Step 3b:

Fmoc group on compound **3a** was deprotected by adding diethylamine (3.8 mL) to the solution of compound **3a** (1 g, 1.17 mmol) in CH_2Cl_2 (3.8 mL). The reaction mixture was stirred at room temperature for 30 min. The resulting solution was concentrated in vacuum to get a thick gummy residue. The crude compound was purified by neutral alumina column chromatography (eluent: 0-50% ethyl acetate in hexane then 0-5% methanol in chloroform) to attain 0.62 g of compound **3b**. LCMS: 630.5 ($\text{M}+\text{H}$)⁺.

Step 3c:

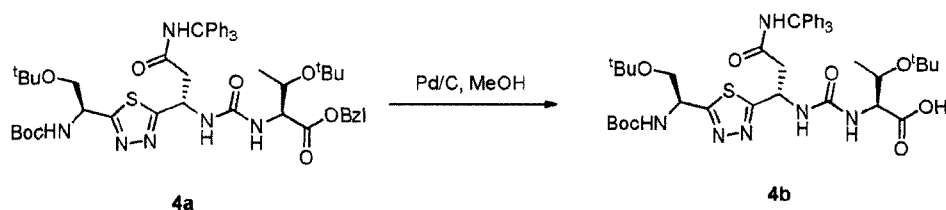
To a solution of compound **3b** (0.6 g) in CH_2Cl_2 (7.5 mL), trifluoroacetic acid (2.5 mL) and catalytic amount of triisopropylsilane were added and stirred at room temperature for 3 h. The resulting solution was concentrated in vacuum to get 0.13 g of compound **3** which was purified by preparative HPLC method described under experimental conditions. LCMS: 232.3 ($\text{M}+\text{H}$)⁺.

Example 4: Synthesis of compound 4**Step 4a:**



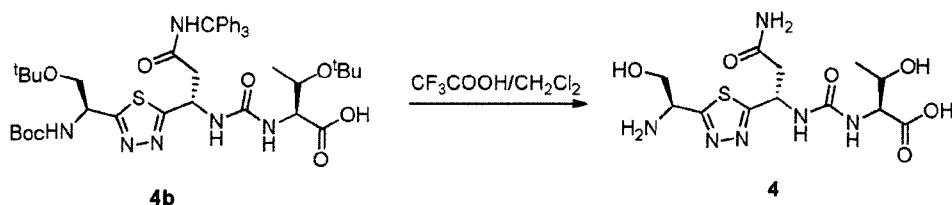
The urea linkage was carried out by coupling of compound **3b** (0.5 g, 7.9 mmol) in THF (10 mL) at room temperature with compound **4e** (0.34 g, 7.9 mmol). The coupling was initiated by the addition of TEA (0.16 g, 15.8 mmol) in THF (10 mL) and the resultant mixture was stirred at room temperature. After 12 h, THF was evaporated from the reaction mass, and partitioned between water and ethyl acetate. The organic layer was washed with water, brine, dried over Na₂SO₄ and evaporated under reduced pressure to yield **4a**, which was further purified by silica gel column chromatography (eluent: 0-50% ethyl acetate in hexane) to get 0.45 g of product **4a** (Yield: 61.64%). LCMS: 921.8 (M+H)⁺.

Step 4b:



To a solution of compound **4a** (0.55 g) in methanol (20 mL), was added 10% Pd-C (0.15 g) under inert atmosphere. The mixture was stirred for 1 h under H₂ atmosphere. The completion of the reaction was confirmed by TLC analysis. The Pd-C catalyst was then removed by filtration through a Celite[®] pad and washed with 20 mL of methanol. The combined organic filtrate on evaporation under reduced pressure resulted in the isolation of the product **4b** (Yield: 0.42 g, 85.71%). LCMS: 831.5 (M+H)⁺.

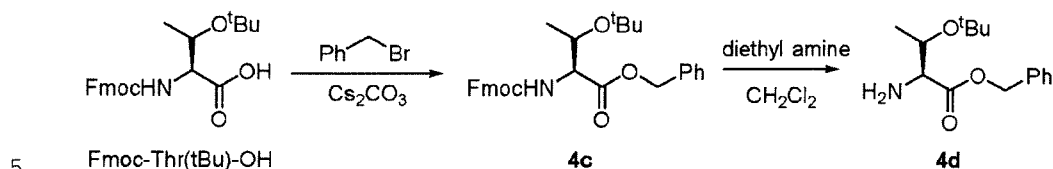
Step 4c:



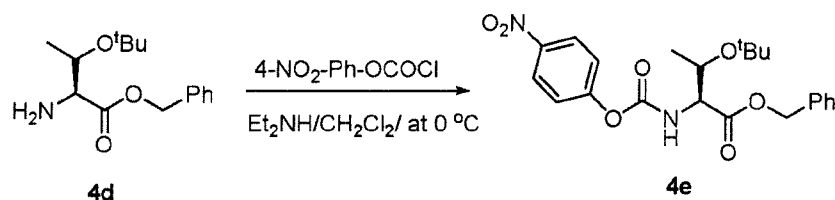
To a solution of compound **4b** (0.2 g, 0.3 mmol) in CH₂Cl₂ (5 mL), trifluoroacetic acid (5 mL) and catalytic amount of triisopropylsilane were added and stirred at room

temperature for 3h. The resulting solution was concentrated in vacuum and the solid material was purified by preparative HPLC method described under experimental conditions (Yield: 0.065 g). HPLC: $t_R = 14.1$ min.; LCMS: 377.3 (M+H)⁺.

Synthesis of compound 4e, (NO₂-C₆H₄-OCO-Thr(O^tBu)-Bzl):



- To a solution of compound Fmoc-Thr(^tBu)-OH (15 g, 37.73 mmol) in 100 mL of DMF, Cs₂CO₃ (14.75 g, 45.2 mmol) was added and the resulting mixture was cooled to 0 °C. To the cooled reaction mixture benzyl bromide (7.74 g, 45.2 mmol) was added and the solution was stirred at ice cold temperature for 30 min and then at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure and diluted with ethyl acetate. The organic layer was washed with water followed by brine solution and dried over Na₂SO₄. The filtered solution was concentrated and purified by silica gel column chromatography (eluent: 0-30% ethyl acetate in hexane) to get 18 g of **4c** as white solid. LCMS: 433.1 (M-O^tBu)⁺, 397.2 (M-OBzl)⁺.
- 15 Fmoc group on compound **4c** (25 g, 51.3 mmol) was deprotected by adding diethylamine (100 mL) to compound **4d** (25 g, 51.3 mmol) in CH₂Cl₂ (100 mL) for 1 h with stirring at room temperature. The resulting solution was concentrated in vacuum and the thick residue was purified by neutral alumina column chromatography (eluent: 0-50% ethyl acetate in hexane then 0-5% methanol in chloroform) to afford 10.6 g of compound **4d**. LCMS: 266.5 (M+H)⁺.
- 20

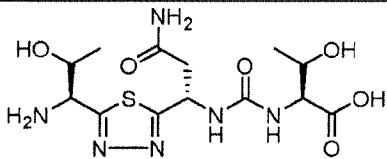
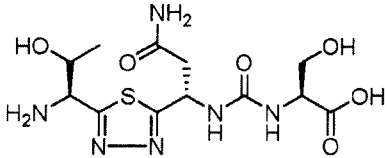
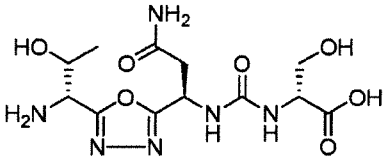
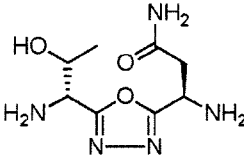
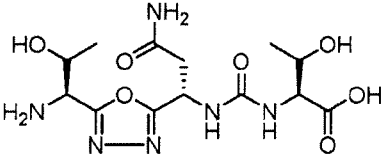
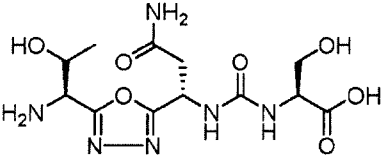


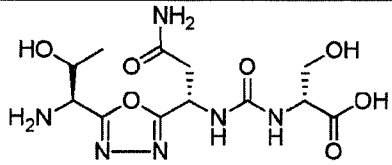
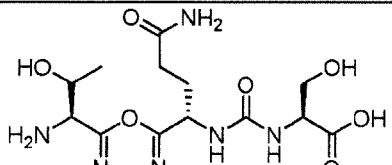
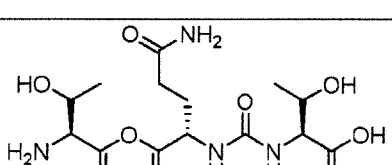
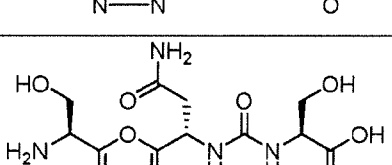
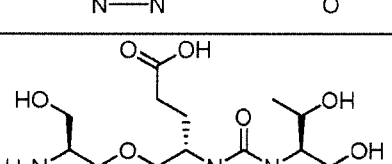
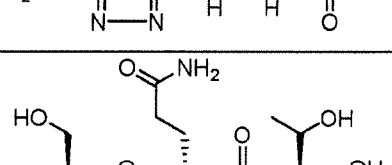
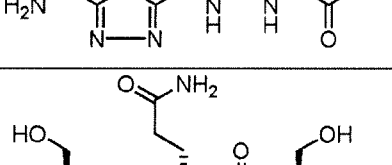
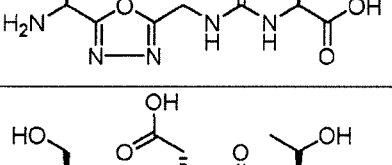
- To a solution of compound **4d** (1.5 g, 5.65 mmol) in CH₂Cl₂ (25 mL) was added TEA (1.14 g, 11.3 mmol) and the solution was stirred at room temperature for 5-10 min. To this mixture a solution of 4-nitrophenyl chloroformate (1.4 g, 6.78 mmol) in CH₂Cl₂ (10 mL) was added and the resultant mixture was stirred at room temperature for 12 h. The completion of the reaction was confirmed by TLC analysis. After completion of reaction it was diluted with DCM and washed with 1.0 M of sodium bisulphate solution followed
- 25

by 1.0 M sodium carbonate solution. The organic layer was dried over Na₂SO₄, filtered and evaporated under reduced pressure to yield crude compound **4e**, which was further purified by silica gel column chromatography (eluent: 0-20% ethyl acetate in hexane) to yield 0.7 g of product **4e**. ¹H NMR (DMSO-*d*⁶, 300 MHz): δ 1.04 (s, 9H), 1.16 (d, 3H), 4.11 (m, 1H), 5.11 (m, 3H), 6.91 (d, 2H), 7.40 (m, 5H), 8.10 (d, 2H), 8.26 (br s, 1H).

The compounds in table 3 below were prepared based on the experimental procedures described above.

Table 3

Compound No.	Structure	LCMS (M+H) ⁺	HPLC t _R in min
5.		391.1	12.43
6.		377.1	-
7.		361.1	12.21
8.		230.1	12.95
9.		375.4	11.55
10.		361.2	11.91

11.		361.1	12.08
12.		375.2	11.5
13.		389.1	11.10
14.		347.1	12.58
15.		376.1	12.20
16.		375.2	11.91
17.		361.2	12.34
18.		362.1	12.50

19.		348.1	12.83
20.		391.1	-

The compounds shown in below table 4, which can be prepared by following similar procedure as described above with suitable modification known to the one ordinary skilled in the art are also included in the scope of the present application.

5

Table 4

Comp ound No.	Structure	Comp ound No.	Structure
21.		26.	
22.		27.	
23.		28.	
24.		29.	
25.			-

Rescue of mouse splenocyte proliferation in the presence of recombinant PD-L1/PD-L2:

Recombinant mouse PD-L1 (rm-PDL-1, cat no: 1019-B7-100 and R&D Systems) were
5 used as the source of PD-L1.

Requirement:

Mouse splenocytes harvested from 6-8 weeks old C57 BL6 mice; RPMI 1640 (GIBCO, Cat # 11875); DMEM with high glucose (GIBCO, Cat # D6429); Fetal Bovine Serum [Hyclone, Cat # SH30071.03]; Penicillin (10000unit/ml)-Streptomycin(10,000µg/ml)
10 Liquid (GIBCO, Cat # 15140-122); MEM Sodium Pyruvate solution 100mM (100x), Liquid (GIBCO, Cat # 11360); Nonessential amino acid (GIBCO, Cat # 11140); L-Glutamine (GIBCO, Cat # 25030); Anti-CD3 antibody (eBiosciences – 16-0032); Anti-CD28 antibody (eBiosciences – 16-0281); ACK lysis buffer (1mL) (GIBCO, Cat # -A10492); Histopaque (density-1.083 gm/mL) (SIGMA 10831); Trypan blue solution
15 (SIGMA-T8154); 2 mL Norm Ject Luer Lock syringe- (Sigma 2014-12); 40 µM nylon cell strainer (BD FALCON 35230); Hemacytometer (Bright line-SIGMA Z359629); FACS Buffer (PBS/0.1% BSA); Phosphate Buffered Saline (PBS) pH 7.2 (HiMedia TS1006) with 0.1% Bovine Serum Albumin (BSA) (SIGMA A7050) and sodium azide (SIGMA 08591); 5 mM stock solution of CFSE: CFSE stock solution was prepared by
20 diluting lyophilized CFSE with 180 µL of Dimethyl sulfoxide (DMSO C₂H₆SO, SIGMA-D-5879) and aliquoted in to tubes for further use. Working concentrations were titrated from 10 µM to 1 µM. (eBioscience-650850-85); 0.05% Trypsin and 0.02% EDTA (SIGMA 59417C); 96-well format ELISA plates (Corning CLS3390); BD FACS caliber (E6016); Recombinant mouse B7-H1/PDL1 Fc Chimera, (rm-PD-L1 cat no:
25 1019-B7-100).

Protocol

Splenocyte preparation and culturing:

Splenocytes harvested in a 50 mL falcon tube by mashing mouse spleen in a 40 µm cell strainer were further treated with 1 mL ACK lysis buffer for 5 min at room temperature.
30 After washing with 9 mL of RPMI complete media, cells were re-suspended in 3 mL of 1xPBS in a 15 mL tube. 3 mL of Histopaque was added carefully to the bottom of the tube without disturbing overlaying splenocyte suspension. After centrifuging at 800xg for 20 min at room temperature, the opaque layer of splenocytes was collected carefully

without disturbing / mixing the layers. Splenocytes were washed twice with cold 1xPBS followed by total cell counting using Trypan Blue exclusion method and used further for cell based assays.

Splenocytes were cultured in RPMI complete media (RPMI + 10% fetal bovine serum + 1 mM sodium pyruvate + 10,000units/ml penicillin and 10,000µg/ml streptomycin) and maintained in a CO₂ incubator with 5% CO₂ at 37°C.

CFSE Proliferation assay:

CFSE is a dye that passively diffuses into cells and binds to intracellular proteins. 1x10⁶ cells/mL of harvested splenocytes were treated with 5 µM of CFSE in pre-warmed 1xPBS/0.1% BSA solution for 10 min at 37°C. Excess CFSE was quenched using 5 volumes of ice-cold culture media to the cells and incubated on ice for 5 min. CFSE labelled splenocytes were further given three washes with ice cold complete RPMI media. CFSE labelled 1x10⁵ splenocytes added to wells containing either MDA-MB231 cells (1x10⁵ cells cultured in high glucose DMEM medium) or recombinant human PDL-1 (100 ng/mL) and test compounds. Splenocytes were stimulated with anti-mouse CD3 and anti- mouse CD28 antibody (1 µg/mL each), and the culture was further incubated for 72 h at 37°C with 5% CO₂. Cells were harvested and washed thrice with ice cold FACS buffer and % proliferation was analyzed by flow cytometry with 488 nm excitation and 521 nm emission filters.

Data compilation, processing and inference:

Percent splenocyte proliferation was analyzed using cell quest FACS program and percent rescue of splenocyte proliferation by compound was estimated after deduction of % background proliferation value and normalising to % stimulated splenocyte proliferation (positive control) as 100%.

Stimulated splenocytes: Splenocytes + anti-CD3/CD28 stimulation

Background proliferation: Splenocytes + anti-CD3/CD28 + PD-L1

Compound proliferation: Splenocytes + anti-CD3/CD28 + PD-L1 + Compound

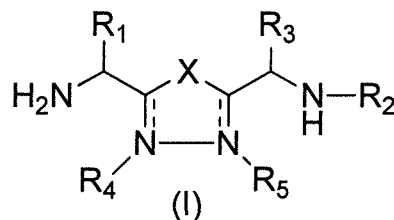
Compound effect is examined by adding required concentration of compound to anti-CD3/CD28 stimulated splenocytes in presence of ligand (PDL-1)

Table 5

Compound No.	Percent rescue of splenocyte proliferation (@100 nM compound concentration)	Compound No.	Percent rescue of splenocyte proliferation (@100 nM compound concentration)
1	61.2	13	75
2	80.3	14	53
3	48.4	15	69
4	60	16	56
9	74	17	53
10	58	18	68
12	92	-	-

CLAIMS

1. A compound of formula (I)



- 5 wherein,

R_1 represents a side chain of an amino acid selected from Ser, Thr, Phe, Ala and Asn;

X is S or O;

R_2 is hydrogen or -CO-Aaa;

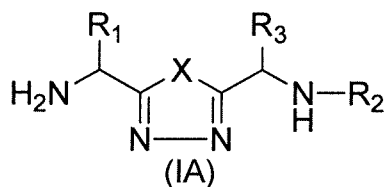
- 10 Aaa is an amino acid residue selected from Ser, Asn and Thr; wherein a C-terminus thereof is a free terminus, is amidated or is esterified;

R_3 represents a side chain of an amino acid selected from Ser, Ala, Glu, Gln, Asn and Asp;

---- is an optional bond;

- 15 R_4 and R_5 independently are hydrogen or absent;
or a pharmaceutically acceptable salt thereof.

2. The compound according to claim 1, having a structure of formula (IA):



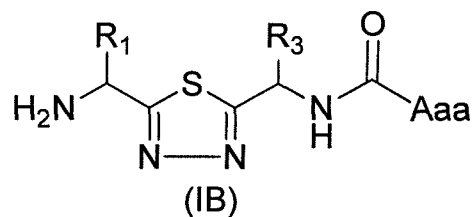
20

3. The compound according to claim 1 or 2, wherein X is S.

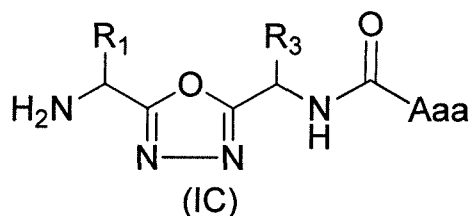
4. The compound according to claim 1 or 2, wherein X is O.

- 25 5. The compound according to any one of claims 1 to 4, wherein R_2 is hydrogen.

6. The compound according to claim 1 or 2, having the structure of formula (IB):



7. The compound according to claim 1 or 2, having the structure of formula (IC):



5

8. The compound according to any one of claims 1 to 4, 6, and 7, wherein R₂ is -CO-Aaa.

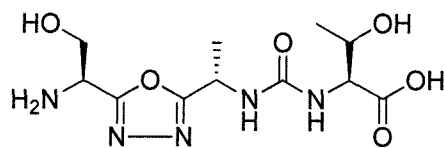
- 10 9. The compound according to any one of claims 1 to 4 and 6 to 8, wherein Aaa is an amino acid residue Ser or Thr; wherein the C-terminus is a free terminus.

10. The compound according to any one of claims 1 to 9, wherein R₁ represents the side chain of an amino acid residue Ser or Thr.

15

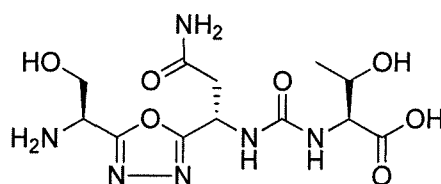
11. The compound according to any one of claims 1 to 10, wherein R₃ represents the side chain of the amino acid residue selected from Glu, Gln, Asn, and Asp.

12. The compound according to claim 1, having the following structure:

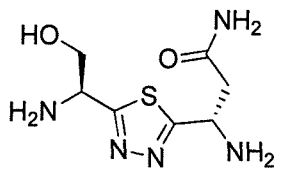


20

13. The compound according to claim 1, having the following structure:

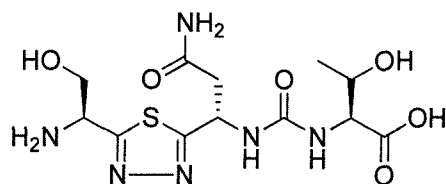


14. The compound according to claim 1, having the following structure:

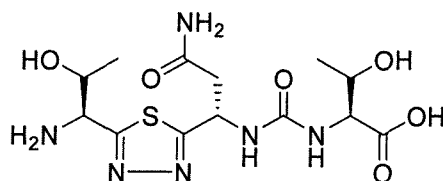


5

15. The compound according to claim 1, having the following structure:

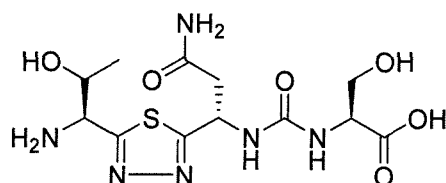


16. The compound according to claim 1, having the following structure:

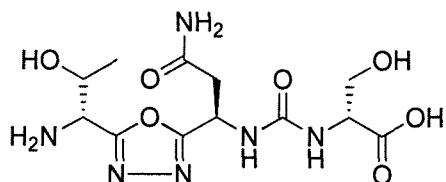


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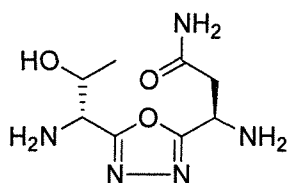
17. The compound according to claim 1, having the following structure:



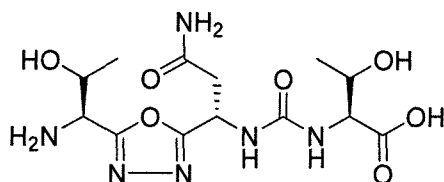
- 15 18. The compound according to claim 1, having the following structure:



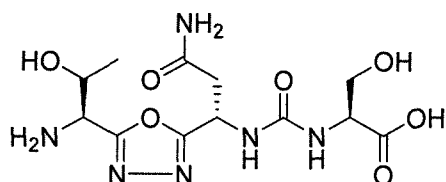
19. The compound according to claim 1, having the following structure:



- 5 20. The compound according to claim 1, having the following structure:

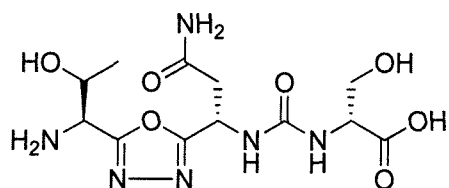


21. The compound according to claim 1, having the following structure:

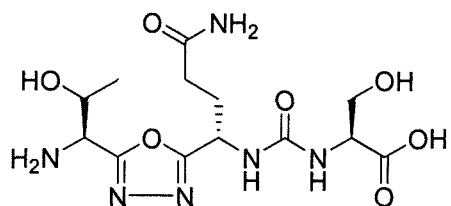


10

22. The compound according to claim 1, having the following structure:

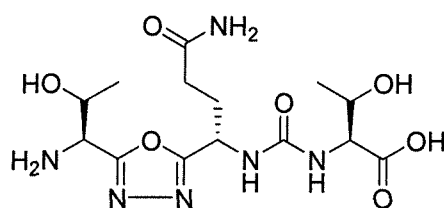


23. The compound according to claim 1, having the following structure:

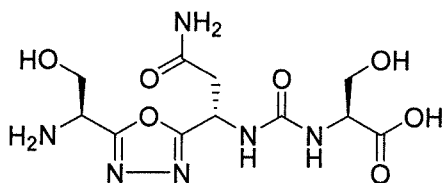


15

24. The compound according to claim 1, having the following structure:

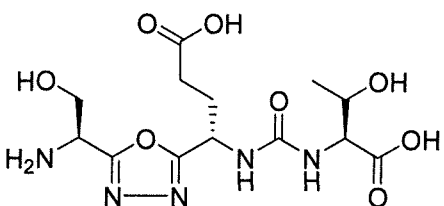


25. The compound according to claim 1, having the following structure:

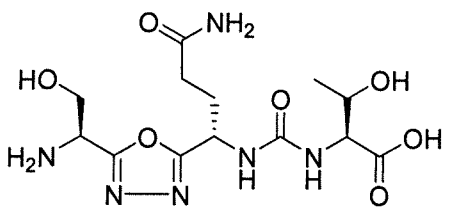


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26. The compound according to claim 1, having the following structure:

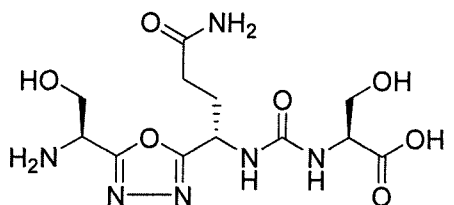


27. The compound according to claim 1, having the following structure:

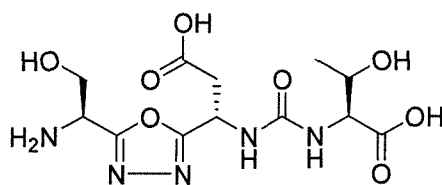


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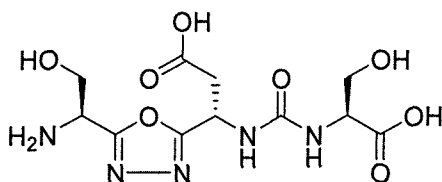
28. The compound according to claim 1, having the following structure:



- 15 29. The compound according to claim 1, having the following structure:

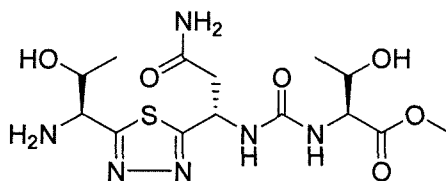


30. The compound according to claim 1, having the following structure:

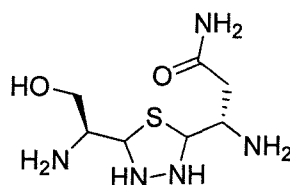


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31. The compound according to claim 1, having the following structure:

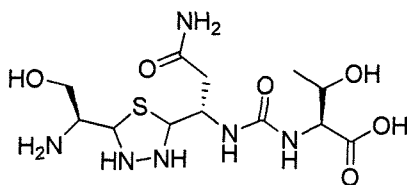


32. The compound according to claim 1, having the following structure:

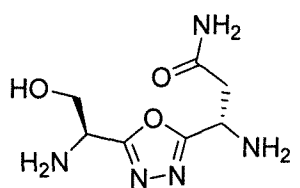


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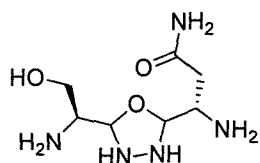
33. The compound according to claim 1, having the following structure:



- 15 34. The compound according to claim 1, having the following structure:

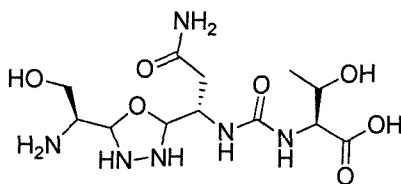


35. The compound according to claim 1, having the following structure:

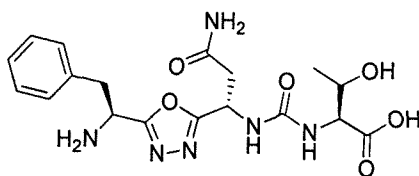


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36. The compound according to claim 1, having the following structure:

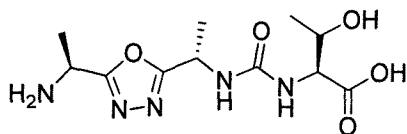


37. The compound according to claim 1, having the following structure:

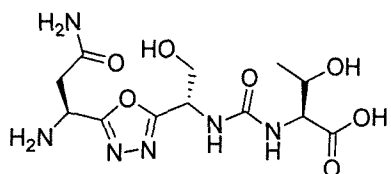


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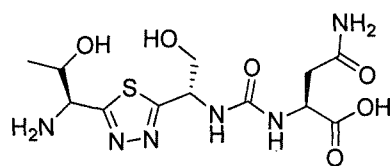
38. The compound according to claim 1, having the following structure:



- 15 39. The compound according to claim 1, having the following structure:



40. The compound according to claim 1, having the following structure:



41. A pharmaceutical composition comprising a pharmaceutically acceptable carrier
5 or excipient and at least one compound according to any one of claims 1 to 40 or a
pharmaceutically acceptable salt thereof.

42. The pharmaceutical composition according to claim 41, further comprising at
least one agent selected from an anticancer agent, a chemotherapy agent, and an
10 antiproliferative compound.

43. Use of a compound according to any one of claims 1 to 40 or a pharmaceutically
acceptable salt thereof, for the manufacture of a medicament for modulating an immune
response mediated by PD-1 signaling pathway in a cancer.

15

44. Use of a compound according to any one of claims 1 to 40 or a pharmaceutically
acceptable salt thereof, for the manufacture of a medicament for the treatment of cancer.

45. Use according to claim 44, wherein the cancer is selected from breast cancer,
20 colon cancer, lung cancer, melanoma, prostate cancer, and renal cancer.

46. Use according to claim 44, wherein the cancer is selected from bone cancer,
cancer of the head or neck, pancreatic cancer, skin cancer, cutaneous or intraocular
malignant melanoma, carcinoma of the endometrium, carcinoma of the cervix,
25 carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's
lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the
endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of
the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis,
chronic or acute leukemias including acute myeloid leukemia, chronic myeloid
30 leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of

childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell
5 lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

47. Use of a compound according to any one of claims 1 to 40 or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for modulating an immune
10 response mediated by PD-1 signaling pathway in an infectious disease.

48. Use of a compound according to any one of claims 1 to 40 or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for modulating an immune response mediated by PD-1 signaling pathway in a bacterial infectious disease, a viral
15 infectious disease or a fungal infectious disease.

49. Use according to claim 47, wherein the infectious disease is selected from HIV, Influenza, Herpes, *Giardia*, Malaria, *Leishmania*, the pathogenic infection by the virus Hepatitis (A, B, & C), herpes virus (e.g., VZV, HSV-I, HAV-6, HSV-II, and CMV,
20 Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus, pathogenic infection by the bacteria *chlamydia*, rickettsial bacteria,
25 mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, *klebsiella*, *proteus*, *serratia*, *pseudomonas*, *E. coli*, *legionella*, diphtheria, *salmonella*, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme's disease bacteria, pathogenic infection by the fungi *Candida* (*albicans*, *krusei*, *glabrata*, *tropicalis*, etc.), *Cryptococcus neoformans*, *Aspergillus* (*fumigatus*, *niger*,
30 etc.), Genus *Mucorales* (*mucor*, *absidia*, *rhizophus*), *Sporothrix schenkii*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis* and *Histoplasma capsulatum*, and pathogenic infection by the parasites *Entamoeba histolytica*,

Balantidium coli, *Naegleria fowleri*, *Acanthamoeba* sp., *Giardia lamblia*, *Cryptosporidium* sp., *Pneumocystis carinii*, *Plasmodium vivax*, *Babesia microti*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondi*, or *Nippostrongylus brasiliensis*.