Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2925698 C 2021/11/09

(11)(21) 2 925 698

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2014/11/27
(87) Date publication PCT/PCT Publication Date: 2015/06/04
(45) Date de délivrance/Issue Date: 2021/11/09
(85) Entrée phase nationale/National Entry: 2016/03/29
(86) N° demande PCT/PCT Application No.: IB 2014/066402
(87) N° publication PCT/PCT Publication No.: 2015/079411
(30) Priorité/Priority: 2013/11/28 (US61/910,060)

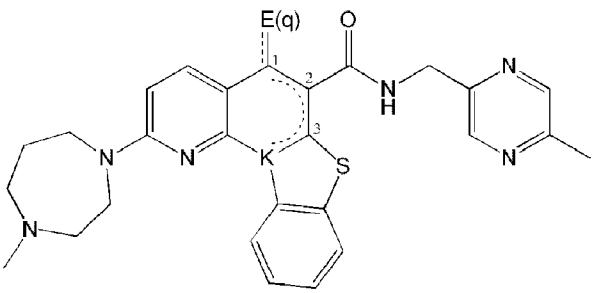
(51) Cl.Int./Int.Cl. *C07D 413/14* (2006.01), *A61K 31/425* (2006.01), *A61K 31/43* (2006.01), *A61K 31/47* (2006.01)

(72) Inventeurs/Inventors:
ACHIRON, ANAT, IL;
MASHIACH, ROI, IL

(73) Propriétaire/Owner:
TEL HASHOMER MEDICAL RESEARCH INFRASTRUCTURE AND SERVICES LTD., IL

(74) Agent: INTEGRAL IP

(54) Titre : INHIBITEURS DE L'ARN POLYMERASE I ET LEURS UTILISATIONS
(54) Title: RNA POLYMERASE I INHIBITORS AND USES THEREOF



Formula I

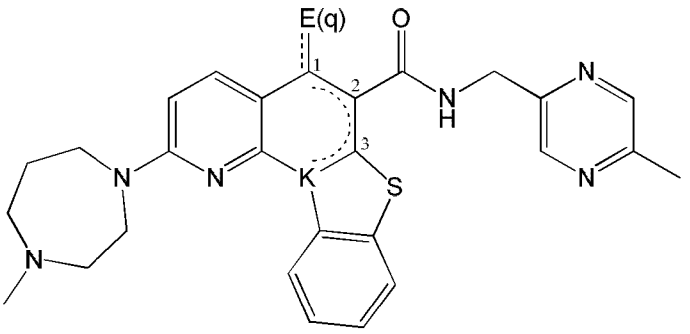
(57) Abrégé/Abstract:
Provided are novel compounds collectively represented by Fommla I:

(see formula I)

wherein ------ or the dashed line each independently indicates an optionally unsaturated bond, depending on the nature and valency of E; E forms a chemical moiety other then carbonyl, capable of interfering with a hydrogen binding capacity of the compound; q equals 1 or 2; and K is N or depending on the nature and valency of E. Further provided are uses of these compounds in inhibiting an activity of RNA polymerase I, and in treating diseases or disorders modulated by RNA polymerase I, preferably autoimmune diseases such as multiple sclerosis and proliferative diseases or disorders.

Abstract

Provided are novel compounds collectively represented by Formula I:



Formula I

wherein ------ or the dashed line each independently indicates an optionally unsaturated bond, depending on the nature and valency of E; E forms a chemical moiety other then carbonyl, capable of interfering with a hydrogen binding capacity of the compound; q equals 1 or 2; and K is N or $N^{(+)}$, depending on the nature and valency of E. Further provided are uses of these compounds in inhibiting an activity of RNA polymerase I, and in treating diseases or disorders modulated by RNA polymerase I, preferably autoimmune diseases such as multiple sclerosis and proliferative diseases or disorders.

Date Reçue/Date Received 2021-04-30

RNA POLYMERASE I INHIBITORS AND USES THEREOF

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to therapy and, more particularly, but not exclusively, to novel RNA Polymerase I inhibitors and to uses thereof in methods of treating medical conditions including, for example, autoimmune diseases multiple sclerosis and proliferative diseases such as cancer.

Autoimmune diseases are caused by an autoimmune response, *i.e.,* an immune response directed to a substance in the body of the subject. The characteristics of the autoimmune diseases vary and depend on the site affected by the autoimmune response.

Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS) affecting young adults (disease onset between 20 to 40 years of age) and is the third leading cause for disability after trauma and rheumatic diseases, with an estimated annual cost 34,000 USD per patient (total life time cost of 2.2 million USD per patient).

The disease is characterized by destruction of myelin, associated with death of oligodendrocytes and axonal loss. The main pathologic finding in MS is the presence of infiltrating mononuclear cells, predominantly T lymphocytes and macrophages, which surpass the blood brain barrier and induce an active inflammation within the brain and spinal cord. The neurological symptoms that characterize MS include complete or partial vision loss, diplopia, sensory symptoms, motor weakness that can worsen to complete paralysis, bladder dysfunction and cognitive deficits, which eventually may lead to a significant disability. The associated multiple inflammatory foci lead to myelin destruction, plaques of demyelination, gliosis and axonal loss within the brain and spinal cord and are the reasons which contribute to the clinical manifestations of neurological disability.

The etiology of MS is not fully understood. The disease develops in genetically predisposed subjects exposed to yet undefined environmental factors, and the pathogenesis involves autoimmune mechanisms associated with autoreactive T cells against myelin antigens. It is well established that not one dominant gene determines genetic susceptibility to develop MS, but rather many genes, each with different influence, are involved.

Clinically, in 85 % of MS patients the illness is initiated with a relapsing-remitting course (RRMS), and in about 10-15 % of MS patients have an a-priori primary progressive course (PPMS) without relapses. RRMS is characterized by inflammatory attacks associated with neurological deficits with periods of remissions between the relapses that vary in time. After a period of 10 years, about 50 % of RRMS patients will progress to a secondary progressive MS (SPMS) course, characterized by permanent neurological dysfunction, with or without relapses and progressive disability.

Benign MS (BMS) is a clinical variant of RRMS in which the patients develop low neurological disability if at all after a disease duration of at least 10 years. Accordingly, this group of MS patients do not experience devastating accumulating disability over-time and when these patients are examined neurologically and scored by the Expanded Disability Status Scale (EDSS) they receive a score that is equal to or lower than 3.0. This low EDSS score signifies mild disability and when this low disability occurs more than 10 years after disease onset, the course of MS is defined as benign. Prediction of patients that will have BMS is currently impossible and the definition of these patients is retrospective. The molecular events accountable for the BMS variant of disease are not understood.

WO 2008/081435 discloses methods and kits for predicting the prognosis of a subject diagnosed with multiple sclerosis and methods of selecting a treatment regimen of a subject diagnosed with multiple sclerosis.

Achiron A, et al., 2007 [Clinical and Experimental Immunology, 149: 235-242] describe genes of the zinc-ion binding and cytokine activity regulation pathways which predict outcome in relapsing–remitting multiple sclerosis.

WO 2010/113096 discloses methods of predicting clinical course and treating multiple sclerosis.

Current approved drugs for the treatment of MS are either general anti-inflammatory agents or immunomodulators and consequently result only in moderate beneficial effects suppressing disease activity.

CX-5461 (see, Table 1 hereinunder) is a small molecule that was designed to selectively inhibit rRNA synthesis by inhibiting RNA Polymerase I (POL I or POL1), without affecting mRNA synthesis by RNA Polymerase II (POL II), and without inhibiting DNA replication or protein synthesis (Russell J, Zomerdijk JC. Trends

Biochem Sci 30:87-96, 2005; Drygin D, et al. Annu Rev Pharmacol Toxicol 50:131-156, 2010).

The inhibition of POL1 results in nucleolar stress which causes the release of ribosomal proteins (RP) from the nucleolus and subsequent activation of p53, resulting in cell apoptosis [Kalita K, et al. J Neurochem 105:2286-2299, 2008]. In a previous study [Drygin D, et al. Cancer Res 71:1418-1430, 2011], the antiproliferative activity of CX-5461 was studied in cell lines and it was shown that CX-5461 inhibited POL-I activity in human cancer cell lines.

Recent studies indicate that disruption of the SL1/rDNA complex by CX-5461 results from the interference between SL1 and rDNA. SL1, a protein complex containing TATA binding protein-associated factors, is responsible for POLI1 promoter specificity. SL1 performs important tasks in the transcription complex assembly, mediating specific interactions between the rDNA promoter region and the POL1 enzyme complex, thereby recruiting POL1, together with a collection of POL1-associated factors like RRN3 to rDNA (Cavanaugh A, et al. *Gene Expr* 14:131-147, 2008).

U.S. Patent Application Publication No. 2009/0093465 discloses a family of compounds, including CX-5461, as kinase modulators useful in the treatment of proliferative diseases such as cancer.

Recently, a role for inhibition of RNA polymerase I (POL1) pathway in the regulation of MS disease activity by suppression of inflammation and enhancement of apoptosis of autoreactive lymphocytes has been uncovered. The suggested mechanism by which POL1 pathway inhibition affects the disease process is demonstrated in Background Art FIGs. 1 and 2A-B.

The above findings have supported a basis for direct targeting of RNA Polymerase-I transcription pathway as a strategy for selective induction of apoptosis in MS in order to transform the active disease of RRMS to the preferable BMS subtype. Administration of a specific POL1 inhibitor (POL1-I) was demonstrated to prevent animal Experimental Autoimmune Encephalomyelitis (EAE) when administered at disease induction and to reduce the disease severity when administered at clinical disease onset [Achiron et al. 2013, J Neuroimmunol 263:91-97], thus confirming that a POL1 inhibitor acts specifically by inhibiting the polymerase I associated molecules.

WO 2012/123938 discloses uses of family of compounds, including CX-5461 and derivatives thereof, in the treatment of autoimmune diseases such as MS.

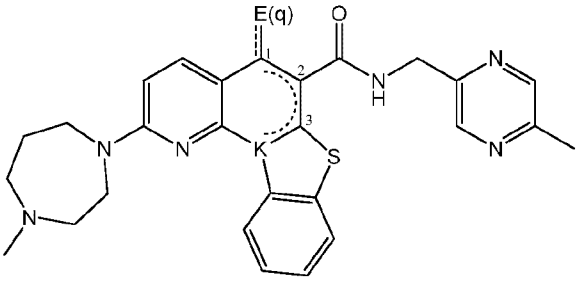
Additional background art includes Leuenroth SJ and Crews CM (Triptolide-induced transcriptional arrest is associated with changes in nuclear substructure. Cancer Res. 2008; 68:5257-5266); Liu Y, et al. (Triptolide, a component of Chinese herbal medicine, modulates the functional phenotype of dendritic cells. Transplantation. 2007; 84:1517-1526); Wang Y, et al. (Triptolide modulates T-cell inflammatory responses and ameliorates experimental autoimmune encephalomyelitis. J Neurosci Res. 2008; 86:2441-2449; EP 0983256; PCT/US1998/008562; WO9852933A1; Alice H. Cavanaugh, et al., 2002 (Rrn3 Phosphorylation is a regulatory checkpoint for ribosome biogenesis J. Biol. Chem., 2002; 277: 27423 – 27432); PCT Pub. No. WO 03/081201.

SUMMARY OF THE INVENTION

Based on the findings that inhibition of RNA Polymerase-I plays a role in regulation of MS and other autoimmune diseases, as well as cell proliferation, the present inventors have searched for POL-1 inhibitors that would exhibit an improved effect as compared to the presently known POL1 inhibitors (e.g., POL1-I and structural analogs thereof).

The present inventors have uncovered that by modifying a structural feature of CX-5461 (POL1-I) or analogs thereof, so as to reduce or even reverse its capability of participating in hydrogen bond formation, inhibitors which exhibit improved performance are obtained.

According to an aspect of some embodiments of the present invention there is provided a compound represented by general Formula I:



Formula I

wherein ------ or the dashed line each independently indicates an optionally unsaturated bond, depending on the nature and valency of E;

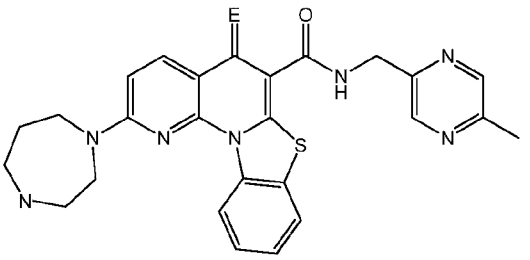
E forms a chemical moiety other then carbonyl, capable of interfering with a hydrogen binding capacity of the compound;

Q equals 1 or 2; and

K is N or $N^{(+)}$, depending on the nature and valency of E.

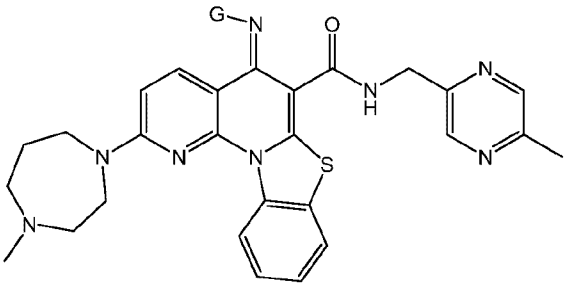
According to some embodiments of the present invention, E forms a chemical moiety selected from the group consisting of thiocarbonyl and a substituted or unsubstituted imine.

According to some embodiments of the present invention, q is 1, K is N, E is linked to carbon 1 of the ring via an unsaturated double bond, and another unsaturated double bond is present between carbons 2 and 3 of the ring, the compound being represented by Formula Ia:



Formula Ia.

According to some embodiments of the present invention, E forms a substituted or unsubstituted imine, the compound being represented by Formula Ib:



Formula Ib,

wherein G is selected from the group consisting of hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, alkoxy, thioalkoxy, thiol, hydroxyl, aryloxy, and thioaryloxy.

According to some embodiments of the present invention, G is aryl.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein, for use in the treatment of an autoimmune disease.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein, for use in the manufacture of a medicament for treating an autoimmune disease.

According to an aspect of some embodiments of the present invention there is provided a method of treating an autoimmune disease in a subject, the method comprising administering to the subject a therapeutically effective amount of the compound of Formula I as described in any one of the respective embodiments herein.

According to some embodiments of the present invention, the autoimmune disease is multiple sclerosis.

According to some embodiments of the present invention, the multiple sclerosis is a relapsing-remitting multiple sclerosis (RRMS) or benign multiple sclerosis (BMS).

According to some embodiments of the present invention, treating the multiple sclerosis comprises changing the course of the disease from the RRMS to BMS.

According to some embodiments of the present invention, the autoimmune disease is treatable by inhibiting an activity of RNA Polymerase I.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein, for use in the treatment of a proliferative disease or disorder.

According to an aspect of some embodiments of the present invention there is provided a use of the compound as described herein, in the manufacture of a medicament for the treatment of a proliferative disease or disorder.

According to an aspect of some embodiments of the present invention there is provided a method of treating a proliferative disease or disorder, the method comprising administering to a subject in need thereof a therapeutically effective amount of the compound as described herein.

According to some embodiments of the present invention, the proliferative disease or disorder is treatable by inhibiting an activity of a protein kinase.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein, for use in inhibiting an activity of RNA Polymerase I and/or for treating a disease or disorder treatable by inhibiting an activity of RNA Polymerase I.

According to an aspect of some embodiments of the present invention there is provided a use of as compound as described herein, in the manufacture of a medicament for inhibiting an activity of RNA Polymerase I and/or for treating a disease or disorder treatable by inhibiting an activity of RNA Polymerase I.

According to an aspect of some embodiments of the present invention there is provided a method of inhibiting an activity of RNA Polymerase I, the method comprising contacting the RNA Polymerase I with an effective amount of a compound as described herein.

According to some embodiments of the present invention, contacting is effected *in vitro*.

According to some embodiments of the present invention, contacting is effected *in vivo*.

According to some embodiments of the present invention, the method is being for treating a disease treatable by inhibiting an activity of RNA Polymerase I.

According to an aspect of some embodiments of the present invention there is provided a compound as described herein, for use in inhibiting an activity of a protein kinase and/or for treating a disease or disorder treatable by inhibiting an activity of a protein kinase.

According to an aspect of some embodiments of the present invention there is provided a use of a compound as described herein, in the manufacture of a medicament for inhibiting an activity of a kinase and/or for treating a disease or disorder treatable by inhibiting an activity of a protein kinase.

According to an aspect of some embodiments of the present invention there is provided a method of inhibiting an activity of a protein kinase, the method comprising contacting the protein kinase with an effective amount of a compound as described herein.

According to some embodiments of the present invention, contacting is effected *in vitro*.

According to some embodiments of the present invention, contacting is effected *in vivo*.

According to some embodiments of the present invention, the method is being for treating a disease treatable by inhibiting an activity of a protein kinase.

A "compound as described herein" refers to a compound having Formula I as described in any one of its respective embodiments, and further to any other compound described in the following description as being contemplated by embodiments of the present invention.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings and images. With specific reference now to the drawings and images in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings and images makes apparent to those skilled in the art how embodiments of the invention may be practiced. In the drawings:

FIG. 1 (Background Art) presents a schematic illustration of POL1 molecular mechanism, showing the effect of POL1 on apoptosis and proliferation.

FIGs. 2A-2B (Background Art) present schematic illustrations of the effect of POL1 inhibition on multiple sclerosis.

FIGs. 3A-B present chemical structures and synthetic pathways of exemplary compounds according to some embodiments of the present invention.

FIGs. 4A-C present bar graphs showing the effect of exemplary compounds according to some embodiments of the present invention in suppressing proliferation of mouse splenocytes, as determined in an XTT assay.

FIG. 5 presents plots showing the effect of various concentration of Compound **10** (RAM-An) on the EAE clinical score, as observed in an EAE prevention mice model.

FIGs. 6A-B present graphs showing the time-dependent profile of Compound **10** (FIG. 6A) and Compound **1** (FIG. 6B) in mice serum following administration by oral gavage.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to therapy and, more particularly, but not exclusively, to novel RNA Polymerase I inhibitors and to uses thereof in methods of treating medical conditions including, for example, autoimmune diseases multiple sclerosis and proliferative diseases such as cancer.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Embodiments of the present invention stem, at least on part, from previous findings that demonstrated a characterizing gene expression signature in blood sample of RRMS and BMS subjects, whereby the major operating pathway was RNA Polymerase I (POL1). These findings have previously led the present inventors to explore a role for POL1 inhibitors in the treatment, and optionally personalized treatment, of MS.

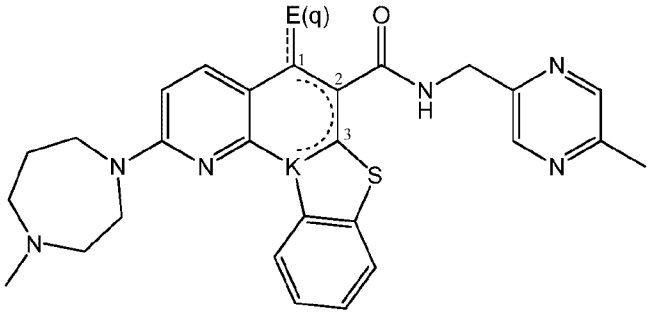
Led by the fact that the current commercial products for the treatment of autoimmune diseases, and particularly MS, are used intramuscularly, intradermaly or as intravenous injections for drug delivery, and lead to uncontrolled plasma peaks, undesired side effects such as flu like reactions and painful local reactions, and thus are accompanied by a high rate of non-compliance to these treatments, the present inventors have explored utilizing inhibitors of POL1-I, which are characterized by oral bioavailability, and improve patients' compliance and benefit patients in the aspect of side effects and pain relief.

As described hereinabove, a POL1 inhibitor, termed CX-5461, and structural analogs thereof, and their use in inhibiting a protein kinase activity and an abbrant cell proliferation, has been previously disclosed. See, for example, U.S. Patent Application Publication No. 2009/0093465.

A family of such POL1 inhibitors, including CX-5461, for use in the treatment of autoimmune diseases has been disclosed in WO 2012/123938.

In a search for POL1 inhibitors that exhibit an improved therapeutic effect, such as, for example, an improved (wider) therapeutic window, the present inventors have devised and successfully prepared and practiced a novel family of POL1 inhibitors, which can be used to treat autoimmune diseases such as multiple sclerosis, proliferative diseases such as cancer, and other medical conditions which are associated with inhibition of POL1 and/or a protein kinase.

According to an aspect of some embodiments of the present invention there are provided compounds which can be collectively represented by Formula I:



Formula I

wherein ------ or the dashed line each independently indicates an optionally unsaturated bond, depending on the nature and valency of E;

E forms a chemical moiety other then carbonyl, capable of interfering with a hydrogen binding capacity of the compound;

Q equals 1 or 2; and

K is N or $N^{(+)}$, depending on the nature and valency of E.

Compounds represented by Formula I feature structural similarity of CX5461 (POL1-I, RAM-0, Compound **1**; See, for example, FIG. 3A and Table 1 hereinbelow),

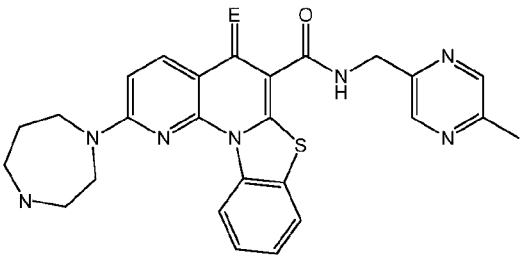
yet the structure of CX5461 is modified so as to longer include a carbonyl (oxo substituent) at a position equivalent to variable E in Formula I.

The variable E therefore represents a chemical group that, when attached to the carbon marked as carbon "1" in Formula I of the quinazoline ring, forms a chemical moiety other than carbonyl (C=O). E is therefore a chemical group other than oxo (=O).

The chemical group of variable E in Formula I herein can be attached to carbon "1" via a double (unsaturated bond), in which case, q is 1. In such cases, the valency of E is such that is suitable to be attached via an unsaturated bond to carbon "1" (as in the case of, for example, an oxo group =O that forms a carbonyl C=O group.

In such cases, the electronic structure of the quinazoline ring of CX-5461 is maintained, such that an unsaturated (double) bond also exists between carbons "2" and "3" of the ring, and K is nitrogen in a neutral form (N).

Compound exhibiting such structures are represented by Formula Ia:

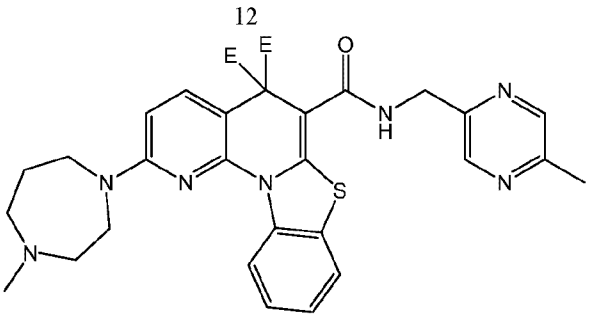


Formula Ia.

Exemplary chemical groups formed by "E" in such cases include, but are not limited to, thiocarbonyl (C=S), formed of thioxo (=S) group; and imine (e.g., C=N-G, with being as defined hereinafter), formed of e.g., a corresponding =N-G group.

Alternatively, the group represented by variable E is attached to carbon "1" via a single bond, and q is 2. Thus each E group is attached to the ring via a single bond (saturated bond). In such cases, the electronic structure of the quinazoline ring is maintained, such that an unsaturated (double) bond also exists between carbons "2" and "3" of the ring, and K is nitrogen in a neutral form (N).

Compound exhibiting such structures are represented by Formula Ic:

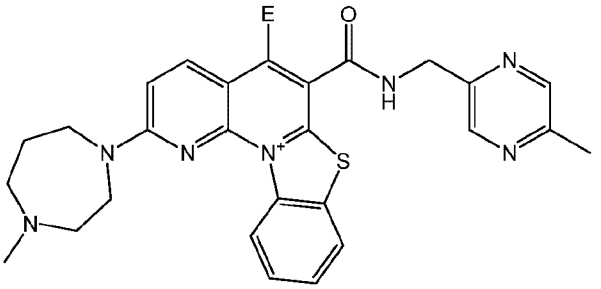


Formula Ic.

Exemplary chemical groups formed by "E" in such cases include, for example, two halides, preferably two fluorides, as explained hereinafter.

Further alternatively, the group represented by variable E is attached to carbon "1" via a single (saturated) bond, and q is 1. In such cases, the electronic structure of the quinazoline ring undergoes a rearrangement (a tautomerization rearrangement), such that an unsaturated bond exists between carbons "1" and "2" of the ring, and between carbon "3" and K, and K is a positively charged nitrogen N^+.

Compound exhibiting such structures are represented by Formula Id:



Formula Id.

Exemplary chemical groups formed by "E" in such cases include, for example, halides, preferably a chloride.

While the above formulae provide an exemplary illustration for some preferred embodiments of the invention, generally, the chemical moiety formed by variable E is selected so as to modulate the hydrogen bonding capacity of the compound.

As used herein and known in the art, a "hydrogen bond" is a relatively weak bond that forms a type of dipole-dipole attraction which occurs when a hydrogen atom

bonded to a strongly electronegative atom exists in the vicinity of another electronegative atom with a lone pair of electrons.

The hydrogen atom in a hydrogen bond is partly shared between two relatively electronegative atoms.

Hydrogen bonds typically have energies of 1–3 kcal mol^{-1} (4–13 kJ mol^{-1}), and their bond distances (measured from the hydrogen atom) typically range from 1.5 to 2.6 Å.

A hydrogen-bond donor is the group that includes both the atom to which the hydrogen is more tightly linked and the hydrogen atom itself, whereas a hydrogen-bond acceptor is the atom less tightly linked to the hydrogen atom. The relatively electronegative atom to which the hydrogen atom is covalently bonded pulls electron density away from the hydrogen atom so that it develops a partial positive charge (δ^+). Thus, it can interact with an atom having a partial negative charge (δ^-) through an electrostatic interaction.

Atoms that typically participate in hydrogen bond interactions, both as donors and acceptors, include oxygen, nitrogen and fluorine. These atoms typically form a part of chemical group or moiety such as, for example, carbonyl, carboxylate, amide, hydroxyl, amine, imine, alkylfluoride, F_2, and more. However, other electronegative atoms and chemical groups or moieties containing same may participate in hydrogen bonding.

By "modulating the hydrogen bonding capacity" it is meant altering the number and/or strength of hydrogen bonds that the compound may form intramolecularly or intermolecularly, as compared to a carbonyl moiety at the same position.

For example, the group formed by variable E can be, for example, a stronger donor for a hydrogen bond compared to carbonyl, a weaker donor for a hydrogen bond, compared to carbonyl, or be a stronger or a weaker acceptor of a hydrogen bond, compared to carbonyl.

Without being bound by any particular theory, it is assumed that hydrogen bonds may form upon a keto-enol-type tautomerization of the amide group attached to carbon "2" in Formula I, which results in a hydroxyl group (-OH), the latter participates in hydrogen bonding.

The hydroxyl group thus formed is a strong donor of a hydrogen bond and may form a hydrogen bond intermolecularly, with, for example, a hydrogen bond acceptor group of a targeted molecule (e.g., a targeted enzyme such as POL1).

The hydroxyl group may also form hydrogen bond with a carbonyl, when it is the substituent of carbon "1", so as to form a six-membered ring structure, by intramolecular hydrogen bonding.

Alternatively, both a carbonyl at carbon "1" and the hydroxyl group may participate in hydrogen bonds with compatible groups of a targeted biomolecule (e.g., a targeted enzyme).

The modification of substituent E so as to no longer include a carbonyl group may therefore alter the compound's hydrogen bonding capacity by, for example, reducing or increasing the probability of hydrogen bond formation intramolecularly, reducing or increasing the probability of hydrogen bond formation intermolecularly, and/or reducing the strength of an intermolecular or intramolecular hydrogen bond.

In some embodiments, group E is selected such that the chemical moiety formed therewith increases the probability of forming a hydrogen bond intermolecularly and reduces the probability of forming a hydrogen bond intramolecularly (e.g., due to the formation of a group that forms a less stable hydrogen bond with the hydroxyl).

In some embodiments, E is such that the energy of a hydrogen bond formed between a highly electronegative atom therein and hydrogen of the neighboring hydroxyl is lower than the energy of a hydrogen bond formed with the same hydroxyl by carbonyl's oxygen.

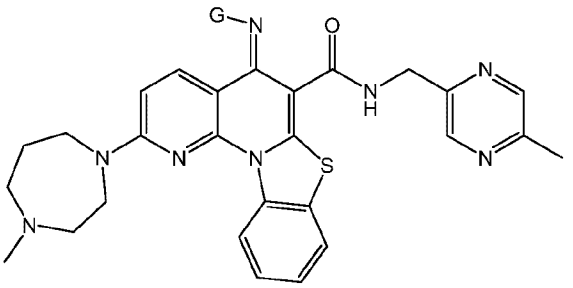
In some embodiments, the energy is lower by at least 0.1 kcal/mol, and can be lower by, for example, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, 1, 1.5, or 2kcal/mol, including any subranges and intermediates between these values. A person skilled in the art would recognize which groups are encompassed by this definition based on art-recognized tables that define the energies of hydrogen bonds formed with a hydroxyl group.

In some embodiments, the electron density on such an electronegative atom is lower than an electron density of carbonyl's oxygen, that is, the atom is less electronegative than the oxygen in carbonyl.

Without being bound by any particular theory, it is assumed that by interfering with the hydrogen bond capacity of the compound, by e.g., reducing the number (e.g.,

from 1 to 0) and/or strength of intramolecular bonds, and at the same time increasing the number and/or strength of intermolecular bonds, the compound may better interact with the targeted biomolecule (e.g., POL1), even more electively, and may further exhibit improved water solubility, which facilitates its administration.

In some embodiments, E is an imine group, which can be substituted or non-substituted, as depicted for compounds represented by Formula Ib:



Formula Ib,

wherein G can be, for example, hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, alkoxy, thioalkoxy, thiol, hydroxyl, aryloxy, or thioaryloxy.

Exemplary such compounds are presented in Table 1 hereinafter as Compounds **3**, **4**, **5**, **6**, **7**, **8** and **10**.

In some embodiments, G is an electron withdrawing group.

Without being bound by any particular theory, it is assumed that electron withdrawing groups reduce the electronegativity of the imine's nitrogen and hence result in a weaker hydrogen bond intramolecular interaction with the presumably formed neighboring hydroxyl described hereinabove, and increase the hydrogen bond intermolecular interactions of the hydroxyl group.

In some embodiments, G is a bulky group as defined herein.

As used herein, the phrase "bulky" describes a group that occupies a large volume. A bulkiness of a group is determined by the number and size of the atoms composing the group, by their arrangement, and by the interactions between the atoms (e.g., bond lengths, repulsive interactions). Typically, lower, linear alkyls are less bulky than branched alkyls; cyclic moieties are more bulky than liner moieties; bicyclic molecules are more bulky than cycloalkyls, etc.

Exemplary suitable electron-withdrawing substituents of an imine include, but are not limited to, substituted or unsubstituted aryls, which, when substituted, preferably are substituted by chemical moieties and at position which strengthen the electron-withdrawing nature of the aryl; heteroaryls in which the heteroatom is positioned such that it exhibits electron-withdrawal with respect to the imine nitrogen; and bulky cycloalkyls substituted by one or more electron withdrawing substituents.

The phrases "electron-withdrawing substituent" or "electron-withdrawing group" are well known to those of skill in the art and are used herein interchangeably as their standard meaning which is a functional group that draws electrons to itself more than a hydrogen atom would if it occupied the same position in the molecule, as described in J. March, Advanced Organic Chemistry, third edition, Pub: John Wiley & Sons, Inc. (1985).

Exemplary electron-withdrawing substituents include, but are not limited to, halogen, pseudohalogen, haloalkyl, haloalicyclic, haloaryl, haloheteroaryl, carbonyl, ester, -C(=O)H and any combination thereof.

In some embodiments, G is aryl and the compound is Compound **10** (see, Table 1 and FIG. 3B).

It is to be noted that an inclusion of moieties that enhance the hydrophobicity of the compound, such as, for example, aryl, are assumed, without being by bond by any particular theory, to enhance the bioavailability of the compound, compared to compounds featuring a carbonyl moiety at the same position.

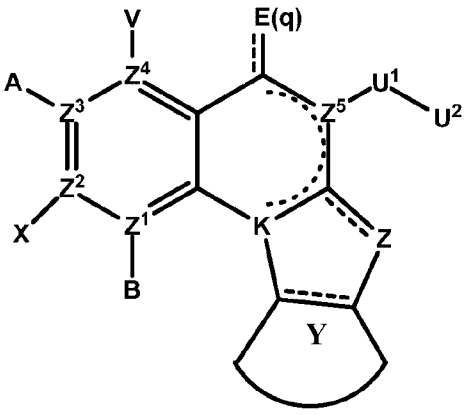
Thus, in some embodiments, there are provided compounds having Formula I as described herein, or Formula Ia or Ib, as described herein, which are characterized by higher hydrophobicity compared to corresponding compound in which E is oxo.

The term "hydrophobic" thus often translates into values such as Log P, which describes the partition coefficient of a substance between an aqueous phase (water) and an oily phase (1-octanol).

In some of these embodiments, the group denoted as E in these formulae increases the LogP of the compound, compared to CX-5461, by at least 0.5, or by at least 0.6, 0.7, 0.8, 0.9, 1, 1.2., 1.5, 2, 3, or 4 and any intermediate value therebetween.

According to some embodiments of the present invention, additional compounds, featuring or encompassing the main structural features described herein for compounds represented by Formula I are encompassed by the present embodiments.

According to some of these embodiments, there are provided compounds represented by Formula II:



Formula II

wherein E, q and K are as defined for any one of the embodiments of Formula I hereinabove;

------ indicates an optionally unsaturated bond;

each of B, X, A or V is absent if Z^1, Z^2, Z^3 and Z^4, respectively, is N; and

each of B, X, A and V is independently H, halo, azido, —CN, —CF_3, —$CONR^1R^2$, —NR^1R^2, -SR^2, -OR^2, -R^3, -W, -L-W, -W^0, -L-N(R) -W^0, A^2 or A^3, when each of Z^1, Z^2, Z^3 and Z^4, respectively, is C;

Z is O, S, CR^4_2, NR^4CR^4, CR^4NR^4, CR^4, NR^4 or N;

each of Z^1, Z^2, Z^3 and Z^4 is independently C or N, provided any three N are non-adjacent;

Z^5 is C; or Z^5 may be N when Z is N;

Y is an optionally substituted 5-6 membered carbocyclic or heterocyclic ring;

U^1 is -C(=T)N(R)-, -C(=T)N(R)O-, -C(=T)-, -SO_2N(R)-, -SO_2N(R)N(R^0)-, -SO_2-, or -SO_3-, where T is O, S, or NH; or U^1 may be a bond when Z^5 is N or U^2 is H;

U^2 is H, or C3-C7 cycloalkyl, C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl or C2-C10 heteroalkenyl group, each of which may be optionally substituted with one or more

halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring; or U^2 is -W, -L-W, -L-N(R)-W^0, A^2 or A^3;

in each -NR^1R^2, R^1 and R^2 together with N may form an optionally substituted azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

R^1 is H or C1-C6 alkyl, optionally substituted with one or more halogens, or =O;

R^2 is H, or C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring;

R^3 is an optionally substituted C1-C10 alkyl, C2-C10 alkenyl, C5-C10 aryl, or C6-C12 arylalkyl, or a heteroform of one of these, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-6 membered carbocyclic or heterocyclic ring;

each R^4 is independently H, or C1-C6 alkyl; or R^4 may be -W, -L-W or -L-N(R)-W^0;

each R and R^0 is independently H or C1-C6 alkyl;

L is a C1-C10 alkylene, C1-C10 heteroalkylene, C2-C10 alkenylene or C2-C10 heteroalkenylene linker, each of which may be optionally substituted with one or more substituents selected from the group consisting of halogen, oxo (=O), or C1-C6 alkyl;

W is an optionally substituted 4-7 membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

W^0 is an optionally substituted 3-4 membered carbocyclic ring, or a C1-C6 alkyl group substituted with from 1 to 4 fluorine atoms;

provided one of U^2, V, A, X and B is a secondary amine A^2 or a tertiary amine A^3, wherein

the secondary amine A^2 is -NH-W^0, and

the tertiary amine A^3 is a fully saturated and optionally substituted six-membered or seven-membered azacyclic ring optionally containing an additional heteroatom selected from N, O or S as a ring member, or the tertiary amine A^3 is a partially unsaturated or aromatic optionally substituted five-membered azacyclic ring, optionally containing an additional heteroatom selected from N, O or S as a ring member.

According to some embodiments of the invention, Z^1 is N, and each of Z^2, Z^3 and Z^4 is C.

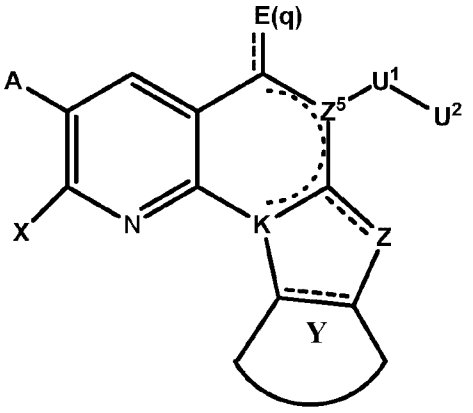
According to some embodiments of the invention, U is —W or -L-W, where W is an optionally substituted 5-6 membered unsaturated or aromatic azacyclic ring, optionally containing an additional heteroatom selected from N, O and S; or W is an optionally substituted 5-7 membered saturated azacyclic ring containing an additional heteroatom selected from N and S.

According to some embodiments of the invention, U^2 is -L-N(R)—W^0.

According to some embodiments of the invention, Y is an optionally substituted phenyl ring.

According to some embodiments of the invention, the compound with the proviso that when Z^1 is N, Z^2 and Z^4 are C, Z^5 is C, U^1 is -C(O)NH-, U^2 is -L-W, and L is an ethylene linker, one of V, A, and X is independently an optionally substituted aryl, heteroaryl, or 7-membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member, if W is pyrrolidin-1-yl, N-methyl-pyrrolidin-2-yl, piperidin-1-yl or morpholin-1-yl.

According to any one of the embodiments of the invention, compounds having a general structure represented by Formula III, are also contemplated:



Formula III

wherein E, q and K are as defined for any one of the embodiments of Formula I hereinabove;

and wherein:

---- indicates an optionally unsaturated bond;

each of A and X is independently H, halo, azido, -CN, -CF_3, -$CONR^1R^2$, -NR^1R^2, -SR^2, -OR^2, -R^3, -W, -L-W, -W^0, -L-N(R)-W^0, A^2 or A^3;

Z is O,S, CR^4_2, NR^4CR^4, CR^4NR^4 or NR^4;

Y is an optionally substituted 5-6 membered carbocyclic or heterocyclic ring;

U^1 is -C(=T)N(R)-, -C(=T)N(R)O-, -C(=T)-, -SO_2N(R)-, -SO_2N(R)N(R^0)-, -SO_2-, or -SO_3-, where T is O, S, or NH; or U^1 may be a bond when U2 is H;

U^2 is H, or C3-C7 cycloalkyl, C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl or C2-C10 heteroalkenyl group, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring; or U^2 is -W, -L-W, -L-N(R)-W^0, A^2 or A^3;

in each -NR^1R^2, R^1 and R^2 together with N may form an optionally substituted azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

R^1 is H or C1-C6 alkyl, optionally substituted with one or more halogens, or =O;

R2 is H, or C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring;

R^3 is an optionally substituted C1-C10 alkyl, C2-C10 alkenyl, C5-C10 aryl, or C6-C12 arylalkyl, or a heteroform of one of these, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-6 membered carbocyclic or heterocyclic ring;

each R^4 is independently H, or C1-C6 alkyl; or R^4 may be -W, -L-W or -L-N(R)-W0;

each R and R^0 is independently H or C1-C6 alkyl;

L is a C1-C10 alkylene, C1-C10 heteroalkylene, C2-C10 alkenylene or C2-C10 heteroalkenylene linker, each of which may be optionally substituted with one or more substituents selected from the group consisting of halogen, oxo (=O), or C1-C6 alkyl;

W is an optionally substituted 4-7 membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

W^0 is an optionally substituted 3-4 membered carbocyclic ring, or a C1-C6 alkyl group substituted with from 1 to 4 fluorine atoms;

provided that one of U^2, A, and X is a secondary amine A^2 or a tertiary amine A^3, wherein

the secondary amine A^2 is -NH-W^0, and

the tertiary amine A^3 is a fully saturated and optionally substituted six-membered or seven-membered azacyclic ring optionally containing an additional heteroatom selected from N, O or S as a ring member, or the tertiary amine A^3 is a partially unsaturated or aromatic optionally substituted five-membered azacyclic ring optionally containing an additional heteroatom selected from N, O or S as a ring member.

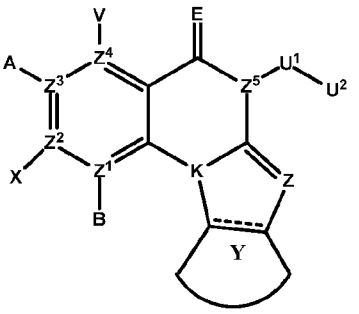
According to some embodiments of the invention, with the proviso that when U^1 is -C(O)NH-, U^2 is -L-W, and L is an ethylene linker, one of A and X is independently an optionally substituted aryl, heteroaryl, or 7-membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member, if W is pyrrolidin-1-yl, N-methyl-pyrrolidin-2-yl, piperidin-1-yl or morpholin-1-yl.

According to some embodiments of the invention, at least one of A and X is a tertiary amine A^3.

According to some embodiments of the invention, A^3 is selected from the group consisting of imidazole, imidazoline, pyrroline, piperidine, piperazine, morpholine, thiomorpholine and homopiperazine.

According to some embodiments of the invention, U^1 is a —C(=T)N(R)—, T is O, and U^2 is -L-W or -L-N(R)—W^0.

According any one of the embodiments of the invention, compounds having a general structure represented by Formula IV, are also contemplated:



Formula IV

wherein E is as defined for any one of the respective embodiments of Formula I, and K is N; and

wherein ---- indicates an optionally unsaturated bond; and

each of B, X, A or V is absent if Z^1, Z^2, Z^3 and Z^4, respectively, is N; and

each of B, X, A and V is independently H, halo, azido, -CN, $-CF_3$, $-CONR^1R^2$, $-NR^1R^2$, $-SR^2$, $-OR^2$, $-R^3$, -W, -L-W, $-W^0$, -L-N(R)-W^0, A^2 or A^3, when each of Z^1, Z^2, Z^3 and Z^4, respectively, is C;

each of Z^1, Z^2, Z^3 and Z^4 is independently C or N, provided any three N are non-adjacent;

Y is an optionally substituted 5-6 membered carbocyclic or heterocyclic ring;

U^1 is -C(=T)N(R)-, -C(=T)N(R)O-, -C(=T)-, $-SO_2N(R)-$, $-SO_2N(R)N(R^0)-$, $-SO_2-$, or $-SO_3-$, where T is O, S, or NH; or U^1 may be a bond when Z^5 is N or U^2 is H;

U^2 is H, or C3-C7 cycloalkyl, C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl or C2-C10 heteroalkenyl group, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring; or U^2 is -W, -L-W, -L-N(R) -W^0, A^2 or A^3;

in each $-NR^1R^2$, R^1 and R^2 together with N may form an optionally substituted azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

R^1 is H or C1-C6 alkyl, optionally substituted with one or more halogens, or =O;

R^2 is H, or C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring;

R^3 is an optionally substituted C1-C10 alkyl, C2-C10 alkenyl, C5-C10 aryl, or C6-C12 arylalkyl, or a heteroform of one of these, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-6 membered carbocyclic or heterocyclic ring;

each R^4 is independently H, or C1-C6 alkyl; or R^4 may be -W, -L-W or -L-N(R)-W^0;

each R and R^0 is independently H or C1-C6 alkyl;

L is a C1-C10 alkylene, C1-C10 heteroalkylene, C2-C10 alkenylene or C2-C10 heteroalkenylene linker, each of which may be optionally substituted with one or more substituents selected from the group consisting of halogen, oxo (=O), or C1-C6 alkyl;

W is an optionally substituted 4-7 membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

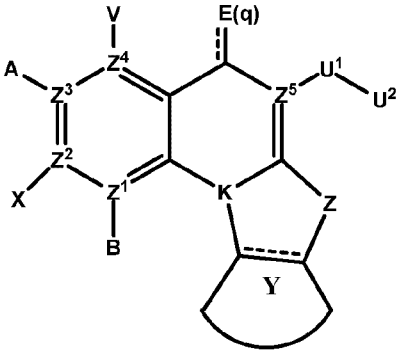
W^0 is an optionally substituted 3-4 membered carbocyclic ring, or a C1-C6 alkyl group substituted with from 1 to 4 fluorine atoms;

provided that one of U^2, V, A, X and B is a secondary amine A^2 or a tertiary amine A3, wherein

the secondary amine A^2 is -NH-W^0, and

the tertiary amine A^3 is a fully saturated and optionally substituted six-membered or seven-membered azacyclic ring optionally containing an additional heteroatom selected from N, O or S as a ring member, or the tertiary amine A^3 is a partially unsaturated or aromatic optionally substituted five-membered azacyclic ring, optionally containing an additional heteroatom selected from N, O or S as a ring member.

According to any one of the embodiments of the invention, compounds having a general structure represented by Formula V, are also contemplated:



Formula V

wherein E, q and K are as defined for any one of the embodiments of Formula I hereinabove;

and wherein:

---- indicates an optionally unsaturated bond;

A and V independently are H, halo, azido, -CN, -CF_3, -$CONR^1R^2$, -NR^1R^2, -SR^2, -OR^2, -R^3, -W, -L-W, -W^0, -L-N(R)-W^0, A^2 or A^3;

Z is O, S, CR^4_2, NR^4CR^4, CR^4NR^4 or NR^4;

Y is an optionally substituted 5-6 membered carbocyclic or heterocyclic ring;

U^1 is -C(=T)N(R)-, -C(=T)N(R)O-, -C(=T)-, -SO_2N(R)-, -SO_2N(R)N(R^0)-, -SO_2-, or -SO_3-, where T is O, S, or NH; or U^1 may be a bond when U2 is H;

U^2 is H, or C3-C7 cycloalkyl, C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl or C2-C10 heteroalkenyl group, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring; or U^2 is -W, -L-W or -L-N(R)-W^0, A^2 or A^3;

in each -NR^1R^2, R^1 and R^2 together with N may form an optionally substituted azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

R^1 is H or C1-C6 alkyl, optionally substituted with one or more halogens, or =O;

R^2 is H, or C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring;

R^3 is an optionally substituted C1-C10 alkyl, C2-C10 alkenyl, C5-C10 aryl, or C6-C12 arylalkyl, or a heteroform of one of these, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-6 membered carbocyclic or heterocyclic ring;

each R^4 is independently H, or C1-C6 alkyl; or R4 may be -W, -L-W or -L-N(R)-W^0;

each R and R^0 is independently H or C1-C6 alkyl;

L is a C1-C10 alkylene, C1-C10 heteroalkylene, C2-C10 alkenylene or C2-C10 heteroalkenylene linker, each of which may be optionally substituted with one or more substituents selected from the group consisting of halogen, oxo (=O), or C1-C6 alkyl;

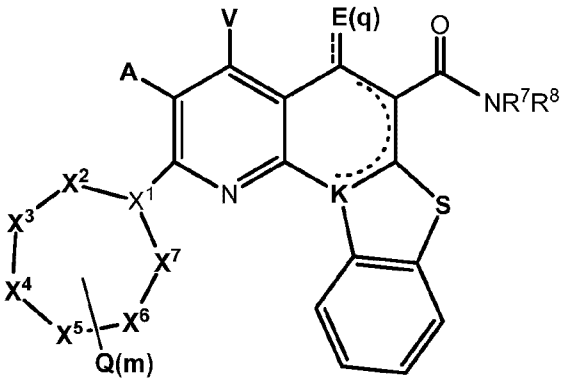
W is an optionally substituted 4-7 membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

W^0 is an optionally substituted 3-4 membered carbocyclic ring, or a C1-C6 alkyl group substituted with from 1 to 4 fluorine atoms;

provided one of U^2, A, and V is a secondary amine A^2 or a tertiary amine A3, wherein the secondary amine A^2 is -NH-W0, and

the tertiary amine A^3 is a fully saturated and optionally substituted six-membered or seven-membered azacyclic ring optionally containing an additional heteroatom selected from N, O or S as a ring member, or the tertiary amine A^3 is a partially unsaturated or aromatic optionally substituted five-membered azacyclic ring optionally containing an additional heteroatom selected from N, O or S as a ring member.

According any one of the embodiments of the invention, compounds having a general structure represented by Formula VI, are also contemplated:



Formula VI

wherein E, q and K are as defined for any one of the embodiments of Formula I hereinabove;

and wherein:

X^1 is CH or N;

X^2, X^3, X^4, X^5, X^6 and X^7 independently are NR^4, CH_2, CHQ or $C(Q)_2$, provided that: (i) zero, one or two of X^2, X^3, X^4, X^5, X^6 and X^7 are NR^4; (ii) when X^1 is N, both of X^2 and X^7 are not NR^4; (iii) when X^1 is N, X^3 and X^6 are not NR^4; and (iv) when X^1 is CH and two of X^2, X^3, X^4, X^5, X^6 and X^7 are NR^4, the two NR^4 are located at adjacent ring positions or are separated by two or more other ring positions;

A and V independently are H, halo, azido, -CN, $-CF_3$, $-CONR^1R^2$, $-NR^1R^2$, $-SR^2$, $-OR^2$, $-R^3$, -W, -L-W, $-W^0$, or -L-N(R)-W^0;

each Q is independently halo, azido, -CN, $-CF_3$, $-CONR^1R^2$, $-NR^1R^2$, $-SR^2$, $-OR^2$, $-R^3$, -W, -L-W, $-W^0$, or -L-N(R)-W^0;

in each $—NR^1R^2$, R^1 and R^2 together with N may form an optionally substituted azacyclic ring, optionally containing one additional heteroatom selected from N, O and S as a ring member;

R^1 is H or C1-C6 alkyl, optionally substituted with one or more halogens, or =O;

R is H, or C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring;

R^3 is an optionally substituted C1-C10 alkyl, C2-C10 alkenyl, C5-C10 aryl, or C6-C12 arylalkyl, or a heteroform of one of these, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-6 membered carbocyclic or heterocyclic ring;

each R^4 is independently H, or C1-C6 alkyl; or R4 may be -W, -L-W or -L-N(R)-W^0;

each R is independently H or C1-C6 alkyl;

R^7 is H and R^8 is C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring; or in —NR^7R^8, R^7 and R^8 together with N may form an optionally substituted azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

m is 0, 1, 2, 3 or 4;

n is 0, 1, 2, 3, 4, or 5;

L is a C1-C10 alkylene, C1-C10 heteroalkylene, C2-C10 alkenylene or C2-C10 heteroalkenylene linker, each of which may be optionally substituted with one or more substituents selected from the group consisting of halogen, oxo (=O), or C1-C6 alkyl;

W is an optionally substituted 4-7 membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member; and

W^0 is an optionally substituted 3-4 membered carbocyclic ring, or a C1-C6 alkyl group substituted with from 1 to 4 fluorine atoms.

According to some embodiments of the invention, X^1 is CH and two of X^2, X^3, X^4, X^5, X^6 and X^7 are NR^4.

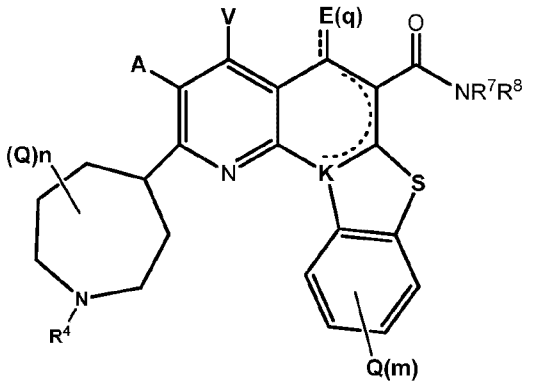
According to some embodiments of the invention, wherein X^1 is CH and one of X^2, X^3, X^4, X^5, X^6 and X^7 are NR^4.

According to some embodiments of the invention, X^1 is CH and none of X^2, X^3, X^4, X^5, X^6 and X^7 are NR^4.

According to some embodiments of the invention, wherein X^1 is N and none of X^2, X^3, X^4, X^5, X^6 and X^7 are NR^4.

According to some embodiments of the invention, X^1 is N and one of X^4 or X^5 is NR^4.

According any one of the embodiments of the invention, compounds having a general structure represented by Formula VIII, are also contemplated:



Formula (VIII)

wherein E, q and K are as defined for any one of the embodiments of Formula I hereinabove;

and wherein:

A and V independently are H, halo, azido, -CN, -CF_3, -$CONR^1R^2$, -NR^1R^2, -SR^2, -OR^2, -R^3, -W, -L-W, -W^0, or -L-N(R)-W^0;

each Q is independently halo, azido, -CN, -CF_3, -$CONR^1R^2$, -NR^1R^2, -SR^2, -OR^2, -R^3, -W, -L-W, -W^0, or -L-N(R)-W^0;

in each —NR^1R^2, R^1 and R^2 together with N may form an optionally substituted azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

R^1 is H or C1-C6 alkyl, optionally substituted with one or more halogens, or =O;

R^2 is H, or C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring;

R^3 is an optionally substituted C1-C10 alkyl, C2-C10 alkenyl, C5-C10 aryl, or C6-C12 arylalkyl, or a heteroform of one of these, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-6 membered carbocyclic or heterocyclic ring;

each R^4 is independently H, or C1-C6 alkyl; or R4 may be -W, -L-W or -L-N(R)-W^0;

each R is independently H or C1-C6 alkyl;

R^7 is H and R^8 is C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring; or in —NR^7R^8, R^7 and R^8 together with N may form an optionally substituted azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

m is 0, 1, 2, 3 or 4;

n is 0, 1, 2, 3, 4 or 5;

p is 0, 1, 2 or 3;

L is a C1-C10 alkylene, C1-C10 heteroalkylene, C2-C10 alkenylene or C2-C10 heteroalkenylene linker, each of which may be optionally substituted with one or more substituents selected from the group consisting of halogen, oxo (=O), or C1-C6 alkyl;

W is an optionally substituted 4-7 membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member; and

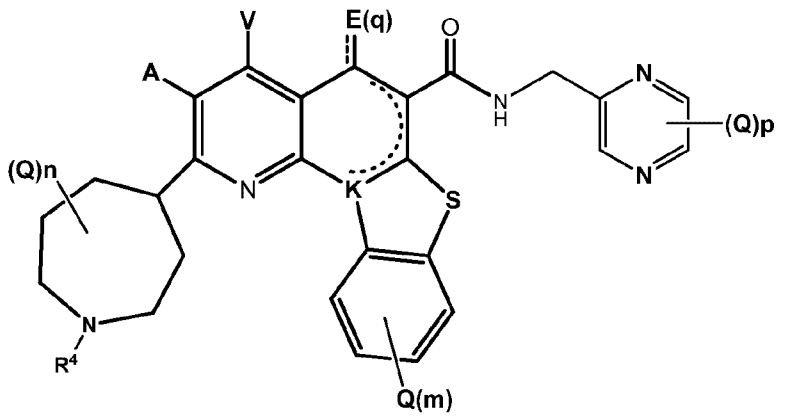
W^0 is an optionally substituted 3-4 membered carbocyclic ring, or a C1-C6 alkyl group substituted with from 1 to 4 fluorine atoms.

According to some embodiments of the invention, R^7 is H and R^8 is a C_{1-4} alkyl substituted with an optionally substituted aromatic heterocyclic ring.

According to some embodiments of the invention, the optionally substituted aromatic heterocyclic ring is selected from pyridine, pyrimidine, pyrazine, imidazole, pyrrolidine, and thiazole.

According to some embodiments of the invention, R^7 and R^8 together with N in -NR^7R^8 form an optionally substituted azacyclic ring selected from the group consisting of morpholine, thiomorpholine, piperidine or piperazine ring.

According to any one of the embodiments of the invention, compounds having a general structure represented by Formula VII are also contemplated:



Formula VII

wherein E, q and K are as defined for any one of the embodiments of Formula I hereinabove;

and wherein:

A and V independently are H, halo, azido, -CN, -CF_3, -CONR1R2, -NR1R2, -SR^2, -OR^2, -R^3, -W, -L-W, -W^0, or -L-N(R)-W^0;

each Q is independently halo, azido, -CN, -CF_3, -$CONR^1R^2$, -NR^1R^2, -SR^2, -OR^2, -R^3, -W, -L-W, -W^0, or -L-N(R)-W^0;

in each —NR^1R^2, R^1 and R^2 together with N may form an optionally substituted azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member;

R^1 is H or C1-C6 alkyl, optionally substituted with one or more halogens, or =O;

R2 is H, or C1-C10 alkyl, C1-C10 heteroalkyl, C2-C10 alkenyl, or C2-C10 heteroalkenyl, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-7 membered carbocyclic or heterocyclic ring;

R^3 is an optionally substituted C1-C10 alkyl, C2-C10 alkenyl, C5-C10 aryl, or C6-C12 arylalkyl, or a heteroform of one of these, each of which may be optionally substituted with one or more halogens, =O, or an optionally substituted 3-6 membered carbocyclic or heterocyclic ring;

each R^4 is independently H, or C1-C6 alkyl; or R^4 may be -W, -L-W or -L-N(R)-W^0;

each R is independently H or C1-C6 alkyl;

m is 0, 1, 2, 3 or 4;

n is 0, 1, 2, 3, 4 or 5;

p is 0, 1, 2 or 3;

L is a C1-C10 alkylene, C1-C10 heteroalkylene, C2-C10 alkenylene or C2-C10 heteroalkenylene linker, each of which may be optionally substituted with one or more substituents selected from the group consisting of halogen, oxo (=O), or C1-C6 alkyl;

W is an optionally substituted 4-7 membered azacyclic ring, optionally containing an additional heteroatom selected from N, O and S as a ring member; and

W^0 is an optionally substituted 3-4 membered carbocyclic ring, or a C1-C6 alkyl group substituted with from 1 to 4 fluorine atoms.

According to some embodiments of the invention, A and V are independently H or halo.

According to some embodiments of the invention, R^4 is H or C1-4 alkyl.

According to some embodiments of the invention, m and n are each 0.

According to some embodiments of the invention, p is 0 or 1.

Methods of synthesizing the compounds of some embodiments of the invention are described in Example 1 in the Examples section the follows.

According to some embodiments, compounds represented by Formula I as described herein, or by any one of Formulae II-VIII are prepared by converting a compound encompassed by these formulae into a corresponding chloride such as depicted for Compound **2** herein (see, Table 1) and the chloride is thereafter reacted with a suitable precursor (e.g., an amine) to form the desired compound (e.g., a corresponding imine).

For use as pharmaceutical agents, the compound of some embodiments of the invention is sterile.

According to some embodiments of the invention, the compound is purified using known methods.

According to some embodiments of the invention, the compound has 95-99.9% purity.

For any of the embodiments described herein, the compound may be in a form of a salt, for example, a pharmaceutically acceptable salt, and/or in a form of a prodrug.

As used herein, the phrase "pharmaceutically acceptable salt" refers to a charged species of the parent compound and its counter-ion, which is typically used to modify the solubility characteristics of the parent compound and/or to reduce any significant

irritation to an organism by the parent compound, while not abrogating the biological activity and properties of the administered compound.

In the context of some of the present embodiments, a pharmaceutically acceptable salt of the compounds described herein may optionally be an acid addition salt comprising at least one basic (e.g., amine) group of the compound which is in a positively charged form (e.g., an ammonium ion), in combination with at least one counter-ion, derived from the selected acid, that forms a pharmaceutically acceptable salt.

The acid addition salts of the compounds described herein may therefore be complexes formed between one or more amino groups of the drug and one or more equivalents of an acid.

The acid addition salts may include a variety of organic and inorganic acids, such as, but not limited to, hydrochloric acid which affords a hydrochloric acid addition salt, hydrobromic acid which affords a hydrobromic acid addition salt, acetic acid which affords an acetic acid addition salt, ascorbic acid which affords an ascorbic acid addition salt, benzenesulfonic acid which affords a besylate addition salt, camphorsulfonic acid which affords a camphorsulfonic acid addition salt, citric acid which affords a citric acid addition salt, maleic acid which affords a maleic acid addition salt, malic acid which affords a malic acid addition salt, methanesulfonic acid which affords a methanesulfonic acid (mesylate) addition salt, naphthalenesulfonic acid which affords a naphthalenesulfonic acid addition salt, oxalic acid which affords an oxalic acid addition salt, phosphoric acid which affords a phosphoric acid addition salt, toluenesulfonic acid which affords a p-toluenesulfonic acid addition salt, succinic acid which affords a succinic acid addition salt, sulfuric acid which affords a sulfuric acid addition salt, tartaric acid which affords a tartaric acid addition salt and trifluoroacetic acid which affords a trifluoroacetic acid addition salt. Each of these acid addition salts can be either a mono-addition salt or a poly-addition salt, as these terms are defined herein.

Depending on the stoichiometric proportions between the basic or acidic charged group(s) in the compound (e.g., amine group(s)) and the counter-ion in the salt, the acid or base additions salts can be either mono-addition salts or poly-addition salts.

The phrase "mono-addition salt", as used herein, refers to a salt in which the stoichiometric ratio between the counter-ion and charged form of the compound is 1:1, such that the addition salt includes one molar equivalent of the counter-ion per one molar equivalent of the compound.

The phrase "poly-addition salt", as used herein, refers to a salt in which the stoichiometric ratio between the counter-ion and the charged form of the compound is greater than 1:1 and is, for example, 2:1, 3:1, 4:1 and so on, such that the addition salt includes two or more molar equivalents of the counter-ion per one molar equivalent of the compound.

As used herein, the term "prodrug" refers to a compound which is converted in the body to an active compound (e.g., a SYNJ2 inhibitor described herein). A prodrug is typically designed to facilitate administration, e.g., by enhancing absorption. A prodrug may comprise, for example, the active compound modified with ester groups, for example, wherein one or more hydroxy groups of the active compound is modified by an acyl (e.g., acetyl) group to form an ester group, and/or wherein one or more carboxylic acid of the active compound is modified by an alkyl (e.g., ethyl) group to form an ester group.

Further, each of the compounds described herein, including the salts thereof, can be in a form of a solvate or a hydrate thereof.

The term "solvate" refers to a complex of variable stoichiometry (e.g., di-, tri-, tetra-, penta-, hexa-, and so on), which is formed by a solute (the heterocyclic compounds described herein) and a solvent, whereby the solvent does not interfere with the biological activity of the solute.

The term "hydrate" refers to a solvate, as defined hereinabove, where the solvent is water.

The present embodiments further encompass any stereoisomers (enantiomers and diastereomers) of the compounds described herein, except in embodiments wherein a specific stereoisomer is explicitly required, as well as any isomorph thereof.

As used herein throughout, the term "alkyl" refers to a saturated aliphatic hydrocarbon including straight chain and branched chain groups. Preferably, the alkyl group has 1 to 20 carbon atoms. Whenever a numerical range; *e.g.*, "1-20", is stated herein, it implies that the group, in this case the alkyl group, may contain 1 carbon

atom, 2 carbon atoms, 3 carbon atoms, etc., up to and including 20 carbon atoms. More preferably, the alkyl is a medium size alkyl having 1 to 10 carbon atoms. Most preferably, unless otherwise indicated, the alkyl is a lower alkyl having 1 to 4 carbon atoms. The alkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, cycloalkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, hydrazine, and amino, as these terms are defined herein.

A "cycloalkyl" group refers to an all-carbon monocyclic or fused ring (i.e., rings which share an adjacent pair of carbon atoms) group wherein one of more of the rings does not have a completely conjugated pi-electron system. Examples, without limitation, of cycloalkyl groups are cyclopropane, cyclobutane, cyclopentane, cyclopentene, cyclohexane, cyclohexadiene, cycloheptane, cycloheptatriene, and adamantane. A cycloalkyl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, alkenyl, alkynyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, hydrazine, and amino, as these terms are defined herein.

An "alkenyl" group refers to an alkyl group which consists of at least two carbon atoms and at least one carbon-carbon double bond.

An "alkynyl" group refers to an alkyl group which consists of at least two carbon atoms and at least one carbon-carbon triple bond.

An "aryl" group refers to an all-carbon monocyclic or fused-ring polycyclic (i.e., rings which share adjacent pairs of carbon atoms) groups having a completely conjugated pi-electron system. Examples, without limitation, of aryl groups are phenyl, naphthalenyl and anthracenyl. The aryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy,

thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, hydrazine, and amino, as these terms are defined herein.

A "heteroaryl" group refers to a monocyclic or fused ring (i.e., rings which share an adjacent pair of atoms) group having in the ring(s) one or more atoms, such as, for example, nitrogen, oxygen and sulfur and, in addition, having a completely conjugated pi-electron system. Examples, without limitation, of heteroaryl groups include pyrrole, furane, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrimidine, quinoline, isoquinoline and purine. The heteroaryl group may be substituted or unsubstituted. When substituted, the substituent group can be, for example, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, hydrazine, and amino, as these terms are defined herein.

A "heteroalicyclic" group refers to a monocyclic or fused ring group having in the ring(s) one or more atoms such as nitrogen, oxygen and sulfur. The rings may also have one or more double bonds. However, the rings do not have a completely conjugated pi-electron system. The heteroalicyclic may be substituted or unsubstituted. When substituted, the substituted group can be, for example, lone pair electrons, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heteroalicyclic, halo, hydroxy, alkoxy, aryloxy, thiohydroxy, thioalkoxy, thioaryloxy, sulfinyl, sulfonyl, cyano, nitro, azide, phosphonyl, phosphinyl, oxo, carbonyl, thiocarbonyl, urea, thiourea, O-carbamyl, N-carbamyl, O-thiocarbamyl, N-thiocarbamyl, C-amido, N-amido, C-carboxy, O-carboxy, sulfonamido, hydrazine, and amino, as these terms are defined herein. Representative examples are piperidine, piperazine, tetrahydrofuran, tetrahydropyran, morpholine and the like.

A "hydroxy" group refers to an -OH group.

As used herein, the terms “amine” and “amino” refer to either a –NR’R’’ group, wherein R’ and R’’ are selected from the group consisting of hydrogen, alkyl, cycloalkyl, heteroalicyclic (bonded through a ring carbon), aryl and heteroaryl (bonded

through a ring carbon). R' and R'' are bound via a carbon atom thereof. Optionally, R' and R'' are selected from the group consisting of hydrogen and alkyl comprising 1 to 4 carbon atoms. Optionally, R' and R'' are hydrogen.

An "azide" group refers to a $-N=N^{+}=N^{-}$ group.

An "alkoxy" group refers to both an -O-alkyl and an -O-cycloalkyl group, as defined herein.

An "aryloxy" group refers to both an -O-aryl and an -O-heteroaryl group, as defined herein.

A "thiohydroxy" or "thiol" group refers to a -SH group.

A "thioalkoxy" group refers to both an -S-alkyl group, and an -S-cycloalkyl group, as defined herein.

A "thioaryloxy" group refers to both an -S-aryl and an -S-heteroaryl group, as defined herein.

A "disulfide" group refers to both a –S-thioalkoxy and a –S-thioaryloxy group.

A disulfide bond describes a –S-S- bond.

A "carbonyl" group refers to a -C(=O)-R' group, where R' is defined as hereinabove, or where R' and C form a part of a cyclic moiety such as cycloalkyl, aryl, heteroaryl and heteroalicyclic, as defined herein.

A "thiocarbonyl" group refers to a -C(=S)-R' group, where R' is as defined herein.

A "C-carboxy" group refers to a -C(=O)-O-R' groups, where R' is as defined herein.

An "O-carboxy" group refers to an R'C(=O)-O- group, where R' is as defined herein.

An "oxo" group refers to a =O group.

A "thioxo" group refers to a =S group.

A "carboxylate" or "carboxyl" encompasses both C-carboxy and O-carboxy groups, as defined herein.

A "carboxylic acid" group refers to a C-carboxy group in which R' is hydrogen.

A "thiocarboxy" or "thiocarboxylate" group refers to both –C(=S)-O-R' and -O-C(=S)R' groups.

An "ester" refers to a C-carboxy group wherein R' is not hydrogen.

An ester bond refers to a –O-C(=O)- bond.

A "halo" group refers to fluorine, chlorine, bromine or iodine.

A "sulfinyl" group refers to an -S(=O)-R' group, where R' is as defined herein.

A "sulfonyl" group refers to an -$S(=O)_2$-R' group, where R' is as defined herein.

A "sulfonate" group refers to an –$S(=O)_2$-O-R' group, where R' is as defined herein.

A "sulfate" group refers to an –O-$S(=O)_2$-O-R' group, where R' is as defined as herein.

A "sulfonamide" or "sulfonamido" group encompasses both S-sulfonamido and N-sulfonamido groups, as defined herein.

An "S-sulfonamido" group refers to a -$S(=O)_2$-NR'R'' group, with each of R' and R'' as defined herein.

An "N-sulfonamido" group refers to an R'$S(=O)_2$-NR'' group, where each of R' and R'' is as defined herein.

An "O-carbamyl" group refers to an -OC(=O)-NR'R'' group, where each of R' and R'' is as defined herein.

An "N-carbamyl" group refers to an R'OC(=O)-NR''- group, where each of R' and R'' is as defined herein.

A "carbamyl" or "carbamate" group encompasses O-carbamyl and N-carbamyl groups.

A carbamate bond describes a –O-C(=O)-NR'- bond, where R' is as described herein.

An "O-thiocarbamyl" group refers to an -OC(=S)-NR'R'' group, where each of R' and R'' is as defined herein.

An "N-thiocarbamyl" group refers to an R'OC(=S)NR''- group, where each of R' and R'' is as defined herein.

A "thiocarbamyl" or "thiocarbamate" group encompasses O-thiocarbamyl and N-thiocarbamyl groups.

A thiocarbamate bond describes a –O-C(=S)-NR'- bond, where R' is as described herein.

A "C-amido" group refers to a -C(=O)-NR'R'' group, where each of R' and R'' is as defined herein.

An "N-amido" group refers to an R'C(=O)-NR''- group, where each of R' and R'' is as defined herein.

An "amide" group encompasses both C-amido and N-amido groups.

An amide bond describes a –NR'-C(=O)- bond, where R' is as defined herein.

A "urea" group refers to an –N(R')-C(=O)-NR''R''' group, where each of R' and R'' is as defined herein, and R''' is defined as R' and R'' are defined herein.

A "nitro" group refers to an $-NO_2$ group.

A "cyano" group refers to a -C≡N group.

The term "hydrazine" describes a –N(R')-N(R'')R''' group, with each of R', R'' and R''' as defined hereinabove.

Treatment of Autoimmune Diseases:

According to an aspect of some embodiments of the present invention any one of the compounds as described herein is for use in the treatment of an autoimmune disease in a subject in need thereof.

According to an aspect of some embodiments of the present invention any one of the compounds as described herein is for use in the manufacture of a medicament for treating an autoimmune disease in a subject in need thereof.

According to an aspect of some embodiments of the present invention there is provided a method of treating an autoimmune disease, which is effected by administering to the subject a therapeutically effective amount of any one of the compounds as described herein.

As used in the context of this aspect of the present embodiments, the phrase "treating" refers to inhibiting or arresting the development of the autoimmune disease (e.g., multiple sclerosis) and/or causing the reduction, remission, or regression of the autoimmune disease and/or optimally curing the autoimmune disease. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of autoimmune disease, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of the autoimmune disease.

As used herein, the term "subject" includes mammals, preferably human beings at any age which suffer from the pathology (the autoimmune disease) or which have been diagnosed as being afflicted by the pathology.

According to some embodiments of the invention, the term "subject" encompasses individuals who are at risk to develop the pathology or are suspected of having the pathology. As used herein the phrase "autoimmune disease" refers to any disease caused by an autoimmune response, *i.e.*, an immune response directed to a substance in the body of the subject.

It should be noted that since autoimmunity can affect any organ or tissue of the subject, e.g., the brain, skin, kidney, lungs, liver, heart, or thyroid of the subject, the clinical expression of the disease depends upon the site affected.

Following is a non-limiting list of autoimmune diseases or disorders (including autoimmune-related diseases or disorders) which can be treated by the compound of some embodiments of the invention: Acute Disseminated Encephalomyelitis (ADEM); Acute necrotizing hemorrhagic leukoencephalitis; Addison's disease; Agammaglobulinemia; Alopecia areata; Amyloidosis; Ankylosing spondylitis; Anti-GBM/Anti-TBM nephritis; Antiphospholipid syndrome (APS); Autoimmune angioedema; Autoimmune aplastic anemia; Autoimmune dysautonomia; Autoimmune hepatitis; Autoimmune hyperlipidemia; Autoimmune immunodeficiency; Autoimmune inner ear disease (AIED); Autoimmune myocarditis; Autoimmune pancreatitis; Autoimmune retinopathy; Autoimmune thrombocytopenic purpura (ATP); Autoimmune thyroid disease; Autoimmune urticaria; Axonal & neuronal neuropathies; Balo disease; Behcet's disease; Bullous pemphigoid; Cardiomyopathy; Castleman disease; Celiac disease; Chagas disease; Chronic inflammatory demyelinating polyneuropathy (CIDP); Chronic recurrent multifocal ostomyelitis (CRMO); Churg-Strauss syndrome; Cicatricial pemphigoid/benign mucosal pemphigoid; Crohn's disease; Cogans syndrome; Cold agglutinin disease; Congenital heart block; Coxsackie myocarditis; CREST disease; Essential mixed cryoglobulinemia; Demyelinating neuropathies; Dermatitis herpetiformis; Dermatomyositis; Devic's disease (neuromyelitis optica); Discoid lupus; Dressler's syndrome; Endometriosis; Eosinophilic fasciitis; Erythema nodosum; Experimental allergic encephalomyelitis; Evans syndrome; Fibrosing alveolitis; Giant cell arteritis (temporal arteritis); Glomerulonephritis; Goodpasture's syndrome; Granulomatosis with Polyangiitis (GPA) see Wegener's; Graves' disease; Guillain-Barre syndrome; Hashimoto's encephalitis; Hashimoto's thyroiditis; Hemolytic anemia; Henoch-Schonlein purpura; Herpes gestationis; Hypogammaglobulinemia; Idiopathic

thrombocytopenic purpura (ITP); IgA nephropathy; IgG4-related sclerosing disease; Immunoregulatory lipoproteins; Inclusion body myositis; Insulin-dependent diabetes (type1); Interstitial cystitis; Juvenile arthritis; Juvenile diabetes; Kawasaki syndrome; Lambert-Eaton syndrome; Leukocytoclastic vasculitis; Lichen planus; Lichen sclerosus; Ligneous conjunctivitis; Linear IgA disease (LAD); Lupus (SLE); Lyme disease, chronic; Meniere's disease; Microscopic polyangiitis; Mixed connective tissue disease (MCTD); Mooren's ulcer; Mucha-Habermann disease; Multiple sclerosis; Myasthenia gravis; Myositis; Narcolepsy; Neuromyelitis optica (Devic's); Neutropenia; Ocular cicatricial pemphigoid; Optic neuritis; Palindromic rheumatism; PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcus); Paraneoplastic cerebellar degeneration; Paroxysmal nocturnal hemoglobinuria (PNH); Parry Romberg syndrome; Parsonnage-Turner syndrome; Pars planitis (peripheral uveitis); Pemphigus; Peripheral neuropathy; Perivenous encephalomyelitis; Pernicious anemia; POEMS syndrome; Polyarteritis nodosa; Type I, II, & III autoimmune polyglandular syndromes; Polymyalgia rheumatica; Polymyositis; Postmyocardial infarction syndrome; Postpericardiotomy syndrome; Progesterone dermatitis; Primary biliary cirrhosis; Primary sclerosing cholangitis; Psoriasis; Psoriatic arthritis; Idiopathic pulmonary fibrosis; Pyoderma gangrenosum; Pure red cell aplasia; Raynauds phenomenon; Reflex sympathetic dystrophy; Reiter's syndrome; Relapsing polychondritis; Restless legs syndrome; Retroperitoneal fibrosis; Rheumatic fever; Rheumatoid arthritis; Sarcoidosis; Schmidt syndrome; Scleritis; Scleroderma; Sjogren's syndrome; Sperm & testicular autoimmunity; Stiff person syndrome; Subacute bacterial endocarditis (SBE); Susac's syndrome; Sympathetic ophthalmia; Takayasu's arteritis; Temporal arteritis/Giant cell arteritis; Thrombocytopenic purpura (TTP); Tolosa-Hunt syndrome; Transverse myelitis; Ulcerative colitis; Undifferentiated connective tissue disease (UCTD); Uveitis; Vasculitis; Vesiculobullous dermatosis; Vitiligo; Wegener's granulomatosis (now termed Granulomatosis with Polyangiitis (GPA).

According to some embodiments of the invention, the autoimmune disease is multiple sclerosis.

According to some embodiments of the invention, the subject is diagnosed with multiple sclerosis.

The diagnosis of "multiple sclerosis" can be made when a subject has experienced at least one neurological attack affecting the central nervous system (CNS) accompanied by demyelinating lesions within the brain or spinal cord, which may have, but not necessarily confirmed by magnetic resonance imaging (MRI). The neurological attack can involve acute or sub-acute neurological symptomatology (attack) manifested by various clinical presentations like unilateral loss of vision, vertigo, ataxia, incoordination, gait difficulties, sensory impairment characterized by paresthesia, dysesthesia, sensory loss, urinary disturbances until incontinence, diplopia, dysarthria, various degrees of motor weakness until paralysis, cognitive decline either as a monosymptomatic or in combination. The symptoms usually remain for several days to few weeks, and then partially or completely resolve.

Further details on the diagnosis of multiple sclerosis according to 2010 McDonald Criteria for Diagnosis of MS are provided in Polman CH., et al., 2011 ("Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria" Annals of Neurology, vol. 69 (2): pages 292-302).

For example, the diagnosis of multiple sclerosis can be made upon (I): Clinical presentation of ≥2 attacks, with objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack; (II): Clinical presentation of ≥2 attacks, with objective clinical evidence of 1 lesion, additional data have to include dissemination in space, demonstrated by: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord); (III): Clinical presentation of 1 attack, with objective clinical evidence of ≥2 lesions, additional data have to include dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; (IV): Clinical presentation of 1 attack, additional data have to include dissemination in space and time, demonstrated by: For DIS: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord) and for DIT: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up

Date Reçue/Date Received 2021-04-30

MRI, irrespective of its timing with reference to a baseline scan; (V): Clinical presentation of Insidious neurological progression suggestive of MS (PPMS), additional data have to include 1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria: 1. Evidence for DIS in the brain based on ≥1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions 2. Evidence for DIS in the spinal cord based on ≥2 T2 lesions in the cord 3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index).

According to some embodiments of the invention, the subject has relapsing-remitting multiple sclerosis (RRMS).

According to some embodiments of the invention, the subject has a primary progressive multiple sclerosis (PPMS).

According to some embodiments of the invention, the subject has a secondary progressive MS (SPMS).

According to some embodiments of the invention, the subject has benign multiple sclerosis (BMS).

According to some embodiments of the invention, the subject has a progressive-relapsing course of MS.

According to some embodiments of the invention, treating the subject refers to changing the disease course of the subject from a typical RRMS course to a BMS course.

According to some embodiments of the invention, treating the subject refers to suppressing the activity of typical RRMS course.

According to some embodiments of the invention, administering the compound is performed after diagnosing the subject as having the autoimmune disease.

According to some embodiments of the invention, the autoimmune disease is multiple sclerosis and the diagnosis comprises appearance of brain lesions characteristics of the multiple sclerosis.

According to some embodiments of the invention, the compound prevents the appearance of additional neurological attack(s) and/or brain lesion(s) as compared to the number of neurological attack(s) and/or brain lesion(s) present at time of diagnosing multiple sclerosis.

According to an aspect of some embodiments of the present invention the compounds as described herein, in any one of the embodiments thereof are useful in inhibiting an activity of RNA Polymerase I, or in modulating a RNA Polymerase I pathway. These compounds are therefore useful in the treatment of any disease or disorder that is associated the RNA Polymerase I or which is treatable by modulating (e.g., inhibiting), a RNA Polymerase I activity or pathway, as is described in further detail hereinafter.

Such diseases and disorders include, in addition to autoimmune diseases as described herein, also proliferative diseases or disorders, as described herein, and any other medical conditions which would be recognized by any person skilled in the art.

Treatment of proliferative diseases or disorders:

According to some embodiments of the invention, any of the compounds described herein are useful in treating a proliferative disease or disorder and/or in modulating (e.g., inhibiting) a protein kinase activity.

As used herein the phrase "proliferative disease" refers to diseases manifested by abnormal cell proliferation, and includes, for example, benign tumors, pre-malignant tumors, and malignant tumors, such as cancer.

As used herein the terms "cancer" and "malignant tumor" are interchangeably used. The term refers to a malignant growth or tumor caused by abnormal and uncontrolled cell proliferation (cell division). Exemplary cancers include, without limitation, cancers of the colorectum, breast, lung, liver, pancreas, lymph node, colon, prostate, brain, head and neck, skin, liver, kidney, blood and heart (e.g., leukemia, lymphoma, carcinoma).

The terms "treat" and "treating" and "treatment" as used herein refer to ameliorating, alleviating, lessening, and removing symptoms of a disease or condition. In some embodiments, "treating" is effected by a compound as described herein, which, when administered to a subject in need thereof, exhibit a biological effect such as apoptosis of certain cells (e.g., cancer cells), reduction of proliferation of certain cells, or lead to ameliorating, alleviating, lessening, or removing symptoms of a disease or condition. "The terms "treat" and "treating" and "treatment" as used herein in some embodiments, also can refer to reducing or stopping a cell proliferation rate (e.g., slowing or halting tumor growth) or reducing the number of proliferating cancer cells

(e.g., removing part or all of a tumor). The terms "treat" and "treating" and "treatment" as used herein also are applicable to reducing a titre of a microorganism in a system (i.e., cell, tissue, or subject) infected with a microorganism, reducing the rate of microbial propagation, reducing the number of symptoms or an effect of a symptom associated with the microbial infection, and/or removing detectable amounts of the microbe from the system. Examples of microorganism include but are not limited to virus, bacterium and fungus.

As used herein, the term "apoptosis" refers to an intrinsic cell self-destruction or suicide program. In response to a triggering stimulus, cells undergo a cascade of events including cell shrinkage, blebbing of cell membranes and chromatic condensation and fragmentation. These events culminate in cell conversion to clusters of membrane-bound particles (apoptotic bodies), which are thereafter engulfed by macrophages.

Also provided herein are methods and uses of any one of the compounds described herein, for modulating the activity of a protein kinase, which are effected by contacting a system comprising the protein kinase with a compound as described herein in an amount effective for modulating (e.g., inhibiting) the activity of the kinase. The system in such embodiments can be a cell-free system or a system comprising cells. Also provided are methods and uses utilizing the compounds as described herein for reducing cell proliferation, and optionally inducing apoptosis, which are effected by contacting cells with a compound as described herein in an amount effective to reduce proliferation of the cells. The cells in such embodiments can be in a cell line, in a tissue or in a subject (e.g., a research animal or human).

Protein kinases are a family of enzyme which catalyze the transfer of a gamma phosphate from adenosine triphosphate to a serine or threonine amino acid (serine/threonine protein kinase), tyrosine amino acid (tyrosine protein kinase), tyrosine, serine or threonine (dual specificity protein kinase) or histidine amino acid (histidine protein kinase) in a peptide or protein substrate. Thus, included herein are methods and uses which are effected by contacting a system comprising a protein kinase with a compound as described herein in an amount effective for modulating (e.g., inhibiting) the activity of the protein kinase. In some embodiments, the activity of the protein kinase is the catalytic activity of the protein (e.g., catalyzing the transfer of a gamma

phosphate from adenosine triphosphate to a peptide or protein substrate). Systems in such embodiments can be a cell-free system or a system comprising cells (e.g., in vitro).

In some embodiments, the protein kinase is a serine-threonine protein kinase or a tyrosine protein kinase. In some embodiments, the protein kinase is a protein kinase fragment having compound-binding activity.

In some embodiments, the protein kinase is, or contains a subunit (e.g., catalytic subunit, SH2 domain, SH3 domain) of, CK2, Pim subfamily protein kinase (e.g., PIM1, PIM2, PIM3) or Flt subfamily protein kinase (e.g, FLT1, FLT3, FLT4).

In some embodiments the protein kinase is a recombinant protein. The protein kinase can be from any source, such as cells from a mammal, ape or human, for example. In some embodiments, the protein kinase is a human protein kinase.

In some embodiments, any of the compounds described herein is also useful in the treatment of a condition related to inflammation or pain. Conditions associated with inflammation and pain include without limitation, acid reflux, heartburn, acne, allergies and sensitivities, Alzheimer's disease, asthma, atherosclerosis, bronchitis, carditis, celiac disease, chronic pain, Crohn's disease, cirrhosis, colitis, dementia, dermatitis, diabetes, dry eyes, edema, emphysema, eczema, fibromyalgia, gastroenteritis, gingivitis, heart disease, hepatitis, high blood pressure, insulin resistance, interstitial cystitis, joint pain/arthritis/rheumatoid arthritis, metabolic syndrome (syndrome X), myositis, nephritis, obesity, osteopenia, osteoporosis, Parkinson's disease, periodontal disease, polyarteritis, polychondritis, psoriasis, scleroderma, sinusitis, Sjogren's syndrome, spastic colon, systemic candidiasis, tendonitis, urinary tract infections, vaginitis, inflammatory cancer (e.g., inflammatory breast cancer) and the like.

In some embodiments, any of the compounds described herein is also useful for modulating angiogenesis in a subject, and for treating a condition associated with aberrant angiogenesis in a subject.

Pharmaceutical Compositions:

In any one of the methods and uses described herein, and any one of the embodiments thereof, a compound as described herein can be administered to the subject *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the compound of some embodiments of the invention accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

According to some embodiments of the invention, the compound is administered by oral administration.

Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intracerebral injection or intracerebroventricular infusion); pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting

Date Reçue/Date Received 2021-04-30

from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size limitation imposed by a limitation inherent in the endogenous transport systems, potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

The term "tissue" refers to part of an organism consisting of cells designed to perform a function or functions. Examples include, but are not limited to, brain tissue, retina, skin tissue, hepatic tissue, pancreatic tissue, bone, cartilage, connective tissue, blood tissue, muscle tissue, cardiac tissue brain tissue, vascular tissue, renal tissue, pulmonary tissue, gonadal tissue, hematopoietic tissue.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an

aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of some embodiments of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients (the compound of some embodiments of the invention) effective to prevent, alleviate or ameliorate symptoms of

a disorder (e.g., an autoimmune disease such as multiple sclerosis) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide tissue or blood levels of the active ingredient which are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

The doses shown herein with respect to the mouse animal model can be converted for the treatment other species such as human and other animals diagnosed with the autoimmune disease. Figure 11 shows an art accepted conversion Table approved by the FDA (Reagan-Shaw S., et al., FASEB J. 22:659-661 (2007)).

The human equivalent dose is calculated as follows: HED (mg/kg) = Animal dose (mg/kg) multiplied by (Animal K_m/human K_m).

According to some embodiments of the invention, the compound is provided at an amount equivalent to a range of from about 3 – 30 mg/kg/day in mice, including any intermediate subranges and values therebetween.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is detailed herein.

Monitoring Treatment Efficacy:

The teachings of the invention can be also used to determine efficiency of the compound of some embodiments of the invention in treating the autoimmune disease (e.g., multiple sclerosis) by determining the effect of the compound on the expression level of the at least one gene of the RNA polymerase I pathway. This can be used to develop a tailored treatment of an autoimmune disease by monitoring drug efficacy. This system is based on measuring the level of genes of the RNA polymerase I pathway during treatment with the compound and the ability to perform an ongoing fine-tuning drug efficacy assessment.

Thus, according to an aspect of some embodiments of the invention, there is provided a method of monitoring treatment efficiency of the compound of some embodiments of the invention, the method comprising:

(a) treating the subject with the compound according to the method of some embodiments of the invention, and

(b) comparing a level of expression of least one gene involved in the RNA polymerase I pathway in a cell of the subject following treating with the compound to a level of expression of the at least one gene in a cell of the subject prior to treating the subject with the compound,

(i) wherein a decrease above a predetermined threshold in the level of expression of the at least one gene following treating with the compound relative to the level of expression of the at least one gene prior to treating with the compound indicates that the compound is efficient for treating the subject;

(ii) wherein an increase above a predetermined threshold in the level of expression of the at least one gene following treating with the compound relative to the level of expression of the at least one gene prior to treating with the compound indicates that the compound is not efficient for treating the subject; or

(iii) wherein when a level of expression of the at least one gene following treating with the compound is identical or changed below a predetermined threshold as compared to prior to treating with the compound then the treatment is not efficient for treating the subject;

thereby monitoring treatment efficiency of the subject having disease or disorder as described herein.

As used herein, the phrase "level of expression" refers to the degree of gene expression and/or gene product activity in a specific cell. For example, up-regulation or down-regulation of various genes can affect the level of the gene product (*i.e.*, RNA and/or protein) in a specific cell.

It should be noted that the level of expression can be determined in arbitrary absolute units, or in normalized units (relative to known expression levels of a control reference). For example, when using DNA chips, the expression levels are normalized according to the chips' internal controls or by using quantile normalization such as RMA.

As used herein the phrase "a cell of the subject" refers to at least one cell (e.g., an isolated cell), cell culture, cell content and/or cell secreted content which contains RNA and/or proteins of the subject. Examples include a blood cell, a cell obtained from any tissue biopsy [e.g., cerebrospinal fluid, (CSF), brain biopsy], a bone marrow cell, body fluids such as plasma, serum, saliva, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, sputum and milk. According to an embodiment of the invention, the cell is a blood cell (e.g., white blood cells, macrophages, B- and T-lymphocytes, monocytes, neutrophiles, eosinophiles, and basophiles) which can be obtained using a syringe needle from a vein of the subject. It should be noted that the cell may be isolated from the subject (e.g., for *in vitro* detection) or may optionally comprise a cell that has not been physically removed from the subject (e.g., *in vivo* detection).

According to some embodiments of the invention, the white blood cell comprises peripheral blood mononuclear cells (PBMC). The phrase, "peripheral blood mononuclear cells (PBMCs)" as used herein, refers to a mixture of monocytes and lymphocytes. Several methods for isolating white blood cells are known in the art. For example, PBMCs can be isolated from whole blood samples using density gradient centrifugation procedures. Typically, anticoagulated whole blood is layered over the separating medium. At the end of the centrifugation step, the following layers are visually observed from top to bottom: plasma/platelets, PBMCs, separating medium and erythrocytes/granulocytes. The PBMC layer is then removed and washed to remove contaminants (e.g., red blood cells) prior to determining the expression level of the polynucleotide(s) therein.

According to some embodiments of the invention, the level of expression of the gene(s) of the invention is determined using an RNA and/or a protein detection method.

According to some embodiments of the invention, the RNA or protein molecules are extracted from the cell of the subject.

Methods of extracting RNA or protein molecules from cells of a subject are well known in the art. Once obtained, the RNA or protein molecules can be characterized for the expression and/or activity level of various RNA and/or protein molecules using methods known in the arts.

According to some embodiments of the invention, detection of the expression level of the RNA of the POL1 pathway is performed using a probe which specifically hybridizes to a polynucleotide expressed from the gene of the POL1 pathway (e.g., including any alternative spliced form which is known in the art).

Non-limiting examples of methods of detecting RNA molecules in a cell sample include Northern blot analysis, RT-PCR, RNA *in situ* hybridization (using e.g., DNA or RNA probes to hybridize RNA molecules present in the cells or tissue sections), *in situ* RT-PCR (e.g., as described in Nuovo GJ, et al. Am J Surg Pathol. 1993, 17: 683-90; Komminoth P, et al. Pathol Res Pract. 1994, 190: 1017-25), and oligonucleotide microarray (e.g., by hybridization of polynucleotide sequences derived from a sample to oligonucleotides attached to a solid surface [e.g., a glass wafer) with addressable location, such as Affymetrix microarray (Affymetrix®, Santa Clara, CA)].

For example, the level of RRN3 in a sample can be determined by RT-PCR using primers available from Santa Cruz Biotechnology Inc. (sc-106866-PR), or Taqman Gene Expression Assay HS00607907_ml (Applied Biosystems, Foster City, CA, USA), according to manufacturer's recommendation.

According to some embodiments of the invention, detection of the expression level of the protein of the POL1 pathway is performed using an antibody which specifically binds to a polypeptide expressed from the gene of the Pol I pathway (e.g., including any variants thereof which is known in the art).

Non-limiting examples of methods of detecting the level and/or activity of specific protein molecules in a cell sample include Enzyme linked immunosorbent assay (ELISA), Western blot analysis, radio-immunoassay (RIA), Fluorescence activated cell sorting (FACS), immunohistochemical analysis, *in situ* activity assay (using e.g., a chromogenic substrate applied on the cells containing an active enzyme), in vitro activity assays (in which the activity of a particular enzyme is measured in a protein mixture extracted from the cells). For example, in case the detection of the expression level of a secreted protein is desired, ELISA assay may be performed on a sample of fluid obtained from the subject (e.g., serum), which contains cell-secreted content.

As described above, the level of expression of least one gene involved in the RNA polymerase I pathway in a cell of the subject following treating with the compound

is compared to the level of expression of the at least one gene in a cell of the subject prior to treating the subject with the compound.

As used herein the phrase "following treating with the compound" refers to any time period after administering the compound to the subject, e.g., from a few minutes to hours, or from a few days to weeks or months after drug administration.

According to some embodiments of the invention the level of expression is determined following administration of the first dose of the compound.

According to some embodiments of the invention the level of expression is determined following administration of any dose of the compound.

As used herein the phrase "prior to treating with the compound" refers to any time period prior administering the compound to the subject, e.g., from a few minutes to hours, or from a few days to weeks or months prior to drug administration.

According to some embodiments of the invention the level of expression is determined prior any dose of the compound (e.g., when the subject is naïve to treatment).

According to some embodiments of the invention prior to treating refers to when the subject is first diagnosed with autoimmune disease, e.g., multiple sclerosis.

According to some embodiments of the invention prior to treating refers to when the subject is suspected of having the autoimmune disease (e.g., multiple sclerosis), or diagnosed with probable autoimmune disease (e.g., probable multiple sclerosis).

According to some embodiments of the invention prior to treating refers to upon the onset of the autoimmune disease.

According to some embodiments of the invention the effect of the treatment on the subject can be evaluated by monitoring the level of expression of at least one of the polynucleotides described hereinabove. For example, downregulation in the level of RRN3 in the subject following treatment can be indicative of the positive effect of the treatment on the subject, *e.g.*, switching from a typical RRMS to a BMS course of multiple sclerosis.

As described above, a decrease above a predetermined threshold in the level of expression of the at least one gene following treating with the compound relative to the level of expression of the at least one gene prior to treating with the compound indicates that the compound is efficient for treating the subject.

As used herein the phrase "a decrease above a predetermined threshold" refers to a decrease in the level of expression in the cell of the subject following treating with the compound which is higher than a predetermined threshold such as a about 10 %, e.g., higher than about 20 %, e.g., higher than about 30 %, e.g., higher than about 40 %, e.g., higher than about 50 %, e.g., higher than about 60 %, higher than about 70 %, higher than about 80 %, higher than about 90 %, higher than about 2 times, higher than about three times, higher than about four time, higher than about five times, higher than about six times, higher than about seven times, higher than about eight times, higher than about nine times, higher than about 20 times, higher than about 50 times, higher than about 100 times, higher than about 200 times, higher than about 350, higher than about 500 times, higher than about 1000 times, or more relative to the level of expression prior to treating with the compound.

As described, an increase above a predetermined threshold in the level of expression of the at least one gene following treating with the compound relative to the level of expression of the at least one gene prior to treating with the compound indicates that the compound is not efficient for treating the subject.

As used herein the phrase "an increase above a predetermined threshold" refers to an increase in the level of expression in the cell of the subject following treating with the compound, which is higher than a predetermined threshold such as about 10 %, e.g., higher than about 20 %, e.g., higher than about 30 %, e.g., higher than about 40 %, e.g., higher than about 50 %, e.g., higher than about 60 %, higher than about 70 %, higher than about 80 %, higher than about 90 %, higher than about 2 times, higher than about three times, higher than about four time, higher than about five times, higher than about six times, higher than about seven times, higher than about eight times, higher than about nine times, higher than about 20 times, higher than about 50 times, higher than about 100 times, higher than about 200 times, higher than about 350, higher than about 500 times, higher than about 1000 times, or more relative to the level of expression of the at least one gene prior to treating with the compound.

As described, a level of expression of the at least one gene following treating with the compound which is identical or changed below a predetermined threshold as compared to prior to treating with the compound is indicative that the treatment is not efficient for treating the subject.

As used herein the phrase "changed below a predetermined threshold as compared to prior to treating with the compound" refers to an increase or a decrease in the level of expression in the cell of the subject following treating with the compound, which is lower than a predetermined threshold, such as lower than about 10 times, e.g., lower than about 9 times, e.g., lower than about 8 times, e.g., lower than about 7 times, e.g., lower than about 6 times, e.g., lower than about 5 times, e.g., lower than about 4 times, e.g., lower than about 3 times, e.g., lower than about 2 times, e.g., lower than about 90%, e.g., lower than about 80%, e.g., lower than about 70%, e.g., lower than about 60%, e.g., lower than about 50%, e.g., lower than about 40%, e.g., lower than about 30%, e.g., lower than about 20%, e.g., lower than about 10%, e.g., lower than about 9%, e.g., lower than about 8%, e.g., lower than about 7%, e.g., lower than about 6%, e.g., lower than about 5%, e.g., lower than about 4%, e.g., lower than about 3%, e.g., lower than about 2%, e.g., lower than about 1% relative to the level of expression of the at least one gene prior to treating with the compound.

Non-limiting examples of genes involved in the RNA polymerase I pathway which can be used according to the method of the invention include RRN3, LRPPRC, POLR1B, POLR1C, POLR1D, POLR2A, POLR2B, POLR2C, POLR2D, POLR2E, POLR2E, POLR2F, POLR2G, POLR2H, POLR2I, POLR2J, POLR2J2, MGC13098, POLR2K, POLR2L, POLR3B, POLR3C, POLR3D, POLR3E, POLR3F, POLR3G, POLR3K, POLRMT, POLRMT and POLS.

Sequence information regarding gene products (*i.e.*, RNA transcripts and polypeptide sequences) of the genes of RNA polymerase I pathway and of probes which can be used for detection thereof can be found according to the following access numbers:

Affymetrix ProbSet	***Representative Public ID / SEQ ID NO:***	***Representative polypeptide Public ID / SEQ ID NO:***	***Gene Symbol***	***Gene Title***
216902_s_at	AF001549 / 40; NM_018427 / 41	NP_060897/81	RRN3	RRN3 RNA polymerase I transcription factor homolog
211971_s_at	AI653608 / 42; NM_133259 / 43	NP_573566/82	LRPPRC	leucine-rich PPR-motif containing
220113_x_at	NM_019014 / 44	NP_001131076/ 83/NP_061887/1 20	POLR1B	polymerase (RNA) I polypeptide B, 128kDa

Affymetrix ProbSet	*Representative Public ID / SEQ ID NO:*	*Representative polypeptide Public ID / SEQ ID NO:*	*Gene Symbol*	*Gene Title*
207515_s_at	NM_004875 / 45	NP_976035/84	POLR1C	polymerase (RNA) I polypeptide C, 30kDa
209317_at	AF008442 / 46	NP_976035/85	POLR1C	polymerase (RNA) I polypeptide C, 30kDa
218258_at	NM_015972 / 47	NP_057056/86/ NP_689918/121	POLR1D	polymerase (RNA) I polypeptide D, 16kDa
202725_at	NM_000937 / 48	NP_000928/87	POLR2A	polymerase (RNA) II (DNA directed) polypeptide A, 220kDa
217420_s_at	M21610 / 49	NP_000928/88	POLR2A	polymerase (RNA) II (DNA directed) polypeptide A, 220kDa
201803_at	NM_000938 / 50	NP_000929/89	POLR2B	polymerase (RNA) II (DNA directed) polypeptide B, 140kDa
208996_s_at	BC000409 / 51	NP_116558/90	POLR2C	polymerase (RNA) II (DNA directed) polypeptide C, 33kDa
214263_x_at	AI192781 / 52	NP_116558/91	POLR2C	polymerase (RNA) II (DNA directed) polypeptide C, 33kDa
216282_x_at	AJ224143 / 53	NP_116558/92	POLR2C	polymerase (RNA) II (DNA directed) polypeptide C, 33kDa
203664_s_at	NM_004805 / 54	NP_004796/93	POLR2D	polymerase (RNA) II (DNA directed) polypeptide D
214144_at	BF432147 / 55	NP_004796/94	POLR2D	polymerase (RNA) II (DNA directed) polypeptide D
213887_s_at	AI554759 / 56	NP_002686/95	POLR2E	polymerase (RNA) II (DNA directed) polypeptide E, 25kDa
217854_s_at	BC004441 / 57	NP_002686/96	POLR2E	polymerase (RNA) II (DNA directed) polypeptide E, 25kDa
209511_at	BC003582 / 58	NP_068809/97	POLR2F	polymerase (RNA) II (DNA directed) polypeptide F
202306_at	NM_002696 / 59	NP_002687/98	POLR2G	polymerase (RNA) II (DNA directed) polypeptide G
209302_at	U37689 / 60	NP_006223/99	POLR2H	polymerase (RNA) II (DNA directed) polypeptide H
212955_s_at	AL037557 / 61	NP_006224/100	POLR2I	polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa
212782_x_at	BG335629 / 62	NP_006225/101	POLR2J	polymerase (RNA) II (DNA directed) polypeptide J, 13.3kDa
216242_x_at	AW402635 / 63	NP_116581/102	POLR2J2	DNA directed RNA polymerase II polypeptide J-related gene

Affymetrix ProbSet	*Representative Public ID / SEQ ID NO:*	*Representative polypeptide Public ID / SEQ ID NO:*	*Gene Symbol*	*Gene Title*
214740_at	BE676209 / 64	NP_001091084/ 103/NP_006225/ 122/NP_116581/ 125	POLR2J2 /// MGC13098	DNA directed RNA polymerase II polypeptide J-related gene /// hypothetical prote
202634_at	AL558030 / 65	NP_005025/104	POLR2K	polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa
202635_s_at	NM_005034 / 66	NP_005025/105	POLR2K	polymerase (RNA) II (DNA directed) polypeptide K, 7.0kDa
202586_at	AA772747 / 67	NP_066951/106	POLR2L	polymerase (RNA) II (DNA directed) polypeptide L, 7.6kDa
211730_s_at	BC005903 / 68	NP_066951/107	POLR2L	polymerase (RNA) II (DNA directed) polypeptide L, 7.6kDa /// polymerase (RNA) II
219459_at	NM_018082 / 69	NP_001154180/ 108/NP_060552/ 123	POLR3B	polymerase (RNA) III (DNA directed) polypeptide B
209382_at	U93867 / 70	NP_006459/109	POLR3C	polymerase (RNA) III (DNA directed) polypeptide C (62kD)
210573_s_at	BC004424 / 71	NP_006459/110	POLR3C	polymerase (RNA) III (DNA directed) polypeptide C (62kD)
208361_s_at	NM_001722 / 72	NP_001713/111	POLR3D	polymerase (RNA) III (DNA directed) polypeptide D, 44kDa
218016_s_at	NM_018119 / 73	NP_060589/112	POLR3E	polymerase (RNA) III (DNA directed) polypeptide E (80kD)
205218_at	NM_006466 / 74	NP_006457/113	POLR3F	polymerase (RNA) III (DNA directed) polypeptide F, 39 kDa
206653_at	BF062139 / 75	NP_006458/114	POLR3G	Polymerase (RNA) III (DNA directed) polypeptide G (32kD)
206654_s_at	NM_006467 / 76	NP_006458/115	POLR3G	polymerase (RNA) III (DNA directed) polypeptide G (32kD)
218866_s_at	AF060223 / 77	NP_057394/116	POLR3K	polymerase (RNA) III (DNA directed) polypeptide K, 12.3 kDa
203782_s_at	NM_005035 / 78	NP_005026/117	POLRMT	polymerase (RNA) mitochondrial (DNA directed)
203783_x_at	BF057617 / 79	NP_005026/118	POLRMT	polymerase (RNA) mitochondrial (DNA directed)
202466_at	NM_006999 / 80	NP_001165276/ 119/NP_001165 277/124/NP_008 930/126	PAPD7	polymerase (DNA directed) sigma

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway is selected from the group consisting of POLR1D, LRPPRC, RRN3 and NCL.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway is RRN3.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway is LRPPRC.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway is POLR1D.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway comprises RRN3 and POLR1D.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway comprises RRN3 and LRPPRC.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway comprises POLR1D and LRPPRC.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway comprises RRN3, LRPPRC and POLR1D.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway is RRN3 and NCL.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway is LRPPRC and NCL.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway is POLR1D and NCL.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway comprises RRN3, POLR1D and NCL.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway comprises RRN3, LRPPRC and NCL.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway comprises POLR1D, LRPPRC and NCL.

According to some embodiments of the invention, the at least one gene involved in the RNA polymerase 1 pathway comprises RRN3, LRPPRC, POLR1D and NCL.

Qualifying the compound as being suitable for treating the autoimmune disease in the subject can be also performed by an *in-vitro* method.

As used herein the term "about" refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994);

"Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader.

EXAMPLE 1

Chemical Syntheses and characterization of POL1 inhibitors

Materials and Methods:

2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-oxo-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (also referred to herein interchangeably as CX-5461, POL1-I, RAM-0 or Compound **1**; see, chemical structure as presented in FIG. 3A and Table 1 below) was synthesized according to known procedures (see, for example, U.S. Patent Application Publication No. 2009/0093465 and WO 2012/123938).

All of the reagents were obtained from Sigma Aldrich.

^{1}H NMR analyses were performed using a Bruker Avance DPX-400 Ultra shield or alternatively Bruker Avance DMX-500. All the chemical shifts are referenced to the residual solvent signal.

All MS analyses were performed on a Thermo Scientific LCQ Fleet mass spectrometer with an ESI source. All the spectra were recorded in the positive mode (unless mentioned otherwise) and were analyzed by the Thermo Scientific Xcalibur software.

General Synthetic Procedure:

POL1-I (CX-5461, Compound **1**) is refluxed in phosphoryl chloride for several hours to afford the chlorinated analog 5-chloro-2-(4-methyl-1,4-diazepan-1-yl)-6-(((5-methylpyrazin-2-yl)methyl)carbamoyl)benzo[4,5]thiazolo[3,2-a][1,8]naphthyridin-12-ium (also referred to herein interchangeably as POL1-I/1, RAM-2, RAM Cl or Compound **2**; see, Figure 3A and Table 1 below).

The phosphoryl chloride is thereafter removed by evaporation and the crude product **2** is dissolved or suspended in an alcoholic solvent (e.g., methanol or ethanol). An amine or thiol compound, as desired, is then added and the resulting reaction mixture is stirred, possibly under reflux, until reaction completion. The solvent is then removed by evaporation and the resulting crude is purified, typically by preparative HPLC.

The chemical structure of the obtained product was verified by MS [ESI] and/or ^{1}H NMR, as detailed hereinbelow.

An exemplary synthetic pathway of exemplary compounds according to some embodiments of the present invention is presented in FIG. 3.

5-chloro-2-(4-methyl-1,4-diazepan-1-yl)-6-(((5-methylpyrazin-2-yl)methyl) carbamoyl)benzo[4,5]thiazolo[3,2-a][1,8]naphthyridin-12-ium (POL1-I/1; Compound 2):

MS [ESI]: calcd. 532.1 found [M+H] 532.2.

Preparation of 2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-thioxo-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (referred to herein interchangeably as POL1-I/2; RAM-3; or Compound 3):

2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-oxo-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (Compound **1**) (100 mg)

was suspended in 3 mL of phosphoryl chloride, and the obtained mixture was refluxed for 3 hours. The phosphoryl chloride was thereafter removed by evaporation and the obtained crude product was dissolved in MeOH. Sodium hydrosulfide was then added (100 mg) and the resulting solution was stirred for 5 minutes. The obtained compound **3** was purified by preparative HPLC to yield 82.4 mg 80 % yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 13.02 (t, J = 5.58 Hz, 1H), 9.50 (d, J = 7.01 Hz, 1H), 9.23 (d, J = 9.38 Hz, 1H), 8.64 (d, J = 1.13 Hz, 1H), 8.43 (s, 1H), 7.75 (m, 1H), 7.45 (m, 2H), 6.82 (d, J = 9.42 Hz, 1H), 4.89 (d, J = 5.61 Hz, 2H), 4.17-3.64 (m, 4H), 2.88-2.79 (m, 2H), 2.64-2.57 (m, 2H), 2.56-2.53 (s, 3H), 2.39 (s, 3H), 2.11 (m, 2H) ppm.

MS [ESI]: calcd. 530.6 found [M+H] 530.2

Preparation of 5-imino-2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (referred to herein, interchangeably, as POL1-I/4; RAM-1 or Compound 4):

2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-oxo-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (Compound **1**) (100 mg) was suspended in 3 mL of phosphoryl chloride, and the obtained mixture was refluxed for 3 hours. The phosphoryl chloride was thereafter removed by evaporation and the obtained crude product was dissolved in MeOH. Gaseous ammonia was then bubbled into the methanol for 1 minute and the resulting solution was stirred for 5 minutes. The obtained compound **4** was purified by preparative HPLC to yield 64 mg (64 % yield).

^{1}H NMR (400 MHz, $CDCl_3$ ppm): 9.28 (d, J = 8.21 Hz, 1H), 8.58 (m, 1H), 8.44 (m, 1H), 8.04 (d, J = 8.72 Hz, 1H), 7.64 (dd, J = 7.62, 1.35 Hz, 1H), 7.34 (m, 2H), 6.68 (d, J = 9.23 Hz, 1H), 4.86 (s, 2H), 3.98-3.74 (m, 4H), 2.84 (m, 2H), 2.66-2.60 (m, 2H), 2.54 (s, 3H), 2.41 (s, 3H), 2.12 (m, 2H),

MS [ESI]: calcd. 513.2 found [M+H] 513.3.

Preparation of (E/Z)-2-(4-methyl-1,4-diazepan-1-yl)-5-(methylimino)-N-((5-methylpyrazin-2-yl)methyl)-5H-benzo[4,5]thiazolo[3,2-a] [1,8]naphthyridine-6-carboxamide (referred to herein, interchangeably, as POL1-I/3; or Compound 5):

2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-oxo-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (Compound **1**) (100 mg) was suspended in 3 mL of phosphoryl chloride, and the obtained mixture was refluxed for 3 hours. The phosphoryl chloride was thereafter removed by evaporation and the obtained crude product was dissolved in MeOH. Methylamine was then added (3 mL) and the resulting solution was stirred for 5 minutes. The obtained compound **5** was purified by preparative HPLC to yield 74 mg (73 % yield).

MS [ESI]: calcd. 527.6 found [M+H] 527.3

Preparation of Compounds 6 and 7 (see, Table 1) was performed similarly to Compound **5**, using propylamine and isopropyelamine, respectively, and yielding 82 mg (76 % yield) and 85 mg (79 % yield), respectively.

MS [ESI]: calcd. 555.7 found [M+H] 555.4, MS [ESI]: calcd. 555.7 found [M+H] 555.3

Preparation of Compound 8 (see, Table 1):

2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-oxo-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (Compound **1**) (100 mg) was suspended in 3 mL of phosphoryl chloride, and the obtained mixture was refluxed for 3 hours. The phosphoryl chloride was thereafter removed by evaporation and the obtained crude product was dissolved in MeOH. 3 mL of Triethylamine and 100 mg of [Methoxylamine hydrogen chloride] were then added and the resulting solution was stirred for 5 minutes. The obtained compound **5** was purified by preparative HPLC to yield 34 mg (32 % yield).

MS [ESI]: calcd. 543.6 found [M+H] 543.2

Preparation of Compound 9 (see, Table 1):

2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-oxo-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (Compound **1**) (100 mg) was suspended in 3 mL of phosphoryl chloride, and the obtained mixture was refluxed for 3 hours. The phosphoryl chloride was thereafter removed by evaporation and the

obtained crude product was dissolved in MeOH. 100 mg of urea were added and the resulting solution was left to stir for 4 hours. The title compound was purified by preparative HPLC to yield 78 mg (75% yield)

MS [ESI]: calcd. 537.2 found [M+H] 537.6

Preparation of (E)-2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-(phenylimino)-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (Compound 10; See, Table 1 and Figure 3B):

2-(4-methyl-1,4-diazepan-1-yl)-N-((5-methylpyrazin-2-yl)methyl)-5-oxo-5H-benzo[4,5]thiazolo[3,2-a][1,8]naphthyridine-6-carboxamide (100 mg) was suspended in 3 mL of phosphoryl chloride, and the resulting mixture was refluxed for 3 hours. The phosphoryl chloride was thereafter removed by evaporation and the resulting crude product was dissolved in MeOH. Aniline was then added (2 mL) and the resulting solution was stirred for 5 minutes. The compound was purified by preparative HPLC to yield 56 mg (50 % yield).

MS [ESI]: calcd. 589.7 found [M+H] 589.4

Compound 11 (see, Table 1) was prepared similarly to Compound **10**, using 3-fluoroaniline instead of aniline.

MS [ESI]: calcd. 607.2 found [M+H] 607.5

Solubility:

The solubility of Compounds **1** and **10** was determined by dissolving 50 mg of the tested compound in 0.5 mL of mQ water, at room temperature.

Compound **10** immediately dissolved in the aqueous solution, whereby Compound **1** dissolved only in a pH 4.5 buffered solution after vigorous stirring for 30 minutes.

EXAMPLE 2

Cell viability assay

Cell viability was assessed by the 2,3 bis [2-Methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) assay (Biological Industries, Kibbutz Beit Hemeek, Israel), which measures the reduction of a tetrazolium component (XTT) into soluble formazan product by the mitochondria of viable cells. The intensity of the dye obtained is proportional to the number of metabolic active cells. On the day of

measurement, cells were washed and XTT was added according to the manufacturer's instructions. Plates were incubated at 37 °C for 2–5 hours. The absorbance was read at 450 nm.

Mouse splenocytes were removed and spleenocytes were plated (250,000 cell/well) in DMEM + 10 % FCS+P/S+Q and 10 mg/ml Phytohaemaglutinin (PHA) , in the presence of elevated concentrations (50-400 nM) of RAM-0 (Compound **1**), RAM-1 (Compound **4**), RAM-2 (Compound **2**) or RAM-3 (Compound **3**) for 72 hours. Cells cultured without PHA served as control. Control mice at zero are mice splenocytes with PHA stimulation.

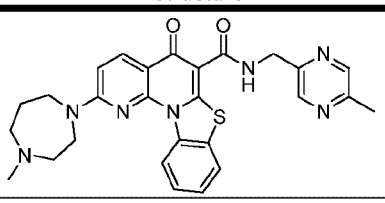
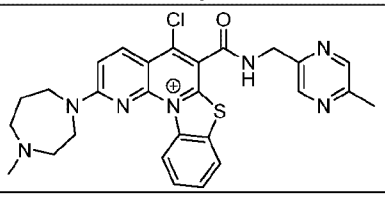
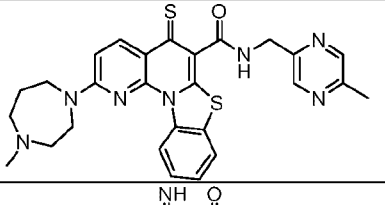
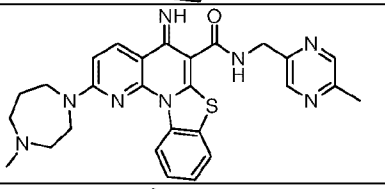
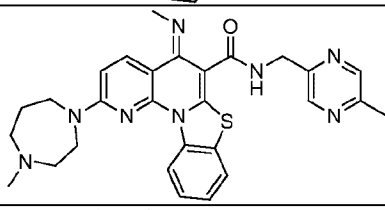
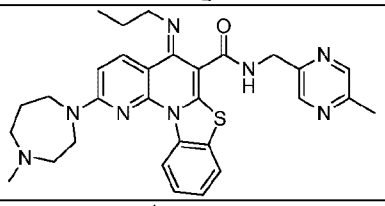
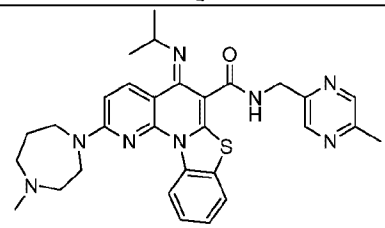
Following incubation, cell viability was determined by XTT assay, as described above. RNA samples from similar cultures were also prepared.

The obtained data is presented in FIG. 4A (for Compound **1** compared with Compounds 2 and 4), in FIG. 4B (for Compound **1** compared with Compound **3**), and FIG. 4C (for Compound **1** compared with Compound **10**).

As shown, PHA stimulation resulted in substantial increase in proliferation as compared to control. As shown in FIG. 4A, Compound **4** (RAM-1) exhibited a dose response curve similar to RAM-0 (Compound **1**), while RAM-2 (Compound **2**) showed no substantial effect. As shown in FIG. 4B, RAM-3 (Compound **3**) was 6-folds more effective in suppressing proliferation compared to Compound **1** (RAM-0), suggesting much lower therapeutic doses of this compound. As shown in FIG. 4C, Compound **10** exhibits an improved performance is suppressing proliferation compared to Compound **1**. As indicated below, this compound was also found to feature a larger therapeutic window and improved solubility and pharmacokinetic properties, compared to Compound **1**.

IC_{50} values as determined in these assays for Compounds **1-4** and **10,** and for all other tested compounds, are presented in Table 1 below.

Table 1

No.	Structure	Mw	Name	XTT
1		513.2	CX-5461; RAM-0; POL1-I	IC_{50} = 50 nM
2		532.7	RAM-1; POL1-I/1	No Effect
3		529.6	RAM-3; POL1-I/2	IC_{50} = 20 nM*
4		512.6	RAM-1; POL1-I/4	IC_{50} = 50 nM
5		526.2	RAM Me	IC_{50} = 50 nM
6		554.2	RAM Pr	IC_{50} = 50 nM
7		554.3	RAM iPr	IC_{50} = 50 nM

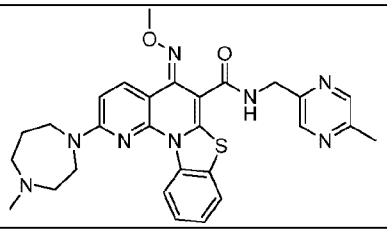
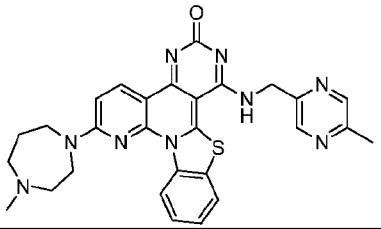
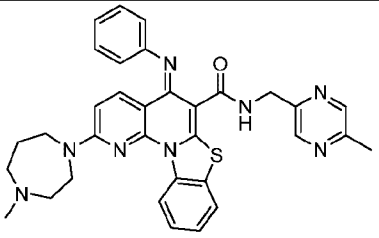
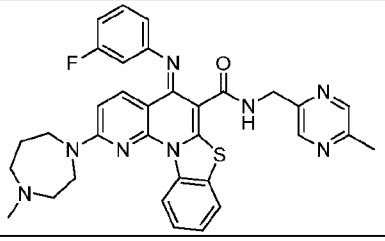
8		542.2	RAM MeAM	IC_{50} = 50 nM
9		555.2	RAM urea	No Effect
10		588.2	RAM An	IC_{50} = 40 nM
11		606.2	RAM 3Fan	ND

Table 1 (Cont.)

* Compound **2**, although effective, was found to decompose in some of the experiments conducted.

EXAMPLE 3

In vivo assays

Experimental methods:

Induction of MOG35-55 EAE (Prevention Model): EAE (Experimental Autoimmune Encephalomyelitis) was induced in 8 week old female C57BL/6J mice (15–20 g, Harlan laboratories, Rehovot, Israel) by immunization with an emulsion containing 300 μg of purified myelin oligodendrocyte glycoprotein (MOG) peptide (MEVGWYRSPFSRVVHLYRNGK, corresponding to residues 35–55; obtained from (Difco, Detroit, MI) in saline and an equal volume of complete Freund's adjuvant containing 5 mg H37RA (Difco, Detroit, MI). 0.2 ml of the inoculum was injected subcutaneously. In addition, 300 ng of Bordetella pertusis toxin (Sigma) in 0.2 ml

saline was injected intraperitoneally at the day of induction and two days later.

Oral gavages with the tested compound, at various concentrations ranging from 3 mg/kg – 30 mg/kg in PBS or 50 mM NaH_2PO_4 (PH 4.5), or with vehicle only, were initiated at day of immunization. Mice were monitored daily for clinical signs of EAE, scored as: 1, flaccid tail; 2, forelimb weakness and poor righting ability; 3, hind limb paralysis; 4, quadriplegia; 5, moribund. Animal reaching a score of 4 were scarified using CO_2.

Treatment was stopped once 30 % of the vehicle-treated animals scored 1 on the EAE score. The experiment was terminated after 28 days.

Toxicity: The lethal dose for 50 % of animals (LD_{50}), was determined for the EAE model, and was estimated in a continuous administration model. The animals were evaluated for signs of acute toxicity and survival during the entire administration period in the EAE model. Various concentrations were evaluated for efficacy, and when 50 % mortality was observed in a specific concentration, this concentration was determined as the LD_{50}.

The therapeutic index (LD_{50}/ED_{50}), which is also referred to herein as Safety Margin (SM), was then determined based on the EAE model. ED_{50} is the minimum effective dose observed for 50 % of the tested animals.

Bioavailability: Determination of the level of the tested compound in serum was done following oral gavage. Blood samples (0.5 mL) were collected and immediately centrifuged at 5,000 rpm for 10 minutes. The serum was separated and stored at -20 °C until fluoprometric analysis by Tecan SpectraFluor, based on the specific excitation/emission values of the tested compound was conducted. The pharmacokinetic parameters including serum maximum concentration (Cmax), the time needed to reach Cmax (Tmax), and half-life (T1/2), were calculated to evaluate oral bioavailability. The serum concentration after oral gavages was calculated according to a calibration curve for each compound in the serum.

The Pharmacokinetic properties of compound **10** were quantified by the LCMS method described below. The concentration of Compound **10** was calculated with a calibration curve. The serum was prepared for analysis using protein precipitation. 15 µL of water were added to 15 µL of serum and a H_2O: MeOH: $CHCl_3$ (90:120:30) solution was added. The supernatant was subjected to LCMS analysis equipped with a

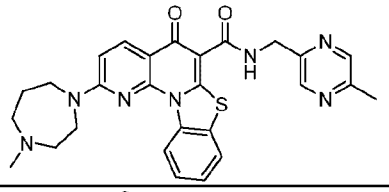
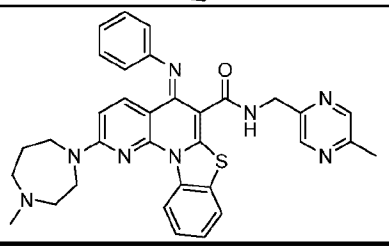
PHENOMENEX ® C-18 RP column.

Statistical analysis: All statistical analyses to evaluate differences between groups are performed by T-test and p value <0.05 is considered significant.

Experimental Results:

Table 2 below presents comparative data for the effective and toxic doses as determined in the EAE prevention model assay described hereinabove, as determined for Compounds **1** and **10**.

Table 2

No.	Structure	EAE		
		ED_{50}	LD_{50}	SM
1 CX-5461; RAM-0; POL1-I		12.5 mg/kg	25 mg/kg	2
10 RAM An		3 mg/kg	30 mg/kg	10

Compounds **2** and **9** were not tested; Compounds 3-7 were found relatively toxic during these preliminary studies.

It is shown in Table 2 that Compound **10** exhibits a substantially superior therapeutic index compared to CX-5641 (Compound **1**).

FIG. 5 presents plots showing the effect of various concentrations of Compound **10** (RAM-An) on the EAE clinical score.

FIGs. 6A-B present the data obtained in the bioavailability assay for Compounds **1** and **10**. As shown therein, Compound **10** exhibits a more favorable pharmacokinetic compound to Compound **1**, as reflected by the higher Cmax, the lower Tmax, and the faster clearance.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations

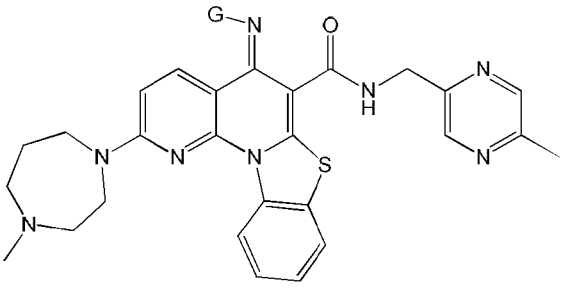
will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

Citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

Date Reçue/Date Received 2021-04-30

WHAT IS CLAIMED IS:

1. A compound represented by Formula Ib:

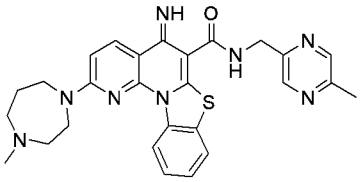


Formula Ib,

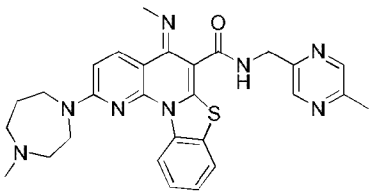
wherein G is selected from the group consisting of hydrogen, an alkyl having 1 to 10 carbon atoms, a cycloalkyl which is an all-carbon monocyclic ring or fused rings, an aryl which is an all-carbon monocyclic ring or fused polycyclic rings having a completely conjugated pi-electron system, and an alkoxy having 1 to 10 carbon atoms.

2. The compound of claim 1, wherein G is said aryl.

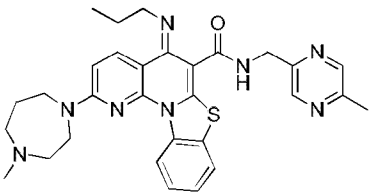
3. The compound of claim 1, being selected from:



Compound **4**

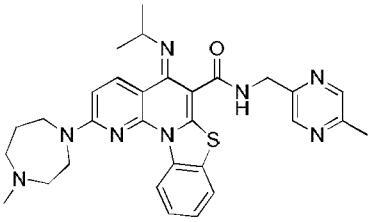


Compound **5**

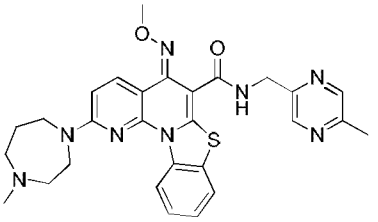


Compound **6**

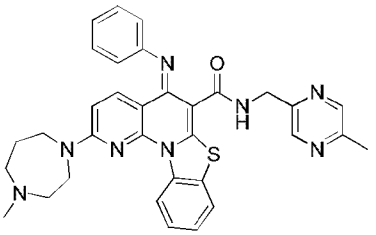
Date Reçue/Date Received 2021-04-30



Compound **7**

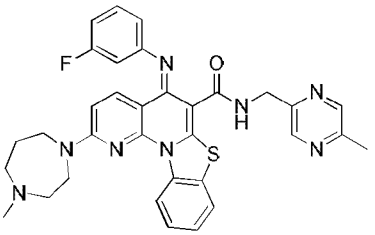


Compound **8**



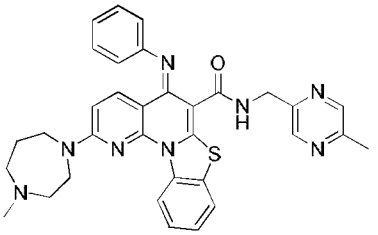
Compound **10**

and



Compound **11**.

4. The compound of claim 1, being:



Compound **10**.

5. The compound of any one of claims 1-4, for use in the treatment of an autoimmune disease.

6. The compound for use of claim 5, wherein the autoimmune disease is multiple sclerosis.

7. The compound for use of claim 6, wherein said multiple sclerosis is a relapsing-remitting multiple sclerosis (RRMS) or benign multiple sclerosis (BMS).

8. The compound for use of claim 7, wherein treating said multiple sclerosis comprises changing the course of the disease from said RRMS to BMS.

9. The compound for use of claim 5, wherein the autoimmune disease is treatable by inhibiting an activity of RNA Polymerase I.

10. The compound of any one of claims 1-4, for use in the manufacture of a medicament for treating an autoimmune disease.

11. The compound for use of claim 10, wherein the autoimmune disease is multiple sclerosis.

12. The compound for use of claim 11, wherein said multiple sclerosis is a relapsing-remitting multiple sclerosis (RRMS) or benign multiple sclerosis (BMS).

13. The compound for use of claim 12, wherein treating said multiple sclerosis comprises changing the course of the disease from said RRMS to BMS.

14. The compound for use of claim 10, wherein the autoimmune disease is treatable by inhibiting an activity of RNA Polymerase I.

15. The compound of any one of claims 1-4, for use in the treatment of a proliferative disease or disorder.

16. The compound for use of claim 15, wherein said proliferative disease or disorder is treatable by inhibiting an activity of a protein kinase.

17. The compound of any one of claims 1-4, for use in the manufacture of a medicament for the treatment of a proliferative disease or disorder.

Date Reçue/Date Received 2021-04-30

18. The compound for use of claim 17, wherein said proliferative disease or disorder is treatable by inhibiting an activity of a protein kinase.

19. The compound of any one of claims 1-4, for use in inhibiting an activity of RNA Polymerase I and/or for treating a disease or disorder treatable by inhibiting an activity of RNA Polymerase I.

20. The compound of any one of claims 1-4, for use in the manufacture of a medicament for inhibiting an activity of RNA Polymerase I and/or for treating a disease or disorder treatable by inhibiting an activity of RNA Polymerase I.

21. A method of inhibiting an activity of RNA Polymerase I, the method comprising contacting the RNA Polymerase I with an effective amount of a compound of any one of claims 1-4, wherein said contacting is effected *in vitro*.

22. The compound of any one of claims 1-4, for use in treating a disease treatable by inhibiting an activity of RNA Polymerase I.

23. The compound of any one of claims 1-4, for use in inhibiting an activity of a protein kinase and/or for treating a disease or disorder treatable by inhibiting an activity of a protein kinase.

24. The compound of any one of claims 1-4, for use in the manufacture of a medicament for inhibiting an activity of a protein kinase and/or for treating a disease or disorder treatable by inhibiting an activity of a protein kinase.

25. A method of inhibiting an activity of a protein kinase, the method comprising contacting the protein kinase with an effective amount of a compound of any one of claims 1-4, wherein said contacting is effected *in vitro*.

Date Reçue/Date Received 2021-04-30

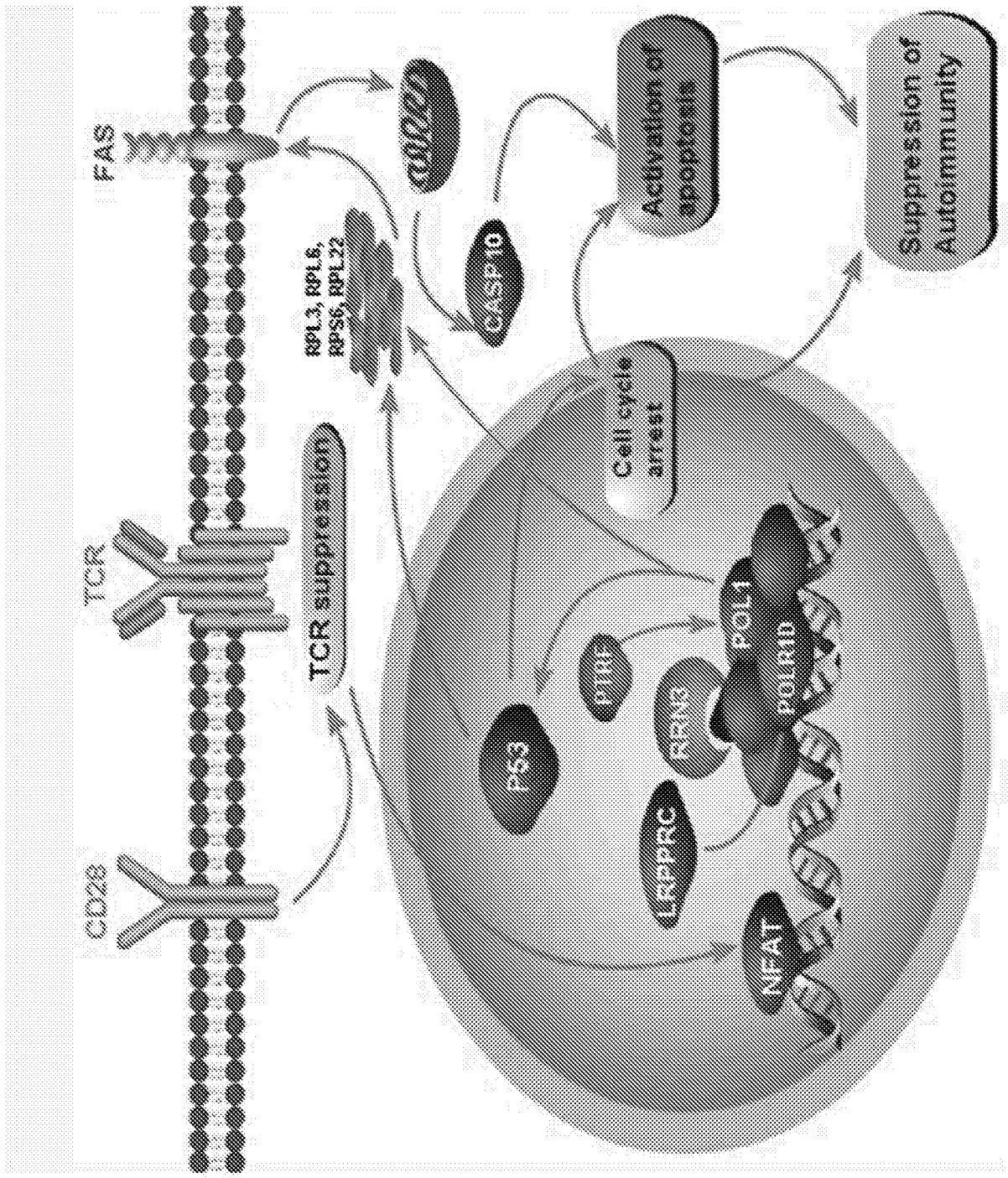


FIG. 1 (Background Art)

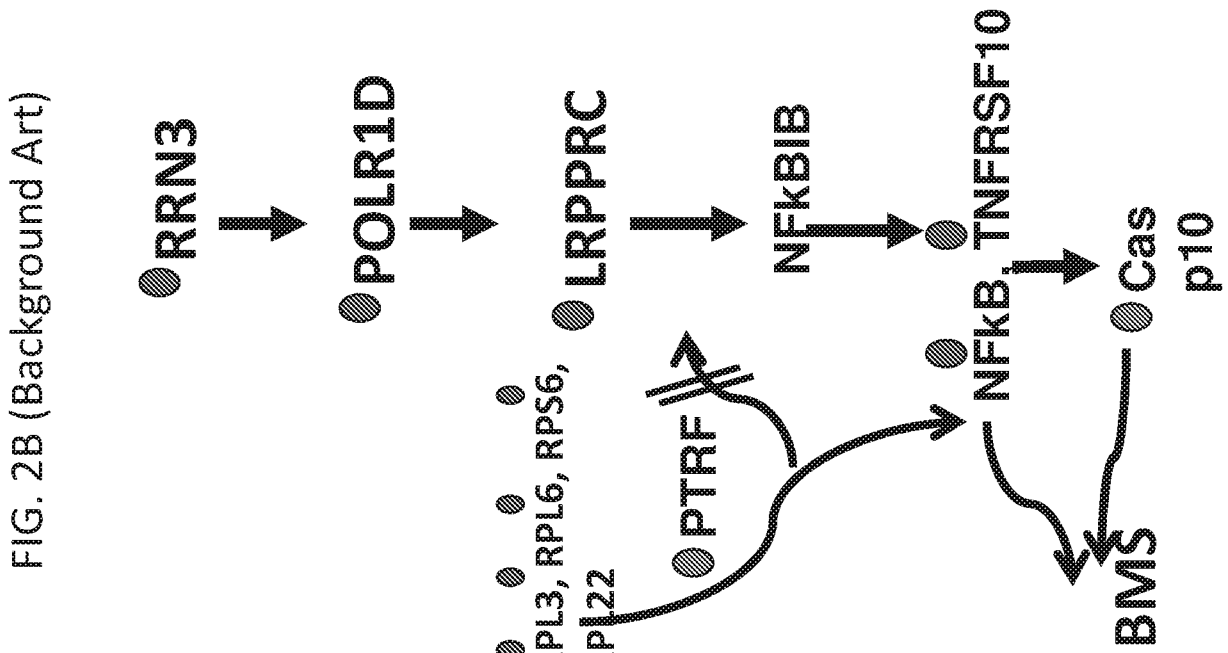


FIG. 2B (Background Art)

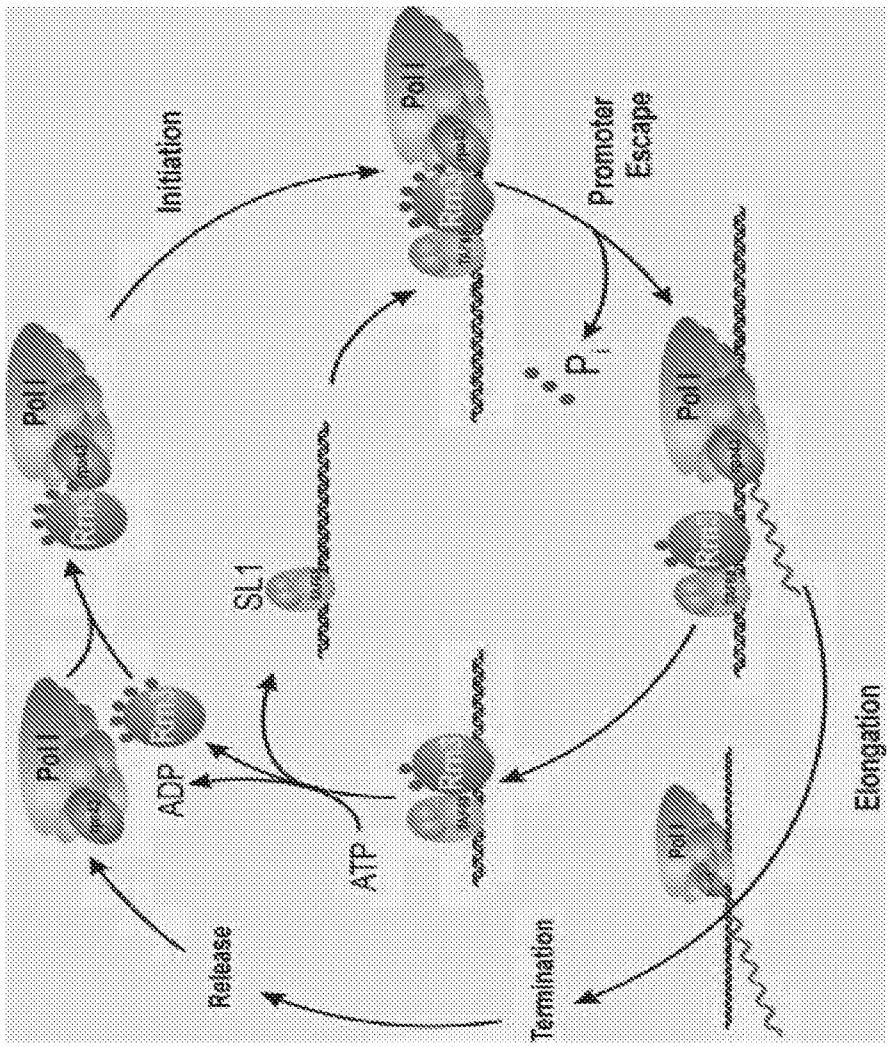


FIG. 2A (Background Art)

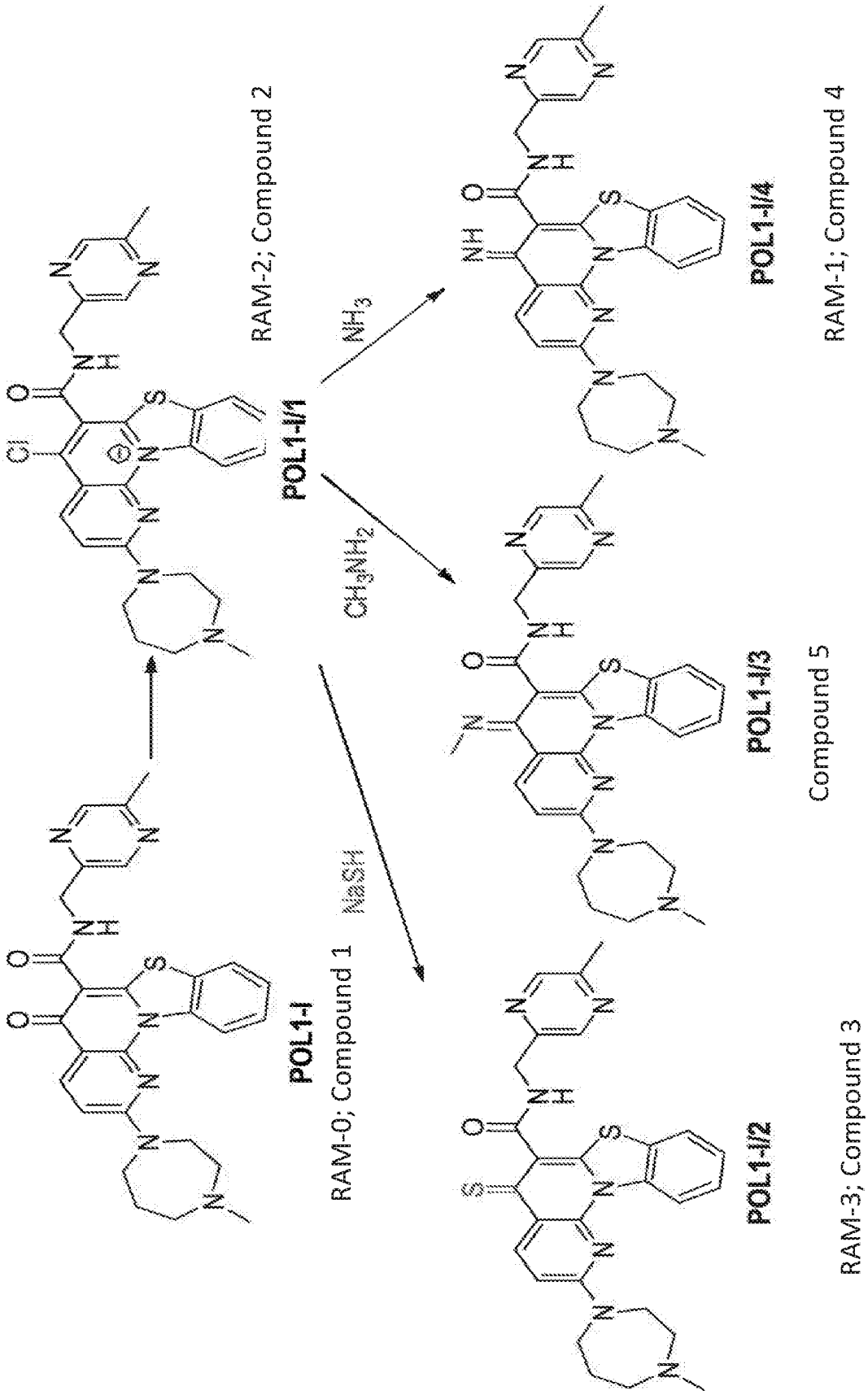

RAM-2; Compound 2
POL1-I/1
NH_3
POL1-I/4
RAM-1; Compound 4
CH_3NH_2
POL1-I/3
Compound 5
POL1-I
RAM-0; Compound 1
NaSH
POL1-I/2
RAM-3; Compound 3

FIG. 3A

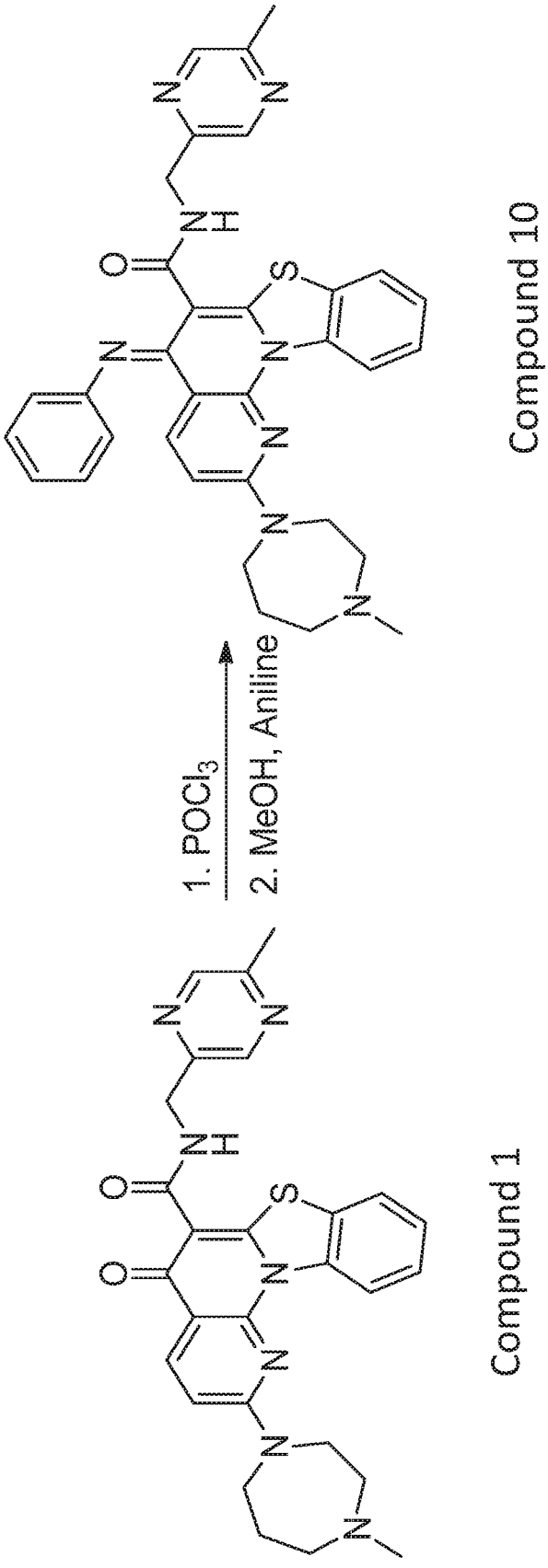


FIG. 3B

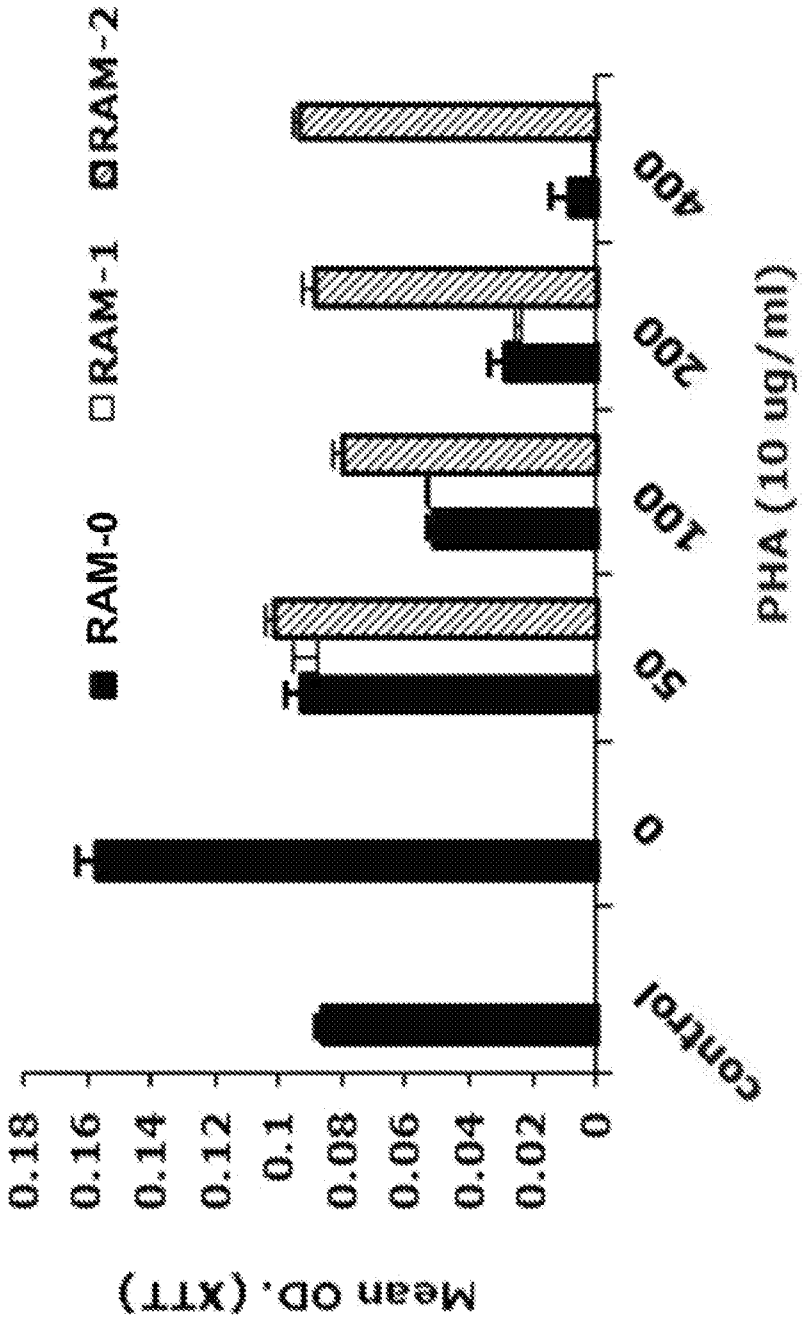

RAM-0
RAM-1
RAM-2
Mean OD. (XTT)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
control
0
50
100
200
400
PHA (10 ug/ml)

FIG. 4A

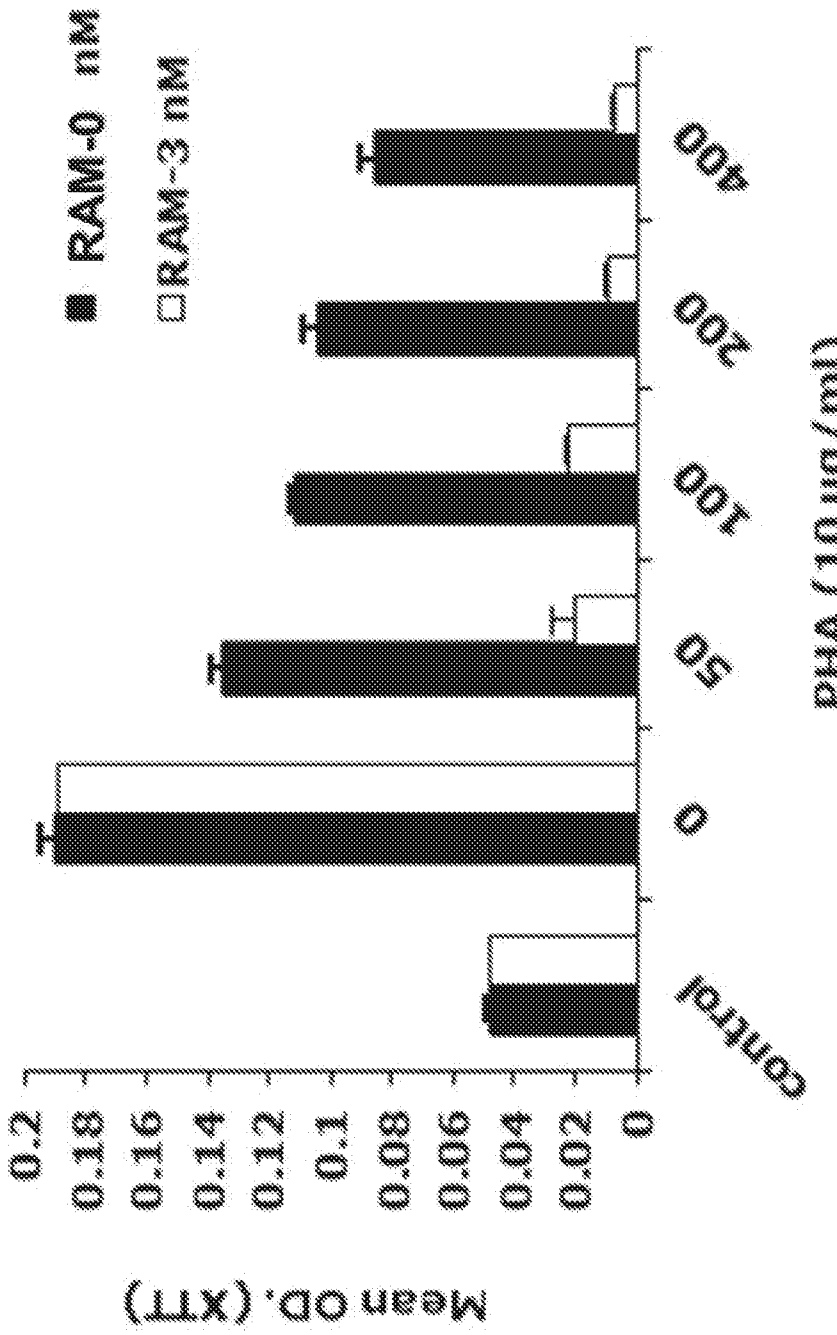

RAM-0 nM
RAM-3 nM
Mean OD. (XTT)
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Control
0
50
100
200
400
PHA (10 ug/ml)

FIG. 4B

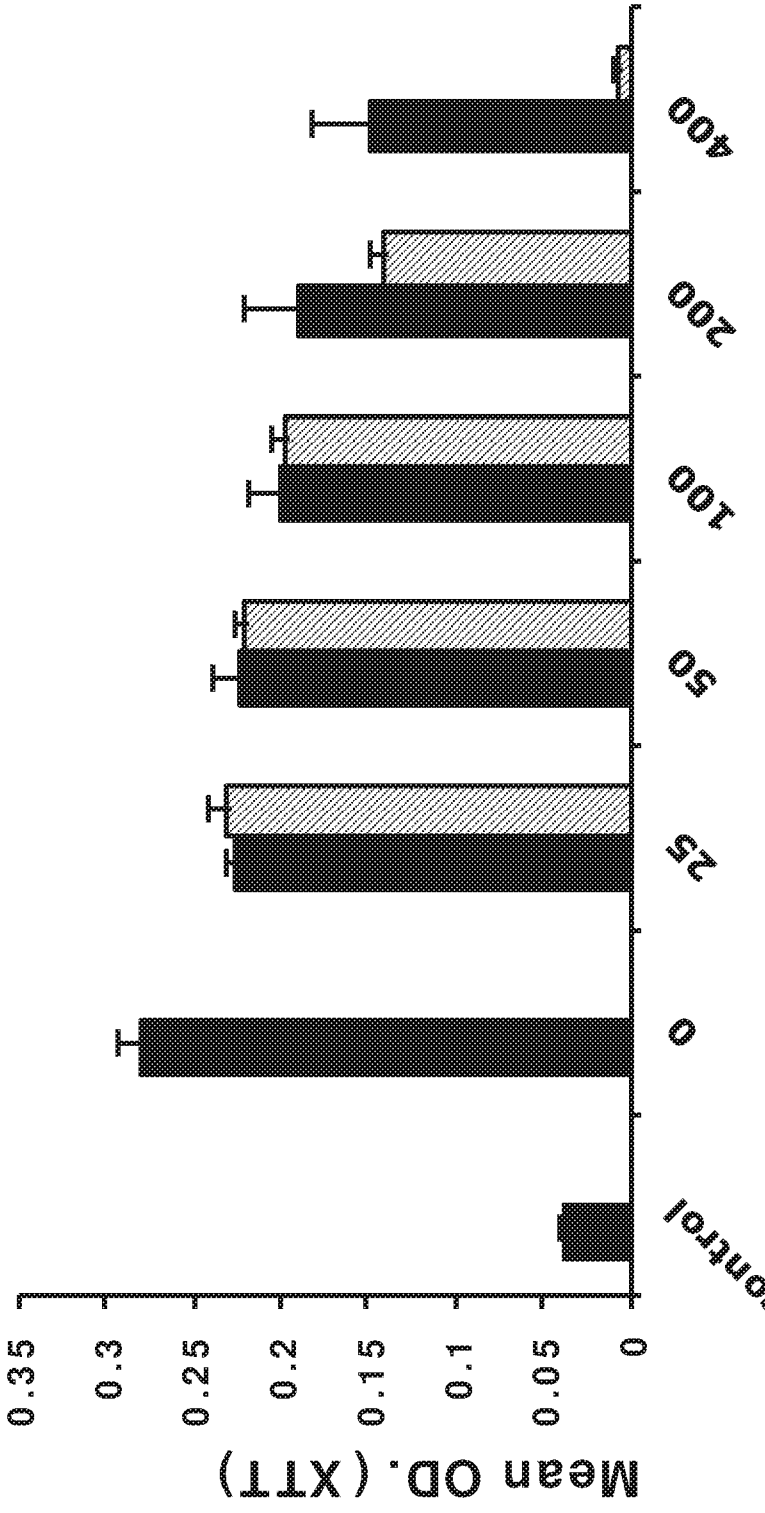

Mean OD. (XTT)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
control
0
25
50
100
200
400
PHA (10 ug/ ml)
CX-5461
RAM An

FIG. 4C

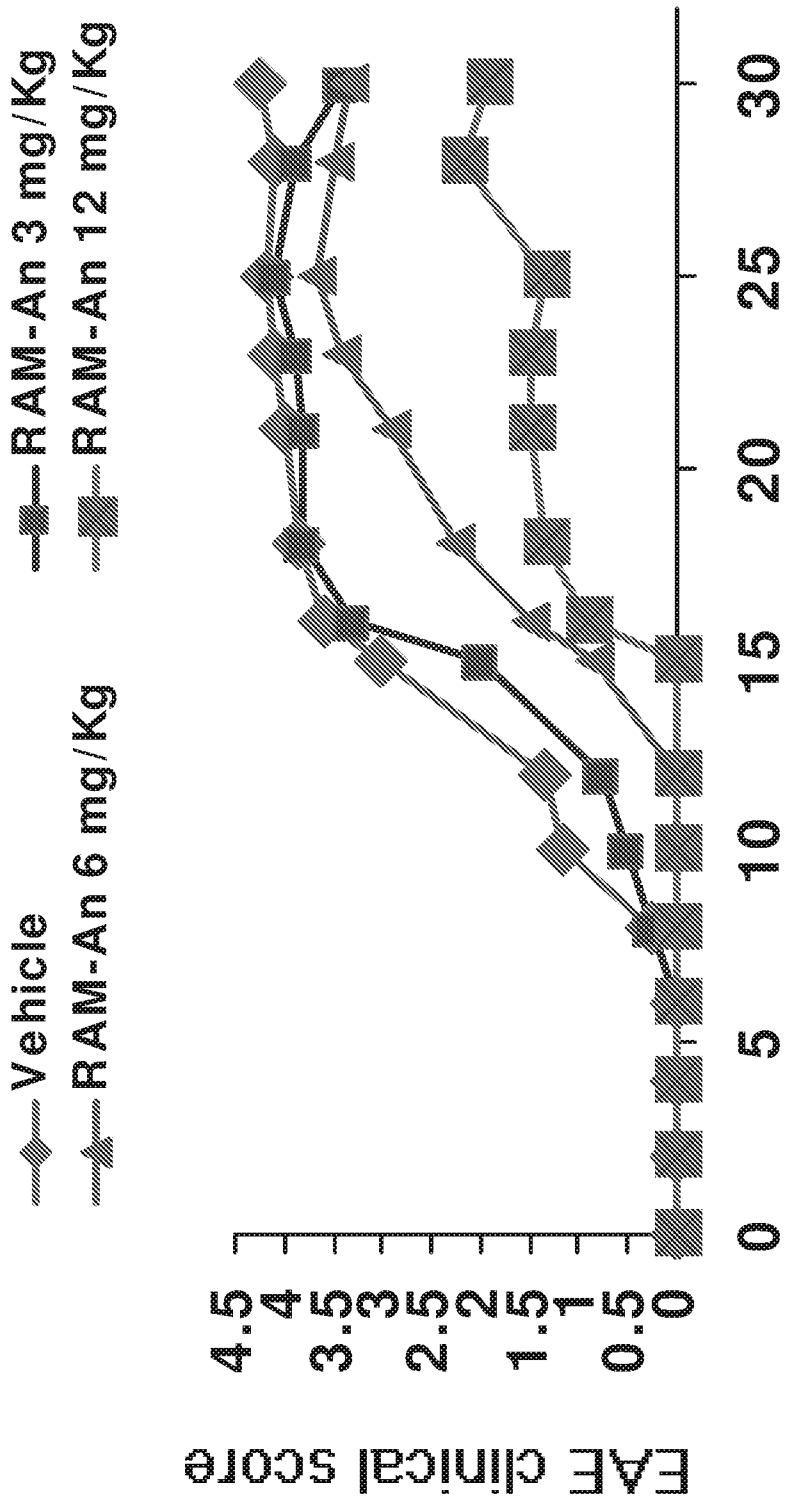

Vehicle
RAM-An 6 mg/Kg
RAM-An 3 mg/Kg
RAM-An 12 mg/Kg
EAE clinical score
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
30
Days post immunization

FIG. 5

FIG. 6A

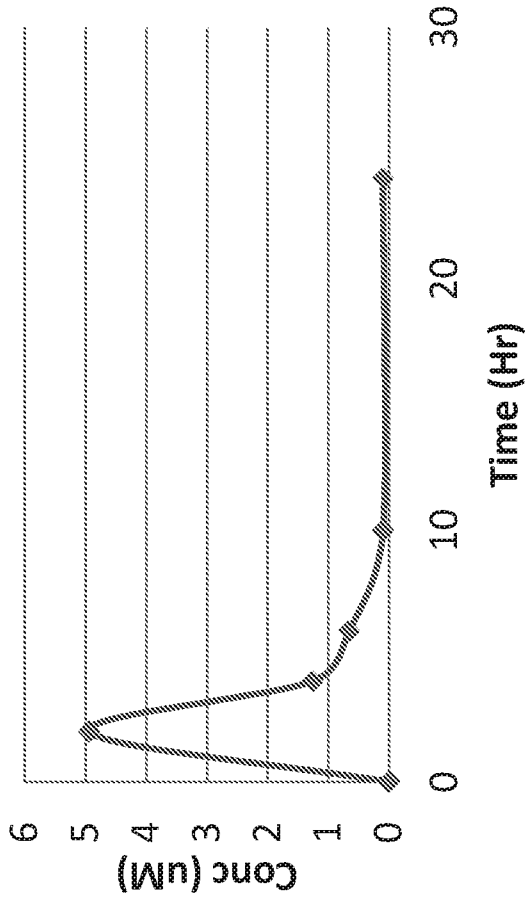
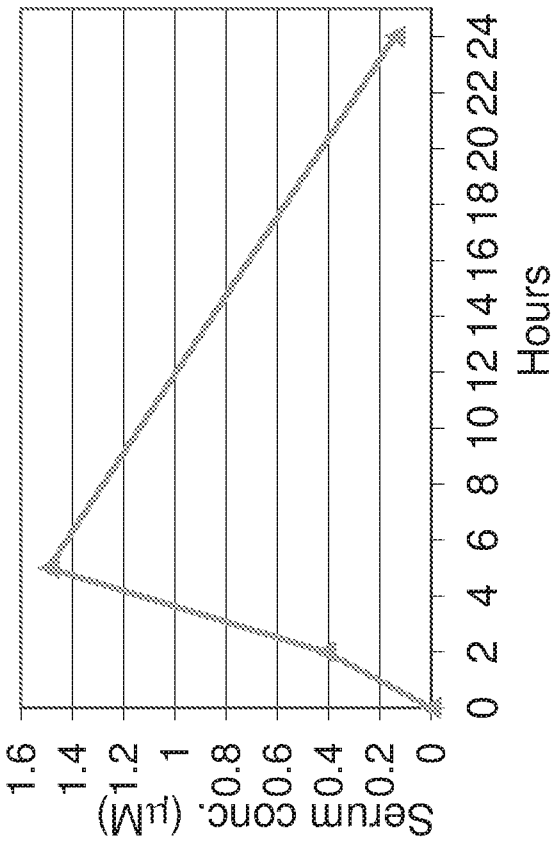
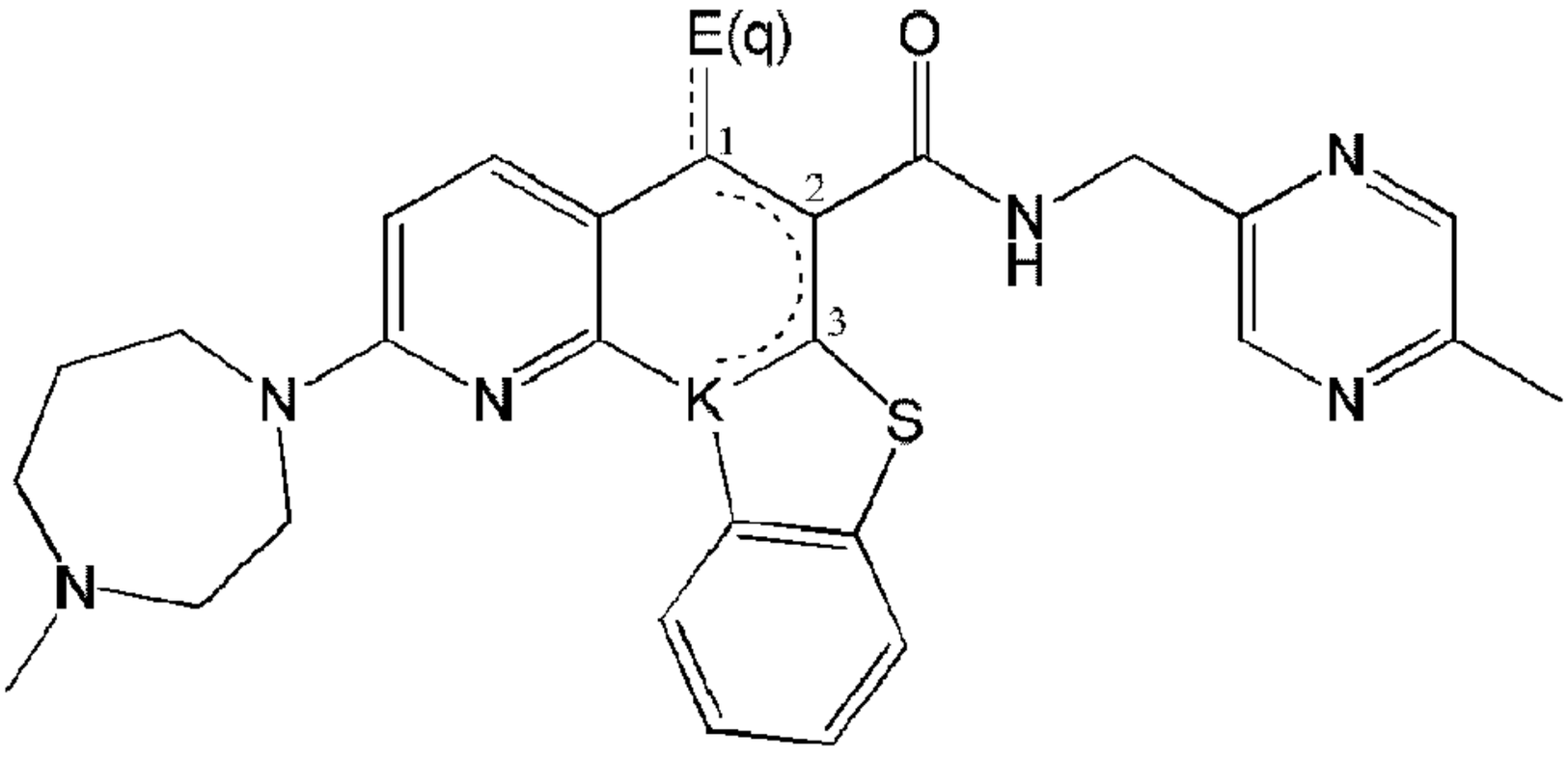

Conc (uM)
0
1
2
3
4
5
6
0
10
20
30
Time (Hr)

FIG. 6B


Serum conc. (μM)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0 2 4 6 8 10 12 14 16 18 20 22 24
Hours



Formula I