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(54) Benævnelse: **Kombination af zanubrutinib med et anti-CD20- eller et anti-PD-1-antistof til anvendelse i behandling af cancer**

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DESCRIPTION

[0001] This application claims the benefit of priority to International Application No. PCT/CN2016/ 096082 filed on August 19, 2016.

FIELD OF THE INVENTION

[0002] Disclosed herein is a method for the delay of progression or treatment of cancer in a subject, comprising administering to the subject in need thereof a Btk inhibitor (in particularly (S)-7-(1-acryloylpiperidin-4-yl)-2-(4-phenoxyphenyl)-4,5,6,7-tetrahydropyrazolo-[1,5-a]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof) in combination with an immune checkpoint inhibitor or a targeted therapy agent. Disclosed herein is also a pharmaceutical combination comprising a Btk inhibitor (in particularly (S)-7-(1-acryloylpiperidin-4-yl)-2-(4-phenoxyphenyl)-4,5,6,7-tetra-hydropyrazolo[1,5-a]pyrimidine-3-carboxamide or a pharmaceutically acceptable salt thereof) in combination with an immune checkpoint inhibitor, or a targeted therapy agent and the use thereof.

[0003] The references to the methods of treatment by therapy or surgery or in vivo diagnosis methods in this description are to be interpreted as references to compounds of the present invention for use in those methods.

[0004] The invention is defined in the claims. Any subject-matter disclosed herein which is outside the scope of the claims is provided for information or reference only.

BACKGROUND OF THE INVENTION

[0005] Bruton's tyrosine kinase (Btk) belongs to the Tec family of cytoplasmic tyrosine kinases, which is the second largest family of non-receptor kinases in humans [Vetrie et al., Nature 361: 226-233, 1993; Bradshaw, Cell Signal. 22: 1175-84, 2010]. It is expressed in all cell lineages of the hematopoietic system, except for T cells and is localized in bone marrow, spleen and lymph node tissue [Smith et al., J. Immunol. 152: 557-565, 1994]. Inactivating mutations in the gene encoding Btk cause X-linked agammaglobulinemia (XLA) in humans and X-linked immunodeficiency (XID) in mice [Conley et al., Annu. Rev. Immunol. 27: 199-227, 2009]. Both diseases are characterized by dramatic defects in B cell development and function, suggesting the essential role of Btk for B cell development and function. In addition, constitutive activation of Btk in B cells results in the accumulation of autoreactive plasma cells [Kersseboom et al., Eur J Immunol. 40:2643-2654, 2010]. Btk is activated by upstream Src-family kinases in BCR signaling pahway. Once activated, Btk in turn phosphorylates phospholipase-Cy (PLCy), leading to Ca^{2+} mobilization and activation of NF-κB and MAP kinase pathways. These proximal signaling events promote expression of genes involved in proliferation and survival

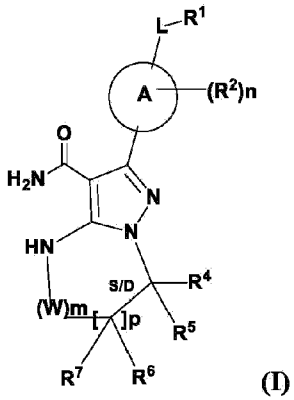
[Humphries et al., J. Biol.Chem. 279: 37651, 2004]. In addition to its essential regulatory role as downstream of BCR, Btk activity also plays a critical role in FcR signaling. Signaling via FcRy associated receptors also promotes Btk-dependent proinflammatory cytokine production by cells such as macrophages [Di Paolo et al., Nat. Chem. Biol. 7: 41-50, 2011]. Btk has been an important target due to its proximal location in the BCR and FcR signaling pathways. Preclinical studies show that Btk deficient mice are resistant to developing collagen-induced arthritis. Moreover, clinical studies of Rituxan, a CD20 antibody to deplete mature B-cells, reveal the key role of B-cells in a number of inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis [Gurcan et al., Int. Immunopharmacol. 9: 10-25, 2009]. In addition, aberrant activating of Btk plays important role in pathogenesis of B-cell lymphomas indicating that inhibition of Btk is useful in the treatment of hematological malignancies [Davis et al., Nature 463: 88-92, 2010].

[0006] In addition, aberrant activation of Btk plays an important role in pathogenesis of B-cell lymphomas indicating that inhibition of Btk is useful in the treatment of hematological malignancies [Davis et al., Nature 463: 88-92, 2010)]. The covalent BTK inhibitor ibrutinib (PCI-32765, Imbruvica®) was approved by the US Food and Drug Administration for the treatment of chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL) and Waldenström's macroglobulinemia (WM).

[0007] WO 2016/087994 A1 describes a method of treating a cancer in a human, comprising the step of administering a therapeutically effective dose of a BTK inhibitor, i.e., ibrutinib, and an anti-CD20 antibody, including Obinutuzumab.

[0008] WO 2015/061752 A1 describes methods of treating cancers and autoimmune disorders, involving use of a BTK inhibitor ibrutinib with anti-PD-1 antibody.

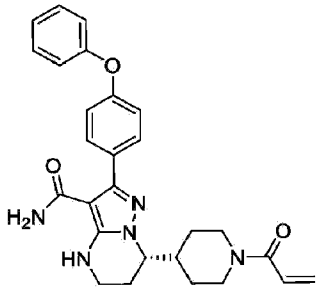
[0009] WO2014/173289A1 disclosed a series of fused heterocyclic compounds having the following general Formula (I) or a stereoisomer thereof, or a pharmaceutically acceptable salt thereof as Btk inhibitors, which have demonstrated potent inhibitory activity against Bruton's tyrosine kinase.



(I)

[0010] The unpublished PCT application PCT/CN2016/095510 disclosed a crystalline form of

the Btk inhibitor in WO 2014/173289 A1, particularly, (S)-7-(1-acryloylpiperidin-4-yl)-2-(4-phenoxyphenyl)-4,5,6,7-tetra-hydropyrazolo[1,5-a]pyrimidine-3 -carboxamide (hereinafter **Compound 1**) for the treatment of cancers with aberrations in the B-cell receptor (BCR) and FcR signaling pathway in which Btk plays important roles. **Compound 1** has demonstrated to have potent and irreversible inhibitory activities against Btk.



Compound 1

[0011] The inventors of the present application have found that the combination of a Btk inhibitor (in particular, the above-mentioned **Compound 1**) with an immunotherapy agent or a targeted therapy agent produces significant inhibition of tumor growth in cancers with aberrations in the B-cell receptor as compared with the monotherapy of each of the above active pharmaceutical agent alone.

SUMMARY OF THE INVENTION

[0012] The invention provides a Btk inhibitor, as defined in the claims, for use in a method for the delay of progression or treatment of cancer in a subject, also as defined in the claims.

[0013] Not explicitly claimed, yet useful for understanding the invention, is a pharmaceutical combination for use in the prevention, delay of progression or treatment of cancer, comprising a Btk inhibitor of Formula **(I)** or a stereoisomer thereof, or a pharmaceutically acceptable salt thereof, in combination with an immune checkpoint inhibitor or a targeted therapy agent.

[0014] Not explicitly claimed, yet useful for understanding the invention, is a use of a pharmaceutical combination in the manufacture of a medicament for use in the prevention, delay of progression or treatment of cancer, said pharmaceutical combination comprising a Btk inhibitor of Formula **(I)** or a stereoisomer thereof, or a pharmaceutically acceptable salt thereof, and an immune checkpoint inhibitor or a targeted therapy agent.

[0015] Not explicitly claimed, yet useful for understanding the invention, is an article of manufacture, or "kit" comprising a first container, a second container and a package insert, wherein the first container comprises at least one dose of a medicament comprising a Btk inhibitor of Formula **(I)** or a stereoisomer thereof, or a pharmaceutically acceptable salt thereof, the second container comprises at least one dose of a medicament comprising an immune checkpoint inhibitor or a targeted therapy agent, and the package insert comprises

instructions for treating cancer a subject using the medicaments.

[0016] The method and pharmaceutical combination disclosed herein, as a combination therapy, produce significantly more efficacious than either single agent.

[0017] In an embodiment, the targeted therapy agent is an anti-CD20 antibody as defined in the claims. In an embodiment, the immune checkpoint inhibitor is an inhibitor of PD-1 as defined in the claims.

[0018] In an embodiment, the cancer is a hematologic cancer. In an embodiment, the hematologic cancer is a leukemia, a lymphoma, a myeloma, a non-Hodgkin's lymphoma (NHL), a Hodgkin's lymphoma (HL), or a B-cell malignancy. In an embodiment, the hematologic cancer is a B-cell malignancy. In an embodiment, the B-cell malignancy is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Waldenstrom macroglobulinemia (WM), Hairy cell leukemia (HCL), Burkitt's-like leukemia (BL), B cell prolymphocytic leukemia (B-PLL), diffuse large B cell lymphoma (DLBCL), germinal center B-cell diffuse large B-cell lymphoma (GCB-DLBCL), non-germinal center B-cell diffuse large B-cell lymphoma (non-GCB DLBCL), DLBCL with undetermined subtype, primary central nervous system lymphoma (PCNSL), secondary central nervous system lymphoma (SCNSL) of breast or testicular origin, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, or a combination thereof. In an embodiment, the B-cell malignancy is diffuse large B-cell lymphoma (DLBCL). In an embodiment, DLBCL is activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL), GCB-DLBCL or Non-GCB DLBCL. In an embodiment, the B-cell malignancy is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia (B-PLL), non-CLL/SLL lymphoma, follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Waldenstrom's macroglobulinemia (WM), multiple myeloma or a combination thereof. In an embodiment, the B-cell malignancy is a relapsed or refractory (R/R) B-cell malignancy. In an embodiment, the relapsed or refractory B-cell malignancy is diffuse large B-cell lymphoma (DLBCL). In an embodiment, the relapsed or refractory DLBCL is activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL), GCB-DLBCL or Non-GCB DLBCL. In an embodiment, the relapsed or refractory (R/R) B-cell malignancy is diffuse large B-cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia (B-PLL), non-CLL / SLL lymphoma, follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Waldenstrom's macroglobulinemia (WM), multiple myeloma, or a combination thereof. In an embodiment, the B-cell malignancy is a metastasized B-cell malignancy. In an embodiment, the metastasized B-cell malignancy is diffuse large B-cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), B cell prolymphocytic

leukemia (B-PLL), non-CLL/SLL lymphoma, follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Waldenstrom's macroglobulinemia (WM), multiple myeloma or a combination thereof.

[0019] In an embodiment, the cancer is a sarcoma, or carcinoma. In an embodiment, the cancer is selected from anal cancer; appendix cancer; bile duct cancer (i.e., cholangiocarcinoma); bladder cancer; breast cancer; cervical cancer; colon cancer; cancer of Unknown Primary (CUP); esophageal cancer; eye cancer; fallopian tube cancer; gastroenterological cancer; kidney cancer; liver cancer; lung cancer; medulloblastoma; melanoma; oral cancer; ovarian cancer; pancreatic cancer; parathyroid disease; penile cancer; pituitary tumor; prostate cancer; rectal cancer; skin cancer; stomach cancer; testicular cancer; throat cancer; thyroid cancer; uterine cancer; vaginal cancer; vulvar cancer; or a combination thereof. In an embodiment, the cancer is selected from bladder cancer, breast cancer, colon cancer, gastroenterological cancer, kidney cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, proximal or distal bile duct cancer, melanoma, or a combination thereof. In an embodiment, the colon cancer is adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, squamous cell-carcinoma, mucinous adenocarcinoma, Signet ring cell adenocarcinoma, or a combination thereof. In an embodiment, the cancer is a relapsed or refractory cancer. In an embodiment, the relapsed or refractory cancer is selected from bladder cancer, breast cancer, colon cancer, gastroenterological cancer, kidney cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, proximal or distal bile duct cancer, melanoma, or a combination thereof. In an embodiment, the cancer is a metastasized cancer. In an embodiment, the metastasized cancer is selected from bladder cancer, breast cancer, colon cancer, gastroenterological cancer, kidney cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, proximal or distal bile duct cancer, and melanoma.

[0020] The BTK inhibitor is (S)-7-(1-acryloylpiperidin-4-yl)-2-(4-phenoxyphenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine-3-carboxamide (**Compound 1),** or a pharmaceutically acceptable salt thereof. In an embodiment of each of the above five aspects, the Btk inhibitor and the immune checkpoint inhibitor, or a targeted therapy agent, are administered simultaneously, sequentially or intermittently.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021]

FIG. 1 shows the combination effect of **Compound 1** and anti-CD20 mAb (obinutuzumab) on tumor growth in REC-1/NK92MI MCL xenograft model.

FIG. 2 shows the combination effect of **Compound 1** and anti-CD20 mAb (obinutuzumab) on tumor growth in human TMD-8 DLBCL xenograft model.

FIG. 3 shows the combination effects of anti-CD20 mAb (rituximab) and BTK inhibitor

(including **Compound 1** and ibrutinib) on tumor growth in human REC-1 MCL xenograft model.

FIG. 4 shows the combination effects of anti-CD20 mAb (rituximab) and BTK inhibitor (including **Compound 1** and ibrutinib) on tumor weight in human REC-1 MCL xenograft model (on Day 14).

FIG. 5 shows the combination effects of anti-PD-1 mAb (**Mab 1**) and **Compound 1** on tumor volume in human A431 epidermoid carcinoma allogeneic model.

FIG. 6 shows the combination effects of **Compound 1** and anti-PD-1 mAb (pembrolizumab) on tumor volume in human A431 epidermoid carcinoma allogeneic model.

FIG. 7 shows the effect of BTK inhibitor (including **Compound 1** and ibrutinib) on anti-CD20 mAb (obinutuzumab) induced ADCC effect.

FIG. 8 shows an X-ray diffraction pattern of **Compound 1** in a crystalline form.

FIG. 9 shows ^{1}H-NMR of the crystalline form of **Compound 1.**

FIG. 10 shows ^{13}C-NMR of the crystalline form of **Compound 1.**

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations (1):

[0022]

ABC-DLBCL	Activated B-cell diffuse large B-cell lymphoma
A2AR	Adenosine A2A receptor
B-PLL	B cell prolymphocytic leukemia
Btk	Bruton's Tyrosine Kinase
BTLA	B and T Lymphocyte Attenuator, CD272
CDR	Complementarity Determining Region
CLL	chronic lymphocytic leukemia
CTLA-4	Cytotoxic T-Lymphocyte-Associated protein 4, CD152
DLBCL	diffuse large B-cell lymphoma
DMEM	Dulbecco minimum essential medium
HVEM	Herpesvirus Entry Mediator
non-CLL/SLL	non-chronic lymphocytic leukemia / small lymphocytic lymphoma

IDO	Indoleamine 2,3-dioxygenase
TDO	Tryptophan 2,3-dioxygenase
IG	immunoglobulin G
i.p.	Intraperitoneal or Intraperitoneally
KIR	Killer-cell Immunoglobulin-like Receptor
LAG3	Lymphocyte Activation Gene-3
mAb	Monoclonal antibodies
NK	Natural killer
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate Buffered Saline
PD-1	Programmed Death 1 protein, Pdcd-1, CD279
p.o.	"by mouth" or "per os"
QD	Once daily
Q4D	Once every four days
QW	Once weekly
Q2W	Once every two weeks
Q3W	Once every three weeks
SLL	small lymphocytic lymphoma
TIM-3	T-cell Immunoglobulin domain and Mucin domain 3
Vh	Heavy chain variable region
V1	Light chain variable region
VISTA	V-domain Ig suppressor of T-cell activation

Abbreviations (2):

[0023]

ACN	acetonitrile
AcOH	Acetic acid
D-DBTA	(2S, 3S)-Dibenzoyl tartaric acid
DCM	Dichloromethane
DMF	*N*,*N*-dimethylformamide
DMF-DMA	*N*,*N*-dimethylformamide dimethyl acetal
EA	Ethyl Acetate
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EtOAc	ethyl acetate
HOBt	Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
L-DBTA	(2R, 3R)-Dibenzoyl tartaric acid
MeCN	Acetonitrile
MeOH	Methanol
MeMgBr	Methyl Magnesium Bromide
MsOH	Methanesulfonic Acid
MTBE	Methyl tertiary butyl ether
NLT	not less than
NMR	Nuclear Magnetic Resonance
NMT	not more than
Pd	Palladium
pH	Hydrogen ion concentration
RT	Room Temperature
TEA	Triethylamine
XRPD	X-ray Powder Diffraction

Definitions

[0024] Unless specifically defined elsewhere in this document, all other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs.

[0025] As used herein, including the appended claims, the singular forms of words such as **"a", "an",** and **"the",** include their corresponding plural references unless the context clearly indicates otherwise.

[0026] The term **"or"** is used to mean, and is used interchangeably with, the term "and/or" unless the context clearly dictates otherwise.

[0027] Throughout this specification and the claims which follow, unless the context requires otherwise, the word **"comprise",** and variations such as **"comprises"** and **"comprising",** will be understood to imply the inclusion of an active agent (e.g., a mAb or a Btk inhibitor) or a stated amino acid sequence, but not the exclusion of any other active ingredient or amino acid sequence. When used herein the term "comprising" can be interchangeable with the term **"containing"** or **"including".**

[0028] The term **"alkyl"** refers to a hydrocarbon group selected from linear and branched saturated hydrocarbon groups of 1-18, or 1-12, or 1-6 carbon atoms. Examples of the alkyl group include methyl, ethyl, 1 -propyl or n-propyl ("n-Pr"), 2-propyl or isopropyl ("i-Pr"), 1-butyl or n-butyl ("n-Bu"), 2-methyl-l -propyl or isobutyl ("i-Bu"), 1-methylpropyl or s-butyl ("s-Bu"), and 1,1-dimethylethyl or t-butyl ("t-Bu"). Other examples of the alkyl group include 1-pentyl, 2-pentyl, 3-pentyl, 2-methyl-2-butyl, 3-methyl-2-butyl, 3-methyl-1-butyl, 2-methyl-l -butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl and 3,3-dimethyl-2-butyl groups. Lower alkyl means 1-8, preferably 1-6, more preferably 1-4 carbon atoms; lower alkenyl or alkynyl means 2-8, 2-6 or 2-4 carbon atoms.

[0029] The term **"alkenyl"** refers to a hydrocarbon group selected from linear and branched hydrocarbon groups comprising at least one C=C double bond and of 2-18, or 2-12, or 2-6 carbon atoms. Examples of the alkenyl group may be selected from ethenyl or vinyl, prop-1-enyl, prop-2-enyl, 2-methylprop-l-enyl, but-l-enyl, but-2-enyl, but-3-enyl, buta-l,3-dienyl, 2-methylbuta-l,3-diene, hex-l-enyl, hex-2-enyl, hex-3-enyl, hex-4-enyl, and hexa-l,3-dienyl groups.

[0030] The term **"alkynyl"** refers to a hydrocarbon group selected from linear and branched hydrocarbon group, comprising at least one C=C triple bond and of 2-18, or 2-12, or 2-6 carbon atoms. Examples of the alkynyl group include ethynyl, 1-propynyl, 2-propynyl (propargyl), 1-butynyl, 2-butynyl, and 3-butynyl groups.

[0031] The term **"cycloalkyl"** refers to a hydrocarbon group selected from saturated and partially unsaturated cyclic hydrocarbon groups, comprising monocyclic and polycyclic (e.g., bicyclic and tricyclic) groups. For example, the cycloalkyl group may be of 3-12, or 3-8, or 3-6 carbon atoms. Even further for example, the cycloalkyl group may be a monocyclic group of 3-12, or 3-8, or 3-6 carbon atoms. Examples of the monocyclic cycloalkyl group include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-l-enyl, 1-cyclopent-2-enyl, l-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-l-enyl, l-cyclohex-2-enyl, l-cyclohex-3-enyl, cyclohexadienyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl, and cyclododecyl groups. Examples of the bicyclic cycloalkyl groups include those having 7-12 ring atoms arranged as a bicycle ring selected from [4,4], [4,5], [5,5], [5,6] and [6,6] ring systems, or as a bridged bicyclic ring selected from bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, and bicyclo[3.2.2]nonane. The ring may be saturated or have at least one double bond (i.e. partially unsaturated), but is not fully conjugated, and is not aromatic, as aromatic is defined herein.

[0032] The term **"Aryl"** herein refers to a group selected from:5- and 6-membered carbocyclic aromatic rings, for example, phenyl; bicyclic ring systems such as 7-12 membered bicyclic ring systems wherein at least one ring is carbocyclic and aromatic, selected, for example, from naphthalene, and indane; and tricyclic ring systems such as 10-15 membered tricyclic ring systems wherein at least one ring is carbocyclic and aromatic, for example, fluorene. For example, the aryl group is selected from 5- and 6-membered carbocyclic aromatic rings fused to a 5- to 7-membered cycloalkyl or heterocyclic ring optionally comprising at least one

heteroatom selected from N, O, and S, provided that the point of attachment is at the carbocyclic aromatic ring when the carbocyclic aromatic ring is fused with a heterocyclic ring, and the point of attachment can be at the carbocyclic aromatic ring or at the cycloalkyl group when the carbocyclic aromatic ring is fused with a cycloalkyl group. Bivalent radicals formed from substituted benzene derivatives and having the free valences at ring atoms are named as substituted phenylene radicals. Bivalent radicals derived from univalent polycyclic hydrocarbon radicals whose names end in "-yl" by removal of one hydrogen atom from the carbon atom with the free valence are named by adding "-idene" to the name of the corresponding univalent radical, e.g., a naphthyl group with two points of attachment is termed naphthylidene. Aryl, however, does not encompass or overlap with heteroaryl, separately defined below. Hence, if one or more carbocyclic aromatic rings are fused with a heterocyclic aromatic ring, the resulting ring system is heteroaryl, not aryl, as defined herein.

[0033] The term **"halogen"** or **"halo"** refers to F, Cl, Br or I.

[0034] The term **"heteroalkyl"** refers to alkyl comprising at least one heteroatom.

[0035] The term **"heteroaryl"** refers to a group selected from: 5- to 7-membered aromatic, monocyclic rings comprising 1, 2, 3 or 4 heteroatoms selected from N, O, and S, with the remaining ring atoms being carbon; 8- to 12-membered bicyclic rings comprising 1, 2, 3 or 4 heteroatoms, selected from N, O, and S, with the remaining ring atoms being carbon and wherein at least one ring is aromatic and at least one heteroatom is present in the aromatic ring; and 11- to 14-membered tricyclic rings comprising 1, 2, 3 or 4 heteroatoms, selected from N, O, and S, with the remaining ring atoms being carbon and wherein at least one ring is aromatic and at least one heteroatom is present in an aromatic ring. For example, the heteroaryl group includes a 5- to 7-membered heterocyclic aromatic ring fused to a 5- to 7-membered cycloalkyl ring. For such fused, bicyclic heteroaryl ring systems wherein only one of the rings comprises at least one heteroatom, the point of attachment may be at the heteroaromatic ring or at the cycloalkyl ring. When the total number of S and O atoms in the heteroaryl group exceeds 1, those heteroatoms are not adjacent to one another. In some embodiments, the total number of S and O atoms in the heteroaryl group is not more than 2. In some embodiments, the total number of S and O atoms in the aromatic heterocycle is not more than 1. Examples of the heteroaryl group include, but are not limited to, (as numbered from the linkage position assigned priority 1) pyridyl (such as 2-pyridyl, 3-pyridyl, or 4-pyridyl), cinnolinyl, pyrazinyl, 2,4-pyrimidinyl, 3,5-pyrimidinyl, 2,4-imidazolyl, imidazopyridinyl, isoxazolyl, oxazolyl, thiazolyl, isothiazolyl,thiadiazolyl, tetrazolyl, thienyl, triazinyl,benzothienyl, furyl, benzofuryl, benzoimidazolyl, indolyl, isoindolyl, indolinyl, phthalazinyl, pyrazinyl, pyridazinyl, pyrrolyl, triazolyl, quinolinyl, isoquinolinyl, pyrazolyl, pyrrolopyridinyl (such as lH-pyrrolo[2,3-b]pyridin-5-yl), pyrazolopyridinyl (such as lH-pyrazolo[3,4-b]pyridin-5-yl), benzoxazolyl (such as benzo[d]oxazol-6-yl), pteridinyl, purinyl, 1-oxa-2,3-diazolyl, l-oxa-2,4-diazolyl, l-oxa-2,5-diazolyl, l-oxa-3,4-diazolyl, l-thia-2,3-diazolyl, l-thia-2,4-diazolyl, l-thia-2,5-diazolyl, 1-thia-3,4-diazolyl, furazanyl, benzofurazanyl, benzothiophenyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl, furopyridinyl, benzothiazolyl (such as benzo[d]thiazol-6-yl), indazolyl (such as lH-indazol-5-yl) and 5,6,7,8 -tetrahydroisoquinoline.

[0036] The term "heterocyclic" or "heterocycle" or "heterocyclyl" refers to a ring selected from 4- to 12-membered monocyclic, bicyclic and tricyclic, saturated and partially unsaturated rings comprising at least one carbon atoms in addition to 1, 2, 3 or 4 heteroatoms, selected from oxygen, sulfur, and nitrogen. "Heterocycle" also refers to a 5- to 7-membered heterocyclic ring comprising at least one heteroatom selected from N, O, and S fused with 5-, 6-, and/or 7-membered cycloalkyl, carbocyclic aromatic or heteroaromatic ring, provided that the point of attachment is at the heterocyclic ring when the heterocyclic ring is fused with a carbocyclic aromatic or a heteroaromatic ring, and that the point of attachment can be at the cycloalkyl or heterocyclic ring when the heterocyclic ring is fused with cycloalkyl.

[0037] The "Heterocycle" also refers to an aliphatic spirocyclic ring comprising at least one heteroatom selected from N, O, and S, provided that the point of attachment is at the heterocyclic ring. The rings may be saturated or have at least one double bond (i.e. partially unsaturated). The heterocycle may be substituted with oxo. The point of the attachment may be carbon or heteroatom in the heterocyclic ring. A heterocyle is not a heteroaryl as defined herein. Examples of the heterocycle include, but not limited to, (as numbered from the linkage position assigned priority 1) 1-pyrrolidinyl, 2-pyrrolidinyl, 2,4-imidazolidinyl, 2,3-pyrazolidinyl, 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-piperidinyl, 2,5-piperazinyl, pyranyl, 2-morpholinyl, 3-mo holinyl, oxiranyl, aziridinyl, thiiranyl, azetidinyl, oxetanyl, thietanyl, 1,2-dithietanyl, 1,3-difhietanyl, dihydropyridinyl, tetrahydropyridinyl, thiomorpholinyl, thioxanyl, piperazinyl, homopiperazinyl, homopiperidinyl, azepanyl, oxepanyl, thiepanyl, 1,4-oxathianyl, 1,4-dioxepanyl, 1,4-oxafhiepanyl, 1,4-oxaazepanyl, 1,4-difhiepanyl, 1 ,4-fhiazepanyl and 1,4-diazepane 1,4-dithianyl, 1 ,4-azathianyl, oxazepinyl, diazepinyl, thiazepinyl, dihydrothienyl, dihydropyranyl, dihydrofuranyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, tetrahydrothiopyranyl, 1-pyrrolinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl, 4H-pyranyl, 1 ,4-dioxanyl, 1,3-dioxolanyl, pyrazolinyl, pyrazolidinyl, dithianyl, difhiolanyl, pyrazolidinylimidazolinyl, pyrimidinonyl, l,l-dioxo-thiomo holinyl, 3-azabicyco[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl and azabicyclo[2.2.2]hexanyl. Substituted heterocycle also includes ring systems substituted with one or more oxo moieties, such as piperidinyl N-oxide, morpholinyl-N-oxide, 1-oxo-1 -thiomorpholinyl and 1, 1-dioxo-l-thiomorpholinyl.

[0038] Substituents are selected from: halogen, $-R^a$, $-OR^a$, =O, $=NR^a$, $=N-OR^a$, $-NR^aR^b$, $-SR^a$, $-SiR^aR^aR^b$, $-OC(O)R^a$, $-C(O)R^a$, $-CO_2R^a$, $-CONR^aR^b$, $-OC(O)NR^aR^b$, $-NR^bC(O)R^a$, $-NR^a-C(O)NR^bR^b$, $-NR^a-SO_2NR^b$, $-NR^bCO_2R^a$, $-NH-C(NH_2)=NH$, $-NR^aC(NH_2)=NH$, $-NH-C(NH_2)=NR^a$, $-S(O)R^a$, $-SO_2R^a$, $-SO_2NR^aR^b$, $-NR^bSO_2R$, -CN and $-NO_2$, $-N_3$, $-CH(Ph)_2$, perfluoro(C_1-C_4)alkoxy and perfluoro(C_1-C_4)alkyl, in a number ranging from zero to three, with those groups having zero, one or two substituents being particularly preferred. R^a, R^b and R^c each independently refer to hydrogen, unsubstituted (C_1-C_8)alkyl and heteroalkyl, unsubstituted aryl, aryl substituted with one to three halogens, unsubstituted alkyl, alkoxy or thioalkoxy groups, or aryl-(C_1-C_4)alkyl groups. When R^a and R^b are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6- or 7-membered ring. Hence, - NR^aR^b includes

1-pyrrolidinyl and 4-morpholinyl, "alkyl" includes groups such as trihaloalkyl (e.g., $-CF_3$ and $-CH_2CF_3$), and when the aryl group is 1,2,3,4-tetrahydronaphthalene, it may be substituted with a substituted or unsubstituted (C_3-C_7)spirocycloalkyl group. The (C_3-C_7)spirocycloalkyl group may be substituted in the same manner as defined herein for "cycloalkyl". Preferred substituents are selected from: halogen, $-R^a$, $-OR^a$, =O, $-NR^aR^b$, $-SR^a$, $-SiR^aR^aR^b$, $-OC(O)R^a$, $-C(O)R^a$, $-CO_2R^a$, $-CONR^aR^b$, $-OC(O)NR^aR^b$, $-NR^bC(O)R^a$, $-NR^bCO_2R^a$, $-NR^a-SO_2NR^bR^b$, $-S(O)R^a$, $-SO_2R^a$, $-SO_2NR^aR^b$, $-NR^bSO_2R$, -CN and $-NO_2$, perfluoro(C_1-C_4)alkoxy and perfluoro(C_1-C_4)alkyl, where R^a and R^b are as defined above.

[0039] The term **"fused ring"** herein refers to a polycyclic ring system, e.g., a bicyclic or tricyclic ring system, in which two rings share only two ring atoms and one bond in common. Examples of fused rings may comprise a fused bicyclic cycloalkyl ring such as those having from 7 to 12 ring atoms arranged as a bicyclic ring selected from [4,4], [4,5], [5,5], [5,6] and [6,6] ring systems as mentioned above; a fused bicyclic aryl ring such as 7 to 12 membered bicyclic aryl ring systems as mentioned above, a fused tricyclic aryl ring such as 10 to 15 membered tricyclic aryl ring systems mentioned above; a fused bicyclic heteroaryl ring such as 8- to 12-membered bicyclic heteroaryl rings as mentioned above, a fused tricyclic heteroaryl ring such as 11- to 14-membered tricyclic heteroaryl rings as mentioned above; and a fused bicyclic or tricyclic heterocyclyl ring as mentioned above.

[0040] When compounds contain olefin double bonds, unless specified otherwise, such double bonds are meant to include both E and Z geometric isomers.

[0041] Some of the compounds may exist with different points of attachment of hydrogen, referred to as tautomers. For example, compounds including carbonyl $-CH_2C(O)-$ groups (keto forms) may undergo tautomerism to form hydroxyl $-CH=C(OH)-$ groups (enol forms). Both keto and enol forms, individually as well as mixtures thereof, are also intended to be included where applicable.

[0042] The term **"Pharmaceutically acceptable salts"** include, but are not limited to salts with inorganic acids, selected, for example, from hydrochlorates, phosphates, diphosphates, hydrobromates, sulfates, sulfinates, and nitrates; as well as salts with organic acids, selected, for example, from malates, maleates, fumarates, tartrates, succinates, citrates, lactates, methanesulfonates, p-toluenesulfonates, 2-hydroxyethylsulfonates, benzoates, salicylates, stearates, alkanoates such as acetate, and salts with $HOOC-(CH_2)_n-COOH$, wherein n is selected from 0 to 4. Similarly, examples of pharmaceutically acceptable cations include, but are not limited to, sodium, potassium, calcium, aluminum, lithium, and ammonium.

[0043] In addition, if a compound is obtained as an acid addition salt, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, such as a pharmaceutically acceptable addition salt, may be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in

accordance with conventional procedures for preparing acid addition salts from base compounds. Those skilled in the art will recognize various synthetic methodologies that may be used without undue experimentation to prepare non-toxic pharmaceutically acceptable addition salts.

[0044] The terms **"administration", "administering", "treating"** and **"treatment"** herein, when applied to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, mean contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. The term **"administration"** and **"treatment"** also means in vitro and ex vivo treatments, e.g., of a cell, by a reagent, diagnostic, binding compound, or by another cell. The term "subject" herein includes any organism, preferably an animal, more preferably a mammal (e.g., rat, mouse, dog, cat, rabbit) and most preferably a human.

[0045] An **"effective amount"** refers to an amount of at least one compound and/or at least one stereoisomer thereof, and/or at least one pharmaceutically acceptable salt thereof effective to "treat" a disease or disorder in a subject, and that will elicit, to some significant extent, the biological or medical response of a tissue, system, animal or human that is being sought, such as when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the condition or disorder being treated. The therapeutically effective amount will vary depending on the compound, the disease and its severity and the age, weight, etc., of the mammal to be treated.

[0046] The term **"at least one substituent"** includes, for example, from 1 to 4, such as from 1 to 3, further as 1 or 2, substituents. For example, "at least one substituent R^{16}" herein includes from 1 to 4, such as from 1 to 3, further as 1 or 2, substituents selected from the list of R<16>as described herein.

[0047] The term **"antibody"** herein is used in the broadest sense and specifically covers antibodies (including full length monoclonal antibodies) and antibody fragments so long as they recognize antigen, such as, a target antigen (e.g., CD20) or an immune checkpoint (e.g., PD-1). An antibody molecule is usually monospecific, but may also be described as idiospecific, heterospecific, or polyspecific. Antibody molecules bind by means of specific binding sites to specific antigenic determinants or epitopes on antigens..

[0048] The term **"monoclonal antibody"** or **"mAb"** or **"Mab"** herein means a population of substantially homogeneous antibodies, i.e., the antibody molecules comprised in the population are identical in amino acid sequence except for possible naturally occurring mutations that may be present in minor amounts. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of different antibodies having different amino acid sequences in their variable domains, particularly their CDRs, which are often specific for different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be

construed as requiring production of the antibody by any particular method. Monoclonal antibodies (mAbs) may be obtained by methods known to those skilled in the art. See, for example, U.S. Pat. No. 4,376,110. The mAbs disclosed herein may be of any immunoglobulin class including IgG, IgM, IgD, IgE, IgA, and any subclass thereof. A hybridoma producing a mAb may be cultivated *in vitro* or *in vivo.* High titers of mAbs can be obtained in *in vivo* production where cells from the individual hybridomas are injected intraperitoneally into mice, such as pristine-primed Balb/c mice to produce ascites fluid containing high concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

[0049] In general, the basic antibody structural unit comprises a tetramer. Each tetramer includes two identical pairs of polypeptide chains, each pair having one **"light chain"** (about 25 kDa) and one **"heavy chain"** (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of the heavy chain may define a constant region primarily responsible for effector function. Typically, human light chains are classified as kappa and lambda light chains. Furthermore, human heavy chains are typically classified as α, δ, ε, γ, or μ, and define the antibody's isotypes as IgA, IgD, IgE, IgG, and IgM, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids.

[0050] The variable regions of each light/heavy chain (Vl/Vh) pair form the antibody binding site. Thus, in general, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same.

[0051] Typically, the variable domains of both the heavy and light chains comprise three hypervariable regions, also called **"complementarity determining regions (CDRs)",** which are located between relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chain variable domains comprise FR-1 (or FR1), CDR-1 (or CDR1), FR-2 (FR2), CDR-2 (CDR2), FR-3 (or FR3), CDR-3 (CDR3),, and FR-4 (or FR4). The assignment of amino acids to each domain is, generally, in accordance with the definitions of Sequences of Proteins of Immunological Interest, Kabat, et al., National Institutes of Health, Bethesda, Md. ; 5<m> ed.; NIH Publ. No. 91-3242 (1991*);* Kabat (1978) Adv. Prot. Chem. 32: 1-75*;* Kabat, et al., (1977) J. Biol. Chem. 252: 6609-6616*;* Chothia, et al, (1987) J Mol. Biol. 196:901-917 *or* Chothia, et al, (1989) Nature 342:878-883.

[0052] The term **"hypervariable region"** means the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (i.e., CDR-L1, CDR-L2 and CDR-L3 in the light chain variable domain and CDR-H1, CDR-H2 and CDR-H3 in the heavy chain variable domain). See, Kabat et al. (1991) Sequences of Proteins of Immunological Interest, 5th Ed.

Public Health Service, National Institutes of Health, Bethesda, Md. (defining the CDR regions of an antibody by sequence); see also Chothia and Lesk (1987) J. Mol. Biol. 196: 901-917 (defining the CDR regions of an antibody by structure). The term "framework" or "FR" means those variable domain residues other than the hypervariable region residues defined herein as CDR residues.

[0053] Unless otherwise indicated, **"antibody fragment"** or **"antigen-binding fragment"** means antigen binding fragments of antibodies, i.e. antibody fragments that retain the ability to bind specifically to the antigen bound by the full-length antibody, e.g. fragments that retain one or more CDR regions. Examples of antigen binding fragments include, but not limited to, Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules, e.g., single chain Fv (ScFv); nanobodies and multispecific antibodies formed from antibody fragments.

[0054] An antibody that **"specifically binds to"** a specified target protein is an antibody that exhibits preferential binding to that target as compared to other proteins, but this specificity does not require absolute binding specificity. An antibody is considered "specific" for its intended target if its binding is determinative of the presence of the target protein in a sample, e.g. without producing undesired results such as false positives. Antibodies or binding fragments thereof, useful in the present invention will bind to the target protein with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with non-target proteins. An antibody herein is said to bind specifically to a polypeptide comprising a given amino acid sequence, e.g. the amino acid sequence of a mature human PD-1 molecule, if it binds to polypeptides comprising that sequence but does not bind to proteins lacking that sequence.

[0055] The term **"human antibody"** herein means an antibody that comprises human immunoglobulin protein sequences only. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, **"mouse antibody"** or "rat antibody" mean an antibody that comprises only mouse or rat immunoglobulin protein sequences, respectively.

[0056] The term **"humanized antibody"** means forms of antibodies that contain sequences from non-human (e.g., murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The prefix **"hum", "hu", "Hu"** or **"h"** is added to antibody clone designations when necessary to distinguish humanized antibodies from parental rodent antibodies. The humanized forms of rodent antibodies will generally comprise the same CDR sequences of the parental rodent

antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons.

[0057] The terms **"cancer"** or **"tumor"** herein mean or describe the physiological condition involving abnormal cell growth with the potential to invade or spread to other parts of the body. The **"disease"** refers to any disease, discomfort, illness, symptoms or indications, and can be substituted with the term **"disorder"** or **"condition".**

[0058] In some embodiments, the cancer is a hematologic cancer. In some embodiments, the hematologic cancer is a leukemia, a lymphoma, a myeloma, a non-Hodgkin's lymphoma (NHL), a Hodgkin's lymphoma (HL), or a B-cell malignancy. In some embodiments, the hematologic cancer is a B-cell malignancy. In some embodiments, the B-cell malignancy is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Waldenstrom macroglobulinemia (WM), Hairy cell leukemia (HCL), Burkitt's-like leukemia (BL), B cell prolymphocytic leukemia (B-PLL), diffuse large B cell lymphoma (DLBCL), germinal center B-cell diffuse large B-cell lymphoma (GCB-DLBCL), non-germinal center B-cell diffuse large B-cell lymphoma (non-GCB DLBCL), DLBCL with undetermined subtype, primary central nervous system lymphoma (PCNSL), secondary central nervous system lymphoma (SCNSL) of breast or testicular origin, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, lymphomatoid granulomatosis, or a combination thereof. In some embodiments, the B-cell malignancy is diffuse large B-cell lymphoma (DLBCL). In some embodiments, DLBCL is activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL), GCB-DLBCL or Non-GCB DLBCL. In some embodiments, the B-cell malignancy is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia (B-PLL), non-CLL / SLL lymphoma, follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Waldenstrom's macroglobulinemia (WM), multiple myeloma, or a combination thereof. In some embodiments, the B-cell malignancy is a relapsed or refractory (R/R) B-cell malignancy. In some embodiments, the relapsed or refractory (R/R) B-cell malignancy is diffuse large B-cell lymphoma (DLBCL). In some embodiments, the relapsed or refractory DLBCL is activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL), GCB-DLBCL or Non-GCB DLBCL. In some embodiments, the relapsed or refractory (R/R) B-cell malignancy is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia (B-PLL), non-CLL / SLL lymphoma, follicular lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Waldenstrom's macroglobulinemia (WM), multiple myeloma, or a combination thereof. In some embodiments, the B-cell malignancy is a metastasized B-cell malignancy. In some embodiments, the metastasized B-cell malignancy is diffuse large B-cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia (B-PLL), non-CLL / SLL lymphoma, follicular

lymphoma (FL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), Waldenstrom's macroglobulinemia (WM), multiple myeloma, or a combination thereof. In some embodiments, the cancer is a sarcoma, or carcinoma. In some embodiments, the cancer is selected from anal cancer; appendix cancer; bile duct cancer (i.e., cholangiocarcinoma); bladder cancer; breast cancer; cervical cancer; colon cancer; cancer of Unknown Primary (CUP); esophageal cancer; eye cancer; fallopian tube cancer; gastroenterological cancer; kidney cancer; liver cancer; lung cancer; medulloblastoma; melanoma; oral cancer; ovarian cancer; pancreatic cancer; parathyroid disease; penile cancer; pituitary tumor; prostate cancer; rectal cancer; skin cancer; stomach cancer; testicular cancer; throat cancer; thyroid cancer; uterine cancer; vaginal cancer; or vulvar cancer; or a combination thereof. In some embodiments, the cancer is selected from bladder cancer, breast cancer, colon cancer, gastroenterological cancer, kidney cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, proximal or distal bile duct cancer, melanoma, or a combination thereof. In some embodiments, the colon cancer is adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, squamous cell-carcinoma, mucinous adenocarcinoma, Signet ring cell adenocarcinoma, or a combination thereof. In some embodiments, the cancer is a relapsed or refractory cancer. In some embodiments, the relapsed or refractory cancer is selected from bladder cancer, breast cancer, colon cancer, gastroenterological cancer, kidney cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, proximal or distal bile duct cancer, melanoma, or a combination thereof. In some embodiments, the cancer is a metastasized cancer. In embodiments, the metastasized cancer is selected from bladder cancer, breast cancer, colon cancer, gastroenterological cancer, kidney cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, proximal or distal bile duct cancer, and melanoma.

[0059] The term "CDRs" means complementarity determining region(s) in an immunoglobulin variable region, defined using the Kabat numbering system, unless otherwise indicated.

Immune checkpoint inhibitors

[0060] In some embodiments, the Btk inhibitor is co-administered with an immune checkpoint inhibitor as defined in the claims.

[0061] "Immune checkpoints (checkpoint proteins)"are molecules in the immune system that either turn up a signal (co-stimulatory molecules) or turn down a signal. And they also regulate T-cell activation or function. Many cancers protect themselves from the immune system by inhibiting the T cell signal. An **"immune checkpoint inhibitor",** which totally or partially reduces, inhibits, interferes with or modulates one or more checkpoint proteins, has been increasing considered as targets for cancer immunotherapies. Numerous checkpoint proteins are known, such as PD-1 (Programmed Death 1, CD279) with its ligands PD-L1 (also named CD274 or B7-H1) and PD-L2; TIM-3 (T-cell Immunoglobulin domain and Mucin domain 3, also known as HAVCR2) and its ligand Gal-9; CTLA-4 (Cytotoxic T-Lymphocyte-Associated protein 4, CD152) and its ligands CD80 and CD86; and A2AR (Adenosine A2A receptor); B7-

H3 (CD276); B7-H4 (VTCN1); BTLA (B and T Lymphocyte Attenuator, CD272) and its ligand HVEM (Herpesvirus Entry Mediator); IDO (Indoleamine 2,3-dioxygenase); LAG3 (Lymphocyte Activation Gene-3); VISTA (V-domain Ig suppressor of T-cell activation); KIR (Killer-cell Immunoglobulin-like Receptor). These proteins are responsible for co-stimulatory or inhibitory interactions of T-cell responses. Immune checkpoint proteins regulate and maintain self-tolerance and the duration and amplitude of physiological immune responses. Immune checkpoint inhibitors include antibodies or are derived from antibodies.

[0062] The immune system has multiple inhibitory pathways that are critical for maintaining self-tolerance and modulating immune responses. In T-cells, the amplitude and quality of response is initiated through antigen recognition by the T-cell receptor and is regulated by immune checkpoint proteins that balance co-stimulatory and inhibitory signals.

[0063] PD-1 is an immune checkpoint protein, that limits the activity of T cells in peripheral tissues at the time of an inflammatory response to infection and to limit autoimmunity PD-1 blockade in vitro enhances T-cell proliferation and cytokine production in response to a challenge by specific antigen targets or by allogeneic cells in mixed lymphocyte reactions. A strong correlation between PD-1 expression and response was shown with blockade of PD-1 (Pardoll, Nature Reviews Cancer, 12: 252-264, 2012). PD-1 blockade can be accomplished by a variety of mechanisms including antibodies that bind PD-1 or its ligands. Examples of PD-1 and PD-L1 blockers, also named PD-1 and PD-L1 inhibitors, are described in US7488802; US7943743; US8008449; US8,168,757; US8217149, and WO03042402, WO2008156712, WO2010089411, WO2010036959, WO2011066342, WO2011159877, WO2011082400, WO2011161699, and WO2015035606. In some embodiments the PD-1 inhibitors include an antibody or a fragment antigen binding thereof, which specifically binds to PD-1. In certain other embodiments the PD-1 blockers include anti-PD-1 antibodies and similar binding proteins such as nivolumab (MDX 1106, BMS 936558, ONO-4538, Opdivo®) described in US8008449B2, a fully human IgG4 antibody that binds to and blocks the activation of PD-1 by its ligands PD-L1 and PD-L2; pembrolizumab (lambrolizumab, MK-3475 or SCH 900475, Keytruda®) disclosed in US8168757B2, a humanized monoclonal IgG4 antibody against PD-1; pidilizumab (CT-011), a humanized antibody that binds PD-1; AMP-224, a fusion protein of B7-DC; an antibody Fc portion; BMS-936559 (MDX-1105-01) for PD-L1 (B7-H1) blockade for PD-1 blockade.

[0064] Other immune checkpoint protein is CTLA-4, that down-regulates pathways of T-cell activation (Fong et al., Cancer Res. 69(2):609-615, 2009; Weber Cancer Immunol. Immunother, 58:823-830, 2009). Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation. Inhibitors of CTLA-4 include anti-CTLA-4 antibodies. Anti-CTLA-4 antibodies bind to CTLA-4 and block the interaction of CTLA-4 with its ligands CD80/CD86 expressed on antigen presenting cells and thereby blocking the negative down regulation of the immune responses elicited by the interaction of these molecules. Examples of anti-CTLA-4 antibodies are described in US Patent Nos: 5,811,097; 5,811,097; 5,855,887; 6,051,227; 6,207,157; 6,682,736; 6,984,720; *and* 7,605,238. One anti-CDLA-4 antibody is tremelimumab (ticilimumab, CP-675,206). In one embodiment, the anti-CTLA-4 antibody is ipilimumab (MDX-

010, MDX-101, Yervoy®);) a fully human monoclonal IgG antibody that binds to CTLA-4. Ipilimumab is marketed under the name Yervoy™ and has been approved for the treatment of unresectable or metastatic melanoma. Other immune-checkpoint inhibitors include: LAG-3 inhibitors, such as IMP321, a soluble Ig fusion protein (Brignone et al., 2007, J. Immunol. 179:4202-4211*);* B7 inhibitors, such as B7-H3 and B7-H4 inhibitors, eg., anti-B7-H3 antibody MGA271 (Loo et al., 2012, Clin. Cancer Res. July 15 (18) 3834); TIM3 inhibitors; A2AR inhibitors; BTLA inhibitors; IDO inhibitors, eg., INCB024360, an IDO1 inhibitor; VISTA inhibitors; or KIR inhibitors, such as lirilumab (INN), an antibody binding to KIR2DL1/2L3.

[0065] The anti-PD-1 monoclonal antibody comprises CDR regions according to "317-4B6" in the following table, which also includes other antibodies, not explicitly claimed, that are useful for understanding the invention:

a) mu317	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 11, 12, 13, respectively); and CDR-L1, CDR-L2 and CDR-L3 (SEQ ID NOs: 14, 15, 16, respectively);
b) mu326	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 17, 18, 19, respectively); and CDR-L1, CDR-L2 and CDR-L3 (SEQ ID NOs: 20, 21, 22, respectively);
c) 317-4B6	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 31, 32, 33, respectively); and CDR-L1 , CDR-L2 and CDR-L3 (SEQ ID NOs: 34, 35, 36, respectively);
d) 326-4A3	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 37, 38, 39, respectively); and CDR-L1, CDR-L2 and CDR-L3 (SEQ ID NOs: 40, 41, 42, respectively);
e) 317-1H	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 11, 59, 13, respectively); and CDR-L1, CDR-L2 and CDR-L3 (SEQ ID NOs: 14, 15, 16, respectively);
f) 317-4B2	CDR-HL CDR-H2 and CDR-H3 (SEQ ID NOs: 11, 60, 13, respectively); and CDR-L1 , CDR-L2 and CDR-L3 (SEQ ID NOs: 61, 15, 16, respectively);
g) 317-4B5	CDR-HI, CDR-H2 and CDR-H3 (SEQ ID NOs: 11 , 60, 13, respectively); and CDR-L1 , CDR-L2 and CDR-L3 (SEQ ID NOs: 61, 15, 16, respectively);
h) 317-4B6	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 11, 32, 13, respectively); and CDR-L1, CDR-L2 and CDR-L3 (SEQ ID NOs: 61, 15, 16, respectively);
i) 326-1	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 17, 62, 19, respectively); and CDR-L1, CDR-L2 and CDR-L3 (SEQ ID NOs: 20, 21, 22, respectively);
j) 326-3B1	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 17, 62, 19, respectively); and CDR-L1, CDR-L2 and CDR-L3 (SEQ ID NOs: 20, 21, 22, respectively);
or k) 326-3G1	CDR-H1, CDR-H2 and CDR-H3 (SEQ ID NOs: 17, 62, 19, respectively); and CDR-L1, CDR-I 2 and CDR-L3 (SEQ ID NOs: 20,

	21, 22, respectively).

[0066] Not explicitly claimed, but useful for understanding the invention, is an antibody which comprises a heavy chain variable region (Vh) and a light chain variable region (Vl) that contain any combinations of CDRs listed as follows:

(a)	CDR-H1 (SEQ ID NO 31), CDR-H2 (SEQ ID NO 12, 32, 59 or 60) and CDR-H3 (SEQ ID NO 33), CDR-L1 (SEQ ID NO 14, 34 or 61), CDR-L2 (SEQ ID NO 35) and CDR-L3 (SEQ ID NO 36); or
(b)	CDR-H1 (SEQ ID NO 37), CDR-H2 (SEQ ID NO 18, 38 or 62) and CDR-H3 (SEQ ID NO 39), CDR-L1 (SEQ ID NO 40), CDR-L2 (SEQ ID NO 41) and CDR-L3 (SEQ ID NO 42).

[0067] The anti-PD-1 antibody comprises Vh and Vl domains according to "317-4B6" in the following table, which also includes other antibodies, not explicitly claimed, that are useful for understanding the invention:

a) mu317 (SEQ ID NOs: 4 and 6, respectively);	p) 317-3H1 (SEQ ID NOs: 69 and 26, respectively);
b) mu326 (SEQ ID NOs: 8 and 10, respectively);	q) 317-311 (SEQ ID NOs: 70 and 26, respectively);
c) 317-4B6 (SEQ ID NOs: 24 and 26,	
respectively);	r) 317-4B 1 (SEQ ID NOs: 71 and 26, respectively);
d) 326-4A3 (SEQ ID NOs: 28 and 30, respectively);	s) 317-4B3 (SEQ ID NOs: 72 and 26, respectively);
e) 317-4B2 (SEQ ID NOs: 43 and 44, respectively);	t) 317-4B4 (SEQ ID NOs: 73 and 26, respectively);
f) 317-4B5 (SEQ ID NOs: 45 and 46, respectively);	u) 317-4A2 (SEQ ID NOs: 74 and 26, respectively);
g) 317-1 (SEQ ID NOs: 48 and 50, respectively);	v) 326-3 A 1 (SEQ ID NOs: 75 and 30, respectively);
h) 326-3B1 (SEQ ID NOs: 51 and 52, respectively);	w) 326-3C1 (SEQ ID NOs: 76 and 30, respectively);
i) 326-3GI (SEQ ID NOs: 53 and 54, respectively);	x) 326-3D1 (SEQ ID NOs: 77 and 30, respectively);
j) 326-1 (SEQ ID NOs: 56 and 58, respectively);	y) 326-3E1 (SEQ ID NOs: 78 and 30, respectively);
k) 317-3A1 (SEQ ID NOs: 64 and 26, respectively);	z) 326-3F1 (SEQ ID NOs: 79 and 30, respectively);
l) 317-3C1 (SEQ ID NOs: 65 and 26, respectively);	aa) 326-3B N55D (SEQ ID NOs: 80 and 30, respectively);
m) 317-3E1 (SEQ ID NOs: 66 and 26,	

respectively);	ab) 326-4A1 (SEQ ID NOs: 28 and 81, respectively); or
n) 317-3F1 (SEQ ID NOs: 67 and 26, respectively);	ac) 326-4A2 (SEQ ID NOs: 28 and 82, respectively).
o) 317-3G1 (SEQ ID NOs: 68 and 26, respectively);	

[0068] The anti-PD1 monoclonal antibody comprises an IgG4 constant region as defined in the claims, namely SEQ ID NO: 88.

[0069] Preferably, the anti-PD-1 monoclonal antibody is an antibody which contains a F(ab) or F(ab)2 comprising a domain said above, including a heavy chain variable region (Vh), a light chain variable region (Vl) and a IgG4 heavy chain effector or constant domain .

[0070] The anti-PD-1 monoclonal antibody as defined by the claims is an antibody which comprises a heavy chain variable region (Vh) and a light chain variable region (Vl) (comprising SEQ ID No 24 and SEQ ID No 26, respectively) and a IgG4 heavy chain effector or constant domain (comprising SEQ ID NO 88), hereinafter **Mab 1,** which specifically binds to PD-1, especially PD-1 residues including K45 and I93; or, I93, L95 and P97, and inhibits PD-1-medidated cellular signaling and activities in immune cells, antibodies binding to a set of amino acid residues required for its ligand binding..

[0071] The anti-PD1 monoclonal antibodies and antibody fragments thereof may be prepared in accordance with the disclosure of WO2015/035606 A1. In a preferred embodiment, the anti-PD1 monoclonal antibodies is **Mab 1,** which is administered at a dosage of about 2 mg/kg Q3W to about 200 mg/kg Q3W.

Targeted Therapy Agent

[0072] In some embodiments, the Btk inhibitor is co-administered with an targeted therapy agent as defined in the claims.

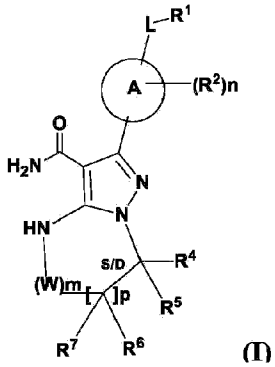
[0073] Targeted therapy is one of the major modalities of medical treatment for cancer, which blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with all rapidly dividing cells. B-lymphocyte antigen CD20 (CD20), which is an important target for the treatment of B-cell malignancies, is an activated-glycosylated phosphoprotein expressed on the surface of all B-cells beginning at the pro-B phase (CD45R+, CD117+) and progressively increasing in concentration until maturity,.

[0074] The target therapy agent is selected from rituximab, ibritumomab tiuxetan tositumonmab, ofatumumab or obinutuzumab.

Btk inhibitors

[0075] "Btk inhibitor" means a compound of Formula (I), or a stereoisomer thereof, or a pharmaceutically acceptable salt thereof.

[0076] The Btk inhibitor as defined by the claims is encompassed by a compound of Formula (I),



(I)

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

A is a 5- or 6-membered aromatic ring comprising 0-3 heteroatoms of N, S or O;

each W is independently $-(CH_2)-$ or $-C(O)-$;

L is a bond, CH_2, NR^{12}, O, or S;

S/D is a single or double bond, and when a double bond, R^5 and R^7 are absent;

m is 0, or an integer of 1-4;

n is 0, or an integer of 1-4, wherein when n is more than 1, each R^2 may be different;

p is 0, or an integer of 1-2, wherein when p is 0, m is non-zero, and when p is more than 1,

each R^6 and each R^7 may be different;

R^1, R^4, R^5, R^6,and R^7 are each independently H, halogen, heteroalkyl, alkyl,alkenyl, cycloalkyl, aryl, saturated or unsaturated heterocyclyl, heteroaryl, alkynyl, -CN, $-NR^{13}R^{14}$, $-OR^{13}$, $-COR^{13}$, $-CO_2R^{13}$, $-CONR^{13}R^{14}$, $-C(=NR^{13})NR^{14}R^{15}$, $-NR^{13}COR^{14}$, $-NR^{13}CONR^{14}R^{15}$, $-NR^{13}CO_2R^{14}$, $-SO_2R^{13}$, $-NR^{13}SO_2NR^{14}R^{15}$, or $-NR^{13}SO_2R^{14}$, wherein the alkyl, alkenyl, alkynyl, cycloalkyl, heteroaryl, aryl, and saturated or unsaturated heterocyclyl are optionally substituted with at least one substituent R^{16}, wherein (R^4 and R^5), or (R^4 and R^6), or (R^6 and R^7), or (R^6 and R^6 when p is 2), together with the atoms to which they are attached, can form a ring selected from cycloalkyl, saturated or unsaturated heterocycle, aryl, and heteroaryl rings optionally substituted with at least one substituent R^{16};

R^2 is halogen, alkyl, -S-alkyl, -CN, $-NR^{13}R^{14}$, $-OR^{13}$, $-COR^{13}$, $-CO_2R^{13}$, $-CONR^{13}R^{14}$, $-C(=NR^{13})NR^{14}R^{15}$, $-NR^{13}COR^{14}$, $-NR^{13}CONR^{14}R^{15}$, $-NR^{13}CO_2R^{14}$, $-SO_2R^{13}$, $-NR^{13}SO_2NR^{14}R^{15}$, or $-NR^{13}SO_2R^{14}$;

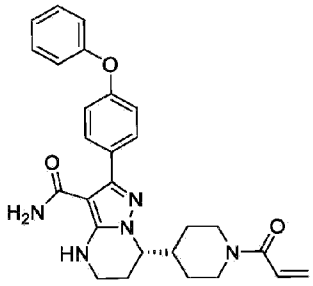
R^{12} is H or lower alkyl;

R^{13}, R^{14} and R^{15} are each independently H, heteroalkyl, alkyl, alkenyl, alkynyl, cycloalkyl, saturated or unsaturated heterocyclyl, aryl, or heteroaryl; wherein (R^{13} and R^{14}), and/or (R^{14} and R^{15}) together with the atom(s) to which they are attached, each can form a ring selected from cycloalkyl, saturated or unsaturated heterocycle, aryl, and heteroaryl rings optionally substituted with at least one substituent R^{16};

R^{16} is halogen, subsituted or unsubstitued alkyl, substituted or unsubstituted alkenyl, subsituted or unsubstitued alkynyl, subsituted or unsubstitued cycloalkyl, subsituted or unsubstitued aryl, subsituted or unsubstitued heteroaryl, subsituted or unsubstitued heterocyclyl, oxo, -CN, $-OR^a$, $-NR^aR^b$, $-COR^a$, $-CO_2R^a$, $-CONR^aR^b$, $-C(=NR^a)NR^bR^c$, $-NR^aCOR^b$, $-NR^aCONR^aR^b$, $-NR^aCO_2R^b$, $-SO_2R^a$, $-SO_2$aryl, $-NR^aSO_2NR^bR^c$, or $-NR^aSO_2R^b$, wherein R^a, R^b, and R^c are independently hydrogen, halogen, subsituted or unsubstitued alkyl, substituted or unsubstituted alkenyl, subsituted or unsubstitued alkynyl, subsituted or unsubstitued cycloalkyl, subsituted or unsubstitued aryl, subsituted or unsubstitued heteroaryl, subsituted or unsubstitued heterocyclyl,

wherein (R^a and R^b), and/or (R^b and R^c) together with the atoms to which they are attached, can form a ring selected from cycloalkyl, saturated or unsaturated heterocycle, aryl, and heteroaryl rings.

[0077] The Btk inhibitor as defined by the claims is a compound of Formula (**II**)--i.e., **Compound 1,**



(II)

or a pharmaceutically acceptable salt thereof.

[0078] The Btk inhibitor disclosed herein, such as the compound of Formula **(II),** may be

synthesized by synthetic routes disclosed in WO 2014/173289 A1 and unpublished PCT application PCT/CN2016/095510. The Btk inhibitor, i.e., **Compound 1,** disclosed herein, may be prepared in accordance with the procedures in PCT/CN2016/095510.

Combination therapy

[0079] The combination therapy may be administered as a simultaneous, or separate or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations. The combined administration includes co-administration, using separate formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

[0080] Suitable dosages for any of the above co-administered agents are those presently used and may be lowered due to the combined action (synergy) of the Btk inhibitor and the targeted therapy agent or the immune checkpoint inhibitor, such as to increase the therapeutic index or mitigate toxicity or other side-effects or consequences.

[0081] In a particular embodiment of anti-cancer therapy, the Btk inhibitor and the targeted therapy agent or the immune checkpoint inhibitor may be further combined with surgical therapy and radiotherapy.

[0082] In an embodiment of each of the above five aspects, the amounts of the Btk inhibitor and the targeted therapy agent or the immune checkpoint inhibitor disclosed herein and the relative timings of administration be determined by the individual needs of the patient to be treated, administration route, severity of disease or illness, dosing schedule, as well as evaluation and judgment of the designated doctor.

[0083] The Btk inhibitor and the targeted therapy agent or the immune checkpoint inhibitor disclosed herein may be administered in various known manners, such as orally, topically, rectally, parenterally, by inhalation spray, or via an implanted reservoir, although the most suitable route in any given case will depend on the particular host, and nature and severity of the conditions for which the active ingredient is being administered. The term "parenteral" as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

[0084] In one embodiment of each of the above five aspects, the Btk inhibitor and the targeted therapy agent or the immune checkpoint inhibitor disclosed herein may be administered in different route. In a preferred embodiment, the Btk inhibitor is administered orally, and the targeted therapy agent or the immune checkpoint inhibitor is administered parenterally such as subcutaneously, intracutaneously, intravenously or intraperitoneally. In a preferred embodiment, the BTK inhibitor is administered once a day, two times per day, three times per day, four times per day, or five times per day, and is administered at a dosage of about 80

mg/day to about 640 mg/day. In a preferred embodiment, the BTK inhibitor is administered at a dose of 320 mg QD or 160 mg BID.

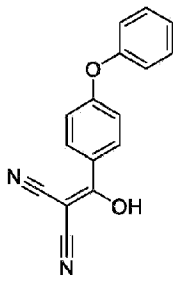
EXAMPLE

[0085] The present invention is further exemplified by the following examples that illustrate the invention.

Example 1 Preparation of (S)-7-(1-acryloylpiperidin-4-yl)-2-(4-phenoxyphenyl) -4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine-3-carboxamide (Compound 1) and Crystalline Form A Thereof

Step 1: Synthesis of BG-2

[0086]

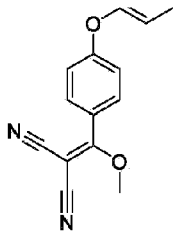


BG-2

[0087] Under nitrogen atmosphere, to a solution of EA (5 v), HOBT (1.2 eq.), EDCI (1.2 eq.), 4-phenoxybenzoic acid (BG-1, 80 Kg, 1.0 eq.) and malononitrile (1.2 eq.) was added TEA (2.4 eq.) at 10°C. The mixture was then stirred at RT until the reaction was completed. The mixture was then centrifuged and the cake was washed with EA. The filtrate was washed with aqueous $NaHCO_3$ twice and NH_4Cl. The organic phase was washed with 1.5 N H_2SO_4 twice and stirred. Concentrated, precipitated from methanol and purified water. The solid was collected by centrifugation and dried under vacuum. This gave 79.9 Kg of BG-2. ^1H NMR (DMSO-d_6) δ 7.62 (d, *J* = 8.6 Hz, 2H), 7.46-7.38 (m, 2H), 7.18 (t, *J* = 7.4 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 2H), 6.94 (d, *J* = 8.6 Hz, 2H).

Step 2: Synthesis of BG-3

[0088]

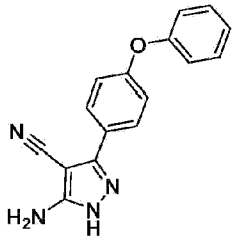


BG-3

[0089] Under nitrogen atmosphere, a solution of BG-2 (79.9 kg, 1.0 eq.) in MeCN (5.0 v) was added into trimethoxymethane (12.0 v) at 85°C. The resultant mixture was stirred until the reaction was completed. Sampled for HPLC analysis. Concentrated under vacuum. The residue was precipitated from i-PrOH and hexane. The mixture was centrifuged, and the cake was washed with hexane and dried under vacuum. This gave 71.7 Kg of product. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.70 (d, *J* = 8.4 Hz, 2H), 7.52-7.45 (m, 2H), 7.28 (t, *J* = 7.6 Hz, 1H), 7.22-7.06 (m, 4H), 3.93 (s, 3H).

Step 3: Synthesis of BG-4

[0090]

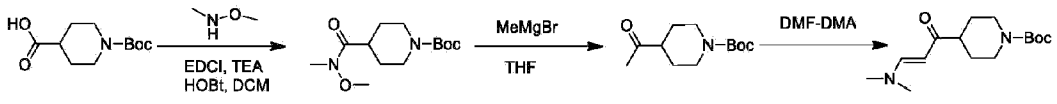


BG-4

[0091] Under nitrogen atmosphere, to a solution of BG-3 (71.6 kg, 1.0 eq.) in ethanol (2.5 v) hydrazinium hydroxide (1.0 eq) in ethanol (0.6 v) was charged dropwise to the reactor below 15°C. The solution was heated to RT and stirred until the reaction was completed. Water (4.0 v) was added to the reactor. The solution was then cooled to 5°C, centrifuged and the cake was washed with water (1.0 v). The cake was dried under vacuum. This gave 66.9 Kg of product. ^{1}H NMR (DMSO-d_6) δ 12.11 (br s, 1H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.46-7.39 (m, 2H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.12-7.04 (m, 4H), 6.43 (br s, 2H).

Steps 4 to 6: Synthesis of BG-8

[0092]

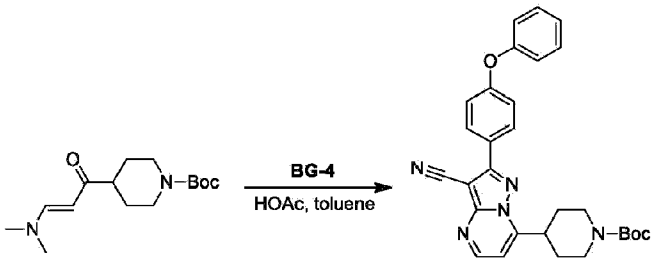


BG-5 BG-6 BG-7 BG-8

[0093] To a mixture of DCM (8.0 v), BG-5 (80.0 Kg, 1.0 eq.), *N,O*-dimethylhydroxylamine hydrochloride (1.2 eq.), HOBt (1.2 eq.) and EDCI (1.2 eq.), TEA (2.6 eq.) was charged dropwise below 15°C. the mixture was stirred at RT until the reaction was completed, centrifuged and the cake was washed with DCM (1.0 v) twice. The filtrate was washed with 20% aqueous NH_4Cl (3 × 4.0 v). The filtrate was concentrated under vacuum to give the crude product BG-6, which was used in the next step without further purification. The residue was dissolved in toluene (5.0 v) and THF (1.0 v), cooled to 10 °C, charged dropwise MeMgBr (1.4 eq.) at 10°C and then stirred at RT until the reaction was completed. The solution was cooled below 10°C. Saturated aqueous NH_4Cl was charged dropwise below 10°C. The mixure was centrifuged, separated, filtrated, and the organic phase was washed with aqueous NaCl twice. The organic phase was concentrated to give the crude product, which was used in the next step without further purification. The residue in DMF (2.5 v) and DMF-DMA (2.5 v) was stirred at 110°C until the reaction was completed. The reaction mixture was cooled, concentrated and then DCM was added. The final mixture was washed with saturated aqueous NH_4Cl. The organic layer was cconcentrated and precipitated by charging hexane. The mixture was ccentrifuged and the cake was collected. The cake was dried under vacuum. This gave 82.2 Kg of the desired product. ^1H NMR (DMSO-d_6) δ 7.49 (d, *J* = 12.6 Hz, 1H), 5.01 (d, *J* = 12.6 Hz, 1H), 3.99-3.82 (m, 2H), 3.14-2.94 (m, 2H), 2.89-2.61 (m, 6H), 2.49-2.37 (m, 1H), 1.66-1.56 (m, 2H), 1.39 (s, 9H), 1.39-1.20 (m, 2H).

Step 7: Synthesis of BG-9

[0094]



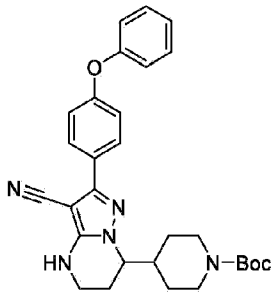
BG-8 BG-9

[0095] Under nitrogen atmosphere, a mixture of toluene (8.0 v), AcOH (0.5 v), BG-8 (1.2 eq.) and BG-4 (66.9 Kg 1.0 eq.) was heated to 95°C and stirred until the reaction was completed. The mixture was cooled, concentrated and precipitated from methanol. The mixture was centrifuged and the cake was washed with methanol. The cake was dried under vacuum. This gave 107.8 Kg of product.^1H NMR (DMSO-d_6) δ 8.78 (d, *J* = 4.6 Hz, 1H), 8.15-8.07 (m, 2H), 7.51-7.41 (m, 2H), 7.34 (d, *J* = 4.6 Hz, 1H), 7.27-7.19 (m, 3H), 7.17-7.10 (m, 2H), 4.24-4.02

(m, 2H), 3.81-3.69 (m, 1H), 3.12-3.82 (m, 2H), 2.15-2.04 (m, 2H), 1.76-1.60 (m, 2H), 1.43 (s, 9H).

Step 8: Synthesis of BG-10

[0096]

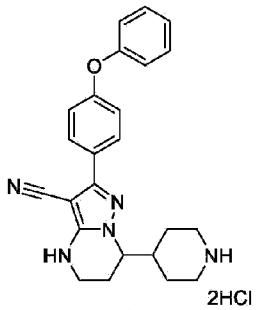


BG-10

[0097] To a mixture of THF (10.0 v), BG-9 (13.0 Kg, 1.0 eq.) and D-DBTA (1.0 eq) under N_2 was charged Pd/C (10% w/w), hydrogen gas was introduced into the reactor and the hydrogen pressure was maintained to 1.8 MPa. The reactor was heated to 40°C slowly and stirred until the reaction was completed. The mixture was then cooled, filtered, and the cake was washed with THF. The filtrate was collected, and concentrated under vacuum. DCM was added. The residue was washed with aq. $NaHCO_3$, concentrated and precipitated from MTBE and hexane, then centrifuged. The cake was collected and dried under vacuum to give the desired compound (yield:94.8% and purity:98.5%). ^{1}H-NMR (DMSO-d_6) δ 7.82-7.76 (m, 2H), 7.56-7.51 (m, 1H), 7.45-7.37 (m, 2H), 7.21-7.14 (m, 1H), 7.12-7.03 (m, 4H), 4.09-3.91 (m, 3H), 3.30-3.22 (m, 2H), 2.82-2.55 (m, 2H), 2.18-1.99 (m, 2H), 1.98-1.86 (m, 1H), 1.69-1.58 (m, 1H), 1.56-1.45 (m, 1H), 1.38 (s, 9H), 1.32-1.13 (m, 2H).

Step 9: Synthesis of BG-11

[0098]

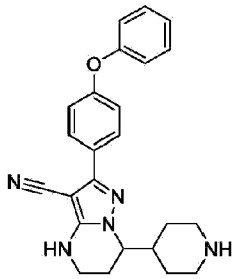


BG-11

[0099] To a solution of BG-10 (100.0 Kg 1.0 eq.) in DCM (6.0 v) was added dropwise HCl in EtOH (20.9% w/w, 2.0 v) under nitrogen atmosphere. The mixture is stirred until the reaction was completed. MTBE (4.0 v) was added to the solution, cooled. The cakes was collected by centrifugation and washed with hexane (2.0 V), then the cake was slurried in hexane (5 v), and centrifuged again. The cake was washed with hexane (2.0 V) and dried under vacuum. This gave 85.2 Kg product. ^{1}H-NMR (DMSO-d_6) δ 9.25-8.85 (m, 2H), 7.84-7.70 (m, 2H), 7.47-7.37 (m, 2H), 7.18 (t, *J* = 7.4 Hz, 1H), 7.12-7.03 (m, 4H), 5.73 (br s, 2H), 4.12-4.03 (m, 1H), 3.25-3.19 (m, 4H), 2.90-2.73 (m, 2H), 2.28-2.12 (m, 1H), 2.10-2.00 (m, 1H), 1.99-1.86 (m, 1H), 1.84-1.52 (m, 4H).

Step 10: Synthesis of BG-11A

[0100]

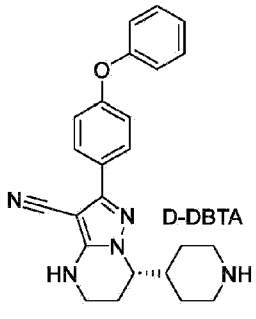


BG-11A

[0101] A mixture of BG-11 (85.0 Kg, 1.0 eq) in water (6.0 v) and NaOH (3.0 eq) was stirred until the reaction was completed at RT. The cake was collected and slurried in MTBE (6.0 v). The mixture was then centrifuged to collect the cake. The cake was dried under vacuum. This gave 71.3 Kg product. ^{1}H-NMR (DMSO-d_6) δ 7.82-7.74 (m, 2H), 7.54-7.49 (m, 1H), 7.45-7.38 (m, 2H), 7.21-7.14 (m, 1H), 7.12-7.04 (m, 4H), 4.03-3.95 (m, 1H), 3.29-3.21 (m, 2H), 3.00-2.87 (m, 2H), 2.46-2.31 (m, 2H), 2.11-1.83 (m, 3H), 1.58-1.12 (m, 4H).

Step 11: Synthesis of BG- I I B

[0102]

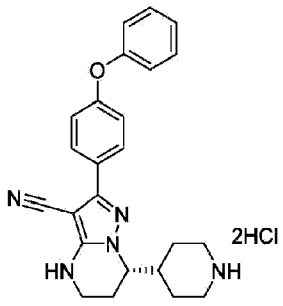


BG-11B

[0103] A mixture of enthanol/water/acetic acid (7:3:1, 46 v) and BG-11A (30 kg, 1.0 eq.) in a reactor was heated to 70±5 °C under nitrogen atmosphere, then a solution of D-DBTA (1.20 eq.) in ethanol/water/acetic acid (7:3:1, 4 v) was added dropwise with the temperature not less than 65°C. The resulting solution was stirred for 16 hrs at 60-65 °C, then cooled to RT. The solid was collected by centrifugation and washed with ethanol (2.0 v). The cake was slurried in the mixed solvent of ethanol/water/AcOH (7:3:1, 20 v) for 16 hrs at 55 °C and cooled to RT. The solid was collected by centrifugation, washed with ethanol (2.0 v). The cake was dried under vacuum (Yield: 37.9%).^{1}H-NMR (DMSO-d_6) δ 8.76 (br s, 2H), 7.99-7.89 (m, 4H), 7.83-7.75 (m, 2H), 7.66-7.57 (m, 3H), 7.52-7.45 (m, 4H), 7.45-7.39 (m, 2H), 7.21-7.14 (m, 1H), 7.13-7.03 (m, 4H), 5.64 (s, 2H), 4.08-4.00 (m, 1H), 3.29-3.19 (m, 4H), 2.85-2.72 (m, 2H), 2.21-1.40 (m, 7H).

Step 12: Synthesis of BG-11C

[0104]



BG-11C

[0105] To a mixture of dichloromethane (15.0 v) and 20.0% aqueous KOH (3.0 v) was added bachwise BG-11B (48.0 kg, 1.0 eq.) under nitrogen atmosphere at RT. After the reaction was completed, the organic layer was collected and the water layer was extracted with dichloromethane (5.0 v). The organic layers were combined. Con. HCl (0.36 v) was added to the above organic layers at RT. The resulting mixture was stirred until the reaction was completed. The solid was collected by centrifugation and washed with dichloromethane (1.0 v). The collected solid was slurried with MTBE (6.0 v). The solid was collected by centrifugation and washed with MTBE (1.0 v), then was dried under vacuum. This gave 31.5 Kg product (Yield:100 %).

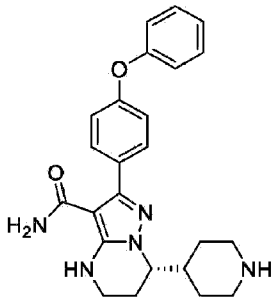
Step 12: Synthesis of BG- I I D (Alternative intermediate)

[0106] ACN (5.0 v), soft water (10.0 v), KOH (5.0 eq) was charged to a reactor and stirred for at least 15 min. BG-11B (1.0 eq) was charge to the reactor in portion-wise. The mixture was

stirred until the reaction was completed. The cake was collected by centrifugation, slurried in ACN (1.0 v) and soft water (5.0 v), and dried under vacuum to give the product.

Step 13: Synthesis of BG-12

[0107]

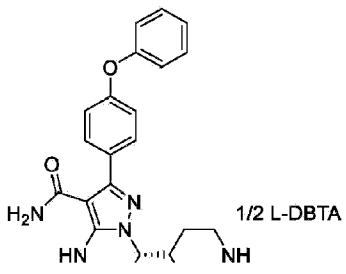


BG-12

[0108] A solution of BG-11C (15.0 Kg 1.0 eq.) in MsOH (2.5 v) was stirred at 85°C under nitrogen atmosphere until the reaction was completed. After cooling to 5 °C. purified water (4.0 v) was added dropwise to the system and kept the temperature not more than 35°C (temperature increased obviously). The resulting solution was stirred for 16 hrs at 30°C, and then washed with DCM (2×3.0 v). The aqueous phase was collected. DCM (6.0 v) was added to the aqueous phase, the mixture was cooled to 5°C. The pH value was adjusted to 11~12 with 20% aqueous NaOH (temperature increased obviously) with stirring with the temperature not more than 30 °C. The organic phase was separated and collected. The aqueous was extracted with DCM (3.0 v). The organic layers were combined and concentrated. MTBE (4.0 v) was added to the residue. The mixture was then concentrated and precipitated from *n*-heptane. The solid was collected by centrifugation and dried in a vacuum oven. This gave 12.55 Kg product (Yield: 94.9%). ^{1}H-NMR (DMSO-d_6) δ 7.52-7.46 (m, 2H), 7.45-7.38 (m, 2H), 7.21-7.13 (m, 1H), 7.12-7.03 (m, 4H), 6.64 (s, 1H), 3.99-3.90 (m, 1H), 3.29-3.22 (m, 2H), 3.03-2.90 (m, 2H), 2.48-2.36 (m, 2H), 2.03 (dd, *J* = 13.9, 5.6 Hz, 2H), 2.14-1.99 (m, 1H), 1.97-1.85 (m, 1H), 1.65-1.15 (m, 3H).

Step 14: Synthesis of BG-13

[0109]

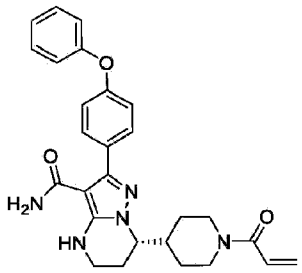


BG-13

[0110] A mixture of MeOH (13.5 v), purified water (4.5 v) and BG-12 (8.5 Kg, 1.0 eq.) in a reactor was heated to 50°C under N_2 atmosphere. To the mixture was charged dropwise a solution of L-DBTA (0.7 eq) in MeOH/purified water (1.5 v/0.5 v) while keeping the temperature at 50 °C. After addition, the mixture was stirred for at least 2 hrs at 50 °C, and then cooled to RT and stirred for at least 16 hrs at RT. The cake was collected by Centrifugation and was washed with MeOH (2.0 v). The cake was dried in a vacuum oven. This gave 9.08 Kg product (Yield: 74.8%).

Step 15: Synthesis of (S)-7-(1-acryloylpiperidin-4-yl)-2-(4-phenoxyphenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine-3-carboxamide (Compound 1)

[0111]

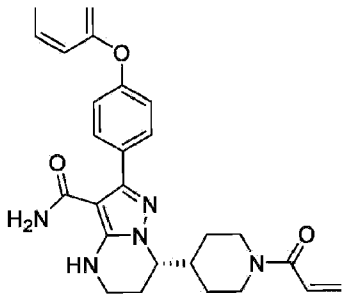


[0112] Under N_2 atmosphere, ACN (12.0 v), water (12.5 v), BG-13 (8.0 Kg, 1.0 eq), and $NaHCO_3$ (2.5 eq.) were added to a reactor. The mixture was then cooled to -5-0 °C. To the mixture, the solution of acryloyl chloride (1.1 eq.) in MeCN (0.5 v) was added dropwise and stirred until the reaction was completed. EA (6.0 v) was then added to the reactor, and stirred. The organic phase was collected. The aqueous layer was further extracted with EA (3.0 v). The organic phases were combined and washed with brine. The organic layer was collected and concentrated.

[0113] The residue was purified by silica gel (2 wt) column, eluted with 3% w/w methanol in DCM (21.0 v). The **Compound 1** solution was collected and concentrated under vacuum. The residue was precipitated from EA/MTBE (2.0 v). The cake was collected by centrifugation as the product.

Step 15: Synthesis of (S)-7-(1-acryloylpiperidin-4-yl)-2-(4-phenoxyphenyl) -4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine-3-carboxamide (Compound 1, alternative method)

[0114]



[0115] A mixture of CH_3CN (10.0 v), purified water (5.0 v), NaOH (1.5 eq.) and BG-13 (1.0 eq.) was stirred to get a clear solution. EtOAc (6.0 v) was then charged to the reaction and separated. The organic phase was collected and washed with 15% brine (3.0 v) twice. The organic phase prepared above was concentrated and the solvent was swapped to CH_3CN (residue volume: NMT 5.0 v). CH_3CN (7.5 v) and purified water (12.5 v) were charged and cooled to 15-20°C. L-(+)-tartaric acid (0.5 eq) and $NaHCO_3$ (2.5 eq.) were charged to the reaction mixture. A solution of acryloyl chloride (1.1 eq.) in CH_3CN (0.5 v) was charged drop-wise to the reaction mixture. After the reaction was completed, EtOAc (6.0 v) was charged to the reaction mixture and organic layer was collected. Aqueous phase was further extracted with EA (3.0 v). The organic layers were combined, washed with 15% brine (5.0 v) and concentrated. The solvent was swapped to DCM (volume of residue: 1.5-2.0 v) and purified by silica gel column (silica gel: 100-200 mush, 2.0 w/ w; eluent: 3% w/ w MeOH in DCM (about 50 v). The collected solution was concentrated and swapped to EtOAc (4.0 v). MTBE (6.4 v) was charged drop-wise to residue at 50°C. The mixture was then cooled to 5°C and the cake was collected centrifugation.

Step 16: Preparation of Crystalline Form A of Compound 1

[0116] The above cake was dissolved in 7.0 volumes of DCM, and then swapped to solvent EA. After recrystallization from EA/MTBE, the cakes was collected by centrifugation, and was dried under vacuum. This gave 4.44 Kg product (Yield: 70.2%).

[0117] The product was then characterized by X-ray powder diffraction (XRPD) pattern method, which was generated on a PANalytical Empyrean X-ray powder diffractometer with the XRPD parameters as follows: X-Ray wavelength (Cu, kα, Kα1 (Å): 1.540598, Kα2(Å): 1.544426; Kα2/Kα1 intensity ratio: 0.50); X-Ray tube setting (45 Kv, 40mA); divergence slit (automatic); scan mode (Continuous); scan range (°2TH) (3°-40); step size (°2TH) (0.0131); scan speed (°/min) (about 10). The XRPD result found the resultant product as a crystalline shown in **FIG. 8.**

[0118] The proton nuclear magnetic resonance (^{1}H-NMR) shown as in **FIG. 9** was collected on a Bruker 400M NMR Spectrometer in DMSO-d_6. ^{1}H-NMR (DMSO-d_6) δ 7.50 (d, *J* = 8.6 Hz,

2H), 7.46-7.38 (m, 2H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.08 (d, *J* = 7.6 Hz, 2H), 7.05 (d, *J* = 8.8 Hz, 2H), 6.85-6.72 (m, 1H), 6.67 (s, 1H), 6.07 (dd, *J* = 16.8, 2.2 Hz, 1H), 5.64 (dd, *J* = 10.4 Hz, 2.2 Hz, 1H), 4.55-4.38 (m, 1H), 4.17-3.94 (m, 2H), 3.33-3.22 (m, 2H), 3.08-2.88 (m, 1H), 2.67-2.51 (m, 1H), 2.36-2.15 (m, 1H), 2.12-1.82 (m, 2H), 1.79-1.65 (m, 1H), 1.63-1.49 (m, 1H), 1.38-1.08 (m, 2H).

[0119] The carbon nuclear magnetic resonance (^{13}C-NMR) shown as in **FIG. 10** was collected on a Bruker 400M NMR Spectrometer in DMSO-d_6. ^{13}C-NMR spectra for Crystalline Form A of **Compound 1.**

Example 2 Effect of the combination of anti-CD20 mAb and BTK inhibitor in human REC-1/NK92MI mantle cell lymphoma xenograft model

Method

[0120] REC-1 cells were cultured in RPMI1640 complete medium supplemented with 10% (v/v) fetal bovine serum, and 100 μg/mL of penicillin and streptomycin. NK92MI/CD16V cell line, overexpressing CD16 and FcRy chain, was established from parental cell line NK92MI. The cells were maintained in MEM alpha supplemented with 0.2 mM inositol; 0.1 mM 2-mercaptoethanol; 0.02 mM folic acid; 1×NEAA; adjust to a final concentration of 20% (v/v) fetal bovine serum and 100 μg/mL of penicillin and streptomycin. NOD/SCID mice were pretreated with cyclophosphamide (prepared in saline, 150 mg/kg, i.p.) and disulfiram (prepared in 0.8% Tween-80 in saline, 125 mg/kg, p.o., one hour after each dose of cyclophosphamide) once daily for two days.

[0121] On the day of implantation, culture medium was replaced with fresh medium. Four hours later, media was removed and cells were collected as described above. Cells were re-suspended in cold (4 °C) PBS and same volume of matrigel was added to give a final concentration of 5×10^7 cells/mL for REC-1 and 1×10^7 cells/mL for NK92MI/CD16V, respectively. Re-suspended cells were placed on ice prior to inoculation. The right flank region of each mouse was cleaned with 75% ethanol prior to cell inoculation. Animals were then coinjected subcutaneously with 1×10^7 REC-1 cells and 2×10^6 NK92MI/CD16V cells in 200 μl of cell suspension in the right front flank via a 26-gauge needle.

[0122] On day 3 after inoculation, animals were randomly divided into four groups with 12 mice per group. The groups consisted of a control group (no drug treatment), 2.5 mg/kg of **Compound 1,** 2 mg/kg of obinutuzumab (Gazyva®, obtained from Roch), and the combination of **Compound 1** and obinutuzumab (2.5 mg/kg and 2 mg/kg, respectively). Treatments were administered in a volume of 10 mL/kg body weight, assessed immediately before dosing and the volume dosed was adjusted accordingly. **Compound 1** was administered by oral gavage

(p.o.) twice daily (BID) and obinutuzumab was administered by intraperitoneal (i.p.) injection once weekly (QW). After implantation, primary tumor volume was measured in two dimensions using a calliper.

[0123] Individual body weight was recorded twice weekly, with mice being monitored daily for clinical signs of toxicity for the duration of the study. Mice were euthanized using carbon dioxide when their tumor volume reached 2,500 mm^3 after twice measurements, the tumor was ulcerated, or body weight loss exceeded 20%.

[0124] Tumor volume was calculated using the formula: $V = 0.5 \times (a \times b^2)$ where a and b are the long and short diameters of the tumor, respectively. Tumor growth inhibition (TGI) was calculated using the following formula:

$$\%\ TGI = 100 \times [1 - (treated_t / placebo_t)]$$

$treated_t$ = treated tumor volume at time t

$placebo_t$ = placebo tumor volume at time t

Result

[0125] In vivo efficacy of **Compound 1** and obinutuzumab was examined in human REC-1/NK92MI MCL xenograft model. **Compound 1,** as a single agent, was shown to be active in this model, with 72% TGI on day 23, while obinutuzumab, as a single agent, was shown to have no anti-tumor effect. The combination of these two agents induced 98% TGI on day 23 which was significantly more efficacious than either single agent (**FIG. 1**).

Example 3 Effect of the combination of anti-CD20 mAb and BTK inhibitor in human TMD-8 DLBCL xenograft model

Method

[0126] TMD-8 DLBCL cells were cultured in RPMI1640 complete medium supplemented with 10% (v/v) fetal bovine serum, and 100 µg/mL of penicillin and streptomycin. On the day of implantation, culture medium was replaced with fresh medium. Four hours later, media was removed and cells were collected as described above. Cells were re-suspended in cold (4 °C) PBS and same volume of matrigel was added to give a final concentration of 5×10^7 cells/mL for TMD-8 cells. Re-suspended cells were placed on ice prior to inoculation. The right flank region of each mouse was cleaned with 75% ethanol prior to cell inoculation. Animals were then injected subcutaneously with 1×10^7 TMD-8 cells in 200 µl of cell suspension in the right front flank via a 26-gauge needle.

[0127] On day 7 after inoculation, animals were randomly divided into 4 groups with 10 mice per group. The groups consisted of a control group (no drug treatment), 7.5 mg/kg of **Compound 1,** 10 mg/kg of obinutuzumab (Gazyva®, obtained from Roch), and the combination of **Compound 1** and obinutuzumab. Treatments were administered in a volume of 10 mL/kg body weight, assessed immediately before dosing and the volume dosed was adjusted accordingly. **Compound 1** was administered by oral gavage (p.o.) twice daily (BID) and obinutuzumab was administered by intraperitoneal (i.p.) injection once every week (QW). After implantation, primary tumor volume was measured in two dimensions using a calliper.

[0128] Mice were euthanized using carbon dioxide when their tumor volume reached 2,500 mm^3 after twice measurements, the tumor was ulcerated, or body weight loss exceeded 20%.

[0129] Tumor volume was calculated using the formula: $V = 0.5 \times (a \times b^2)$ where a and b are the long and short diameters of the tumor, respectively. Tumor growth inhibition (TGI) was calculated using the following formula:

$$\%\ TGI = 100 \times [1 - (treated_t / placebo_t)]$$

$treated_t$ = treated tumor volume at time t

$placebo_t$ = placebo tumor volume at time t

Result

[0130] On day 46, treatments of **Compound 1** and obinutuzumab resulted in 81% and 61% of tumor growth inhibition (TGI), respectively. Objective responses were observed with **Compound 1** at 7.5 mg/kg (1CR/4PR/5) and obinutuzumab at 10 mg/kg (0CR/2PR/2). The combination of **Compound 1** and obinutuzumab was more efficacious than each single agent treatment and resulted in 111 % TGI with 90% (1CR/8PR/9) overall response rate (**FIG. 2)..**

Example 4 Effect of the combination of anti-CD20 mAb and BTK inhibitor in human REC-1 MCL xenograft model

Method

[0131] REC-1 cells were cultured in RPMI1640 complete medium supplemented with 10% (v/v) fetal bovine serum, and 100 µg/mL of penicillin and streptomycin. On the day of implantation, culture medium was replaced with fresh medium. Four hours later, media was removed and cells were collected as described above. Cells were re-suspended in cold (4 °C) PBS and same volume of matrigel was added to give a final concentration of 5×10^7 cells/mL for REC-1.

Re-suspended cells were placed on ice prior to inoculation. The right flank region of each mouse was cleaned with 75% ethanol prior to cell inoculation. Animals were then injected subcutaneously with 1×10^7 REC-1 cells in 200 µl of cell suspension in the right front flank via a 26-gauge needle.

[0132] On day 9 after inoculation, animals were randomly divided into 6 groups with 7 mice per group. The groups consisted of a control group (no drug treatment), 25 mg/kg of Ibrutinib, 7.5 mg/kg of **Compound 1,** 200 µg/dose of rituximab, the combination of **Compound 1** and rituximab (7.5 mg/kg and 200 µg/dose, respectively), and the combination of Ibrutinib and rituximab (25 mg/kg and 200 µg/dose, respectively). **Compound 1** and ibrutinib were administered in a volume of 10 mL/kg body weight, assessed immediately before dosing and the volume dosed was adjusted accordingly. **Compound 1** and Ibrutinib were administered by oral gavage (p.o.) twice daily (BID) and rituximab was administered by intraperitoneal (i.p.) injection once every four days (Q4D). After implantation, primary tumor volume was measured in two dimensions using a calliper.

[0133] Individual body weight was recorded twice weekly, with mice being monitored daily for clinical signs of toxicity for the duration of the study. Mice were euthanized using carbon dioxide when their tumor volume reached 2,500 mm^3 after twice measurements, the tumor was ulcerated, or body weight loss exceeded 20%.

[0134] Tumor volume was calculated using the formula: $V = 0.5 \times (a \times b^2)$ where a and b are the long and short diameters of the tumor, respectively. Tumor growth inhibition (TGI) was calculated using the following formula:

$$\%\ TGI = 100 \times [1 - (treated_t / placebo_t)]$$

$treated_t$ = treated tumor volume at time t

$placebo_t$ = placebo tumor volume at time t

Result

[0135] As shown in **FIG. 3,** the tumor growth was retarded in rituximab, **Compound 1** and Ibrutinib treatment groups. **Compound 1** achieved similar anti-tumor effect as Ibrutinib, with 85% and 83% of TGI, respectively. The combination of **Compound 1** and rituximab was more efficacious than each single agent, whereas combination of Ibrutinib with rituximab showed no obvious combo effect. On day14, **Compound 1** in combination with rituximab caused significant lower tumor weight when compared with combinational treatment of Ibrutinib and rituximab (**FIG. 4**).

Example 5 Effect of the combination of anti-PD-1 mAb and Btk inhibitor in human A431 epidermoid carcinoma allogeneic model

Method

[0136] On the day of implantation, human peripheral blood mononuclear cells (PBMCs) were isolated from 150 mL blood donated by a healthy volunteer. Briefly, peripheral blood was collected into vacuum blood collection tubes containing sodium heparin. PBMCs were separated by density gradient centrifugation using Histopaque-1077 and washed one time by Dulbecco's Phosphate Buffered Saline (DPBS). The cell pellet was suspended with DPBS at appropriate volume to give a final concentration of 1×10^8 cells/mL and placed on ice prior to inoculation.

[0137] A431 cells were cultured in DMEM complete medium supplemented with 10% (v/v) fetal bovine serum, and 100 µg/mL of penicillin and streptomycin. On the day of implantation, culture medium was replaced with fresh medium. Five hours later, media was removed and cells were collected as described above, except that cells were re-suspended in cold (4 °C) DPBS to give a final concentration of 5×10^7 cells/mL and placed on ice prior to inoculation. Mix the A431 cells, PBMCs and matrigel at the ratio of 1:1:2. NOD/SCID mice were pretreated with cyclophosphamide (prepared in saline, 150 mg/kg, i.p.) and disulfiram (prepared in 0.8% Tween-80 in saline, 125 mg/kg, p.o., one hour after each dose of cyclophosphamide) once daily for one day. The right axilla region of each mouse was cleaned with 70% ethanol prior to cell inoculation. Each animal was injected subcutaneously with 2.5×10^6 A431 cells and 5×10^6 PBMC (200 µl cell mixture in 50% matrigel) in the right front flank via a 26-gauge needle 24 hours after the second dose of cyclophosphamide.

[0138] Starting from day 0 after cell inoculation, animals were randomly divided into four groups with 10-11 mice per group. The groups consisted of a control group (no drug treatment), 15 mg/kg of **Compound 1, 10** mg/kg of **Mab 1,** and the combination of **Compound 1** and **Mab 1** (15 mg/kg and 10 mg/kg, respectively). Treatments were administered in a volume of 10 mL/kg body weight, assessed immediately before dosing and the volume dosed was adjusted accordingly. **Compound 1** was administered by oral gavage (p.o.) twice daily (BID) and **Mab 1** was administered by intraperitoneal (i.p.) injection once weekly (QW). After implantation, primary tumor volume was measured in two dimensions using a calliper.

[0139] Individual body weight was recorded twice weekly, with mice being monitored daily for clinical signs of toxicity for the duration of the study. Mice were euthanized using carbon dioxide when their tumor volume reached 2,500 mm^3, the tumor was ulcerated, or body weight loss exceeded 20%.

[0140] Tumor volume was calculated using the formula: $V = 0.5 \times (a \times b^2)$ where a and b are the long and short diameters of the tumor, respectively.

Result

[0141] In vivo efficacy of **Compound 1** and **Mab 1** was examined in human A431 epidermoid carcinoma allogeneic model. **Mab 1,** as a single agent, showed marginal effect in this model while **Compound 1** showed no anti-tumor effect in this model. As shown in **FIG. 5,** the combination of these two agents demonstrated better efficacy than either single agent.

Example 6 Effect of the combination of anti-PD-1 mAb and BTK inhibitor in human A431 epidermoid carcinoma allogeneic model

Method

[0142] On the day of implantation, human peripheral blood mononuclear cells (PBMCs) were isolated from 150 mL blood donated by a healthy volunteer. Briefly, peripheral blood was collected into vacuum blood collection tubes containing sodium heparin. PBMCs were separated by density gradient centrifugation using Histopaque-1077 and washed one time by Dulbecco's Phosphate Buffered Saline (DPBS). The cell pellet was suspended with DPBS at appropriate volume to give a final concentration of 5×10^7 cells/mL and placed on ice prior to inoculation.

[0143] A431 cells were cultured in DMEM complete medium supplemented with 10% (v/v) fetal bovine serum, and 100 µg/mL of penicillin and streptomycin. On the day of implantation, culture medium was replaced with fresh medium. Five hours later, media was removed and cells were collected as described above, except that cells were re-suspended in cold (4 °C) DPBS to give a final concentration of 5×10^7 cells/mL and placed on ice prior to inoculation. Mix the A431 cells, PBMCs and matrigel at the ratio of 1:1:2. NOD/SCID mice were pretreated with cyclophosphamide (prepared in saline, 150 mg/kg, i.p.) and disulfiram (prepared in 0.8% Tween-80 in saline, 125 mg/kg, p.o., one hour after each dose of cyclophosphamide) once daily for one day. The right axilla region of each mouse was cleaned with 70% ethanol prior to cell inoculation. Each animal was injected subcutaneously with 2.5×10^6 A431 cells and 2.5×10^6 PBMC (200 µl cell mixture in 50% matrigel) in the right front flank via a 26-gauge needle 24 hours after the second dose of cyclophosphamide.

[0144] Starting from day 0 after cell inoculation, animals were randomly divided into four groups with 10-11 mice per group. The groups consisted of a control group (no drug treatment), 15 mg/kg of **Compound 1,** 10 mg/kg of pembrolizumab, and the combination of **Compound 1** and pembrolizumab (15 mg/kg and 10 mg/kg, respectively). Treatments were administered in a volume of 10 mL/kg body weight, assessed immediately before dosing and the volume dosed was adjusted accordingly. **Compound 1** was administered by oral gavage

(p.o.) twice daily (BID) and pembrolizumab was administered by intraperitoneal (i.p.) injection once weekly (QW). After implantation, primary tumor volume was measured in two dimensions using a calliper.

[0145] Individual body weight was recorded twice weekly, with mice being monitored daily for clinical signs of toxicity for the duration of the study. Mice were euthanized using carbon dioxide when their tumor volume reached 2,500 mm^3, the tumor was ulcerated, or body weight loss exceeded 20%.

[0146] Tumor volume was calculated using the formula: $V = 0.5 \times (a \times b^2)$ where a and b are the long and short diameters of the tumor, respectively.

Result

[0147] In vivo efficacy of **Compound 1** and pembrolizumab was examined in human A431 epidermoid carcinoma allogeneic model. Neither **Compound 1** nor pembrolizumab showed anti-tumor effect in this model. As shown in **FIG. 6,** the combination of these two agents showed no combo effect.

Example 7A Selectivity for BTK against ITK of BTK inhibitor

Method:

Biochemical IC50 determination of BTK and ITK

[0148] Compound 1 and Ibrutinib were tested for inhibition of BTK kinase in assays based on the time-resolved fluorescence-resonance energy transfer (TR-FRET) methodology. Briefly, the assays were carried out in 384-well low volume black plates in a reaction mixture containing BTK kinase, 5μM ATP, 2μM peptide substrate and 0-10 μM compound in buffer containing 50 mM Tris pH7.4, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.005% Tween-20, 20 nM SEB and 0.01% BSA. The kinase was incubated with compound for 60 minutes at room temperature and the reaction was initiated by the addition of ATP and peptide substrate. After reaction at room temperature for 60 minutes, an equal volume of stop/detection solution was added. Plates were sealed and incubated at room temperature for 1 hour, and the TR-FRET signals were recorded on a PHERAstar FS plate reader (BMG Labtech).

[0149] The protocol of ITK assay is similar to BTK assay except for the following modification:

3μM ATP and 2μM TK substrate were used in the kinase reaction.

ITK p-PLCγ1 Cellular Assay

[0150] Once activated by T cell receptor (TCR) aggregation, ITK directly phosphorylates PLCγ1 residue Tyr783 and subsequently actives NF-κB pathway, leading to increased production of Interleukin 2 (IL-2). Jurkat cells were treated with indicated concentration of ibrutinib or **Compound 1** for 2 hrs. After treatment, cells were exposed to 10 mM of hydrogen peroxide for 10 min. PLCγ1, p-PLCγ1 (Y783) were detected by western blot analysis. IC50 is calculated using Quantity One and Prism 5 software.

ITK IL-2 production cellular assay

[0151] HuT-78 cells and Hek293/OS8V cells were co-cultured in medium with indicated concentration of ibrutinib or **Compound 1** for 20 hr. Potency of ibrutinib and **Compound 1** were calculated basing on the medium IL-2 level.

BTK occupation cellular assay

[0152] Z-138 cells were treated with increasing concentration of **Compound 1** for 2 hours. The cell lysate were load to ELISA plate pre-immobilized with detection probe. After overnight incubation, plate was washed with PBST for 3 times and probe conjugated BTK protein was detected by a BTK antibody. The potency of compounds was calculated basing on the inhibition of ratio between signal intensity at OD450 nm. IC50 values were calculated with GraphPad Prism software using the sigmoidal dose-response function.

Results:

[0153] In the biochemical assay, selectivity of **Compound 1** for BTK was 187-fold against ITK, whereas selectivity of Ibrutinib for BTK was 17-fold against ITK, indicating that **Compound 1** was more selective than ibrutinib for inhibition of BTK vs. ITK.

[0154] In the PLCγ1 phosphorylation assay (Jurkat cell) and IL-2 production assay (HuT-78 cell), **Compound 1** was shown to be 44-fold and 10-fold less effective than ibrutinib in inhibiting H_2O_2 induced PLCγ1 phosphorylation and IL-2 production, suggesting that **Compound 1** is much weaker in ITK inhibition than ibrutinib.

Table 1 Compound 1 is highly selective for BTK against ITK

Targets	Assays	IC_{50} (nM)	
		Ibrutinib	Compound 1
BTK	BTK cell based Occupation Assay	2.3	2.2
	BTK Biochemical Assay	0.18	0.3
ITK	p-PLCγ1 Cellular Assay	77	3477
	IL-2 production Cellular Assay	260	2536
	ITK Biochemical Assay	3	56

Example 7B Effect of BTK inhibitor on anti-CD20 mAb induced ADCC effect

Method

[0155] Mino cells were cultured in RPMI1640 complete medium supplemented with 10% (v/v) fetal bovine serum, and 100 μg/mLof penicillin and streptomycin. NK92MI/CD16V cell line, overexpressing CD16 and FcRy chain, was established from parental cell line NK92MI. The cells were maintained in MEM alpha supplemented with 0.2 mM inositol; 0.1 mM 2-mercaptoethanol; 0.02 mM folic acid; 1×NEAA; adjust to a final concentration of 20% (v/v) fetal bovine serum and 100 μg/mL of penicillin and streptomycin.

[0156] Mino cells (2×10^4cells/well) as the target cells (T) were plated into 96-well plate in triplicates. Cells were treated with vehicle or various concentrations of BTK inhibitors for one hour, followed by co-seeding with NK92MI cells (4×10^4cells/well, as the effector cells (E)) and co-treatment with vehicle or obinutuzumab (2 ug/well) for additional 24 hrs. After incubation, the cell-free supernatants of each well were collected, and the levels of human IFN-γ were measured using Human IFN-y ELISA Ready-SET-Go kit.

Result

[0157] Obinutuzumab -induced ADCC was assessed by IFN-γ secretion in co-culture of NK cells and Mino cells (**FIG. 7**). Ibrutinib inhibited IFN-γ secretion in a dose-depended manner. At 1 μM, ibrutinib strongly inhibited IFN-y secretion. In contrast, **Compound 1** was much less effective in this assay. At high dose tested (10 μM), it had similar inhibition effect on IFN-y secretion as ibrutinib at 1 μM. The result suggest that **Compound 1** is at least 10-fold weaker than ibrutinib in inhibiting obinutuzumab-induced ADCC. This is consistent with **Compound 1** being more selective BTK inhibitor, with much weaker ITK inhibition activity than ibrutinib in both biochemical and cellular assays.

Example 8 Clinical Trial Phase result on BTK inhibitor combined with anti-CD20 antibody

[0158] The multi-center, open-label Phase 1 trial of **Compound 1** with obinutuzumab in patients with B-cell malignancies is being conducted in Australia and the United States and consists of a dose-escalation phase and a dose-expansion phase in disease-specific cohorts, which include treatment naive (TN) or relapsed/refractory (R/R) chronic lymphocytic (CLL) / small lymphocytic lymphoma (SLL) and R/R follicular lymphoma (FL). The dose-escalation component is testing **Compound 1** at 320 mg once daily (QD) or 160 mg twice daily (BID) in 28-day cycles, in combination with obinutuzumab. And, obinutuzumab was administered in line with standard CLL dosing (three loading doses of 1000 mg weekly followed by 1000 mg on day one of cycles 2-6). The ongoing dose-expansion component is testing doses of **Compound 1** at 160 mg BID with the same obinutuzumab schedule. As of March 31, 2017, 45 patients with CLL/SLL and 17 patients with FL were enrolled in the trial.

[0159] 43 patients with CLL/SLL (18 TN, 25 R/R) and 15 patients with R/R FL had greater than 12 weeks of follow-up and were evaluable for efficacy. In TN CLL/SLL, after a median follow-up of 7.0 months (2.8-11.8 months), the overall response rate (ORR) was 89% with complete responses (CRs) in 22% and partial responses (PRs) in 67% of patients. Stable disease (SD) was observed in 11% of patients. In R/R CLL/SLL, at a median follow-up time of 8.0 months (3.8-14.0 months) the ORR was 92% with CRs in 16% and PRs in 76% of patients. SD was observed in 4% of patients. In R/R FL, at a median follow-up time of 6.2 months (1.2-10.7 months), the ORR was 73% with CRs in 33% and PRs in 40% of patients. Stable disease was observed in 13% of patients. One patient with R/R CLL/SLL had progressive disease (Richter's transformation), and two patients with R/R FL had progressive disease.

[0160] The multi-center, open-label Phase 1 trial of **Compound 1** with obinutuzumab in patients with B-cell malignancies is being conducted in Australia and the United States and consists of a dose-escalation phase and a dose-expansion phase in disease-specific cohorts, which include treatment naive (TN) or relapsed/refractory (R/R) chronic lymphocytic (CLL) / small lymphocytic lymphoma (SLL) and R/R follicular lymphoma (FL). The dose-escalation component is testing **Compound 1** at 320 mg once daily (QD) or 160 mg twice daily (BID) in 28-day cycles, in combination with obinutuzumab. And, obinutuzumab was administered in line with standard CLL dosing (three loading doses of 1000 mg weekly followed by 1000 mg on day one of cycles 2-6). The ongoing dose-expansion component is testing doses of **Compound 1** at 160 mg BID with the same obinutuzumab schedule. As of March 31, 2017, 45 patients with CLL/SLL and 17 patients with FL were enrolled in the trial.

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Patentkrav

1. Btk-inhibitor til anvendelse i en fremgangsmåde til forsinkelse af progression eller behandling af cancer hos et individ, omfattende indgivelse til individet med behov derfor af en terapeutisk virksom mængde af Btk-inhibitoren i kombination med en terapeutisk virksom mængde af et anti-CD20-antistof eller et anti-PD-1-antistof,
hvor Btk-inhibitoren er (S)-7-(1-acryloylpiperidin-4-yl)-2-(4-phenoxyphenyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidin-3 -carboxamid eller et farmaceutisk acceptabelt salt deraf,
hvor anti-CD20-antistoffet er rituximab, ibritumomab-tiuxetan, tositumonmab, ofatumumab eller obinutuzumab, og hvor anti-PD-1-antistoffet er et monoklonalt anti-PD-1-antistof omfattende en aminosyresekvens af den variable region med tung kæde (Vh) af SEQ. ID No. 24, en aminosyresekvens af den variable region med let kæde (Vl) af SEQ ID No. 26, og en aminosyresekvens af det konstante IgG4-domæne af SEQ ID NO 88.

2. Btk-inhibitor til anvendelse ifølge krav 1, hvor anti-CD20-antistoffet er rituximab eller Obinutuzumab.

3. Btk-inhibitor til anvendelse ifølge krav 2, hvor anti-CD20-antistoffet er obinutuzumab, og
hvor Btk-inhibitoren indgives i en dosis på 320 mg dagligt eller 160 mg to gange dagligt i 28-dages cyklusser, og obinutuzumab indgives med tre belastningsdoser på 1000 mg ugentligt efterfulgt af 1000 mg på dag et af cyklus 2-6.

4. Btk-inhibitor til anvendelse ifølge krav 1, hvor anti-PD-1-antistoffet administreres i en dosis på 2 mg/kg Q3W til 200 mg/kg Q3W.

5. Btk-inhibitor til anvendelse ifølge krav 1, hvor canceren er hæmatologisk cancer.

6. Btk-inhibitor til anvendelse ifølge krav 5, hvor den hæmatologiske cancer er valgt blandt leukæmi, lymfom, myelom, non-Hodgkins lymfom (NHL), Hodgkins lymfom (HL) eller B-celle-malignitet.

7. Btk-inhibitor til anvendelse ifølge krav 6, hvor B-celle-maligniteten er kronisk lymfatisk leukæmi (CLL), lille lymfocytisk lymfom (SLL), ikke-CLL/SLL-lymfom, follikulært lymfom (FL), kappecellelymfom (MCL), marginal zone-lymfom (MZL), Waldenstrøms makroglobulinæmi (WM), hårcelleleukæmi (HCL), Burkitts-lignende leukæmi (BL), prolymfocytisk B-celle-leukæmi (B-PLL), diffust storcellet B-celle-lymfom (DLBCL), diffust storcellet B-celle-lymfom (DLBCL) med ubestemt undertype, et primært lymfom af centralnervesystemet (PCNSL), et sekundært lymfom af centralnervesystemet (SCNSL) med oprindelse fra bryst eller testikel, multipel myelom eller en kombination af to eller flere deraf.

8. Btk-inhibitor til anvendelse ifølge krav 7, hvor B-celle-maligniteten er diffust storcellet B-celle-lymfom (DLBCL), diffust storcellet B-celle-lymfom med aktiverede B-celler (ABC-DLBCL), diffust storcellet B-celle-lymfom med kernecentrums-B-celler (GCB-DLBCL) eller diffust storcellet B-celle-lymfom med ikke-kernecentrums-B-celler (Non-GCB DLBCL).

9. Btk-inhibitor til anvendelse ifølge et hvilket som helst af kravene 6 til 8, hvor B-celle-maligniteten er en recidiv eller refraktær (R/R) B-celle-malignitet.

10. Btk-inhibitor til anvendelse ifølge krav 9, hvor den recidive eller refraktære B-celle-malignitet er diffust storcellet B-celle-lymfom (DLBCL), diffust storcellet B-celle-lymfom med aktiverede B-celler (ABC-DLBCL), diffust storcellet B-celle-lymfom med kernecentrums-B-celler (GCB-DLBCL) eller diffust storcellet

B-celle-lymfom med ikke-kernecentrums-B-celler (non-GCB DLCBCL).

11. Btk-inhibitor til anvendelse ifølge krav 9, hvor den recidive eller refraktære B-celle-malignitet er valgt fra kronisk lymfatisk leukæmi (CLL), lille lymfocytisk lymfom (SLL), prolymfocytisk B-celle-leukæmi (B-PLL), ikke-CLL/SLL-lymfom, follikulært lymfom (FL), kappecellelymfom (MCL), marginalzone-lymfom(MZL), Waldenstrøms makroglobulinæmi (WM), multipel myelom eller en kombination deraf.

12. Btk-inhibitor til anvendelse ifølge krav 7, hvor B-celle-maligniteten er en metastaseret B-celle-malignitet.

13. Btk-inhibitor til anvendelse ifølge krav 12, hvor:
(i) den metastaserede B-celle-malignitet er diffust storcellet B-celle-lymfom (DLBCL), diffust storcellet B-celle-lymfom med aktiverede B-celler (ABC-DLBCL), diffust storcellet B-celle-lymfom med kernecentrums-B-celler (GCB-DLBCL) eller diffust storcellet B-celle-lymfom med ikke-kernecentrums-B-celler (non-GCB DLCBCL); eller
(ii) den metastatiske B-celle-malignitet er valgt fra kronisk lymfatisk leukæmi (CLL), lille lymfocytisk lymfom (SLL), prolymfocytisk B-celle-leukæmi (B-PLL), ikke-CLL/SLL-lymfom, follikulært lymfom (FL), kappecellelymfom (MCL), marginalzone-lymfom(MZL), Waldenstrøms makroglobulinæmi (WM), multipel myelom eller en kombination deraf.

14. Btk-inhibitor til anvendelse ifølge krav 1, hvor:
(i) canceren er et sarkom eller karcinom; og/eller
(ii) canceren er valgt blandt blærecancer, brystcancer, coloncancer, gastroenterologisk cancer, nyrecancer, lungecancer, ovariecancer, bugspytkirtelcancer, prostatacancer, proksimal eller distal galdekanalcancer og melanom.

15. Btk-inhibitor til anvendelse ifølge krav 1, hvor BTK-inhibitoren indgives i en dosis på 320 mg dagligt eller 160 mg to gange dagligt.

DRAWINGS

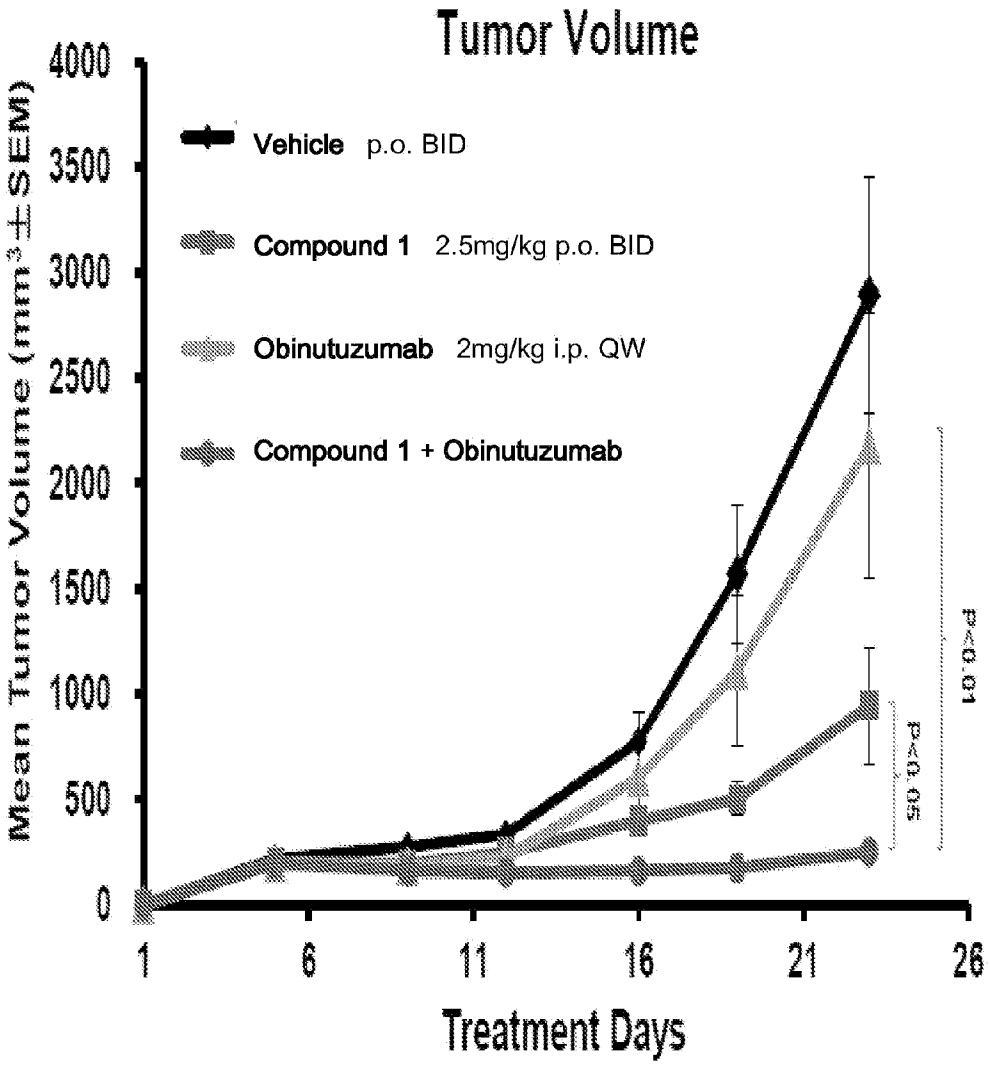


FIG. 1

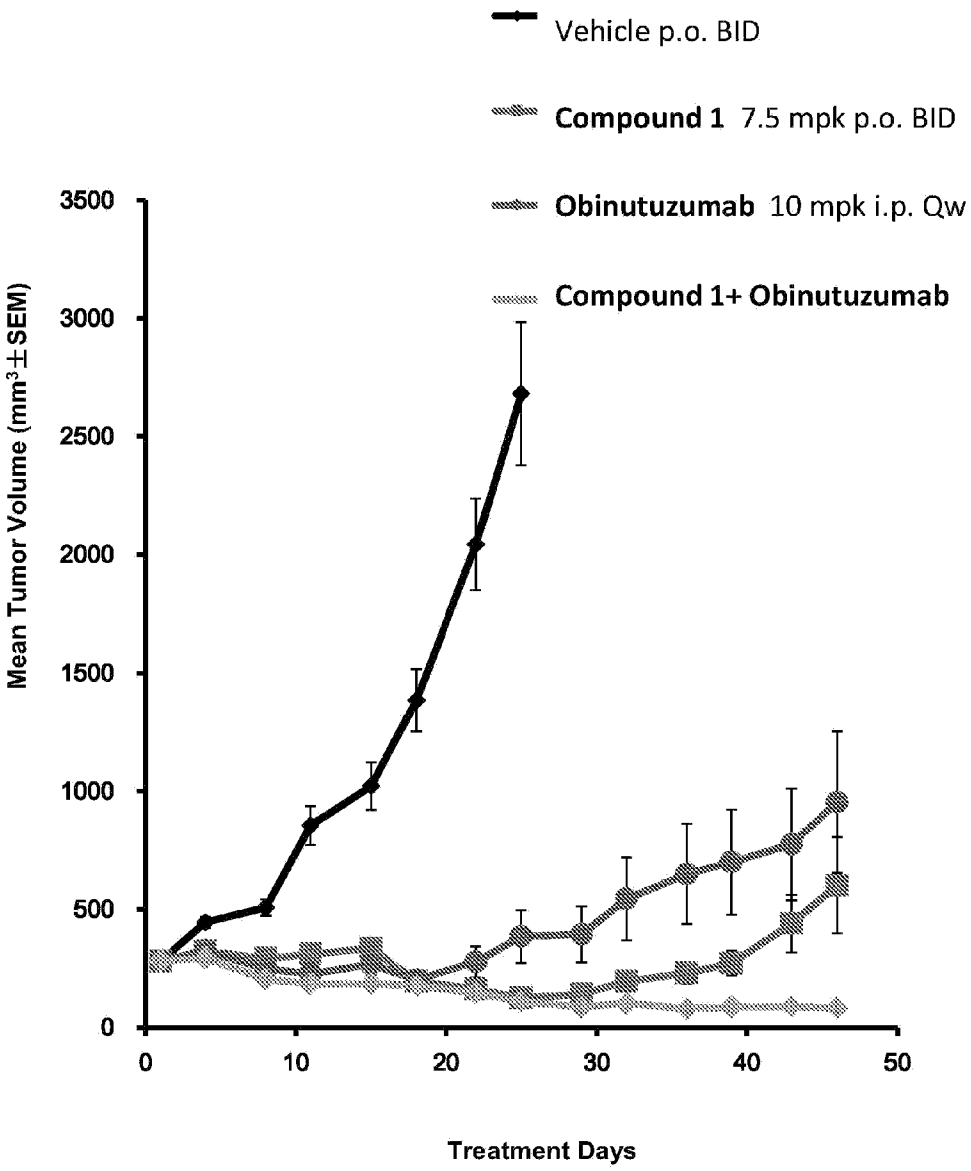

Vehicle p.o. BID
Compound 1 7.5 mpk p.o. BID
Obinutuzumab 10 mpk i.p. Qw
Compound 1+ Obinutuzumab
Mean Tumor Volume (mm3±SEM)
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Treatment Days

FIG. 2

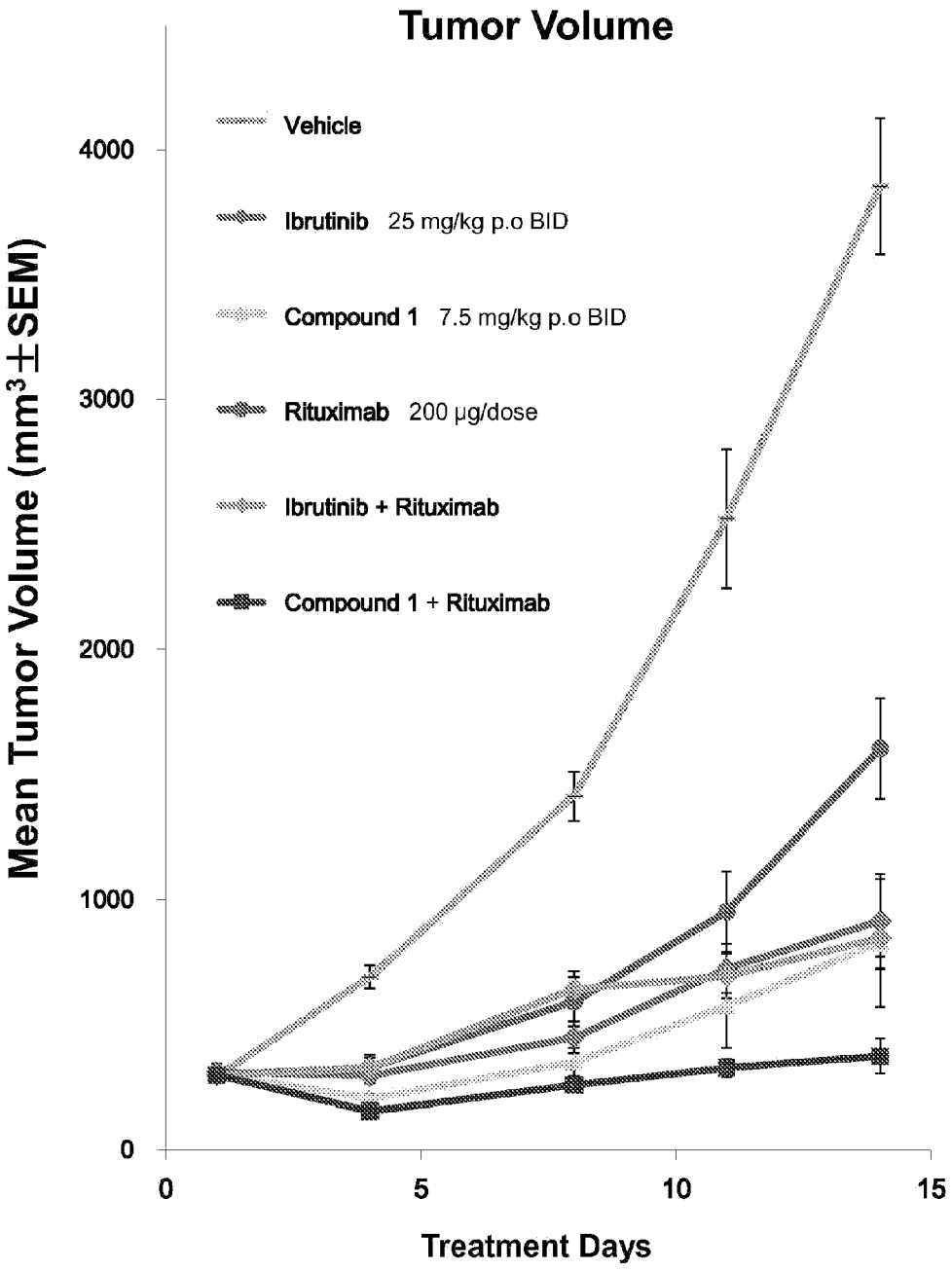

Tumor Volume
Vehicle
Ibrutinib 25 mg/kg p.o BID
Compound 1 7.5 mg/kg p.o BID
Rituximab 200 µg/dose
Ibrutinib + Rituximab
Compound 1 + Rituximab
Mean Tumor Volume (mm3±SEM)
4000
3000
2000
1000
0
0
5
10
15
Treatment Days

FIG. 3

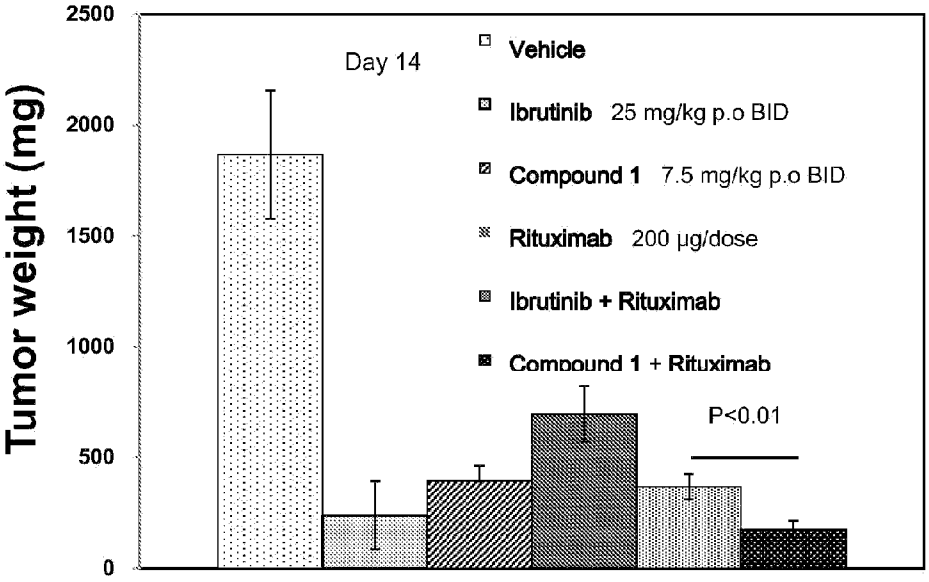

Tumor weight (mg)
2500
2000
1500
1000
500
0
Day 14
Vehicle
Ibrutinib 25 mg/kg p.o BID
Compound 1 7.5 mg/kg p.o BID
Rituximab 200 µg/dose
Ibrutinib + Rituximab
Compound 1 + Rituximab
P<0.01

FIG. 4

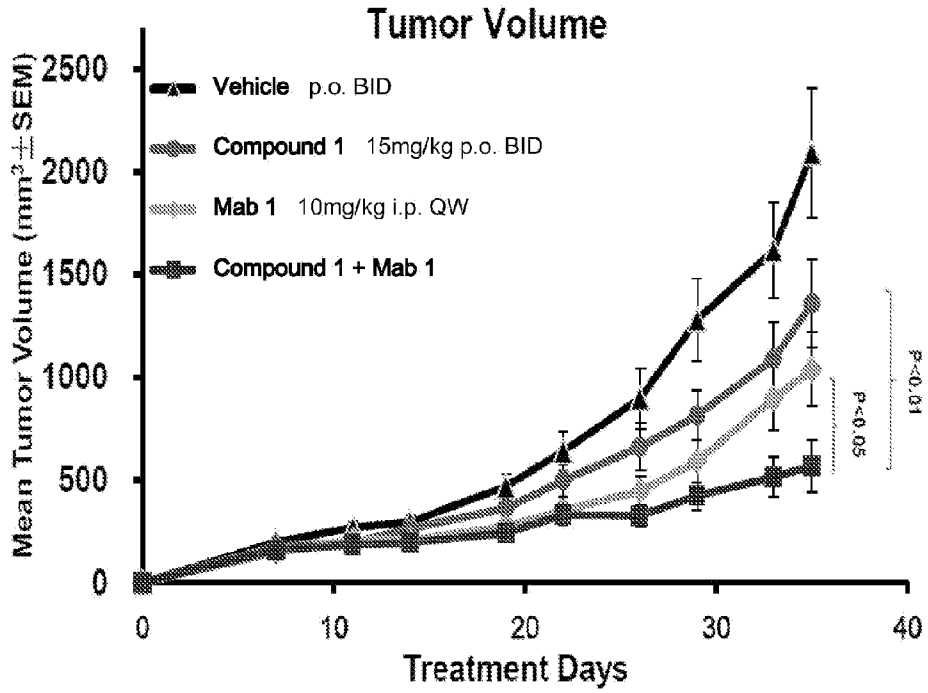

Tumor Volume
Mean Tumor Volume (mm³ ± SEM)
2500
2000
1500
1000
500
0
Vehicle p.o. BID
Compound 1 15mg/kg p.o. BID
Mab 1 10mg/kg i.p. QW
Compound 1 + Mab 1
P<0.01
P<0.05
P<0.01
0
10
20
30
40
Treatment Days

FIG. 5

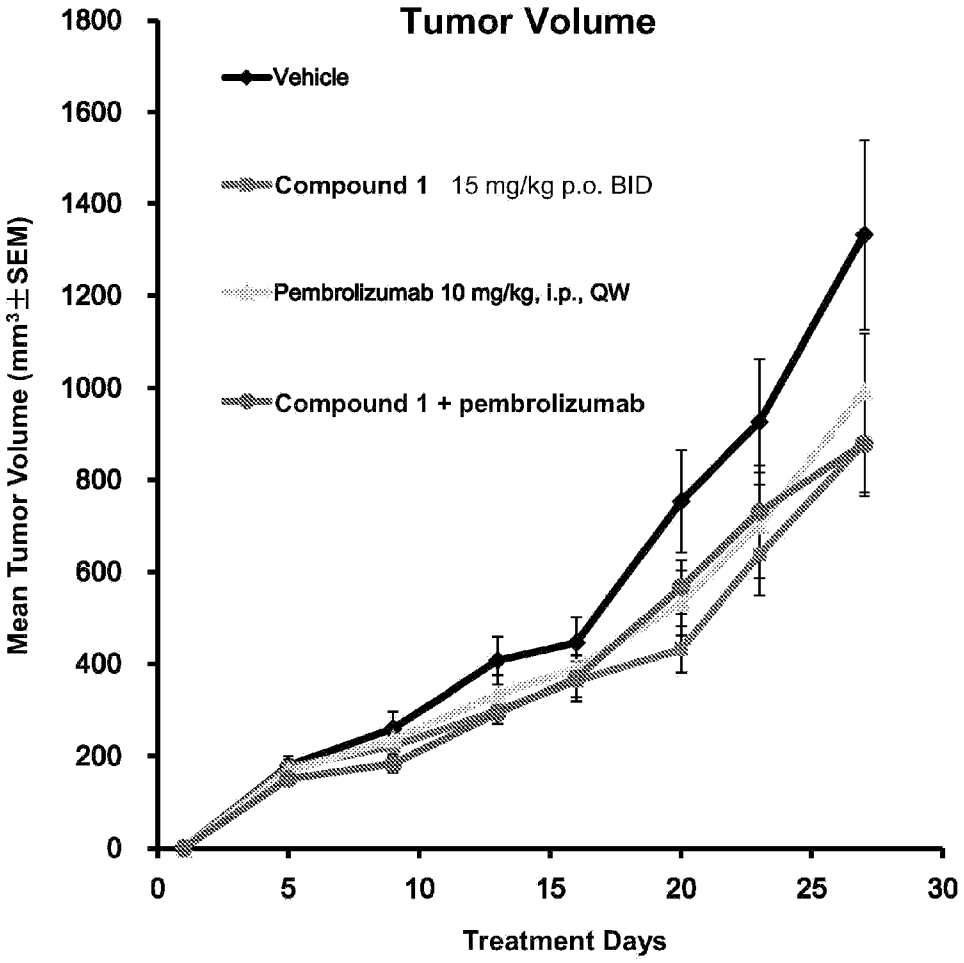
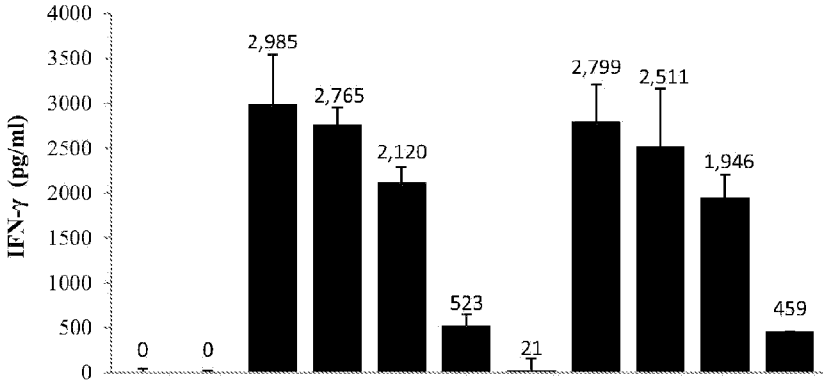

Tumor Volume
Vehicle
Compound 1 15 mg/kg p.o. BID
Pembrolizumab 10 mg/kg, i.p., QW
Compound 1 + pembrolizumab
Mean Tumor Volume (mm3 ± SEM)
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
Treatment Days

FIG. 6



NK92	+	+	+	+	+	+	+	+	+	+	+
Mino	-	+	+	+	+	+	+	+	+	+	+
Obinutuzumab	-	-	+	+	+	+	+	+	+	+	+
Ibrutinib	-	-	-	**0.01**	**0.1**	**1**	**10**	-	-	-	-
Compound 1	-	-	-	-	-	-	-	**0.01**	**0.1**	**1**	**10**

FIG. 7

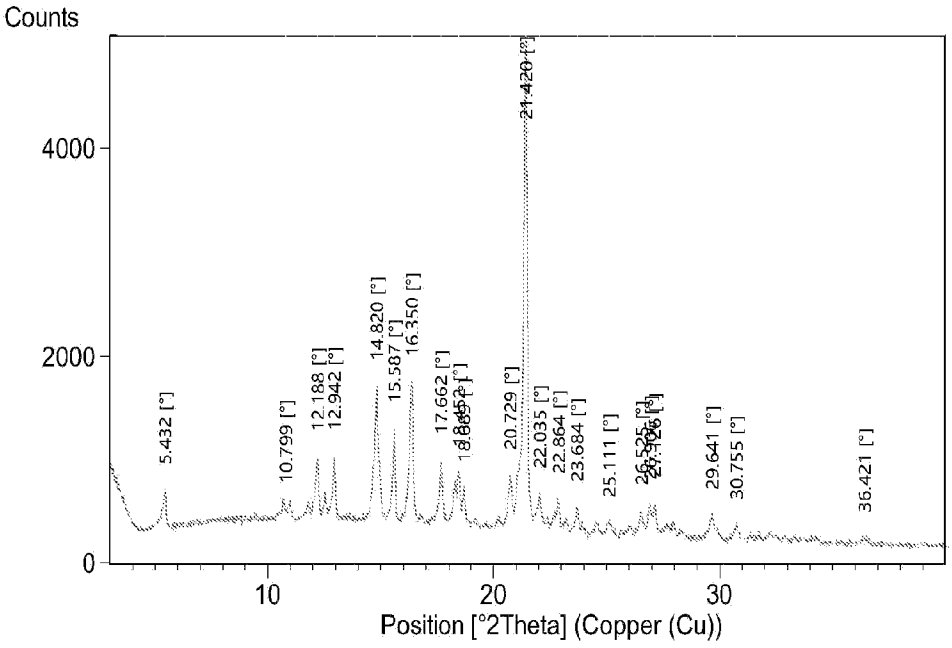


FIG. 8

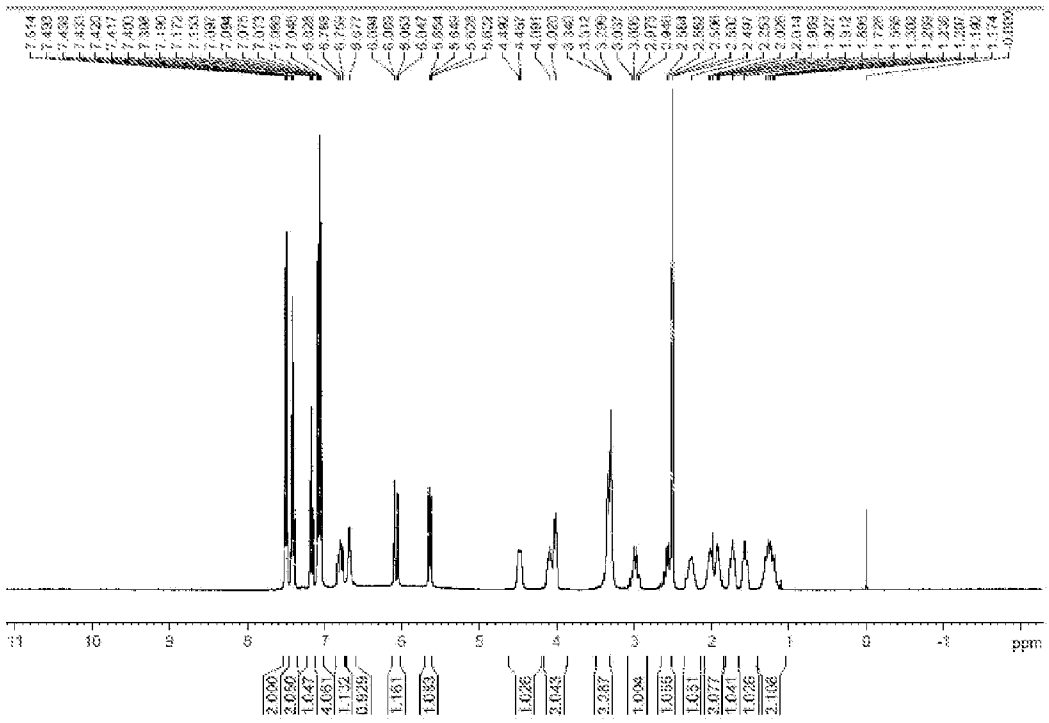


FIG. 9

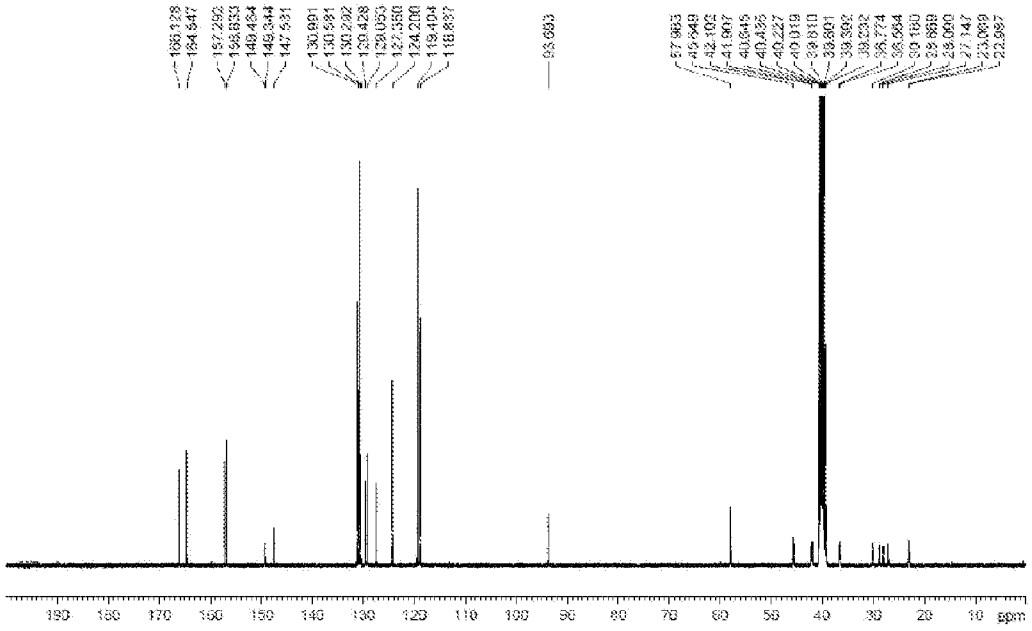


FIG. 10