

6-(6-(((1R,2R,3S,5S)-2-FLUORO-9-AZABICYCLO[3.3.1]NONAN-3-YL)(METHYL)AMINO)PYRIDAZIN-3-YL)-2-METHYLBENZO[D]OXAZOL-5-OL AS A SPLICING MODULATOR FOR THE TREATMENT OF NEUROLOGICAL DISEASES

CROSS REFERENCE

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 63/511,248, filed June 30, 2023, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on June 17, 2024, is named 51503-767_601_SL.xml and is 1,885 bytes in size.

BACKGROUND

[0003] The majority of protein-coding genes in the human genome are composed of multiple exons (coding regions) that are separated by introns (non-coding regions). Gene expression results in a single precursor messenger RNA (pre-mRNA). The intron sequences are subsequently removed from the pre-mRNA by a process called splicing, which results in the mature messenger RNA (mRNA). By including different combinations of exons, alternative splicing gives rise to multiple mRNAs encoding distinct protein isoforms. The spliceosome, an intracellular complex of multiple proteins and ribonucleoproteins, catalyzes splicing.

[0004] Small molecule splicing modulators (SMSMs) overcome many of the problems associated with therapies such as oligonucleotide technologies (antisense, RNA interference, etc.), including lack of oral bioavailability, and lack of blood-brain-barrier penetration, with the latter precluding delivery to the brain or spinal cord after parenteral drug administration for the treatment of diseases (*e.g.*, neurological diseases, brain cancers, etc.).

[0005] SMSMs disclosed in WO2020/163541, such as, 6-(6-(((1R,2R,3S,5S)-2-fluoro-8-azabicyclo[3.2.1]octan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methyl-1,3-benzoxazol-5-ol, are useful in treating and preventing a wide range of diseases and conditions through modulating splicing of pre-mRNAs, including, but not limited to, neurodegenerative diseases, such as Huntington's Disease. SMSMs, however, can also have challenges, such as metabolic profiles in patients, clearance rates, the amount of compound available to exert an effect (*e.g.*, fraction unbound in plasma, half-life of the compound in circulation, etc.).

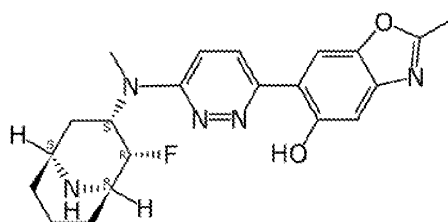
SUMMARY

[0006] Provided herein are small molecule splicing modulators and uses thereof that fulfill this need.

[0007] As mentioned above, WO2020/163541 discloses 6-(6-(((1R,2R,3S,5S)-2-fluoro-8-azabicyclo[3.2.1]octan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methyl-1,3-benzoxazol-5-ol, which is useful in treating and preventing a wide range of diseases and conditions through modulating splicing of pre-mRNAs, including, but not limited to, neurodegenerative diseases, such as Huntington's Disease. Certain parameters for SMSMs such as metabolic profiles in patients, clearance rates, the

amount of compound available to exert an effect (*e.g.*, fraction unbound in plasma, half-life of the compound in circulation, etc.) can be affected by small changes between two similar compounds. Thus, compounds similar to 6-(6-(((1*R*,2*R*,3*S*,5*S*)-2-fluoro-8-azabicyclo[3.2.1]octan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methyl-1,3-benzoxazol-5-ol, but with improved metabolic profiles are useful in developing therapies for neurodegenerative diseases, such as Huntington's Disease.

[0008] In one aspect, described herein is a compound of structure B, or a pharmaceutically acceptable salt or stereoisomer thereof:



Structure B.

[0009] In some aspects, the compound of structure B is 6-(6-(((1*R*,2*R*,3*S*,5*S*)-2-Fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazine-3-yl)-2-methylbenzo[*d*]oxazol-5-ol.

[0010] Also provided herein are pharmaceutical compositions comprising a compound disclosed herein, or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable excipient or carrier.

[0011] Also provided herein are methods of modulating splicing comprising contacting a compound disclosed herein to cells, wherein the compound modulates splicing at a splice site sequence of a pre-mRNA that encodes an mRNA, wherein the mRNA encodes a target protein or a functional RNA.

[0012] Also provided herein are methods of treating a disease or condition comprising administering a compound disclosed herein, or a pharmaceutically acceptable salt or stereoisomer thereof, to a subject in need thereof.

[0013] Also provided herein are uses of a compound disclosed herein, or a pharmaceutically acceptable salt or stereoisomer thereof, in the manufacture of a medicament for the treatment of a condition or disease.

INCORPORATION BY REFERENCE

[0014] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

DETAILED DESCRIPTION

[0015] Certain specific details of this description are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the

present disclosure may be practiced without these details. In other instances, well-known structures have not been shown or described in detail to avoid unnecessarily obscuring descriptions of the embodiments.

[0016] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below.

Definitions

[0017] The terms “compound(s) of this disclosure”, “compound(s) of the present disclosure”, “small molecule splicing modulator(s)”, “splicing modulator(s)”, “compound(s) that modify splicing” and “compound(s) modifying splicing”, or “SMSM” are interchangeably used herein and refer to compounds as disclosed herein and stereoisomers, tautomers, solvates, and salts (*e.g.*, pharmaceutically acceptable salts) thereof.

[0018] Any open valency appearing on a carbon, oxygen, sulfur or nitrogen atom in the structures herein indicates the presence of hydrogen, unless indicated otherwise.

[0019] The terms “administer,” “administering,” “administration,” and the like, as used herein, refer to the methods that may be used to enable delivery of compounds or compositions to the desired site of biological action. These methods include, but are not limited to oral routes (*p.o.*), intraduodenal routes (*i.d.*), parenteral injection (including intravenous (*i.v.*), subcutaneous (*s.c.*), intraperitoneal (*i.p.*), intramuscular (*i.m.*), intravascular or infusion (*inf.*)), topical (*top.*) and rectal (*p.r.*) administration. Those of skill in the art are familiar with administration techniques that can be employed with the compounds and methods described herein. In some embodiments, the compounds and compositions described herein are administered orally.

[0020] The terms “co-administration” or the like, as used herein, are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are administered by the same or different route of administration or at the same or different time.

[0021] The terms “effective amount” or “therapeutically effective amount,” as used herein, refer to a sufficient amount of an agent or a compound being administered which will relieve to some extent one or more of the symptoms of the disease or condition being treated; for example a reduction and/or alleviation of one or more signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an “effective amount” for therapeutic uses can be an amount of an agent that provides a clinically significant decrease in one or more disease symptoms. An appropriate “effective” amount may be determined using techniques, such as a dose escalation study, in individual cases.

[0022] The terms “enhance” or “enhancing,” as used herein, means to increase or prolong either in amount, potency or duration a desired effect. For example, in regard to enhancing splicing of a target,

the term “enhancing” can refer to the ability to increase or prolong splicing, either in amount, potency or duration, of a target.

[0023] The term “subject” or “patient” encompasses mammals. Examples of mammals include, but are not limited to, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. In one aspect, the mammal is a human. The term “animal” as used herein comprises human beings and non-human animals. In one embodiment, a “non-human animal” is a mammal, for example a rodent such as rat or a mouse. In one embodiment, a non-human animal is a mouse.

[0024] The terms “pharmaceutical composition” and “pharmaceutical formulation” (or “formulation”) are used interchangeably and denote a mixture or solution comprising a therapeutically effective amount of an active pharmaceutical ingredient together with one or more pharmaceutically acceptable excipients to be administered to a subject, *e.g.*, a human in need thereof.

[0025] The term “pharmaceutical combination” as used herein, means a product that results from mixing or combining more than one active ingredient and includes both fixed and non-fixed combinations of the active ingredients. The term “fixed combination” means that the active ingredients, *e.g.*, a compound described herein and a co-agent, are both administered to a patient simultaneously in the form of a single entity or dosage. The term “non-fixed combination” means that the active ingredients, *e.g.*, a compound described herein and a co-agent, are administered to a patient as separate entities either simultaneously, concurrently or sequentially with no specific intervening time limits, wherein such administration provides effective levels of the two compounds in the body of the patient. The latter also applies to cocktail therapy, *e.g.*, administration of three or more active ingredients.

[0026] The term “pharmaceutically acceptable” denotes an attribute of a material which is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and neither biologically nor otherwise undesirable and is acceptable for veterinary as well as human pharmaceutical use.

“Pharmaceutically acceptable” can refer to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively nontoxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

[0027] The terms “pharmaceutically acceptable excipient”, “pharmaceutically acceptable carrier” and “therapeutically inert excipient” can be used interchangeably and denote any pharmaceutically acceptable ingredient in a pharmaceutical composition having no therapeutic activity and being non-toxic to the subject administered, such as disintegrators, binders, fillers, solvents, buffers, tonicity

agents, stabilizers, antioxidants, surfactants, carriers, diluents, excipients, preservatives or lubricants used in formulating pharmaceutical products.

[0028] The term “pharmaceutically acceptable salts” denotes salts which are not biologically or otherwise undesirable. Pharmaceutically acceptable salts include both acid and base addition salts. A “pharmaceutically acceptable salt” can refer to a formulation of a compound that does not cause significant irritation to an organism to which it is administered and/or does not abrogate the biological activity and properties of the compound. In some embodiments, pharmaceutically acceptable salts are obtained by reacting an SMSM compound of Formula (I) with an acid. Pharmaceutically acceptable salts are also obtained by reacting a compound of Formula (I) with a base to form a salt.

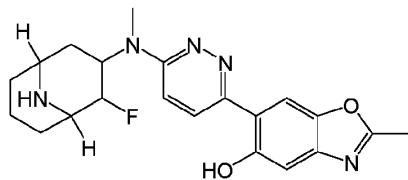
[0029] The term “nucleic acid” as used herein generally refers to one or more nucleobases, nucleosides, or nucleotides, and the term includes polynucleobases, polynucleosides, and polynucleotides.

[0030] As used herein, a “small molecular weight compound” can be used interchangeably with “small molecule” or “small organic molecule”. Small molecules refer to compounds other than peptides or oligonucleotides; and typically have molecular weights of less than about 2000 Daltons, *e.g.*, less than about 900 Daltons.

Small Molecule Splicing Modulators (SMSMs)

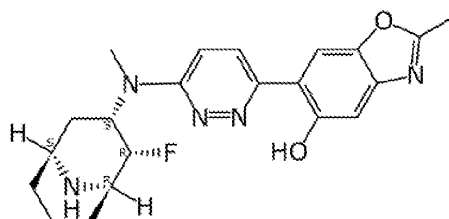
[0031] Described herein are compounds modifying splicing of gene products for use in the treatment, prevention and/or delay of progression of diseases or conditions.

[0032] In one aspect, described herein is a compound that has the structure of



, or a pharmaceutically acceptable salt thereof.

[0033] In one aspect, described herein is a compound that has the structure of structure B, or a pharmaceutically acceptable salt or stereoisomer thereof:

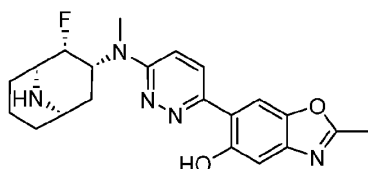


Structure B.

[0034] In one aspect, described herein is a compound that has the structure of Structure B. In one aspect, described herein is a compound that is a pharmaceutically acceptable salt of the compound of

Structure B. In another aspect, described herein is the compound 6-(6-(((1*R*,2*R*,3*S*,5*S*)-2-Fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazine-3-yl)-2-methylbenzo[d]oxazol-5-ol.

[0035] In some embodiments, a stereoisomer of Structure B is a compound of Structure A



Structure A

[0036] In one aspect, described herein is the compound 6-(6-(((1*S*,2*S*,3*R*,5*R*)-2-fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methylbenzo[d]oxazol-5-ol.

[0037] The absolute stereochemistry for Structure A and Structure B is not identified, but relative stereochemistry is known and indicated.

[0038] In one aspect, disclosed herein is a method of modulating splicing comprising contacting a compound of the present disclosure to cells, wherein the compound modulates splicing at a splice site sequence of a pre-mRNA that encodes an mRNA, wherein the mRNA encodes a target protein or a functional RNA.

[0039] In one aspect, disclosed herein is a method of treating a disease or condition comprising administering a compound of the present disclosure.

[0040] The compound described herein may be formed as, and/or used as, a pharmaceutically acceptable salt. The type of pharmaceutical acceptable salts, include, but are not limited to: (1) acid addition salts, formed by reacting the free base form of the compound with a pharmaceutically acceptable: inorganic acid, such as, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, metaphosphoric acid, and the like; or with an organic acid, such as, for example, acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, trifluoroacetic acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, 2-naphthalenesulfonic acid, 4-methylbicyclo-[2.2.2]oct-2-ene-1-carboxylic acid, glucoheptonic acid, 4,4'-methylenebis-(3-hydroxy-2-ene-1-carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, butyric acid, phenylacetic acid, phenylbutyric acid, valproic acid, and the like; (2) salts formed when an acidic proton present in the parent compound is replaced by a metal ion, *e.g.*, an alkali metal ion (*e.g.*, lithium, sodium, potassium), an alkaline earth ion (*e.g.* magnesium, or calcium), or an aluminum ion. In some cases, compounds described herein may coordinate with an organic base, such as, but not limited to, ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine,

dicyclohexylamine, tris(hydroxymethyl)methylamine. In other cases, compounds described herein may form salts with amino acids such as, but not limited to, arginine, lysine, and the like. Acceptable inorganic bases used to form salts with compounds that include an acidic proton, include, but are not limited to, aluminum hydroxide, calcium hydroxide, potassium hydroxide, sodium carbonate, sodium hydroxide, and the like.

[0041] In some embodiments, described herein is a compound modifying splicing of gene products, such as HTT pre-m RNA for use in the treatment, prevention, and/or delay of progression of diseases or conditions (*e.g.*, Huntington's disease). In some embodiments, the present disclosure relates to a pharmaceutical composition comprising a compound described herein for use in the treatment, prevention, and/or delay of progression of Huntington's disease. In some embodiments, a compound described herein can be administered for treatment, prevention, and/or delay of progression of Huntington's disease. In some embodiments, a subject is affected by Huntington's disease associated with the HTT gene. In some embodiments, a subject is affected by Huntington's disease associated with a splicing product of the HTT pre-mRNA. In some embodiments, the splicing product of the HTT pre-mRNA is an aberrant splicing product. In some embodiments, the splicing product of the HTT pre-mRNA encodes an aberrant polypeptide. In some embodiments, the splicing product of the HTT pre-mRNA is an aberrant splicing product resulted from a mutation in the HTT gene. In some embodiments, the splicing product of the HTT pre-mRNA may comprise a string of CAG repeats. In some embodiments, the splicing product of the HTT pre-mRNA may comprise an aberrant expansion of a string of CAG repeats. In some embodiments, the splicing product of the HTT pre-mRNA may comprise an aberrant expansion of a string of CAG repeats resulted from a mutation in the HTT gene.

[0042] In some embodiments, the compound and methods of use described herein can modulate splicing, such as alternative splicing of a polynucleotide encoded by HTT gene. In some embodiments, alternative splicing of the HTT pre-mRNA may lead to the expression of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 isoforms of the huntingtin protein. In some embodiments, the HTT gene may comprise a mutation. In some embodiments, the HTT gene may comprise a mutation associated with expansion of a CAG repeat. In some embodiments, the splice modulating compounds and methods of use described herein can modulate splicing of the HTT pre-mRNA that lead to inclusion of a cryptic exon (*e.g.*, a poison exon) that is normally not included in the HTT spliced product, *e.g.*, mRNA. In some embodiments, the cryptic exon (*e.g.*, a poison exon) included in the HTT spliced product may lead to degradation of the HTT spliced product through nonsense-mediated decay (NMD) mediated RNA degradation. In a preferred embodiment, alternative splicing of the HTT pre-mRNA may lead to inclusion of a cryptic exon that is not normally included in between exon 49 and exon 50 of the HTT mRNA. In a preferred embodiment, alternative splicing of the HTT pre-mRNA may promote the inclusion of a poison exon 49b. In some embodiments, the HTT pre-mRNA comprises the sequence AGAguaaggg (SEQ ID NO: 1). In a preferred embodiment, the compounds described herein bind to the 5'ss sequence AGAguaaggg (SEQ ID NO: 1).

[0043] Described herein is a compound modifying splicing of gene products wherein the compound induces a post-transcriptionally unstable variant or transcript of a gene product. Described herein is a compound modifying splicing of gene products wherein the compound represses a transcript of a gene product. In some embodiments, an HTT transcript harbors a poison exon. In some embodiments, the poison exon results in a frame-shift in a downstream exon, for example in an exon immediately following the poison exon. In some embodiments, the frame-shift in a downstream exon contains an in-frame stop codon that would not be in frame in the absence of inclusion of the poison exon. In some embodiments, the poison exon comprises an in-frame premature termination codon (PTC). In some embodiments, the poison exon triggers NMD and degradation of the transcript. In some embodiments, the gene product is HTT.

Methods of Making Compounds

[0044] The compound of structure B, 6-(6-(((1*R*,2*R*,3*S*,5*S*)-2-Fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazine-3-yl)-2-methylbenzo[*d*]oxazol-5-ol, can be made by the techniques and processes known in the art, for example as described in Example 1.

EXAMPLES

[0045] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein. The compound described herein can be synthesized using standard synthetic techniques or using methods known in the art in combination with methods described herein. Unless otherwise indicated, conventional methods of mass spectroscopy, NMR, HPLC, protein chemistry, biochemistry, recombinant DNA techniques and pharmacology can be employed. The compound can be prepared using standard organic chemistry techniques such as those described in, for example, March's Advanced Organic Chemistry, 6th Edition, John Wiley and Sons, Inc. Alternative reaction conditions for the synthetic transformations described herein may be employed such as variation of solvent, reaction temperature, reaction time, as well as different chemical reagents and other reaction conditions. The starting materials and reagents used for the synthesis of the compound described herein may be synthesized or can be obtained from commercial sources, such as, but not limited to, Sigma-Aldrich, Acros Organics, Fluka, and Fischer Scientific. The starting materials can be available from commercial sources or can be readily prepared. By way of example only, provided are schemes for preparing the Examples described herein.

[0046] Suitable reference books and treatise that detail the synthesis of reactants useful in the preparation of the compound described herein, or provide references to articles that describe the preparation, include for example, "Synthetic Organic Chemistry", John Wiley & Sons, Inc., New York; S. R. Sandler et al., "Organic Functional Group Preparations," 2nd Ed., Academic Press, New York, 1983; H. O. House, "Modern Synthetic Reactions", 2nd Ed., W. A. Benjamin, Inc. Menlo Park, Calif. 1972; T. L. Gilchrist, "Heterocyclic Chemistry", 2nd Ed., John Wiley & Sons, New York, 1992; J. March, "Advanced Organic Chemistry: Reactions, Mechanisms and Structure", 4th Ed.,

Wiley Interscience, New York, 1992. Additional suitable reference books and treatise that detail the synthesis of reactants useful in the preparation of compounds described herein, or provide references to articles that describe the preparation, include for example, Fuhrhop, J. and Penzlin G. "Organic Synthesis: Concepts, Methods, Starting Materials", Second, Revised and Enlarged Edition (1994) John Wiley & Sons ISBN: 3 527-29074-5; Hoffman, R.V. "Organic Chemistry, An Intermediate Text" (1996) Oxford University Press, ISBN 0-19-509618-5; Larock, R. C. "Comprehensive Organic Transformations: A Guide to Functional Group Preparations" 2nd Edition (1999) Wiley-VCH, ISBN: 0-471-19031-4; March, J. "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure" 4th Edition (1992) John Wiley & Sons, ISBN: 0-471-60180-2; Otera, J. (editor) "Modern Carbonyl Chemistry" (2000) Wiley-VCH, ISBN: 3-527-29871-1; Patai, S. "Patai's 1992 Guide to the Chemistry of Functional Groups" (1992) Interscience ISBN: 0-471-93022-9; Solomons, T. W. G. "Organic Chemistry" 7th Edition (2000) John Wiley & Sons, ISBN: 0-471-19095-0; Stowell, J.C., "Intermediate Organic Chemistry" 2nd Edition (1993) Wiley-Interscience, ISBN: 0-471-57456-2; "Industrial Organic Chemicals: Starting Materials and Intermediates: An Ullmann's Encyclopedia" (1999) John Wiley & Sons, ISBN: 3-527-29645-X, in 8 volumes; "Organic Reactions" (1942-2000) John Wiley & Sons, in over 55 volumes; and "Chemistry of Functional Groups" John Wiley & Sons, in 73 volumes.

[0047] In the reactions described, it may be necessary to protect reactive functional groups, for example hydroxy, amino, imino, thio or carboxy groups, where these are desired in the final product, in order to avoid their unwanted participation in reactions. A detailed description of techniques applicable to the creation of protecting groups and their removal are described in Greene and Wuts, Protective Groups in Organic Synthesis, 3rd Ed., John Wiley & Sons, New York, NY, 1999, and Kocienski, Protective Groups, Thieme Verlag, New York, NY, 1994, which are incorporated herein by reference for such disclosure).

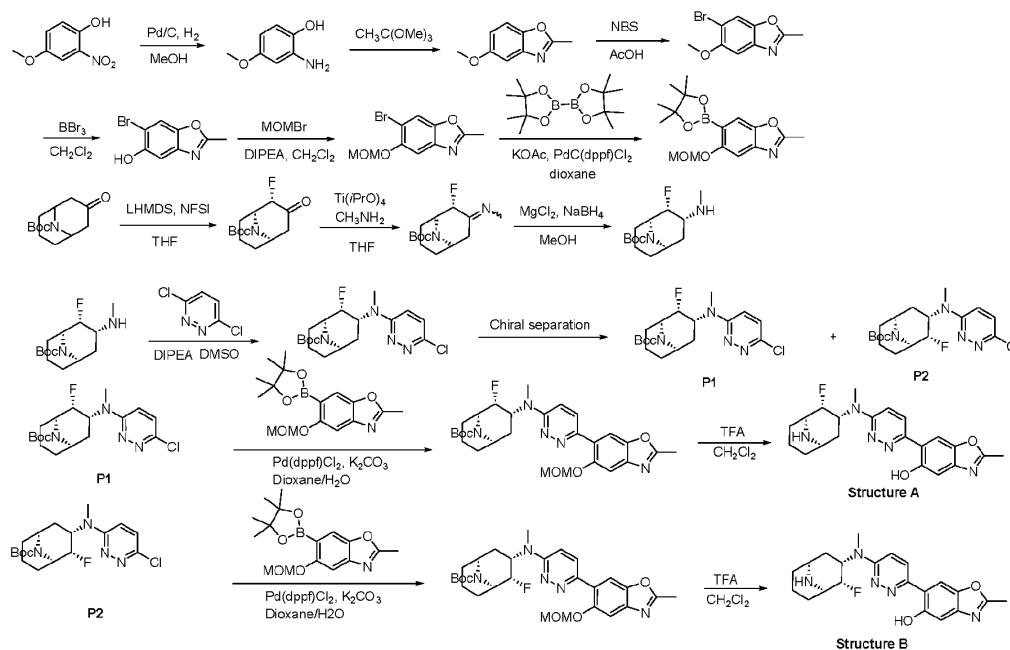
[0048] Examples can be made using known techniques and further chemically modified, in some embodiments, to facilitate intranuclear transfer to, *e.g.*, a splicing complex component, a spliceosome or a pre-mRNA molecule. One of ordinary skill in the art will appreciate the standard medicinal chemistry approaches for chemical modifications for intranuclear transfer (*e.g.*, reducing charge, optimizing size, and/or modifying lipophilicity).

Stereochemistry:

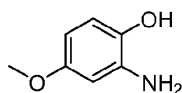
[0049] (±) or racemic indicates that the product is a racemic mixture of enantiomers. For example (±) (1*S*,2*S*,3*R*,5*R*) or racemic (1*S*,2*S*,3*R*,5*R*) indicates that the relative product stereochemistry shown is based on known stereochemistry of similar compounds and or reactions and the product is a racemic mixture of enantiomers of both (1*S*,2*S*,3*R*,5*R*) and (1*R*,2*R*,3*S*,5*S*) stereoisomers. A compound in which the absolute stereochemistry of separated enantiomers is undetermined is represented as being either of the single enantiomers, for example (1*S*,2*S*,3*R*,5*R*) or (1*R*,2*R*,3*S*,5*S*) or drawn as being either

possible single enantiomer. In such cases, the product is pure and a single enantiomer, but absolute stereochemistry is not identified, but relative stereochemistry is known and indicated.

[0050] Example 1: Synthesis of 6-(6-(((1*S*,2*S*,3*R*,5*R*)-2-fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methylbenzo[d]oxazol-5-ol (Structure A) and 6-(6-(((1*R*,2*R*,3*S*,5*S*)-2-fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methylbenzo[d]oxazol-5-ol (Structure B).

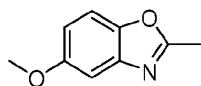


[0051] Synthesis of 2-amino-4-methoxyphenol.



[0052] A mixture of 4-methoxy-2-nitrophenol (25.0 g, 0.15 mol) and Pd/C (2.5 g) in MeOH (500 mL) was stirred, degassed with hydrogen 3 times and then held with stirring at 20 °C under hydrogen for 2 d. The reaction mixture was filtered and the cake was washed with MeOH (350 mL*3). The filtrate was combined and concentrated in vacuum to afford the product 2-amino-4-methoxyphenol as brown solid (20.0 g, yield 97.2 %). LCMS: m/z 140.1 $[M+H]^+$; t_R = 0.93 min.

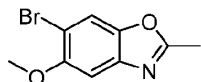
[0053] Synthesis of 5-methoxy-2-methylbenzo[d]oxazole.



[0054] A mixture of 2-amino-4-methoxyphenol (20.0 g, 0.14 mol) in trimethyl orthoacetate (50 mL) was heated to 100 °C with stirring and held for 1 h. The mixture was concentrated and the residue was

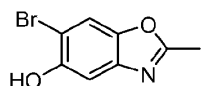
purified by combi-flash (Biotage, Silica gel column, 330 g, 60 mL/min, EA in PE 0 % ~ 35 %, 30 min, 35 %, 12 min, UV 254 280) to give the desired product 5-methoxy-2-methylbenzo[d]oxazole as orange oil (17.5 g, yield 74.6 %). LCMS: m/z 164.1 $[M+H]^+$; t_R = 1.41 min.

[0055] Synthesis of 6-bromo-5-methoxy-2-methylbenzo[d]oxazole.



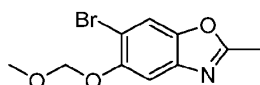
[0056] NBS (19.6 g, 0.11 mol) was added to a mixture of 5-methoxy-2-methylbenzo[d]oxazole (17.5 g, 0.11 mol) in AcOH (150 mL). This resulting mixture was stirred at 20 °C for 18 h. The mixture was quenched with ice water, neutralized with Na₂CO₃ aqueous, extracted with EtOAc (200 mL*3). The extracts were concentrated and the residue was purified by combi-flash (Biotage, Silica gel column, 330 g, 60 mL/min, EA in PE 0 % ~ 10 %, 30 min, 20 %, 15 min, UV 254 280) to give the desired product 6-bromo-5-methoxy-2-methylbenzo[d]oxazole as pink solid (20.5 g, yield 77.0 %). LCMS: m/z 242.1; 243.9 $[M+H]^+$; t_R = 1.70 min. ¹H NMR (500 MHz, CDCl₃-*d*₃) δ 7.68 (s, 1H), 7.17 (s, 1H), 3.93 (s, 3H), 2.61 (s, 3H).

[0057] Synthesis of 6-bromo-2-methylbenzo[d]oxazol-5-ol.



[0058] BBr₃ (210 mL, 1 mol/L, 0.21 mol) was added to a mixture of 6-bromo-5-methoxy-2-methylbenzo[d]oxazole (20.5 g, 0.085 mol) in DCM (30 mL) at 0 °C. This resulting mixture was stirred at 0 °C for 10 min and then warmed to 20 °C with stirring and held for 3 d. The mixture was quenched with ice water, neutralized with NaHCO₃ aqueous, extracted with EtOAc (360 mL*3). The extracts were concentrated and the residue was purified by combi-flash (Biotage, Silica gel column, 330 g, 80 mL/min, EA in PE 0 % ~ 50 %, 30 min, 40 %, 10 min, then MeOH in DCM 20% UV 254 280) to give the desired product 6-bromo-2-methylbenzo[d]oxazol-5-ol as grey solid (19.0 g, yield 98.6 %). LCMS: m/z 228.0; 230.0 $[M+H]^+$; t_R = 1.50 min.

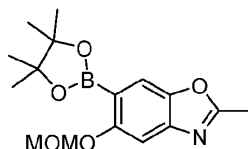
[0059] Synthesis of 6-bromo-5-(methoxymethoxy)-2-methylbenzo[d]oxazole.



[0060] MOMBr (15.6 g, 0.12 mol) was added drop wise to a mixture of 6-bromo-2-methylbenzo[d]oxazol-5-ol (19.0 g, 0.08 mol) and DIPEA (37.7 g, 0.19 mol) in ACN (300 mL) at 5 °C. This resulting mixture was stirred at 5 °C and held for 30 min. The mixture was quenched with ice water, extracted with EtOAc (200 mL*3). The extracts were washed with brine (300 mL) and concentrated. The residue was purified by combi-flash (Biotage, Silica gel column, 330 g, 60 mL/min, EA in PE 0 % ~ 15 %, 20 min, 15 %, 5 min, 15 % ~ 25 %, 10 min, 25 %, 15 min, UV 254 280) to

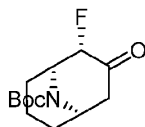
give the desired product 6-bromo-5-(methoxymethoxy)-2-methylbenzo[d]oxazole as pink solid (18.0 g, yield 79.5 %). LCMS: m/z 272.0; 274.0 $[M+H]^+$; t_R = 1.77 min.

[0061] Synthesis of 5-(methoxymethoxy)-2-methyl-6-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl)benzo[d]oxazole.



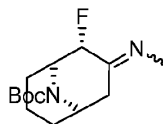
[0062] A mixture of 6-bromo-5-(methoxymethoxy)-2-methylbenzo[d]oxazole (50 g, 0.02 mol), pinacolboron (28.0 g, 0.11 mol), $PdCl_2dppf$ (1.1 g, 1.5 mmol) and KOAc (10.8 g, 0.11 mol) in 1, 4-dioxane (300 mL) was heated to 100 °C with stirring and held for 50 h. The mixture was quenched with ice water, extracted with EtOAc (200 mL*3). The extracts were washed with brine (200 mL) and concentrated. The residue was purified by combi-flash (Biotage, Silica gel column, 20 g, 30 mL/min, EA in PE 0 % ~ 15 %, 20 min, 15 %, 6 min, 15 % ~ 25 %, 15 min, 25 %, 10 min, UV 254 280) to give the desired product 5-(methoxymethoxy)-2-methyl-6-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl)benzo[d]oxazole as pink solid (4.0 g, yield 68.2 %). LCMS: m/z 320.2 $[M+H]^+$; t_R = 1.86 min.

[0063] Synthesis of *tert*-butyl (1*S*,2*S*,5*R*)-2-fluoro-3-oxo-9-azabicyclo[3.3.1]nonane-9-carboxylate.



[0064] LHMDS (94 mL, 94 mmol, 1 N solution in THF) was added to a stirred solution of *tert*-butyl 3-oxo-9-azabicyclo[3.3.1]nonane-9-carboxylate (15 g, 62.8 mmol) in 150 mL of anhydrous THF at -78 °C under nitrogen atmosphere. After stirring for 30 min, NFSI (26.6 g, 75 mmol) in 100 mL of anhydrous THF was added dropwise. The mixture was then stirred at -78 °C for 4 h, quenched with saturated NH_4Cl aqueous solution (30 mL), extracted with EtOAc (80 mL X 3). The combined organic phases were dried over anhydrous Na_2SO_4 , concentrated and purified by silica gel chromatography (0-5% EtOAc/petroleum ether) to give 6.5 g of *tert*-butyl (1*S*,2*S*,5*R*)-2-fluoro-3-oxo-9-azabicyclo[3.3.1]nonane-9-carboxylate as a white solid (40% yield). LCMS: m/z 202.1 $[M-55]^+$; t_R = 1.75 min.

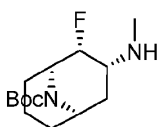
[0065] Synthesis of *tert*-butyl (1*S*,2*S*,5*R*)-2-fluoro-3-(methylimino)-9-azabicyclo[3.3.1]nonane-9-carboxylate.



[0066] Methylamine (58.5 mL, 117 mmol, 2N solution in THF) and $\text{Ti}(\text{iPrO})_4$ (32.8 g, 117 mmol) were added to a stirred solution of *tert*-butyl (1*S*,2*S*,5*R*)-2-fluoro-3-oxo-9-azabicyclo[3.3.1]nonane-9-carboxylate (20 g, 77 mmol) in THF (1 L) under N_2 protection. The reaction mixture was stirred at room temperature for 2 h. Water (1 L) was added to quench the reaction. The mixture was extracted with EtOAc (1 L X 3). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give the crude product (1*S*,2*S*,5*R*)-2-fluoro-3-(methylimino)-9-azabicyclo[3.3.1]nonane-9-carboxylate (20 g, 95% yield), which was directly used in next step.

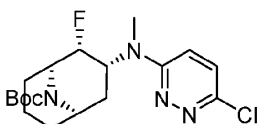
LCMS: m/z 271.2 $[\text{M}+\text{H}]^+$; t_R = 1.58, 1.80 min.

[0067] **Synthesis of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-2-fluoro-3-(methylamino)-9-azabicyclo[3.3.1]nonane-9-carboxylate.**



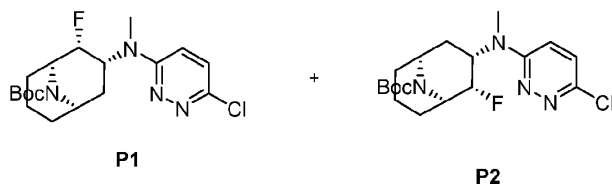
[0068] NaBH_4 (4 g, 104 mmol) was added to a stirred solution of (1*S*,2*S*,5*R*)-2-fluoro-3-(methylimino)-9-azabicyclo[3.3.1]nonane-9-carboxylate (7 g, 26 mmol) and MgCl_2 (2.46 g, 26 mmol) in 30 mL of MeOH. After the addition, the mixture was stirred at room temperature for 2 h. Additional NaBH_4 may be needed till LCMS indicated the imine was consumed completely. 100 mL of water was added to quench the reaction. The resulting mixture was extracted with EtOAc (180 mL X 3). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 , concentrated and purified by silica gel chromatography (0-5% MeOH/ CH_2Cl_2) give 2 g of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-2-fluoro-3-(methylamino)-9-azabicyclo[3.3.1]nonane-9-carboxylate as colorless oil (40% yield), (high polar isomer). LCMS: m/z 273.2 $[\text{M}+\text{H}]^+$; t_R = 1.42 min.

[0069] **Synthesis of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-3-((6-chloropyridazin-3-yl)(methyl)amino)-2-fluoro-9-azabicyclo[3.3.1]nonane-9-carboxylate.**



[0070] A mixture of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-2-fluoro-3-(methylamino)-9-azabicyclo[3.3.1]nonane-9-carboxylate (2 g, 7.35 mmol), 3,6-dichloropyridazine (2.19 g, 14.7 mmol) and DIPEA (3.8 g, 29.4 mmol) in DMSO (10 mL) was stirred at 120 °C for 12 h. After cooling to room temperature, the mixture was quenched with H_2O (100 mL) and extracted with EtOAc (150 mL X 3). The combined organic layers were concentrated and purified with silica gel chromatography (0-50% EtOAc/petroleum ether) to give 1.5 g of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-3-((6-chloropyridazin-3-yl)(methyl)amino)-2-fluoro-9-azabicyclo[3.3.1]nonane-9-carboxylate white solid (54% yield). LCMS: m/z 385.2 $[\text{M}+\text{H}]^+$; t_R = 1.93 min.

[0071] Chiral separation of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-3-((6-chloropyridazin-3-yl)(methyl)amino)-2-fluoro-9-azabicyclo[3.3.1]nonane-9-carboxylate.



1500 mg of racemic intermediate was separated by below chiral condition to give 630 mg of P1 isomer (1.596 min) and 630 mg of P2 isomer (4.811 min).

[0072] Instrument: SFC-150 (Waters)

Column: AD 20*250mm, 10um (Daicel)

Column temperature: 35 °C

Mobile phase: CO₂/MEOH(0.2%Methanol Ammonia) = 65/35

Flow rate: 100 g/min

Back pressure: 100 bar

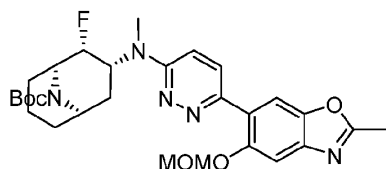
Detection wavelength: 214 nm

Cycle time: 3.5min

Sample solution: 1500mg dissolved in 100ml Methanol

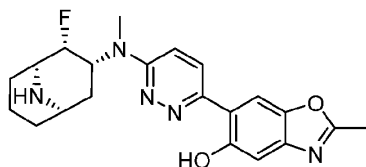
Injection volume: 3ml

[0073] Synthesis of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-2-fluoro-3-((6-(5-(methoxymethoxy)-2-methylbenzo[*d*]oxazol-6-yl)pyridazin-3-yl)(methyl)amino)-9-azabicyclo[3.3.1]nonane-9-carboxylate.



[0074] A mixture of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-3-((6-chloropyridazin-3-yl)(methyl)amino)-2-fluoro-9-azabicyclo[3.3.1]nonane-9-carboxylate (450 mg, 1.17 mmol), 5-(methoxymethoxy)-2-methyl-6-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl)benzo[*d*]oxazole (560 mg, 1.76 mmol), Pd(dppf)Cl₂ (86 mg, 0.117 mmol) and K₂CO₃ (324 mg, 2.34 mmol) in 1,4-Dioxane (15 mL), water (5 ml) was stirred at 110°C for 2 h under N₂ atmosphere. After cooling to room temperature, the mixture was concentrated and purified by silica gel chromatography (0-50% EtOAc/petroleum ether) to give 550 mg of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-2-fluoro-3-((6-(5-(methoxymethoxy)-2-methylbenzo[*d*]oxazol-6-yl)pyridazin-3-yl)(methyl)amino)-9-azabicyclo[3.3.1]nonane-9-carboxylate (86% yield). LCMS: *m/z* 541.9 [M+H]⁺; *t*_R = 1.98 min.

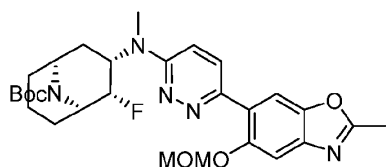
[0075] Synthesis of 6-(6-(((1*S*,2*S*,3*R*,5*R*)-2-fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methylbenzo[d]oxazol-5-ol.



Structure A

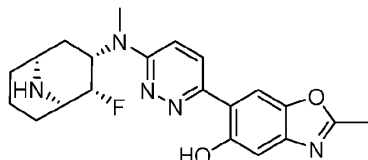
[0076] To a solution of *tert*-butyl (1*S*,2*R*,3*R*,5*R*)-2-fluoro-3-((6-(5-(methoxymethoxy)-2-methylbenzo[d]oxazol-6-yl)pyridazin-3-yl)(methyl)amino)-9-azabicyclo[3.3.1]nonane-9-carboxylate (550 mg, 1.02 mmol) in CH₂Cl₂ (7 mL) was added TFA (3 mL) and the mixture was stirred at room temperature for 2 h, monitored by LCMS. Then the mixture was concentrated and water (10 mL) was added. pH value was adjusted to 8-9 with saturated K₂CO₃ aqueous solution. The product was collected, concentrated and purified by C18 reversed phase column (0-70% 0.01% NH₄HCO₃ in H₂O/CH₃OH) to give 166 mg of 6-(6-(((1*S*,2*S*,3*R*,5*R*)-2-fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methylbenzo[d]oxazol-5-ol (41% yield). ¹H NMR (400 MHz, MeOD-*d*₄) δ 8.17 (d, *J* = 9.9 Hz, 1H), 7.98 (s, 1H), 7.32 (d, *J* = 9.9 Hz, 1H), 7.10 (s, 1H), 6.01 – 5.86 (m, 1H), 5.07 – 4.90 (m, 1H), 3.57 – 3.47 (m, 2H), 3.11 (s, 3H), 2.71 – 2.64 (m, 1H), 2.62 (s, 3H), 2.15 – 2.02 (m, 3H), 1.97 – 1.78 (m, 4H). LCMS: *m/z* 398.1 [M+H]⁺; *t*_R = 1.40 min.

[0077] Synthesis of *tert*-butyl (1*R*,2*S*,3*S*,5*S*)-2-fluoro-3-((6-(5-(methoxymethoxy)-2-methylbenzo[d]oxazol-6-yl)pyridazin-3-yl)(methyl)amino)-9-azabicyclo[3.3.1]nonane-9-carboxylate.



[0078] A mixture of *tert*-butyl (1*R*,2*S*,3*S*,5*S*)-3-((6-chloropyridazin-3-yl)(methyl)amino)-2-fluoro-9-azabicyclo[3.3.1]nonane-9-carboxylate (450 mg, 1.17 mmol), 5-(methoxymethoxy)-2-methyl-6-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl)benzo[d]oxazole (560 mg, 1.76 mmol), Pd(dppf)Cl₂ (86 mg, 0.117 mmol) and K₂CO₃ (324 mg, 2.34 mmol) in 1,4-Dioxane (15 mL), water (5 mL) was stirred at 110 °C for 2 h under N₂ atmosphere. After cooling to room temperature, the mixture was concentrated and purified by silica gel chromatography (0-50% EtOAc/petroleum ether) to give 500 mg of *tert*-butyl (1*R*,2*S*,3*S*,5*S*)-2-fluoro-3-((6-(5-(methoxymethoxy)-2-methylbenzo[d]oxazol-6-yl)pyridazin-3-yl)(methyl)amino)-9-azabicyclo[3.3.1]nonane-9-carboxylate (79% yield). LCMS: *m/z* 541.9 [M+H]⁺; *t*_R = 1.98 min.

[0079] Synthesis of 6-(6-(((1*R*,2*R*,3*S*,5*S*)-2-fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methylbenzo[d]oxazol-5-ol.



Structure B

[0080] To a solution of *tert*-butyl (1*R*,2*S*,3*S*,5*S*)-2-fluoro-3-((6-(5-(methoxymethoxy)-2-methylbenzo[d]oxazol-6-yl)pyridazin-3-yl)(methyl)amino)-9-azabicyclo[3.3.1]nonane-9-carboxylate (500 mg, 0.92 mmol) in CH₂Cl₂ (7 mL) was added TFA (3 mL) and the mixture was stirred at room temperature for 2 h, monitored by LCMS. Then the mixture was concentrated and water (10 mL) was added. pH value was adjusted to 8-9 with saturated K₂CO₃ aqueous solution. The product was collected, concentrated and purified by C18 reversed phase column (0-70% 0.01% NH₄HCO₃ in H₂O/CH₃OH) to give 133 mg of 6-(6-(((1*R*,2*R*,3*S*,5*S*)-2-fluoro-9-azabicyclo[3.3.1]nonan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methylbenzo[d]oxazol-5-ol (36% yield). ¹H NMR (400 MHz, MeOD-*d*₄) δ 8.17 (d, *J* = 9.9 Hz, 1H), 7.98 (s, 1H), 7.32 (d, *J* = 9.9 Hz, 1H), 7.10 (s, 1H), 6.01 – 5.86 (m, 1H), 5.07 – 4.90 (m, 1H), 3.57 – 3.47 (m, 2H), 3.11 (s, 3H), 2.71 – 2.64 (m, 1H), 2.62 (s, 3H), 2.15 – 2.02 (m, 3H), 1.97 – 1.78 (m, 4H). LCMS: *m/z* 398.1 [M+H]⁺; *t*_R = 1.40 min.

[0081] **Example 2: Metabolite ID.**

[0082] Metabolite identification was performed for both 6-(6-(((1*R*,2*R*,3*S*,5*S*)-2-fluoro-8-azabicyclo[3.2.1]octan-3-yl)(methyl)amino)pyridazin-3-yl)-2-methyl-1,3-benzoxazol-5-ol (Compound 117) and Structure B. Human hepatocytes (liverPool™ 10-Doner) from BioIVT (cat. No. X008001) for Compound 117 and (liverPool™ 20-Doner) from BioIVT (cat. No. X008000) for Structure B at 1.0 X 10⁶ cells/ml were incubated with test compound (50 μM Compound 117, 10 μM Structure B) for 240 minutes at 37°C. Incubations were quenched with 2 volumes of acetonitrile (0.1% FA) followed by centrifugation for 15 min at 16,000 g; Supernatant was then analyzed by LC-MS/MS. For UV analysis, 500 μL acetonitrile (0.1% FA) fraction was dried by centrifugal vacuum evaporator and reconstituted with 50 μL water and 50 μL methanol. The following equipment and conditions were used for the analysis:

[0083] Instrumentation: Vanquish UHPLC system (Thermo Fisher Scientific, USA); Vanquish Variable Wavelength (Thermo Fisher Scientific, USA); Thermo Scientific Q Exactive (Thermo Fisher Scientific, USA).

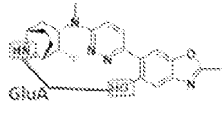
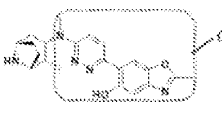
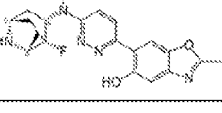
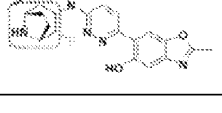
[0084] LC conditions: Column: Waters XSelect HSS T3, 100 x 2.1 mm, 2.5 μm; Solvents: A, water (0.1% formic acid); B, acetonitrile (0.1% formic acid); Flow rate: 500 μL/min; Program for Compound 117: 0-1.5 min, 5%B, 1.5-9 min, 5%-25%B, 9-12 min, 25%-100%B, 12-14 min, 100%B,

14-14.3 min, 100%-5%B, 14.3-15 min, 5%B. Program for Structure B: 0-1.5 min, 5%B, 1.5-9 min, 5%-30%B, 9-12 min, 30%-100%B, 12-14 min, 100%B, 14-14.3 min, 100%-5%B, 14.3-15 min, 5%B.

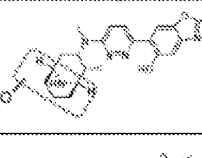
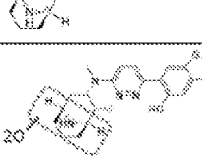
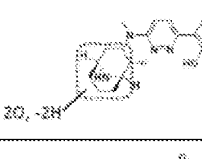
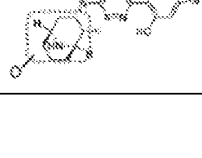
[0085] MS conditions: Ionisation mode: Positive mode; Spray Voltage: 3.5 kV; Aux gas flow rate: 15; Aux gas heater temp: 350°C; Scan type: Full MS/ddMS²; Resolution: 70,000; AGC Target: $3 \times e^6$; NCE/stepped NCE (Full Mass) for Compound 117: 25, 35, 45; NCE/stepped NCE (Full Mass) for Structure B: 30, 35, 40.

[0086] Three metabolites were detected for Compound 117 (**Table 1**), seven metabolites were detected for Structure B (**Table 2**).

[0087] Table 1.

Peak No.	R.T. (min)	Meas. m/z	Mass error (ppm)	Mass Shift	Biotransformation	Normalized peak Area @240 minutes	Proposed Metabolites
1	5.49	280.61090 (z=2)	-1.1	176.03209	Glucorinidation	0.02%	
2	6.97	400.17706	-2.2	15.99491	Oxidation	0.67%	
3	8.79	384.18204	-2.6	0.00000	Parent Compound	83.52%	
4	9.93	400.17719	-1.91	15.99491	Oxidation	15.78%	

[0088] Table 2.

Peak No.	R.T. (min)	Meas. m/z	Mass error (ppm)	Mass Shift	Biotransformation	Normalized peak Area @240 minutes	Proposed Metabolites
1	6.14	208.60809 (z=2)	-0.8	18.01056	Oxidation + Hydrogenation	1.04%	
2	6.65	414.19229	-3.1	15.99491	Oxidation	1.40%	
3	7.48	207.60034 (z=2)	-0.5	15.99491	Oxidation	0.95%	
4	8.02	200.59251 (z=2)	-0.5	1.97926	De-methylation + Oxidation	0.29%	
5	8.18	398.19809	-1.5	0.00000	Parent Compound	87.68%	
6	8.34	430.18732	-2.8	31.98983	Oxidation	0.75%	
7	8.35	428.17157	-3.0	29.97418	Oxidation + De-hydrogenation	0.99%	
8	8.99	414.19266	-2.2	15.99491	Oxidation	6.90%	

[0089] Example 3: Protein Binding Assay.

[0090] Protein binding of Structure B was determined in human, rat and mouse plasma using an equilibrium dialysis method (using 96-well Equilibrium Dialysis Plate (HTDialysis LLC, Gales Ferry, CT) and HTD 96a/b Dialysis Membrane Strips, MWCO 12-14K). Human mixed gender plasma (pH 7.46) was obtained from BioIVT (batch no. HMN575149), Rat SD strain, mixed gender plasma (pH 7.49) was obtained from BioIVT (batch no. RAT463303) and Mouse CD-1 strain, mixed gender plasma (pH 7.23) was obtained from IPHASE (batch no. M21005657).

[0091] A working solution of test compound and control compound (ketoconazole) was prepared in DMSO at a concentration of 1 mM. A basic solution was prepared by dissolving 14.2 g/L Na₂HPO₄ and 8.77 g/L NaCl in deionized water and the solution could be stored at 4°C for up to 7 days. An acidic solution was prepared by dissolving 12.0 g/L NaH₂PO₄ and 8.77 g/L NaCl in deionized water and the solution could be stored at 4°C for up to 7 days. The basic solution was titrated with the acidic solution to pH 7.4 and stored at 4°C for up to 7 days. pH was checked on the day of experiment and was adjusted if outside specification of 7.4 ± 0.1 . The temperature of a water bath was set to 37°C. Frozen Plasma (stored at -80°C) was thawed immediately in a 37°C water bath. The dialysis membranes were soaked in ultrapure water for 60 minutes to separate strips, then in 20% ethanol for 20 minutes, finally in dialysis buffer for 20 minutes. Prepared membranes were loaded into the dialysis device and the device was installed following the manufacturer's guidelines. The air bath was turned on and allow to pre-heat to 37°C. 597 µL of blank plasma solution was added into each vial of a new plastic plate or separate plastic tube by addition of 3 µL of the working solution of test compound, vortex at 1000 rpm for 2 minutes. The final percent volume of organic solvent was 0.5% and the final concentration for test compound was 5 µM. 50 µL of the spiked plasma solution suspension was transferred to a 96-well plate to act as T=0 control sample. All remaining spiked plasma solution is placed in the incubator for the duration of the study. At the same time, the remaining spiked plasma solution sample in the plastic plate or separate plastic tube was incubated for 6 hours at 37°C with 5% CO₂ in the CO₂ incubator.

[0092] At T=6 hours, 50 µL of the original spiked plasma solution suspension was transferred to the 96-well plate for analysis. The dialysis set up was assembled following the manufacturer's instructions. Cells were loaded with 120 µL of plasma sample and dialyzed against equal volume of dialysis buffer (PBS). The assay was performed in duplicate. The unit was covered with a gas permeable lid and incubated for 6 hours at 37°C at 100 rpm with 5% CO₂ on an orbital shaker in the CO₂ incubator. At the end of incubation, the lid was removed and 50 µL of post-dialysis samples from both buffer and plasma solution chambers were transferred into separated 96-well plate for analysis, respectively. 50 µL of plasma solution was added to the buffer samples, and an equal volume of PBS to the collected plasma solution samples. The plate at was shaken at 1000 rpm for 2 minutes and 400 µL of acetonitrile was added containing an appropriate internal standard (IS) to precipitate protein and release compound. Samples were vortexed at 1000 rpm for 10 minutes and then centrifuged for 30

minutes at 3,220 g. 250 μ L of the supernatant was transferred to new 96-well plates and centrifuged again (3,220 g, 30 minutes). 100 μ L of the supernatant was transferred to new 96-well plates for analysis. 100 μ L of distilled water was added to each sample and mixed for analysis by LC-MS/MS. Concentrations of test compound and control compound in the buffer and plasma solution chambers was determined. Percentages of test compound(s) and control compound bound were calculated as follows: % Unbound = (Area ratio buffer chamber / Area ratio plasma solution chamber) $\times$ 100; % Bound = 100 - % Unbound; % Recovery = (Area ratio buffer chamber + Area ratio plasma solution chamber) / (Area ratio Total sample) $\times$ 100; % Remaining = Area ratio 6hr / Area ratio 0hr $\times$ 100. The following chromatography conditions were used:

[0093] LC system: Shimadzu; MS analysis: Triple Quad 5500+ instrument from AB Inc with an ESI interface; Column temperature: 40°C; Injection volume: 1 μ L; Column: XSelect HSS T3 2.5 μ m 2.1 $\times$ 50mm Column; Mobile phase: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B); Elution rate: 0.8 mL/min; with the following program in **Table 3**:

[0094] **Table 3.**

Time (min)	0	0.2	0.5	0.9	0.95	1.2
% B	5	5	95	95	5	5

[0095] The following MS parameters were used: Ion source: Turbo spray; Ionization model: ESI; Scan type: MRM; Collision gas: 9 L/min; Curtain gas: 40 L/min; Nebulize gas: 55 L/min; Auxiliary gas: 55 L/min; Temperature: 500°C; Ionspray voltage: +5500 V. Results for Structure B are in **Table 4**.

[0096] **Table 4.**

Species	Fraction Unbound (Plasma)
Human	0.3255
Rat	0.2760
Mouse	0.2728

[0097] Compound 117 was subjected to a similar protocol. With some variations. The test concentration was 1 μ M, control compounds were warfarin and quinidine. Incubation time was 5 hours. Results for Compound 117 are in **Table 5**.

[0098] **Table 5.**

Species	Fraction unbound (Plasma)
human	0.165
rat	0.213
dog	0.29
monkey	0.277

[0099] Example 4: PK Study in nonhuman primates of Structure B.

[0100] Cynomologous monkeys were administered Structure B at 2 different dose levels. POA was a dose level of 2mg/kg (dosing solution of 0.4mg/ml) and POB was a dose level of 10mg/kg (dosing solution of 2mg/ml). POA was prepared by dissolving 28.8 mg of Structure B in 72.00 mL of 0.5%MC, 0.1%Tween80, 30 mM citrate pH3.5-4 followed by vortexing and sonication to obtain a solution with concentration at 0.4 mg/mL of Structure B.

[0101] POB was prepared by dissolving 109.96 mg of Structure B in 54.980 mL of 0.5%MC, 0.1%Tween80, 30 mM citrate pH3.5-4 followed by vortexing and sonication to obtain a solution with concentration at 2 mg/mL of Structure B.

[0102] HPLC was performed on the samples using the following equipment and parameters. HPLC: Instrument: Shimadzu (DGL-20A5R, Serial No: L20705826727 IX; LC-30AD Serial No: L20555913986 AE and L20555913987 AE; SIL-30AC, Serial No: L20565906455 AE; Rack Changer II Serial No. L20585901289 SS; CTO-30A: Serial No.L20575801653 CD; CBM-20A: Serial No.L20235941035 CD). MS: AB API 5500+ LC/MS/MS instrument (Serial No. EX227122104). Column: Agilent Poroshell 120 EC-C18 4 μ m (50 $\times$ 2.1 mm). Mobile Phase: Solution A: 5% Acetonitrile in Water (0.1%Formic acid); Solution B: 95% Acetonitrile in Water (0.1%Formic acid). Flow rate: 0.6 mL/min, with the following gradient in **Table 6**.

[0103] Table 6.

Time (min)	A (%)	B (%)
0.01	95.0	5.00
0.20	95.0	5.00
1.50	5.00	95.0
1.80	5.00	95.0
1.81	95.0	5.00
2.00	95.0	5.00

[0104] Injection volume: 3 μ L.

[0105] The desired serial concentrations of working solutions were achieved by diluting stock solution of analyte with DMSO. 5 μ L of working solutions (5, 10, 20, 50, 100, 500, 1000, 5000, 10000 ng/mL) were added to 50 μ L of the blank male or female cynomolgus monkeys plasma to achieve calibration standards of 0.5~1000 ng/mL (0.5, 1, 2, 5, 10, 50, 100, 500, 1000 ng/mL) in a total volume of 55 μ L. Five quality control samples at 1 ng/mL, 2 ng/mL, 5 ng/mL, 50 ng/mL and 800 ng/mL for plasma were prepared independently of those used for the calibration curves. These QC samples were prepared on the day of analysis in the same way as calibration standards. 55 μ L of standards, 55 μ L of QC samples and 55 μ L of unknown samples (50 μ L of male and female plasma with 5 μ L of blank solution) were added to 200 μ L of acetonitrile containing IS mixture for

precipitating protein respectively. Then the samples were vortexed for 30 s. After centrifugation at 4 degree Celsius, 3900 rpm for 15 min. The supernatant was diluted 3 times with water. 3 µL of diluted supernatant was injected into the LC/MS/MS system for quantitative analysis.

[0106] Blood samples were taken at the following time points post-does administration: 0.083, 0.17, 0.33, 0.5, 1, 2, 4, 7, 11, 24, and 48 hours.

[0107] Results are shown in **Table 7** for the male cynomolgous monkeys and in **Table 8** for the female cynomolgous monkeys.

[0108] **Table 7.**

Dose	Time (h)	Concentration (ng/mL)
POA	0.083	BLOQ
	0.17	BLOQ
	0.33	BLOQ
	0.5	1.57
	1	13.6
	2	52.6
	4	53.7
	7	47.1
	11	26.1
	24	10.1
	48	2.15
POB	0.083	BLOQ
	0.17	BLOQ
	0.33	10.4
	0.5	44.6
	1	204
	2	291
	4	435
	7	383
	11	222
	24	64.3
	48	1.38

[0109] Table 8.

Dose	Time (h)	Concentration (ng/mL)
POA	0.083	BLOQ
	0.17	BLOQ
	0.33	BLOQ
	0.5	BLOQ
	1	0.601
	2	8.34
	4	24.3
	7	15.7
	11	10.4
	24	3.64
	48	0.898
POB	0.083	BLOQ
	0.17	BLOQ
	0.33	3.56
	0.5	19.2
	1	166
	2	393
	4	451
	7	320
	11	247
	24	80.1
	48	26.6

[0110] BLOQ = Below quantifiable limit of 1 ng/mL for Male; BLOQ = Below quantifiable limit of 0.5 ng/mL for Female. PK Parameters were estimated by non-compartmental model using WinNonlin 6.1. The bioavailability (F%) was calculated as follows: $AUC_{last-PO}/AUC_{INF-PO} > 80\%$:

$$F = (AUC_{INF-PO} \cdot Dose_{IV}) / (\text{mean } AUC_{INF-IV} \cdot Dose_{PO})$$

[0111] $AUC_{last-PO}/AUC_{INF-PO} \leq 80\%$ or AUCINF was not available: $F = (AUC_{last-PO} \cdot Dose_{IV}) / (\text{mean } AUC_{last-IV} \cdot Dose_{PO})$. The PK parameters are set forth in **Table 9** for the male cynomolgous monkeys and **Table 10** for the female cynomolgous monkeys.

[0112] Table 9.

Dose		POA	POB
PK parameters	Unit		
Tl/2	h	10.3	4.95
Tmax	h	4.00	4.00
Cmax	ng/mL	53.7	435
AUClast	h*ng/mL	823	6128
AUCInf	h*ng/mL	856	6138
AUC_%Extrap_obs	%	3.75	0.160
MRTInf_obs	h	14.1	10.7
AUClast/D	h*mg/mL	412	613
F	%	NA	NA

[0113] Table 10.

Dose		POA	POB
PK parameters	Unit		
Tl/2	h	10.6	11.9
Tmax	h	4.00	4.00
Cmax	ng/mL	24.3	451
AUClast	h*ng/mL	295	6869
AUCInf	h*ng/mL	309	7325
AUC_%Extrap_obs	%	4.45	6.21
MRTInf_obs	h	15.0	16.0
AUClast/D	h*mg/mL	148	687
F	%	NA	NA

[0114] Example 5: PK Study in nonhuman primates of Compound 117.

[0115] Female cynomolgous monkeys were administered Compound 117 at 2 different dose levels. POA was a dose level of 1mg/kg (dosing solution of 0.2mg/ml) and POB was a dose level of 3mg/kg (dosing solution of 0.6mg/ml). POA was prepared by dissolving 15.79 mg of Compound 117 in 78.95 mL of deionized water (0.5%MC, 0.1%Tween80), followed by vortexing and sonication to obtain a solution with concentration at 0.2 mg/mL of Compound 117.

[0116] POB was prepared by dissolving 48.85 mg of Compound 117 in 81.417 mL of deionized water (0.5%MC, 0.1%Tween80), followed by vortexing and sonication to obtain a solution with concentration at 3 mg/mL of Compound 117.

[0117] HPLC was performed on the samples using the following equipment and parameters. HPLC: Instrument: Shimadzu (DGL-20A5R, Serial No: L20705518888 IX; LC-30AD Serial No: L20555510784 AE and L20555510780AE; SIL-30AC, Serial No: L20565504983AE; Rack Changer II Serial No. L20585501070 SS; CTO-30A: Serial No. L20575501292 CD; CBM-20A: Serial No. L20235533956 CD). MS: AB API 5500 LC/MS/MS instrument (Serial No. EF20381804). Column: HALO C18 90A 2.7 μ m (50*2.1 mm). Mobile Phase: Solution A: 5% Acetonitrile in Water (0.1%Formic acid); Solution B: 95% Acetonitrile in Water (0.1%Formic acid). Flow rate: 0.6 mL/min, with the following gradient in **Table 11**:

[0118] **Table 11.**

Time (min)	A (%)	B (%)
0.01	100	0.00
0.20	100	0.00
1.90	10.0	90.0
2.20	10.0	90.0
2.21	100	0.00
2.50	100	0.00

[0119] Injection volume: 1 μ L.

[0120] The desired serial concentrations of working solutions were achieved by diluting stock solution of analyte with 50% acetonitrile in water solution. 5 μ L of working solutions (5, 10, 20, 100, 500, 1000, 5000, 10000 ng/mL) were added to 50 μ L of the blank monkey plasma to achieve calibration standards of 0.5~1000 ng/mL (0.5, 1, 2, 10, 50, 100, 500, 1000 ng/mL) in a total volume of 55 μ L. Four quality control samples at 1 ng/mL, 2 ng/mL, 50 ng/mL and 800 ng/mL for plasma were prepared independently of those used for the calibration curves. These QC samples were prepared on the day of analysis in the same way as calibration standards. 55 μ L of standards, 55 μ L of QC samples and 55 μ L of unknown samples (50 μ L of monkey plasma with 5 μ L of blank solution) were added to 200 μ L of acetonitrile containing IS mixture for precipitating protein respectively. Then the samples were vortexed for 30 s. After centrifugation at 4 degree Celsius, 3900 rpm for 15 min. The supernatant was diluted 3 times with water. 1 μ L of diluted supernatant was injected into the LC/MS/MS system for quantitative analysis.

[0121] Blood samples were taken at the following time points post-does administration: 0.083, 0.17, 0.33, 0.5, 1, 2, 4, 7, 11, 24, and 48 hours.

[0122] Results are shown in Table 12.

[0123] Table 12.

Dose	Animal Number	Time (h)	Concentration (ng/ml)
POA	1	0.0833	BLOQ
POA	1	0.17	BLOQ
POA	1	0.33	2.40
POA	1	0.5	9.16
POA	1	1	23.8
POA	1	2	23.1
POA	1	4	20.6
POA	1	7	11
POA	1	11	6
POA	1	24	2.8
POA	1	48	0.5
POA	2	0.083	BLOQ
POA	2	0.17	BLOQ
POA	2	0.33	BLOQ
POA	2	0.5	BLOQ
POA	2	1	1.59
POA	2	2	3.31
POA	2	4	2.1
POA	2	7	1.3
POA	2	11	0.957
POA	2	24	BLOQ
POA	2	48	BLOQ
POA	3	0.083	BLOQ
POA	3	0.17	BLOQ
POA	3	0.33	1.1
POA	3	0.5	2.88
POA	3	1	11.3
POA	3	2	15.7
POA	3	4	14.2
POA	3	7	9.35
POA	3	11	5.25
POA	3	24	1.68

Dose	Animal Number	Time (h)	Concentration (ng/ml)
POA	3	48	BLOQ
POB	4	0.083	BLOQ
POB	4	0.17	BLOQ
POB	4	0.33	3.78
POB	4	0.5	7.97
POB	4	1	22.9
POB	4	2	22.7
POB	4	4	21.7
POB	4	7	15.8
POB	4	11	9.49
POB	4	24	2.91
POB	4	48	BLOQ
POB	5	0.083	BLOQ
POB	5	0.17	0.601
POB	5	0.33	0.742
POB	5	0.5	1.9
POB	5	1	27.6
POB	5	2	36.2
POB	5	4	28.2
POB	5	7	17
POB	5	11	9.36
POB	5	24	3.56
POB	5	48	0.756
POB	6	0.083	BLOQ
POB	6	0.17	BLOQ
POB	6	0.33	2.85
POB	6	0.5	8.87
POB	6	1	56.2
POB	6	2	105
POB	6	4	92.7
POB	6	7	59.1
POB	6	11	42.6
POB	6	24	14.4
POB	6	48	2.42

[0124] BLOQ = Below quantifiable limit of 0.5 ng/mL; PK parameters were estimated by non-compartmental model using WinNonlin 6.1; The bioavailability (F%) was calculated as following: $AUC_{last-PO}/AUC_{inf-PO} > 80\%$: $F = (AUC_{inf-PO} \cdot Dose_{IV}) / (\text{mean } AUC_{inf-IV} \cdot Dose_{PO})$; $AUC_{last-PO}/AUC_{inf-PO} \leq 80\%$ or AUC_{inf} was not available: $F = (AUC_{last-PO} \cdot Dose_{IV}) / (\text{mean } AUC_{last-IV} \cdot Dose_{PO})$; NA = Not available. The PK parameters are set forth in Table 13 as a mean for all the animals given a particular dose.

[0125] Table 13.

Dose		POA	POB
PK parameters	Unit		
T _{1/2}	h	8.1	8.8
T _{max}	h	1.67	1.67
C _{max}	ng/mL	14.3	55
AUC _{last}	h*ng/mL	143	641
AUC _{inf}	h*ng/mL	154	665
AUC_%Extrap_obs	%	15.0	5.2
MRT _{inf_obs}	h	10.7	11.5
AUC _{last} /D	h*mg/mL	143	214
F	%	19.3	28.3

[0126] Example 6. RAT PK following IV administration of Structure B

[0127] Female Sprague Dawley rats were administered Structure B by IV at a dose level of 1mg/kg (dosing solution of 0.2mg/ml). The IV dose was prepared by dissolving 1.06 mg of Structure B in 5.3 mL of 30%HP-β-CD followed by vortexing and sonication to obtain a solution with concentration at 0.2 mg/mL of Structure B.

[0128] HPLC was performed on the samples using the following equipment and parameters. HPLC: Instrument: Shimadzu (DGU-20A5R, Serial No: L20705826739 IX; LC-30AD Serial No: L20555913985 AE and L20555913969 AE; SIL-30AC, Serial No: L20565806434 AE; Rack Changer II Serial No. L20585801286 SS; CTO-30A: Serial No.L20575801652 CD; CBM-20A: Serial No.L20235941033 CD). MS: AB API 5500 LC/MS/MS instrument (Serial No. EX227152104). Column: Agilent Poroshell 120 EC-C8 4 μm (50 × 2.1 mm). Mobile Phase: Solution A: 5% Acetonitrile in Water (0.1%Formic acid); Solution B: 95% Acetonitrile in Water (0.1%Formic acid). Flow rate: 0.6 mL/min, with the following gradient in Table 14:

[0129] Table 14.

Time (min)	A (%)	B (%)
0.01	95.0	5.00
0.20	95.0	5.00
1.50	5.00	95.0
1.80	5.00	95.0
1.81	95.0	5.00
2.00	95.0	5.00

[0130] Injection volume: 5 μ L.

[0131] The desired serial concentrations of working solutions were achieved by diluting stock solution of analyte with 50% acetonitrile in water solution. 5 μ L of working solutions (1, 2, 4, 10, 20, 100, 200, 1000, 2000 ng/mL) were added to 50 μ L of the blank SD Rat plasma to achieve calibration standards of 0.5~1000 ng/mL (0.5, 1, 2, 5, 10, 50, 100, 500, 1000 ng/mL) in a total volume of 55 μ L. Five quality control samples at 1 ng/mL, 2 ng/mL, 5 ng/mL, 50 ng/mL and 800 ng/mL for plasma were prepared independently of those used for the calibration curves. These QC samples were prepared on the day of analysis in the same way as calibration standards. 50 μ L standards, 50 μ L QC samples and 50 μ L unknown samples (50 μ L plasma with 5 μ L blank solution) were added to 200 μ L of acetonitrile containing IS mixture for precipitating protein respectively. Then the samples were vortexed for 30 s. After centrifugation at 4 degree Celsius, 3900 rpm for 15 min, the supernatant was diluted 3 times with water. 5 μ L of diluted supernatant was injected into the LC/MS/MS system for quantitative analysis.

[0132] Blood samples were taken at the following time points post-dose administration: 0.083, 0.17, 0.33, 0.5, 1, 2, 4, 7, 11 and 24 hours.

[0133] Results are shown in Table 15.

[0134] Table 15.

Animal Number	Time (h)	Concentration (ng/ml)
1	0.083	196
1	0.17	141
1	0.33	109
1	0.5	99.5
1	1	69.4
1	2	50.0
1	4	35.7
1	7	25.6
1	11	15.9

Animal Number	Time (h)	Concentration (ng/ml)
1	24	3.29
2	0.083	188
2	0.17	123
2	0.33	103
2	0.5	99.1
2	1	54.2
2	2	39.2
3	4	44.8
3	7	19.6
2	11	13.6
2	24	2.77
3	0.083	185
3	0.17	137
3	0.33	129
3	0.5	94.7
3	1	55.4
3	2	55.3
3	4	38.4
3	7	31.6
3	11	13.2
3	24	2.70
1	0.083	196
1	0.17	141
1	0.33	109
1	0.5	99.5
1	1	69.4
1	2	50.0
1	4	35.7
1	7	25.6
1	11	15.9
1	24	3.29
2	0.083	188
2	0.17	123
2	0.33	103

Animal Number	Time (h)	Concentration (ng/ml)
2	0.5	99.1
2	1	54.2
2	2	39.2
3	4	44.8
3	7	19.6
2	11	13.6
2	24	2.77
3	0.083	185
3	0.17	137
3	0.33	129
3	0.5	94.7
3	1	55.4
3	2	55.3
3	4	38.4
3	7	31.6
3	11	13.2
3	24	2.70

[0135] BLOQ = Below quantifiable limit of 0.5 ng/mL; PK parameters were estimated by non-compartmental model using WinNonlin 6.1; The bioavailability (F%) was calculated as following:

$AUC_{last-PO}/AUC_{INF-PO} > 80\%$: $F = (AUC_{INF-PO} * Dose_{IV}) / (\text{mean } AUC_{INF-IV} * Dose_{PO})$

$AUC_{last-PO}/AUC_{INF-PO} \leq 80\%$ or AUC_{INF} was not available: $F = (AUC_{last-PO} * Dose_{IV}) / (\text{mean } AUC_{last-IV} * Dose_{PO})$; NA = Not available. The PK parameters are set forth in Table 16.

[0136] Table 16.

PK parameters	Unit	Rat 1	Rat 2	Rat 3	Mean	SD	CV(%)
Cl _{obs}	mL/min/kg	28.4	31.4	28.9	29.6	1.6	5.43
T _{1/2}	h	5.74	5.93	4.98	5.55	0.50	9.02
C ₀	ng/mL	268	282	246	266	18	6.73
AUC _{last}	h*ng/mL	559	507	557	541	30	5.47
AUC _{Inf}	h*ng/mL	586	530	576	564	30	5.28
AUC _{%Extrap_obs}	%	4.64	4.47	3.37	4.16	0.69	16.6
MRT _{Inf_obs}	h	6.98	6.76	6.34	6.69	0.32	4.85
AUC _{last/D}	h*mg/mL	559	507	557	541	30	5.47
V _{ss_obs}	L/kg	11.9	12.8	11.0	11.9	0.9	7.34

[0137] Example 7 Rat PK for Compound 117.

[0138] Male Sprague Dawley rats were administered Compound 117 by IV at a dose level of 1mg/kg (dosing solution of 0.5mg/ml). The IV dose was prepared by dissolving 1.17 mg of Compound 117 in 0.117 mL of DMSO followed by vortexing for 2 minutes and sonication for 3 minutes, then 0.117 mL of Solutol HS15 was added and vortexed for 3 minutes and finally 2.106mL of saline was added and vortexed for 3 minutes to obtain a solution with concentration at 0.5 mg/mL of Compound 117.

[0139] Analysis was carried out on samples using the following equipment and parameters: LCMSMS-39 (Triple Quad 6500⁺); Positive ion, ESI; MRM detection. HPLC was performed with a Waters X-Bridge BEH C18 (2.1×50 mm, 1.7 µm) column at 50°C, using solution A: H₂O-0.025% FA-1mM NH₄OAc; solution B: ACN-0.025% FA-1mM NH₄OAc, a flow rate of 0.6 mL/min, with the following gradient set forth in **Table 17**:

[0140] Table 17.

Time (min)	Mobile Phase B (%)
0.300	2.00
1.00	90.0
1.50	90.0
1.51	2.00
2.00	Stop

[0141] 1 µL of supernatant was injected into the LC/MS/MS system for quantitative analysis.

[0142] Blood samples were taken at the following time points post-dose administration: 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hours after administration.

[0143] Results are shown in **Table 18**.

[0144] Table 18.

Animal Number	Time (h)	Concentration (ng/ml)
1	0.083	85.9
1	0.25	77.8
1	0.5	67.7
1	1	45.9
1	2	37.2
1	4	26.5
1	8	14.0
1	24	1.46
2	0.083	91.2

2	0.25	72.6
2	0.5	67.4
2	1	49.6
2	2	37.0
2	4	26.8
2	8	10.5
2	24	1.14
3	0.083	82.0
3	0.25	75.2
3	0.5	74.8
3	1	49.2
3	2	37.7
3	4	22.4
3	8	13.4
3	24	1.09

[0145] PK parameters were estimated by non-compartmental model using WinNonlin 8.2; NA = Not available. The PK parameters are set forth in **Table 19**.

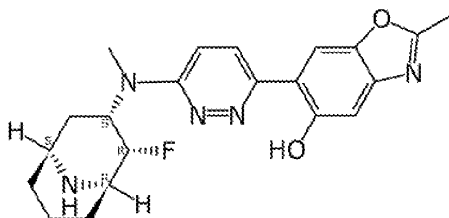
[0146] **Table 19.**

PK parameters	Unit	Rat 1	Rat 2	Rat 3	Mean	SD	CV(%)
CL	L/hr/kg	2.58	2.85	2.72	2.72	0.136	5.02
V _{ss}	L/kg	14.6	14.4	14.3	14.4	0.163	1.13
T _{1/2}	hr	4.82	4.43	4.54	4.59	0.200	4.36
AUC _{last}	hr*ng/mL	377	343	361	361	17.1	4.75
AUC _{INF}	hr*ng/mL	388	351	368	369	18.6	5.03
MRT _{last}	hr	4.99	4.50	4.76	4.75	0.241	5.07
MRT _{INF}	hr	5.67	5.04	5.26	5.32	0.316	5.94
CL	mL/min/kg	43.0	47.5	45.3	45.3	2.27	5.02

CLAIMS

What is claimed is:

1. A compound of structure B, or a pharmaceutically acceptable salt or a stereoisomer thereof:



Structure B.

2. A pharmaceutical composition comprising the compound of claim 1, or a pharmaceutically acceptable salt or stereoisomer thereof, and a pharmaceutically acceptable excipient or carrier.
3. A method of modulating splicing comprising administering to cells the compound of claim 1, or a pharmaceutically acceptable salt or stereoisomer thereof, wherein the compound modulates splicing at a splice site sequence of a pre-mRNA that encodes an mRNA, wherein the mRNA encodes a target protein or a functional RNA.
4. A method of treating a disease or condition comprising administering the compound of claim 1, or a pharmaceutically acceptable salt or stereoisomer thereof, to a subject in need thereof.
5. Use of the compound of claim 1, or a pharmaceutically acceptable salt or stereoisomer thereof, in the manufacture of a medicament for the treatment of a condition or disease.