



(51) International Patent Classification:

C07D 271/06 (2006.01) A61K 31/433 (2006.01)  
C07D 285/08 (2006.01) A61P 31/00 (2006.01)  
A61K 31/4245 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/IB20 14/064281

(22) International Filing Date:

5 September 2014 (05.09.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

4012/CHE/2013 6 September 2013 (06.09.2013) IN

(71) Applicant: AURIGENE DISCOVERY TECHNOLOGIES LIMITED [IN/IN]; 39-40, KIADB Industrial Area, Electronic City Phase II, Hosur Road, Karnataka, Bangalore 560100 (IN).

(72) Inventors: SASIKUMAR, Pottayil Govindan Nair; # 5, Deccan Palms, Ananth Nagar Phase, II, Electronic city post, Bangalore 560100 (IN). RAMACHANDRA, Muralidhara; V173, Adarsh Palm Retreat, Outer Ring, Road, Bellandur Post, Bangalore 560103 (IN). NAREMADDE-PALLI, Seetharamaiah Setty Sudarshan; 33/A, Shenimahathama temple compound, R., K. Layout, K. G. Nagar, Bangalore 560019 (IN).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(H))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: 1,3,4-OXADIAZOLE AND 1,3,4-THIADIAZOLE DERIVATIVES AS IMMUNOMODULATORS

(57) Abstract: The present invention relates to 1,3,4-oxadiazole and 1,3,4-thiadiazole compounds as therapeutic agents capable of inhibiting the programmed cell death 1 (PDI) signalling pathway. The invention also refers to derivatives of the therapeutic agents. The invention also encompasses the use of the said therapeutic agents and derivatives for treatment of disorders via immunopotenti-ation comprising inhibition of immunosuppressive signal induced due to PD-1, PD-L1, or PD-L2 and therapies using them.



## **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).

10 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.

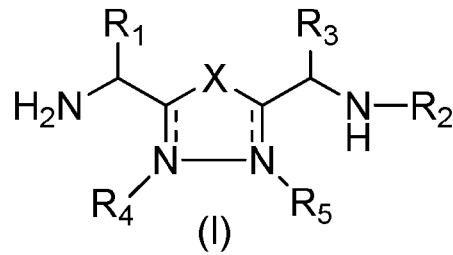
15 Further, International applications, WO2011161699, WO2012/168944, WO2013144704 and WO2013132317 report peptides or peptidomimetic compounds which are capable of suppressing and/or inhibiting the programmed cell death 1 (PD1) signaling pathway.

20 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

25 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,

**R<sub>1</sub>** is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;

X is S or O;

5 **R<sub>2</sub>** 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;

**R<sub>3</sub>** is side chain of an amino acid selected from Ser, Ala, Glu, Gin, Asn or Asp;

— is an optional bond;

10 **R<sub>4</sub>** and **R<sub>5</sub>** 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.

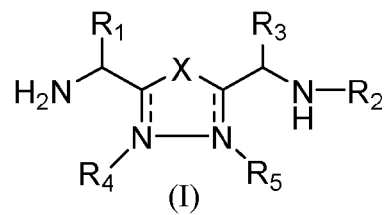
#### DETAILED DESCRIPTION OF THE INVENTION

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

25 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

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.

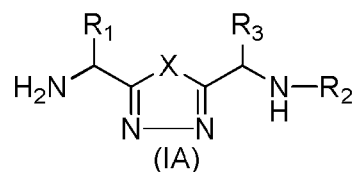
In one embodiment, the present invention relates to compounds of formula (I)



wherein,

- 10 **R<sub>1</sub>** is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;  
**X** is S or O;  
**R<sub>2</sub>** 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<sub>3</sub>** is side chain of an amino acid selected from Ser, Ala, Glu, Gin, Asn or Asp;  
 — is an optional bond;  
**R<sub>4</sub>** and **R<sub>5</sub>** 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

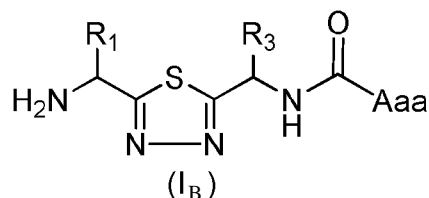
20 (IA)



- or a pharmaceutically acceptable salt or a stereoisomer thereof; wherein,
- R<sub>1</sub>** is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;  
**X** is S or O;
- 25 **R<sub>2</sub>** is hydrogen or -CO-Aaa;  
**R<sub>3</sub>** is side chain of an amino acid selected from Ser, Ala, Glu, Gin, 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)



5

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

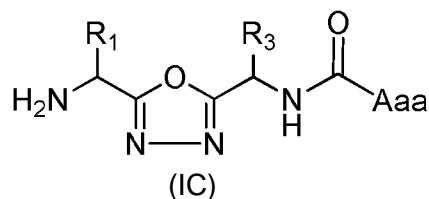
R<sub>1</sub> is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;

R<sub>3</sub> is side chain of an amino acid selected from Ser, Ala, Glu, Gin, 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.

10

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



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

R<sub>1</sub> is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;

R<sub>3</sub> is side chain of an amino acid selected from Ser, Ala, Glu, Gin, 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.

15

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

20

R<sub>1</sub> is side chain of Ser or Thr;

R<sub>2</sub> is -CO-Aaa;

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

R<sub>3</sub> is side chain of Asn, Gin, 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.

25

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  $R_2$  is hydrogen.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which  $R_4$  and  $R_5$  are hydrogen.

10 According to yet another embodiment, specifically provided are compounds of the formula (I) in which  $R_4$  and  $R_5$  are absent.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which  $R_2$  is -CO-Ser.

According to yet another embodiment, specifically provided are compounds of the formula (I) in which  $R_2$  is -CO-Thr.

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

According to yet another embodiment, specifically provided are compounds of the formula (I), (IA), (IB) and (IC) in which  $R_1$  is side chain of Thr.

20 According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IC) in which  $R_1$  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  $R_3$  is side chain of Asn.

According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IB) in which  $R_3$  is side chain of Ser.

25 According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IC) in which  $R_3$  is side chain of Gin.

According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IC) in which  $R_3$  is side chain of Glu.

30 According to yet another embodiment, specifically provided are compounds of the formula (I), (IA) and (IC) in which  $R_3$  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.

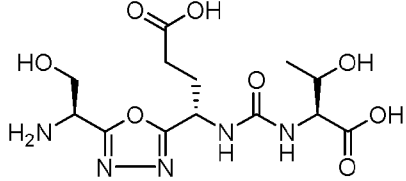
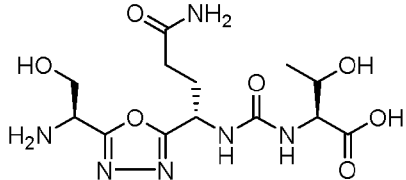
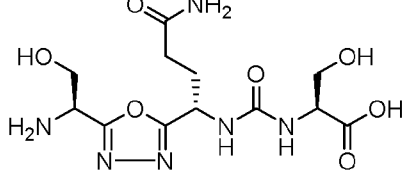
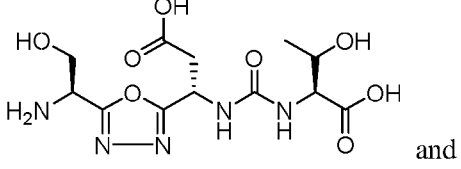
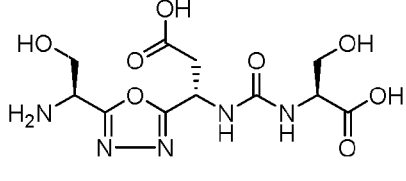
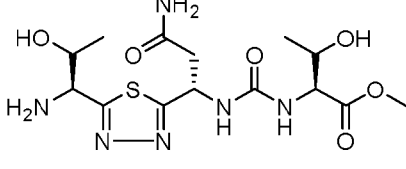
In an embodiment, specific compounds of formula (I) without any limitation are enumerated in Table (1):

**Table 1**

Compound No.	Structure
1.	
2.	
3.	
4.	
5.	
6.	



7.	
8.	
9.	
10.	
11.	
12.	
13.	
14.	

15.	
16.	
17.	
18.	
19.	
20.	

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.

In one embodiment, the present invention provides a method of treatment of  
30 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, pneumococci, 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, Naegleriafowleri, 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 times a day, in the case of an adult, or continuously administered by oral or inhalation  
10 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  
5 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.

10 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  
15 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  
20 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- $\alpha$ ,  $\beta$ , or  $\gamma$ , IL-1, IL-2, IL-3, IL-12, Poly (I:C) and CpG.

25 In another embodiment, the potentiating agents includes cyclophosphamide and analogs of cyclophosphamide, anti-TGF $\beta$  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  
30 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 -N<sup>3/4</sup> 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  
30 geometrical isomers of the compounds of formula (I), wherever they are chiral or when 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 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 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-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 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 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 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);  $\delta$  (delta); % (percentage); brine (NaCl solution);  
 5 CH<sub>2</sub>Cl<sub>2</sub>/DCM (Dichloromethane); br s (Broad singlet); Cs<sub>2</sub>CO<sub>3</sub> (Caesium carbonate); d (Doublet); DMF (Dimethyl formamide); DMSO (Dimethyl sulphoxide); DMSO-d<sub>6</sub> (Deuterated DMSO); EDC.HCl/EDCI (1-(3-Dimethyl aminopropyl)-3-carbodiimide hydrochloride); Et<sub>2</sub>NH (Diethylamine); Fmoc (Fluorenylmethyloxycarbonyl chloride); g or gr (gram); H or H<sub>2</sub> (Hydrogen); H<sub>2</sub>O (Water); HOBt/HOBT (1-Hydroxy  
 10 benzotriazole); HCl (Hydrochloric acid); h or hr (Hours); Hz (Hertz); HPLC (High-performance liquid chromatography); I<sub>2</sub> (Iodine); K<sub>2</sub>CO<sub>3</sub> (Potassium carbonate); LCMS (Liquid chromatography mass spectroscopy); MeOH (Methanol); mmol (Millimoles); M (Molar);  $\mu$ l (Microlitre); mL (Millilitre); mg (Milligram); m (Multiplet); MHz (Megahertz); MS (ES) (Mass spectroscopy-electro spray); min. (Minutes); Na (Sodium);  
 15 NaHCO<sub>3</sub> (Sodium bicarbonate); NH<sub>2</sub>NH<sub>2</sub>·H<sub>2</sub>O (Hydrazine hydrate); NMM (N-methyl morpholine); Na<sub>2</sub>S<sub>0</sub><sub>4</sub> (Sodium sulphate); N<sub>2</sub> (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); 'Bu (tertiary butyl); TEA/Et<sub>3</sub>N (Triethyl amine);  
 20 TLC (Thin Layer Chromatography); THF (Tetrahydrofuran); TIPS (Triisopropylsilane); TFA/CF<sub>3</sub>COOH (Trifluoroacetic acid); t (Triplet); t<sub>R</sub> = (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

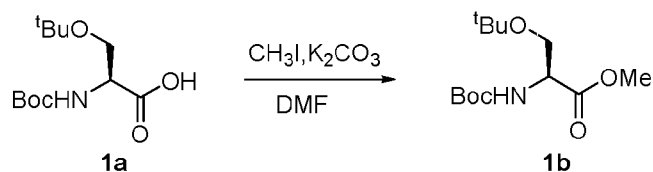
5         **Analytical HPLC method:** Analytical HPLC was performed using on ZIC HILIC 200 A<sup>0</sup> column (4.6 mm x 250 mm, 5 µm), Flow rate: 1.0 mL / min. The elution conditions used are: Buffer A: 5 mmol ammonium acetate, Buffer B: Acetonitrile, Equilibration of the column with 90 % buffer B and elution by a gradient of 90 % to 40 % buffer B during 30 min.

10         **Preparative HPLC Method:** Preparative HPLC was performed using on SeQuant ZIC HILIC 200 A<sup>0</sup> column (10 mm x 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.

15         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.

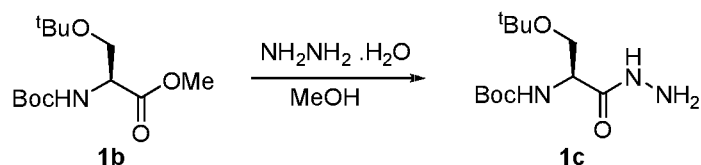
### 20         **Example 1: Synthesis of compound 1**

#### **Step 1a:**



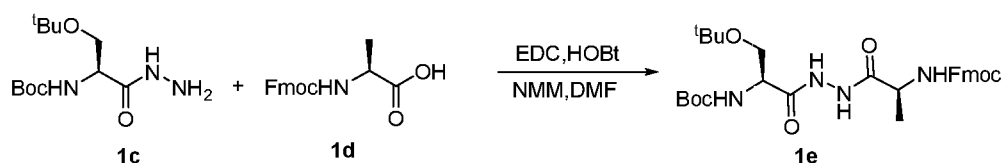
Potassium carbonate (7.9 g, 57.39 mmol) and Methyl iodide (1.3 mL, 21.04 mmol) were added to a solution of compound **1a** (5.0 g, 19.13 mmol) in DMF (35 mL) and stirred at  
25         room temperature for 2 h. The completeness of the reaction was confirmed by TLC analysis. The reaction mixture was partitioned between water and ethyl acetate. Organic layer was washed with water, brine, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under reduced pressure to get 5.0 g of compound **1b** (Yield: 96.1%). LCMS: 176.1 (M-Boc)<sup>+</sup>.

#### **Step 1b:**



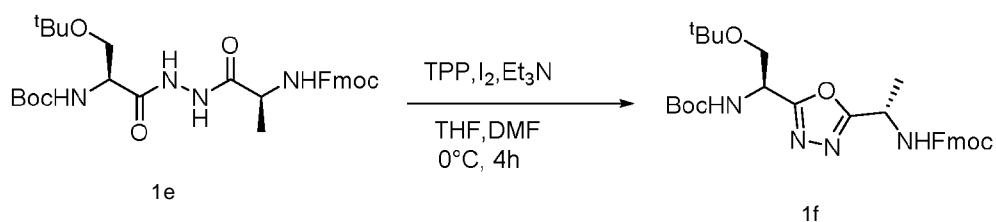
Hydrazine hydrate (7.2 mL) was added to a solution of compound **1b** (5.0 g, 18.16 mmol) in methanol (30 mL) and stirred at room temperature for 2 h. The completeness of the reaction was confirmed by TLC analysis. The reaction mixture was evaporated under reduced pressure, the residue obtained was partitioned between water and ethyl acetate. Organic layer was washed with water, brine, dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure to get 4.0 g of compound **1c** (Yield: 80.0%). LCMS: 276.3 (M+H)<sup>+</sup>.

#### 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)<sup>+</sup>.

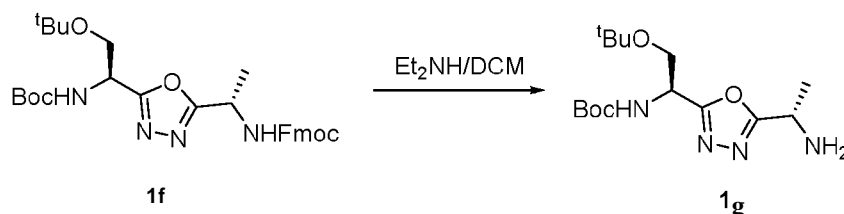
#### Step 1d:



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,  $\text{Et}_3\text{N}$  (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.

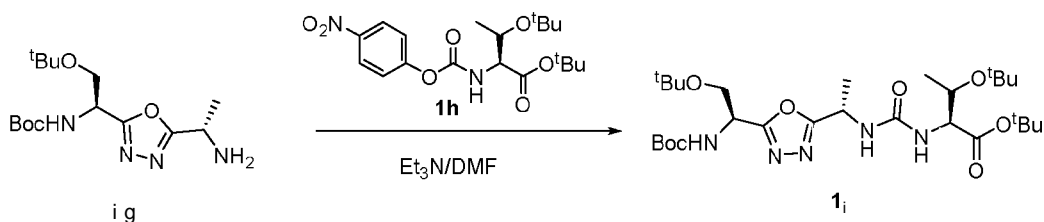
The separated Organic layer was dried over  $\text{Na}_2\text{SO}_4$  and evaporated under reduced pressure to get residue, which was further purified by silica gel column chromatography (eluent: 30% ethyl acetate in hexane) to afford 0.8 g of compound **If** (Yield: 55%). LCMS: 551.3 (M+H)<sup>+</sup>.

5 **Step Ie:**



Fmoc group was deprotected by the addition of diethylamine (20.0 mL) to a solution of compound **If** (0.8 g, 1.45 mmol) in  $\text{CH}_2\text{Cl}_2$  (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 **Ig** (Yield: 80.0%): LCMS: 329.4 (M+H)<sup>+</sup>.

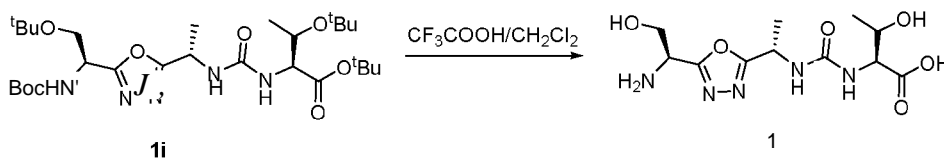
10 **Step If:**



15 Compound **Ig** (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 **Ih** (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

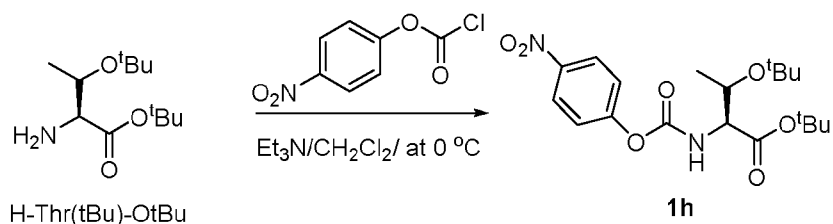
20 was further purified by silica gel column chromatography (eluent: 0-35% ethyl acetate in hexane) to get 0.4 g of product **Ii** (Yield: 59.7%). LCMS: 586.4 (M+H)<sup>+</sup>.

**Step Ig:**



To a solution of compound **1i** (0.4 g, 0.68 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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)<sup>+</sup>; HPLC: t<sub>R</sub>= 10.96 min.

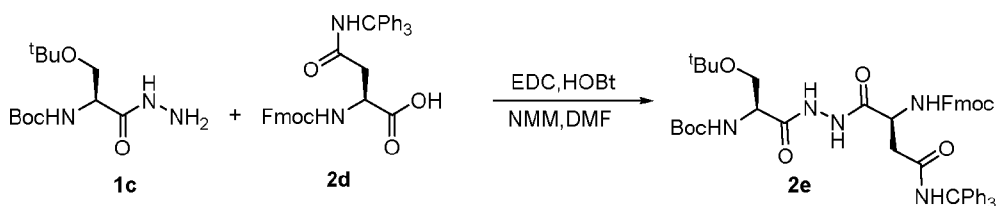
**Synthesis of compound 1h** (NO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-OCO-Thr(tBu)-O<sup>t</sup>Bu):



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)-O<sup>t</sup>Bu (5.0 g, 21.61 mmol) TEA (6.2 mL, 43.22 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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 Na<sub>2</sub>SO<sub>4</sub> 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**. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 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**

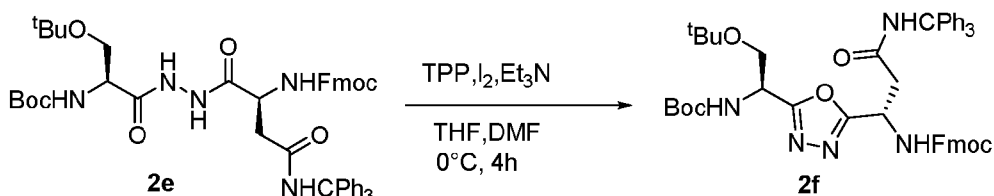
**Step 2a:**



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

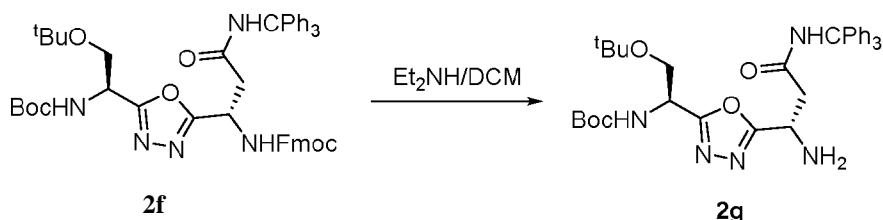
was quenched with ice, the solid precipitated was filtered and dried under vacuum to afford 3.7 g of pure product **2e** (Yield: 59.6%). LCMS: 854.4 (M+H)<sup>+</sup>.

**Step 2b:**



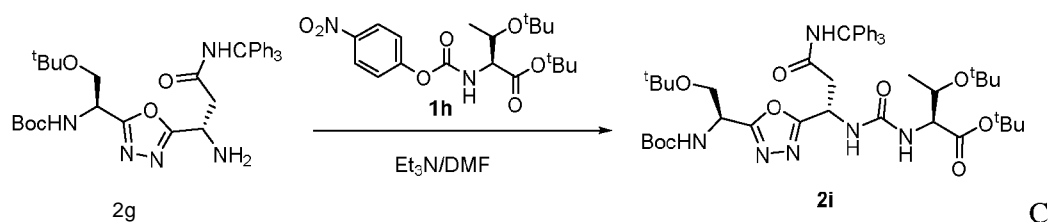
- 5 To a stirred solution of **2e** (3.7 g, 4.33 mmol) dissolved in dry THF (25.0 mL) and DMF (10.0 mL), triphenylphosphine (2.28 g, 8.66 mmol) and iodine (2.2 g, 8.66 mmol) were added at 0°C. After the iodine was completely dissolved, Et<sub>3</sub>N (2.5 mL, 17.32 mmol) was added at same temperature. The reaction mixture was stirred at room temperature for 4 h. The completeness of the reaction was confirmed by TLC analysis. The reaction was
- 10 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 Na<sub>2</sub>SO<sub>4</sub> 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)<sup>+</sup>.

15 **Step 2c:**



- Diethylamine (30.0 mL) was added to a solution of compound **2f** (2.0 g, 1.17 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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
- 20 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)<sup>+</sup>.

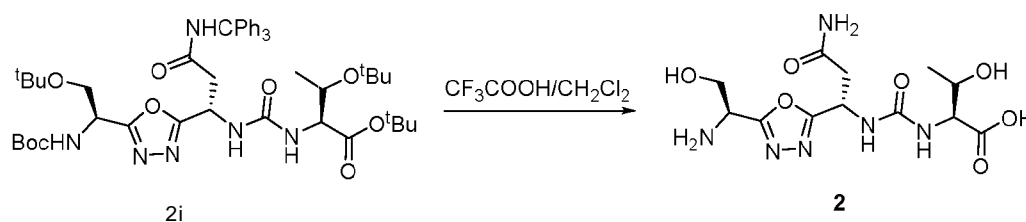
**Step 2d:**



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.

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)<sup>+</sup>.

#### Step 2e:



To a solution of compound **2i** (0.8 g, 0.92 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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<sub>R</sub> = 12.01 min.; LCMS: 361.34 (M+H)<sup>+</sup>.

### Example 3: Synthesis of compound 3

#### Step 3a:

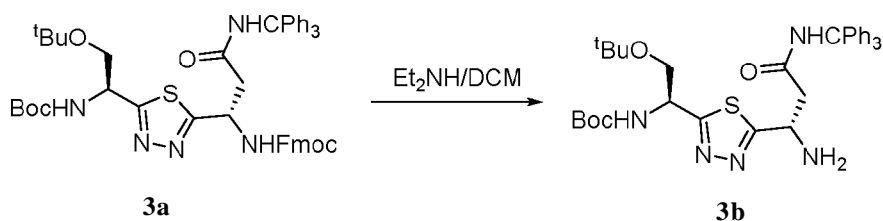


Lawesson's reagent (2.85 g, 7.03 mmol) was added to a solution of compound **2e** (4 g, 4.68 mmol) in THF (40 mL) and stirred at 75°C for 4 h. The completeness of the reaction was confirmed by TLC analysis. The reaction mixture was evaporated under reduced



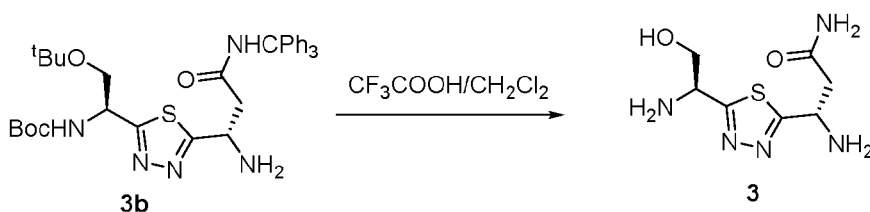
pressure and the obtained residue was partitioned between ice water and ethyl acetate. The organic layer was washed with NaHCO<sub>3</sub> solution followed brine solution. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under reduced pressure to get residue which 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 (M+H)<sup>+</sup>,

**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<sub>2</sub>Cl<sub>2</sub> (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 (M+H)<sup>+</sup>.

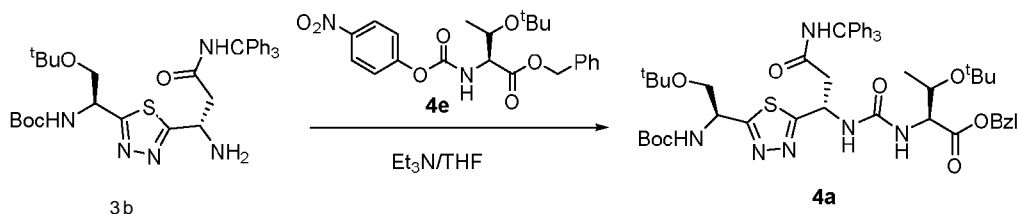
**Step 3c:**



To a solution of compound **3b** (0.6 g) in CH<sub>2</sub>Cl<sub>2</sub> (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 (M+H)<sup>+</sup>.

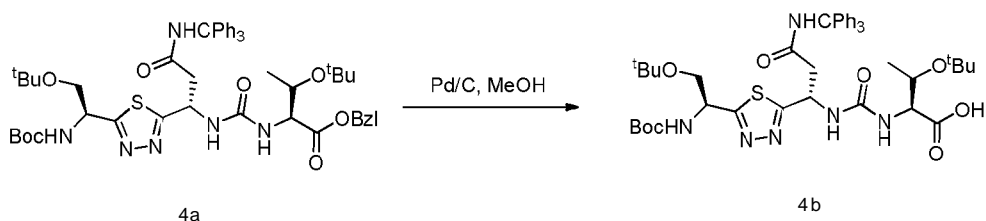
**Example 4: Synthesis of compound 4**

**Step 4a:**



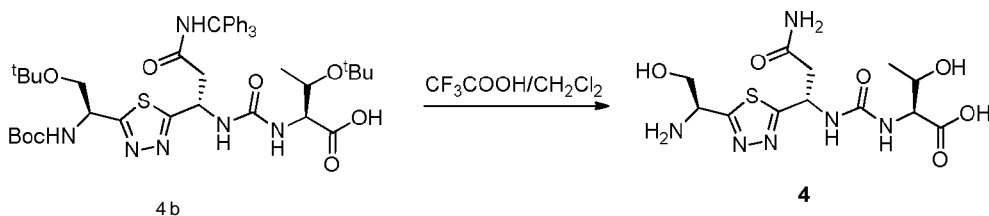
The urea linkage was carried out by coupling of compound **3b** (0.5 g, 7.9 mmol) in THF (10 mL) at room temperature with compound **4e** (0.34 g, 7.9 mmol). The coupling was initiated by the addition of TEA (0.16 g, 15.8 mmol) in THF (10 mL) and the resultant mixture was stirred at room temperature. 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  $\text{Na}_2\text{SO}_4$  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)<sup>+</sup>.

#### 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  $\text{H}_2$  atmosphere. The completion of the reaction was confirmed by TLC analysis. The Pd-C catalyst was then removed by filtration through a Celite<sup>®</sup> 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)<sup>+</sup>.

#### Step 4c:

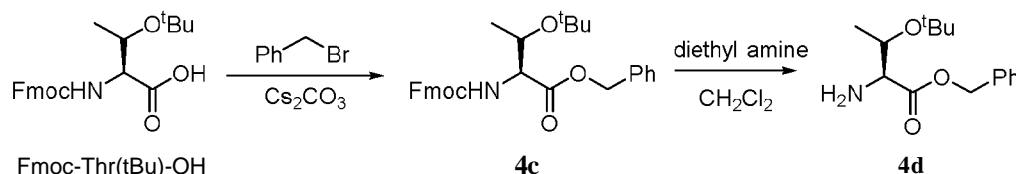


20

To a solution of compound **4b** (0.2 g, 0.3 mmol) in  $\text{CH}_2\text{Cl}_2$  (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)<sup>+</sup>.

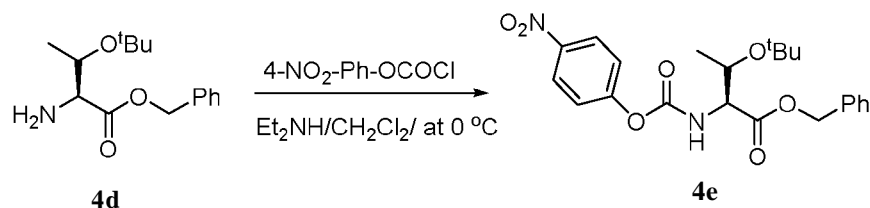
**Synthesis of compound 4e, (NO<sub>2</sub>-C<sub>6</sub>H<sub>4</sub>-OCO-Thr(O<sup>t</sup>Bu)-Bzl):**



To a solution of compound Fmoc-Thr('Bu)-OH (15 g, 37.73 mmol) in 100 mL of DMF, Cs<sub>2</sub>CO<sub>3</sub> (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 10 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 Na<sub>2</sub>SO<sub>4</sub>. 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<sup>t</sup>Bu)<sup>+</sup>, 397.2 (M-OBzl)<sup>+</sup>.

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 CH<sub>2</sub>Cl<sub>2</sub> (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**.

20 LCMS: 266.5 (M+H)<sup>+</sup>.

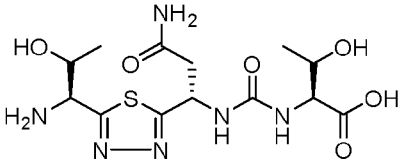
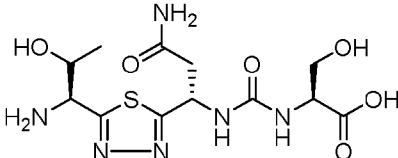
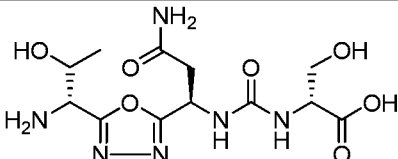
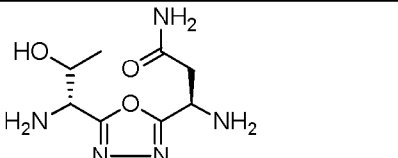
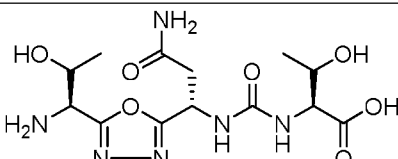
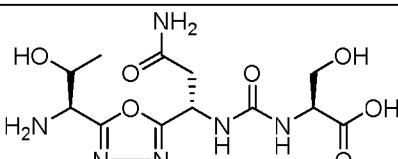


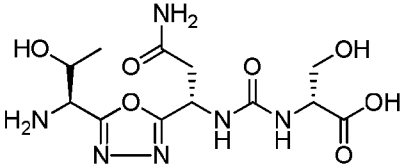
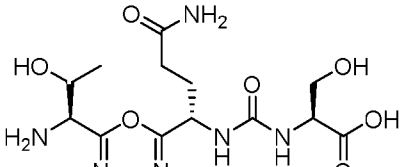
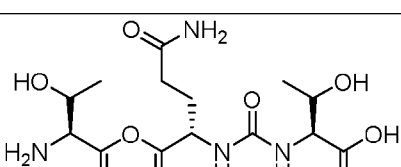
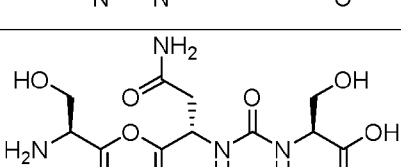
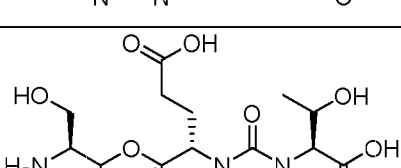
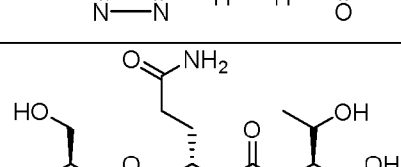
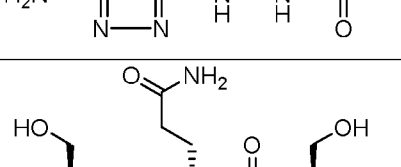
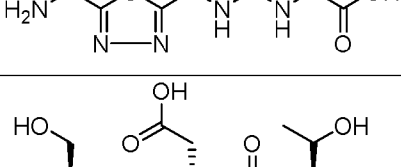
To a solution of compound 4d (1.5 g, 5.65 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) was added TEA (1.14 g, 11.3 mmol) and the solution was stirred at room temperature for 5-10 min. To this mixture a solution of 4-nitrophenyl chloroformate (1.4 g, 6.78 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 25 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

by 1.0 M sodium carbonate solution. The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , 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.  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 300 MHz):  $\delta$  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) <sup>+</sup>	HPLC t <sub>R</sub> 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:**

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

**Requirement:**

10 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(10,000 µg/ml) 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 C<sub>2</sub>H<sub>6</sub>SO, 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 10,000<sup>u</sup>/ml streptomycin) and maintained in a CO<sub>2</sub> incubator with 5% CO<sub>2</sub> at 37°C.

**CFSE Proliferation assay:**

CFSE is a dye that passively diffuses into cells and binds to intracellular proteins. 1x10<sup>6</sup> 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 1x10<sup>5</sup> splenocytes added to wells containing either MDA-MB231 cells (1x10<sup>5</sup> 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% CO<sub>2</sub>. 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%.

25 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)

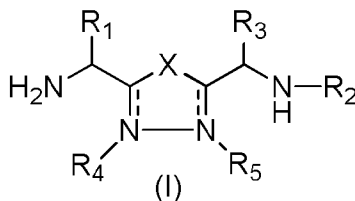
30 **Table 5**



<b>Compound No.</b>	<b>Percent rescue of splenocyte proliferation (@100 nM compound concentration)</b>	<b>Compound No.</b>	<b>Percent rescue of splenocyte proliferation (@100 nM compound concentration)</b>
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	-	-

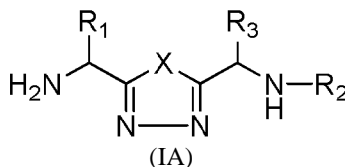
**We claim:**

1. A compound of formula (I)

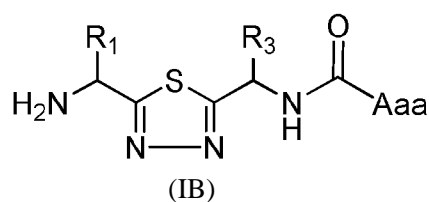


wherein,

- 5           R<sub>1</sub> is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;  
               X is S or O;  
               R<sub>2</sub> 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;
- 10           R<sub>3</sub> is side chain of an amino acid selected from Ser, Ala, Glu, Gin, Asn or Asp;  
               — is an optional bond;  
               R<sub>4</sub> and R<sub>5</sub> independently are hydrogen or absent;  
               or a pharmaceutically acceptable salt or a stereoisomer thereof.
2. The compound according to claim 1 is a compound of formula (IA):



- 15           or a pharmaceutically acceptable salt or a stereoisomer thereof; wherein,  
               R<sub>i</sub> is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;  
               X is S or O;  
               R<sub>2</sub> is hydrogen or -CO-Aaa;
- 20           R<sub>3</sub> is side chain of an amino acid selected from Ser, Ala, Glu, Gin, 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.
3. The compound according to any one of claims 1 to 2, is a compound of formula (IB):



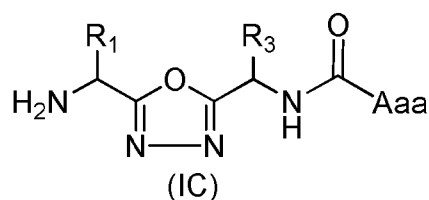
or a pharmaceutically acceptable salt or a stereoisomer thereof; wherein,

$R_1$  is side chain of an amino acid selected from Ser, Thr, Phe, Ala or Asn;

$R_3$  is side chain of an amino acid selected from Ser, Ala, Glu, Gin, Asn or Asp;

5     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.

4.     The compound according to any one of claims 1 to 2, is a compound of formula (IC):



10     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;

$R_3$  is side chain of an amino acid selected from Ser, Ala, Glu, Gin, 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.

15     5.     The compound according to any one of claims 1 to 2, wherein X is O.

6.     The compound according to any one of claims 1 to 2, wherein X is S.

7.     The compound according to claim 1, wherein

$R_1$  is side chain of Ser or Thr;

$R_2$  is -CO-Aaa;

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

$R_3$  is side chain of Asn, Gin, Glu or Asp.

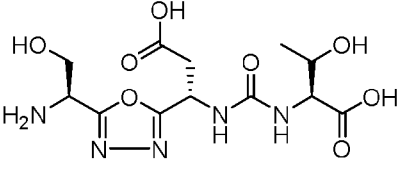
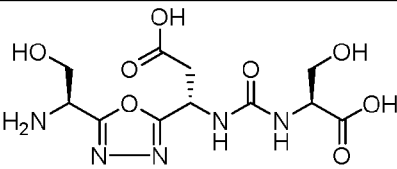
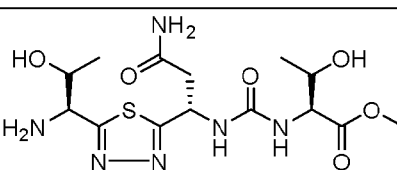
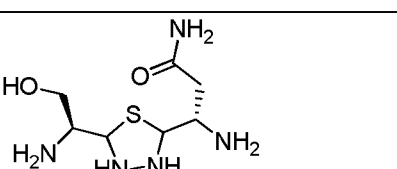
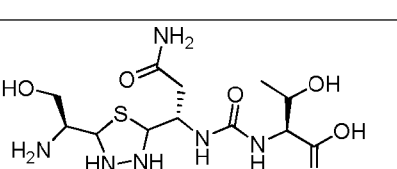
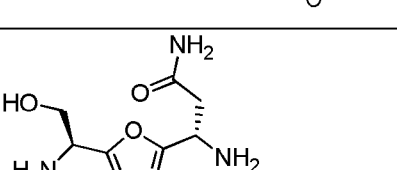
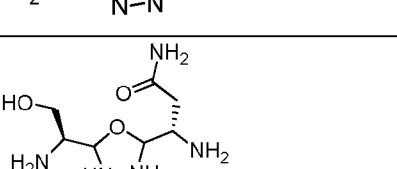
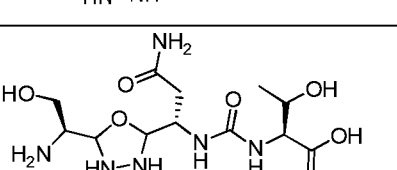
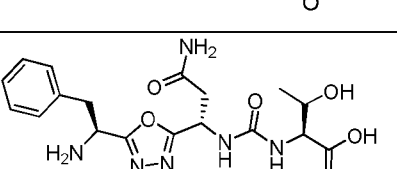
8.     The compound according to any one of claims 1 to 2, wherein  $R_2$  is hydrogen.

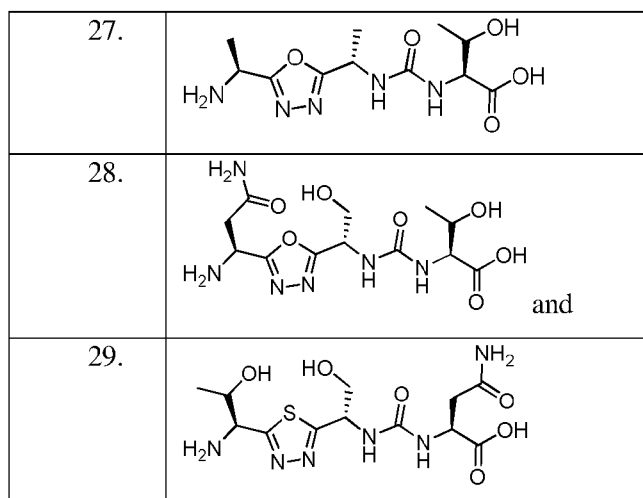
9.     A compound according to claim 1 is selected from the group consisting of

Compound No.	Structure

1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	

10.	
11.	
12.	
13.	
14.	
15.	
16.	
17.	

18.	
19.	
20.	
21.	
22.	
23.	
24.	
25.	
26.	



or a pharmaceutically acceptable salt or a stereoisomer thereof.

10. A pharmaceutical composition comprising at least one compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt or a stereoisomer thereof, and a pharmaceutically acceptable carrier or excipient.
- 5 11. The pharmaceutical composition according to claim 10, comprising at least one additional pharmaceutical agent wherein the said additional pharmaceutical agent is an anticancer agent, chemotherapy agent, or antiproliferative compound.
12. A compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt or a stereoisomer thereof, for use as a medicament.
- 10 13. A compound according to any one of claims 1 to 9, or a pharmaceutically acceptable salt or a stereoisomer thereof, for use as a medicament for the treatment of cancer or infectious disease.
14. A compound for use according to claim 13, wherein the cancer is selected from
- 15 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 esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of
- 20 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 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.

5 15. A compound for use according to claim 13, wherein the infectious disease is a bacterial infectious disease, a viral infectious disease or a fungal infectious disease.

16. 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 a compound according to any one of claims 1 to 9.

10 17. A method of inhibiting growth of tumour cells and/or metastasis in a subject, comprising administering to the subject a therapeutically effective amount of a compound according to any one of claims 1 to 9.

18. The method of claim 15, wherein the tumour cells are of a cancer selected from the group consisting of breast cancer, colon cancer, lung cancer, melanoma, prostate cancer and renal cancer.

15 19. The method of claim 15, wherein the tumour cells are of a cancer selected from the list consisting of 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 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 soft tissue, cancer of the urethra, cancer of the  
20 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 angiogenesis, spinal axis tumour, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell  
25 lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.



20. A method of treating an infectious disease in a subject comprising administering to the subject a therapeutically effective amount of compound according to any one of claims 1 to 9.

5 21. A method of treating bacterial, viral and fungal infections in a subject comprising administering to the subject a therapeutically effective amount of a compound according to any one of claims 1 to 9.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2014/064281

**A. CLASSIFICATION OF SUBJECT MATTER**

C07D 271/06(2006.01)i; C07D 285/08(2006.01)i; A61K 31/4245(2006.01)i; A61K 31/433(2006.01)i; A61P 31/00(2006.01)i; A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C07D271/-, C07D285/-, A61P31/-,A61P35/-

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS;VEN;CNKI;WPI; EPODOC;REG;CAPLUS:oxadiazole, thiadiazole, programmed cell death, PD, infect+, tumor, cancer, immun+, amino acids, AURIGENE DISCOVERY TECHNOLOGIES, substructure search according to formula (I)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009264315 A1 (TEXAS A & M UNIV SYS) 22 October 2009 (2009-10-22) see the whole document, especially description, paragraphs [0004]-[0006], [0035] and [0039]	1-21
A	WO 2012168944 A1 (AURIGENE DISCOVERY TECH LTD) 13 December 2012 (2012-12-13) see the whole document	1-21
A	WO 2011161699 A2 (AURIGENE DISCOVERY TECH LTD) 29 December 2011 (2011-12-29) see the whole document	1-21
A	KO Eunhwa et al. "Universal Peptidomimetics" <i>Journal of the American Chemical Society</i> , Vol. 133, No. 3, 23 December 2010 (2010-12-23), ISSN: ISSN: 0002-7863, pages 462-477	1-21

**I** Further documents are listed in the continuation of Box C.  See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

17 December 2014

Date of mailing of the international search report

26 January 2015

Name and mailing address of the ISA/CN

STATE INTELLECTUAL PROPERTY OFFICE OF THE  
P.R.CHINA(ISA/CN)  
6,Xitucheng Rd., Jimen Bridge, Haidian District, Beijing  
100088 China

Authorized officer

XU,Chi

Facsimile No. (86-10)62019451

Telephone No. (86-10)62086349

**INTERNATIONAL SEARCH REPORT**

International application No.

**PCT/IB2014/064281**

**Box No. II      Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: **16-21**  
because they relate to subject matter not required to be searched by this Authority, namely:  

[1] Claims 16-21 relate to a method of treatment of the human or animal body by therapy (see PCT Rule 39.1.(iv)). However, the search has been carried out and based on the use of the compound in manufacture of medicaments for treating corresponding diseases.
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**INTERNATIONAL SEARCH REPORT**  
**Information on patent family members**

International application No.

**PCT/IB2014/064281**

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2009264315	A1	22 October 2009	US	2012232268	A1	13 September 2012
WO	2012168944	A1	13 December 2012	US	2014199334	A1	17 July 2014
				EP	2717895	A1	16 April 2014
				CN	103732238	A	16 April 2014
WO	201 1161699	A2	29 December 2011	EA	201300005	A1	31 March 2014
				MX	2012014785	A	29 April 2013
				KR	20140002594	A	08 January 2014
				WO	201 1161699	A3	18 May 2012
				AU	2011268484	A1	31 January 2013
				SG	186809	A1	28 February 2013
				CA	2803859	A1	29 December 2011
				JP	2013534922	A	09 September 2013
				CN	103096915	A	08 May 2013
				US	201 1318373	A1	29 December 2011
				EP	2585099	A2	01 May 2013