

METHODS AND COMPOSITIONS OF POLYCYCLIC AND A DUAL ACTING NITROXIDE COMPOUNDS FOR TREATMENT OF FAT CELL ASSOCIATED DISEASES

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/612,864, filed on December 20, 2023. The entire teachings of the above application are incorporated herein by reference.

TECHNICAL FIELD

[0002] The present disclosure describes compounds, compositions of polycyclic, water soluble and a dual acting nitroxide substituted compounds and methods useful as mitogen-activated protein (MAP) kinase-interacting kinases1 and 2 (MNK1 and MNK2) inhibitors, for the treatment of cancer, Alzheimer's disease, autism, fragile X syndrome, obesity and fat cell associated diseases such as liposarcoma, metabolic-associated steatohepatitis (MASH), metabolic-associated fatty liver disease (MAFLD), sarcopenic obesity and polycystic ovary syndrome, and a method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain. The present disclosure produces the unexpected discovery that the compounds discovered herein target adipocytes and demonstrate *in vivo* efficacy, which was accurately predicted at the early, *in vitro* stage using high-throughput M³ technology (MelliCell, Inc.).

BACKGROUND

[0003] Worldwide obesity has nearly tripled in the last 50 years. Obesity and overweight are defined as excessive and abnormal growth of fat cells or body fat that increases the risk of high blood pressure, gallstones, asthma, sleep apnea, obesity hypoventilation syndrome, osteoarthritis, gout and several deadly diseases, including diabetes, coronary heart disease, and signs of damage to other organ systems, most often the liver and kidneys, dysfunction, and in severe cases, polycystic ovary syndrome and even cancers including esophageal, pancreatic, colorectal, breast, uterine, liposarcoma and ovarian. Obesity also increases a risk of premature death from the above causes (Müller, T. *et al*, *Nature Review Drug Discovery*, 21, 201-223, 2022).

[0004] Metabolism is the process that converts calories into energy to fuel the body's function. Metabolic syndrome is a common factor in obesity and contributes to poor health and many diseases. When there are extra calories available than required, the body converts

the extra calories into lipids and stores them in body fats or adipose tissue. The fat cells become enlarged when adipose tissue run out of storage space due to excessive lipids production, and then enlarged fat cells secrete hormones and other chemicals that produce an inflammatory response. Chronic inflammation affects metabolism by contributing to insulin resistance that means the body can no longer use insulin to lower blood glucose and blood lipid levels, which contributes to high cholesterol, triglycerides and eventually into high blood pressure. These combined risk factors (metabolic syndrome) reinforce further weight gain and make it harder to lose weight. Excess body fat can crowd the organs and put stress and strain on them to function properly.

[0005] Sarcopenia is a disease where a decline in skeletal muscle mass and strength occurs. This decline is part of a normal physiological aging process; as well as from several factors that exacerbate this situation, such as low physical activity, inadequate nutrition, neurodegenerative disease, and inflammatory conditions. These factors result in increasing frailty and an increased risk of mortality. Sarcopenia along with obesity (Sarcopenic obesity) has emerged as an important cardiovascular risk factor, particularly in older adults with overweight and obesity due to excess fat mass and reduced skeletal muscle mass and strength.

[0006] Obesity is treated by changing diet, increasing physical activities, counseling, weight loss surgery and using appetite suppressant medications. The appetite suppressant medications can intercept some of the pathways to brain that normally reduce appetite, slow digestion, feel full, and cravings and food intake. The common drugs approved by FDA in this class are Phentermine (ADIPEX-®, LOMAIRA®, SUPRENZA®), Benzphetamine (DIDREX®, REGIMEX®), Diethylpropion (DEPLETITE 2®, RADTUE®, TENUATE®), Phendimetazine (BONTRIL®, MELFIAT®), Bupropion-naltrexone (CONTRAVE®), Lisdexamfetamine dimesylate (VYVANSE®), Cellulose and citric acid (PLENITY®), Liraglutide (SAXENDA®), Semaglutide (WEGOVY®), Tirzepatide (ZEPBOUND®) and combinations of Phentermine-topiramate (QSYMIA®) and SGLT2 inhibitors - glucagon-like-1 receptor agonists, and the drug that reduces absorption of fat from gut, Orlistat (XENICAL®, ALLI®) (Son, J. *et al*, *Diabetes Metab J.*, 44, 802-818, 2020).

[0007] Adipose tissue plays a critical role in regulating the pathophysiological mechanisms of obesity and its related co-morbidities. Obesity and overweight can induce systemic oxidative stress through various biochemical pathways, such as protein kinase C activation, superoxide generation from NADPH oxidases, oxidative phosphorylation, glyceraldehyde auto-oxidation, and polyol and hexosamine pathways (Manna P., *Metab*

Syndr Relat Disord. 13(10): 423–444, 2015). Other factors contributing to oxidative stress in obesity include hyperleptinemia, low antioxidant defense, chronic inflammation, and postprandial reactive oxygen species generation.

[0008] MNK, a Ser/Thr kinase, is the only kinase known to phosphorylate eIF4E at serine 209 which is its only phosphorylation site. Activation of the MNK is firmly established in diet induced obesity models via its stimulation of the endogenous pathway (<https://doi.org/10.1016/j.molmet.2020.101054>. (b) DOI: 10.1038/srep23476 (c) <https://doi.org/10.1038/s41392-022-01149-x>). Disruption of either one or both MNK1 and MNK2 genes has no reported adverse effects. The double knockout (DKO) mice being viable, fertile and without reported abnormalities (DOI: 10.1128/MCB.24.15.6539-6549.2004). MNK-DKO mice were almost completely protected from HFD-induced obesity (3). Higher energy expenditure in MNK-DKO mice was observed, which probably reflects the changes in a number of genes or proteins linked to lipolysis, mitochondrial function/biogenesis, oxidative metabolism, and/or ATP consumption in rodents (<https://doi.org/10.1007/s13311-020-00932-4>). Several selective MNK1/2 inhibitors, such as eFT508, BAY1143269 and ETC-206, show antitumor efficacy in various oncology and anti-inflammatory models. These results show that blocking the eIF4E phosphorylation by selectively inhibiting MNK1/2 can be an effective therapeutic strategy to treat related diseases (Weijun Xu et al., *J. Med. Chem.* 2022, 65, 983–1007; WO 2023/278686 A1, WO 2018/134148 A1, WO 2022/006331 A2, WO 2023/278686 A1, WO 2023/014943 A1, US 2016/0303124 A1, WO 2015/200481 A1, WO 2022/038563 A1, and US 2018/0228803 A1).

[0009] Although weight loss is a key therapeutic objective for patients with type 2 diabetes, only a handful of FDA approved drugs are indicated for weight loss and none target the actual cells that store excess weight, the adipocyte. The lack of safe and effective drugs that target adipocytes stems from the inability of commercial experimental models to adequately predict adipocyte pathophysiology in humans with obesity (Anand, S.S., *PLOS One* 6, e22112, 2011). The conventional *in vitro* adipocyte model used by pharmaceutical companies does not support growth by increasing cell volume (adipocyte hypertrophy). MelliCell, Inc. uses patented high-throughput (M³) technology, that enables adipocyte hypertrophy *in vitro* (Cell system and methods of use. AL Glieberman, BD Pope, DC Bouck. US Patent App. 18/286,077, 2024). This invention produces the unexpected discovery that the compounds discovered herein target adipocytes and demonstrate *in vivo* efficacy, which was accurately predicted at the early, *in vitro* stage using high-throughput M³ technology (MelliCell, Inc.).

BRIEF DESCRIPTION OF THE DRAWINGS

- [0010] FIG. 1 is a dose dependent fat loss in M³ adipocytes after treatment with a prototypical MNK inhibitor hit molecule, MNK IN-IV.
- [0011] FIG. 2 shows dose dependent increase in mitochondrial content in M³ adipocytes after treatment with the MNK inhibitor, MCL5008.
- [0012] FIG. 3 shows the effect of an MNK inhibitor on body weight in combination with tirzepatide in mice with diet induced obesity.
- [0013] FIG. 4 shows MNK inhibition improves blood glucose.
- [0014] FIG. 5 shows the effect of an MNK inhibitor on body weight in combination with retatrutide in mice with diet induced obesity.
- [0015] FIG. 6 shows a Four-month study of MCL5008 in C57BL/6 mice with diet induced obesity.
- [0016] FIG. 7 shows a comparison of fat loss and lean mass preservation after treatment with MCL5008 versus tirzepatide or the combination (tirzepatide + MCL5008) in C57BL/6 mice with diet induced obesity.
- [0017] FIG. 8 shows a follow-on therapy with MCL5008 retained the weight loss effects of tirzepatide after tirzepatide dosing was stopped.
- [0018] FIG. 9 shows western blots of target protein and target expression in fat in HFD fed young mice when treated with MCL5008 (20 mg/kg/PO/QD) for 3 weeks.
- [0019] FIG. 10 shows PK Parameters and tissue distribution data in HFD fed Young Mice (3 weeks study), and Aged Mice (4 months study) treated with MCL5008
- [0020] FIG. 11 shows Hematoxylin & Eosin staining of abdominal fat, and liver from HFD fed aged mice treated with vehicle (water) and MCL5008 (30mg/kg/bid in water). Scale bar: 50µm. Adipocyte size measure from the histological data, N= 6-8 animals/group; **** indicates adj p-value <0.0001 using One-Way ANOVA with Šidák's multiple comparisons test.
- [0021] FIG. 12 shows Leptin ELISA data from serum of mice with diet induced obesity treated with and MCL5000 (10mg/kg/qd) or a combination with tirzepatide. n = 4 animals/group.
- [0022] FIG. 13 shows Serum triglycerides and LDL cholesterol in mice with diet induced obesity treated with MCL5000 (10mg/kg/qd) vs tirzepatide or a combination.
- [0023] FIG. 14 shows a Chiral HPLC of compound If indicated four peaks. The diastereomeric isomers of If (3 g) were separated by chiral super critical fluid

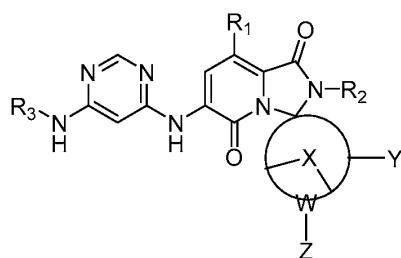
chromatography (SFC) using mobile phase A: water/ 0.1% formic acid and mobile phase B: Acetonitrile/0.1% formic acid.

SUMMARY

[0024] After screening 1,280 compounds, a high-throughput model of mature human adipocytes identified a hit MNK IN IV (FIG. 1) and Tomivosertib (eft508) that target mitogen-activated protein kinase (MAPK)-interacting serine/threonine-protein kinase 1 and 2 (MNK-1 and MNK-2). Provided herein are compounds which are novel MNK 1 and 2 inhibitors of Formula I, II and III based on their biological targets for reversing adipocyte hypertrophy, and the association of those targets with metabolic disease.

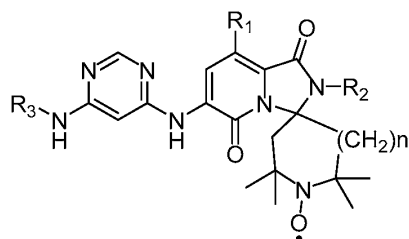
[0025] Cyclic nitroxides (also known as aminoxyls or nitroxyls), are stable free radicals stabilized by adjacent methyl groups at the α position in five, six and seven-membered ring structures. The methyl groups confer stability to the nitroxide radicals, which can quench the radical species. It is shown that the nitroxide radical TEMPOL prevents obesity, hyperlipidemia, elevation of inflammatory cytokines, and modulates atherosclerotic plaque composition in apoE^{-/-} mice (Kim C.H.J. *et al*, *Atherosclerosis*, 240 (1):234-41, 2015).

[0026] Provided herein are substituted polycyclic compounds of the general Formula I

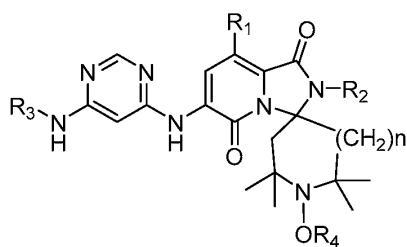


I

and dual acting nitroxide substituted compounds selected from Formula II or Formula III, derived from the polycyclic MNK-1 and 2 inhibitors of Formula I.



II



III

or enantiomers, diastereomers, racemates, or pharmaceutically acceptable salts thereof,

wherein

R1 is selected from F, Cl, Br, I, Me, Et, OMe, OCF₃, NH₂ or OH

R2, R3 and R4 are independently selected from H, CH₂OCOCH₃, CH₂OPO(ONa)₂, COCH₂NMe₂, COCH₂CH₂COOEt, COCH₂CH₂COOH, amide chain linked to the amino acids such as glycine, valine, lysine, arginine, tyrosine, leucine, alanine, cysteine, serine, tryptophan or proline

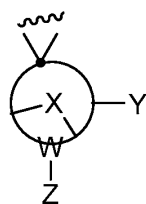
W is selected from N or N-Me

X is selected from N, NR, O, CH₂, CH₂CH₂ or CMe₂, wherein R is H, Me, Et, CHMe₂

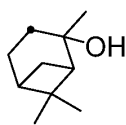
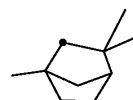
Y is selected from H, =CH₂, Me, F or combination of Me and OH or forming C3-C6 rings (e.g., cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl)

Z is selected from O[•] (O radical), OH, OCOCH₃, OR₂ or OR₃ or OR₄

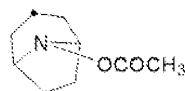
n is 0 to 3.



is selected from the following polycyclic rings in Formula I:

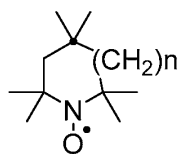
R/R/R -pinanone
derivativetropinone
derivativecamphor
derivativeenchone
derivativemethylene-2-nor
bornanone derivativetricyclic-deca
derivativetricyclic-dec-en
derivative2-adamantanone
derivativehexahydro-1H-4,7-methano
inden-5(6H)-one derivatives

or

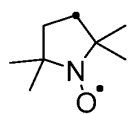
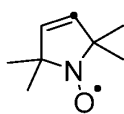
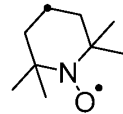
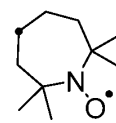


tropinone and tetramethylpiperidine derivatives

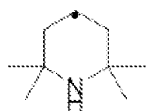
or



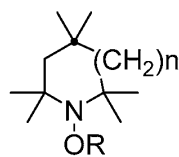
is selected from the following nitroxide rings in Formula II and III:

3-oxo-proxyl
derivative3-oxo-proxyl
derivative4-oxo-TEMPO
derivative4-oxo-TEMAO/
azepane derivative

or



or



R is selected from H, COCH₃, and amino acids, such as glycine,

valine, lysine, arginine, tyrosine, leucine, alanine, cysteine, serine, tryptophan or proline.

[0027] In a related aspect, provided herein are methods for the substituted polycyclic compounds of the general Formula I and dual acting nitroxide substituted compounds of Formula II and III, derived from the polycyclic MNK-1 and 2 inhibitors of Formula I for use in the treatment of cancer, Alzheimer's disease, autism, fragile X syndrome (FXS), obesity and fat cell associated diseases such as liposarcoma, MASH, MAFLD, sarcopenic obesity and polycystic ovary syndrome, and a method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain.

[0028] In another aspect, provided herein is a pharmaceutical composition comprising a compound of the general Formula I, II and III as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, for treatment of obesity and fat cell enlargement associated diseases such as liposarcoma, sarcopenic obesity and polycystic ovary syndrome.

[0029] In yet another aspect, provided herein is the use of a compound of the general Formula I, II and III as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition for treatment of cancer, Alzheimer's disease, autism, fragile X-syndrome (FXS), obesity and fat cell associated diseases such as liposarcoma, MASH, MAFLD, sarcopenic obesity and polycystic ovary syndrome, and a method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain.

[0030] In a further aspect, provided herein is a method for treatment of obesity and fat cell associated diseases such as liposarcoma, sarcopenic obesity and polycystic ovary syndrome in an individual in need thereof, comprising administering to said individual an effective amount of a compound of the general Formula I, II and III as defined above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof.

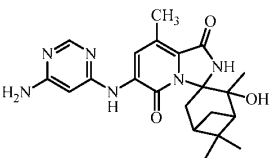
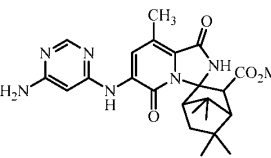
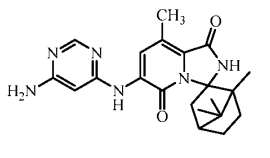
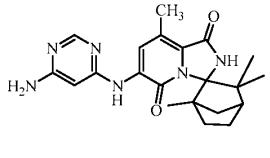
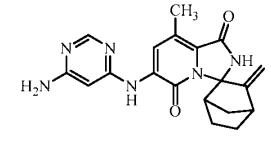
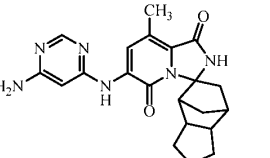
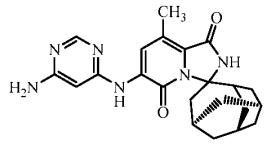
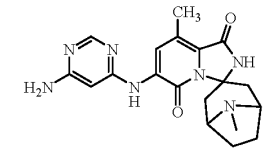
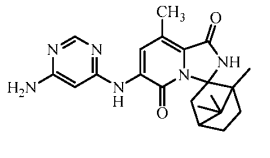
[0031] Other features and advantages of the hemostatic compositions and methods provided herein will be apparent from the following detailed description and examples which should not be construed as limiting. The contents of all references, patents, and published patent applications cited throughout this application are expressly incorporated herein by reference.

DETAILED DESCRIPTION

[0032] The features and advantages of compositions and methods provided herein may be more readily understood by those of ordinary skill in the art upon reading the following detailed description. Embodiments identified herein as exemplary or preferred are intended to be illustrative and not limiting.

[0033] In one aspect, disclosed herein are of the general Formula I, II and III as defined above for use in the treatment of cancer, Alzheimer's disease, autism, Fragile X syndrome (FXS), obesity and fat cell associated diseases such as liposarcoma, MASH, MAFLD, sarcopenic obesity and polycystic ovary syndrome, and a method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain.

Table 1: Structures I indicating the polycyclic compounds

 (R/R)-pinanone derivative) Ia	 (R/R)-kitopinic acid derivative) Ib	 (R)-camphor derivative) Ic
 (fenchone derivative) Id	 (methylene-2-norbornanone) Ie	 (hexahydro-1H-4,7-methanoinden-5(6H)-one derivative) If and Ifa-d isomers
 (2-adamantanone derivative) Ih	 (tropinone derivative) Ii	 (S)-camphor derivative) Ij

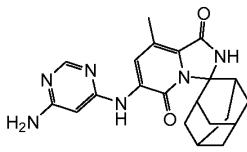
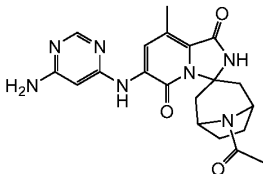
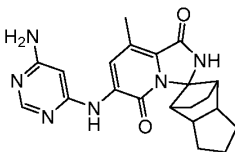
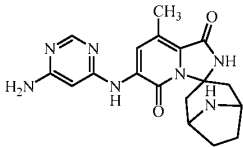
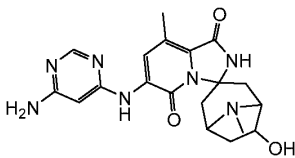
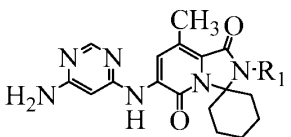
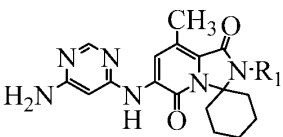
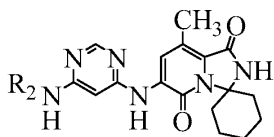
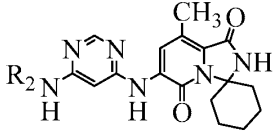
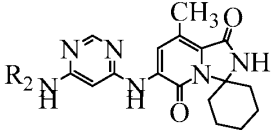
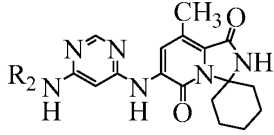
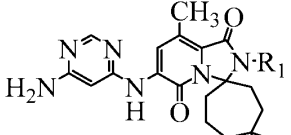
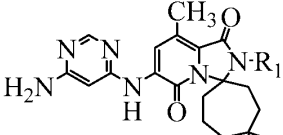
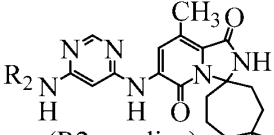
 Ik	 Il	 Im
 (troponone derivative) In	 Io	

Table 2: Structures I indicating the prodrugs

 (R1 = CH₂OPO(ONa)₂) Ip	 (R1 = CH₂OCOCH₃) Iq	 (R2 = valine) Ir
 (R2=COCH₂NMe₂) Is	 (R2=leucine) It	 (R2=arginine) Iu
 (R1 = CH₂OPO(ONa)₂) Iv	 (R1 = CH₂OCOCH₃) Iw	 (R2 = valine) Ix

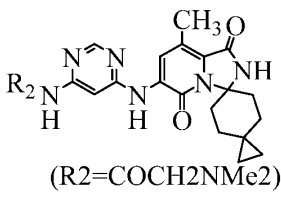
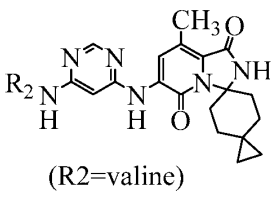
Iv	Iw	
 (R2=COCH2NMe2) Iy	 (R2=valine) Iz	

Table 3: Structures II indicating the dual acting nitroxide compounds

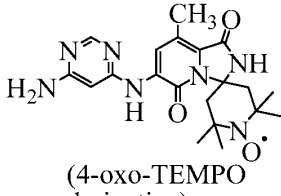
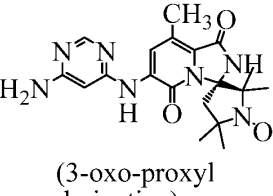
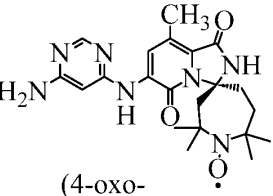
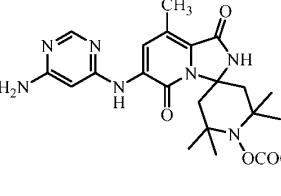
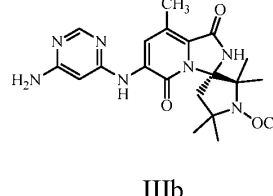
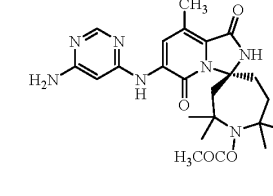
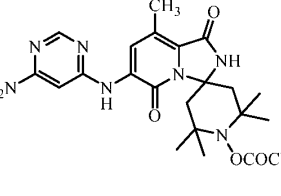
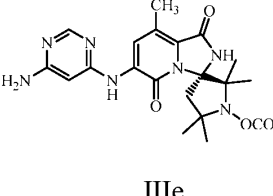
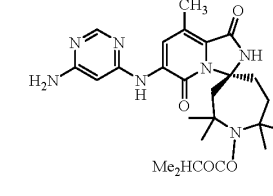
 (4-oxo-TEMPO derivative) IIa -MCL5008	 (3-oxo-proxyl derivative) IIb	 (4-oxo-hexamethylene TEMPO derivative) IIc
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Table 4: Structures III indicating the dual acting nitroxide ester compounds

 IIIa	 IIIb	 IIIc
 III d	 IIIe	 III f

[0034] In some embodiments, the compounds are of the formula I, II and III in **Table 1 - 4**, wherein the compounds are either pure enantiomers or diastereomers.

[0035] In some embodiments, the compound is a compound of the formula I, II and III in Table 1 - 4, wherein the compounds are either cis or trans (E or Z) isomers.

[0036] There has been a long-felt, significant and unmet need for a dual acting MNK inhibitors and redox catalyst class of anti-obesity drugs with enhanced bioavailability and less toxicity, especially a potential hepatotoxicity and penetration of blood-brain barrier, and the prodrugs substituted amino acids of the present invention are an effective approach by which properties of these compounds such as drug pharmacokinetics, pharmacodynamics and toxicology can be modulated.

Table 5: Prodrugs to active molecule

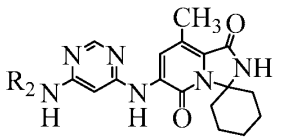
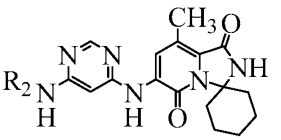
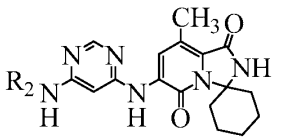
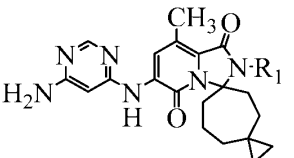
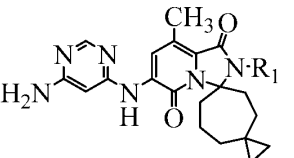
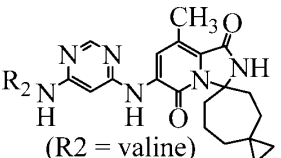
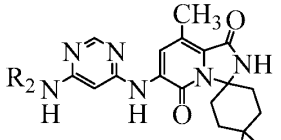
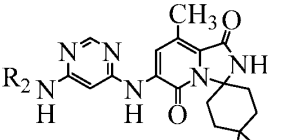
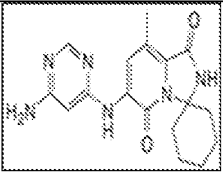
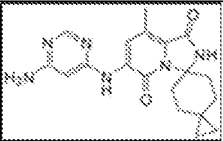
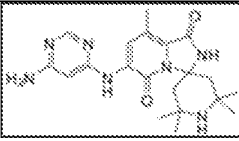
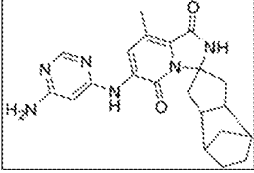
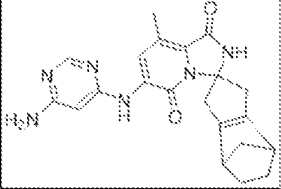
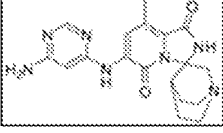
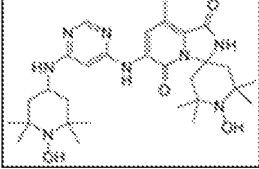
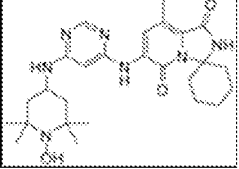
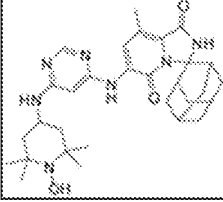
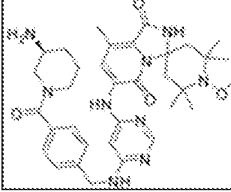
 (R2=COCH2NMe2) Is	 (R2=leucine) It	 (R2=arginine) Iu
 (R1 = CH2OPO(ONa)2) Iv	 (R1 = CH2OCOCH3) Iw	 (R2 = valine) Ix
 (R2=COCH2NMe2) Iy	 (R2=valine) Iz	

Table 6. Active molecules

 Iab (MCL5000)	 Iac	 Iaa
 Iad	 Iae	 Iaf
 Iag	 Iah	 Iai
 Iaj		

[0037] The compounds provided herein may be synthesized according to any technology or procedure known in the art, or as described in experimental section and the prodrugs will be hydrolyzed *in vivo* into the parent drug as displayed in Table 5.

[0038] The compounds of the general formula I, II and III can have one or more asymmetric centers, and can accordingly exist both as enantiomers, *i.e.*, optical isomers (R, S, or racemate, wherein a certain enantiomer may have an optical purity of 90%, 95%, 99% or more) and as diastereoisomers. Specifically, those chiral centers may be, *e.g.*, in each one of the carbon atoms of the macrocyclic ring, of the general formulas I. It should be understood that the present disclosure encompasses the use of all such enantiomers, isomers and mixtures thereof, as well as pharmaceutically acceptable salts thereof.

[0039] Optically active forms of the compounds of the general formula I can be prepared using any method known in the art, *e.g.*, by resolution of the racemic form by recrystallization techniques; by chiral synthesis; by extraction with chiral solvents; or by chromatographic separation using a chiral stationary phase. A non-limiting example of a method for obtaining optically active materials is transport across chiral membranes, *i.e.*, a technique whereby a racemate is placed in contact with a thin membrane barrier, the concentration or pressure differential causes preferential transport across the membrane barrier, and separation occurs as a result of the non-racemic chiral nature of the membrane that allows only one enantiomer of the racemate to pass through. Chiral chromatography, including simulated moving bed chromatography, can also be used. A wide variety of chiral stationary phases are commercially available.

[0040] In another aspect, provided herein is a pharmaceutical composition comprising a compound of the general formula I as defined in any one of the embodiments above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof (herein also referred to as the “*active agent*”), and a pharmaceutically acceptable carrier, for treatment of obesity and fat cell associated diseases such as liposarcoma, sarcopenic obesity and polycystic ovary syndrome. In some embodiments, the pharmaceutical compositions comprise, as an active agent, a compound selected from the compounds of **Table 1 - 4** above, *e.g.*, compound **I**, **II** and **III**, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof.

[0041] The pharmaceutical compositions provided herein can be provided in a variety of formulations, *e.g.*, in a pharmaceutically acceptable form and/or in a salt form, as well as in a variety of dosages.

[0042] In one embodiment, the pharmaceutical composition provided herein comprises a non-toxic pharmaceutically acceptable salt of a compound of the general formula I. Suitable pharmaceutically acceptable salts include acid addition salts such as, without being limited to, the mesylate salt, the maleate salt, the fumarate salt, the tartrate salt, the hydrochloride salt, the hydrobromide salt, the mesylate salt, the *p*-toluenesulfonate salt, the benzenesulfonate salt, the benzoate salt, the acetate salt, the phosphate salt, the sulfate salt, the citrate salt, the carbonate salt, and the succinate salt. Additional pharmaceutically acceptable salts include salts of ammonium (NH_4^+) or an organic cation derived from an amine of the formula R_4N^+ , wherein each one of the Rs independently is selected from H, $\text{C}_1\text{-C}_{10}$, preferably $\text{C}_1\text{-C}_6$ alkyl, such as methyl (Me), ethyl (Et), propyl, isopropyl, *n*-butyl, sec-butyl, isobutyl, *tert*-butyl, *n*-pentyl, 2,2-dimethylpropyl, and *n*-hexyl. Furthermore, where the compounds of the general

formula I carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include metal salts such as alkali metal salts, e.g., lithium, sodium or potassium salts, and alkaline earth metal salts, e.g., calcium or magnesium salts.

[0043] Pharmaceutically acceptable salts of the compounds provided herein can be formed by conventional means, e.g., by reacting a free base form of the active agent, *i.e.*, the compound of the general formula I, with one or more equivalents of the appropriate acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is removed *in vacuo* or by freeze drying, or by exchanging the anion/cation of an existing salt for another anion/cation on a suitable ion exchange resin.

[0044] The pharmaceutical compositions provided herein can be formulated for any suitable route of administration, but they are preferably formulated for parenteral, e.g., oral, intravenous, intraarterial, intramuscular, intraperitoneal, intrathecal, intrapleural, intratracheal, or subcutaneous administration. In certain embodiments, the compositions are formulated for intramuscular injections and are thus suitable, *inter alia*, for emergent use. The dosage will depend on the state of the patient and will be determined as deemed appropriate by the practitioner.

[0045] The pharmaceutical compositions provided herein can be in the form of a sterile injectable aqueous or oleagenous suspension, which may be formulated according to the known art using suitable dispersing, wetting or suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Acceptable vehicles and solvents that may be employed include, without limiting, water, Ringer's solution, polyethylene glycol (PEG), 2-hydroxypropyl- β -cyclodextrin (HPCD), Tween-80, and isotonic sodium chloride solution.

[0046] Pharmaceutical compositions provided herein, when formulated for administration route other than parenteral administration, can be in a form suitable for oral use, e.g., as tablets, troches, lozenges, aqueous, or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs.

[0047] Pharmaceutical compositions intended for oral administration should be formulated so as to inhibit the release of the active agent in the stomach, *i.e.*, delay the release of the active agent until at least a portion of the dosage form has traversed the stomach, in order to avoid the acidity of the gastric contents from hydrolyzing the active agent to its highly water insoluble form, *i.e.*, its corresponding parent molecule. In some embodiments, the compositions are those wherein the active agent is coated by a pH-dependent enteric-

coating polymer. Examples of pH-dependent enteric-coating polymer include, without being limited to, EUDRAGIT® S (poly(methacrylicacid, methylmethacrylate), 1:2), EUDRAGIT® L 55 (poly (methacrylicacid, ethylacrylate), 1:1), KOLLICOAT® (poly(methacrylicacid, ethylacrylate), 1:1), hydroxypropyl methylcellulose phthalate (HPMCP), alginates, carboxymethylcellulose, and combinations thereof. The pH-dependent enteric-coating polymer may be present in the composition in an amount from about 10% to about 95% by weight of the entire composition.

[0048] Pharmaceutical compositions intended for oral administration can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and may further comprise one or more agents selected from sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients, which are suitable for the manufacture of tablets. These excipients may be, *e.g.*, inert diluents such as calcium carbonate, sodium carbonate, lactose, calcium phosphate, or sodium phosphate; granulating and disintegrating agents, *e.g.*, corn starch or alginic acid; binding agents, *e.g.*, starch, gelatin or acacia; and lubricating agents, *e.g.*, magnesium stearate, stearic acid, or talc. The tablets may be either uncoated or coated utilizing known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. The pharmaceutical composition of the invention may also be in the form of oil-in-water emulsion.

[0049] Oral pharmaceutical compositions according to the invention can be formulated for controlled release of the active agent. Such compositions may be formulated as controlled-release matrix, *e.g.*, as controlled-release matrix tablets in which the release of a soluble active agent is controlled by having the active diffuse through a gel formed after the swelling of a hydrophilic polymer brought into contact with dissolving liquid (*in vitro*) or gastro-intestinal fluid (*in vivo*). Many polymers have been described as capable of forming such gel, *e.g.*, derivatives of cellulose, in particular the cellulose ethers such as hydroxypropyl cellulose, hydroxymethyl cellulose, methylcellulose or methyl hydroxypropyl cellulose, and among the different commercial grades of these ethers are those showing fairly high viscosity. In other configurations, the compositions comprise the active agent formulated for controlled release in microencapsulated dosage form, in which small droplets

of the active agent are surrounded by a coating or a membrane to form particles in the range of a few micrometers to a few millimeters.

[0050] Another contemplated formulation is depot systems, based on biodegradable polymers, wherein as the polymer degrades, the active ingredient is slowly released. The most common class of biodegradable polymers is the hydrolytically labile polyesters prepared from lactic acid, glycolic acid, or combinations of these two molecules. Polymers prepared from these individual monomers include poly (D,L-lactide) (PLA), poly (glycolide) (PGA), and the copolymer poly (D,L-lactide-co-glycolide) (PLG).

[0051] In yet another aspect, provided herein is the use of a compound of the general formula I as defined in any one of the embodiments above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical composition for treatment of obesity, PCOS and liposarcoma.

[0052] In a further aspect, the present invention relates to a method for treatment of obesity, PCOS and liposarcoma in an individual in need thereof, comprising administering to said individual an effective amount of a compound of the general formula I as defined in any one of the embodiments above, or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof. In some embodiments, the compound administered according to the methods disclosed herein is selected from the compounds of **Tables 1-2** above or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof.

[0053] The terms “obesity” and “fat cell associated diseases” used herein interchangeably, characterized by terms weight gain and poor health arising from the development of tissue injury in the lung, kidney, pancreas, intestine, and liver.

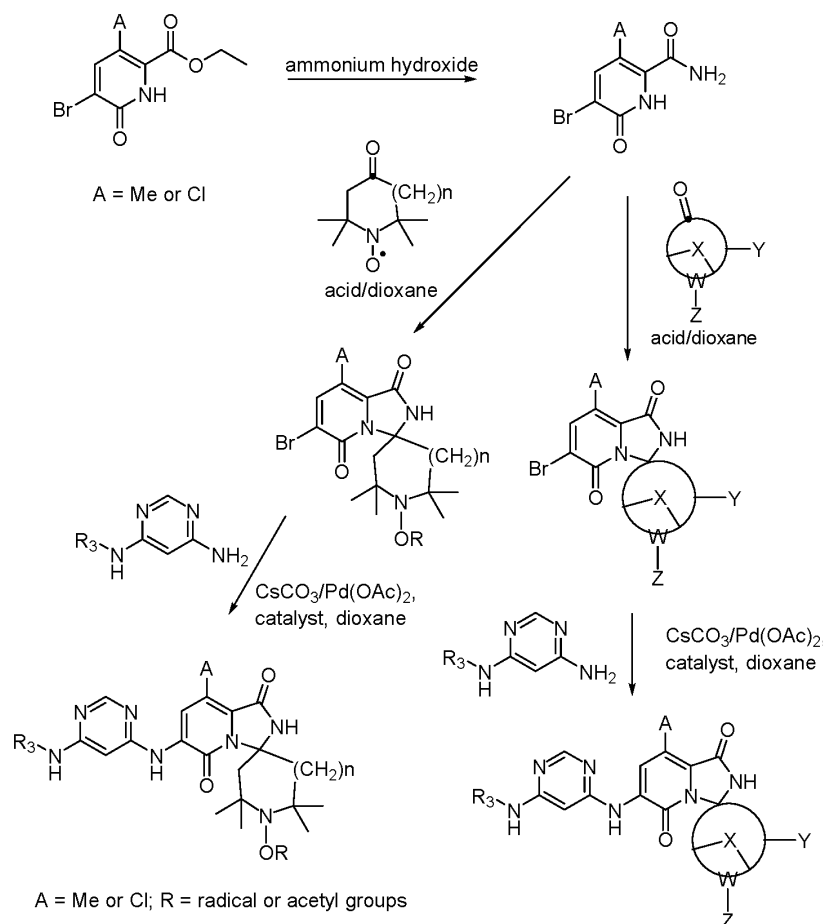
[0054] The term "treatment" as used herein with respect to obesity and fat cell associated diseases such as liposarcoma and polycystic ovary syndrome refers to administration of an active agent after the onset of symptoms of said diseases, and is aimed at inhibiting, *i.e.*, limiting or reducing, or eliminating medical conditions resulting from the infection.

[0055] In a further aspect, provided herein a method for treatment or prevention of liposarcoma and polycystic ovary syndrome by administering a compound of Formula I, II or III.

EXAMPLES

[0056] Compounds I, II and III are synthesized starting from commercially available, ethyl 5-bromo-3-methyl-6-oxo-1,6-dihydropyridine-2-carboxylate and 5-bromo-3-chloro-6-

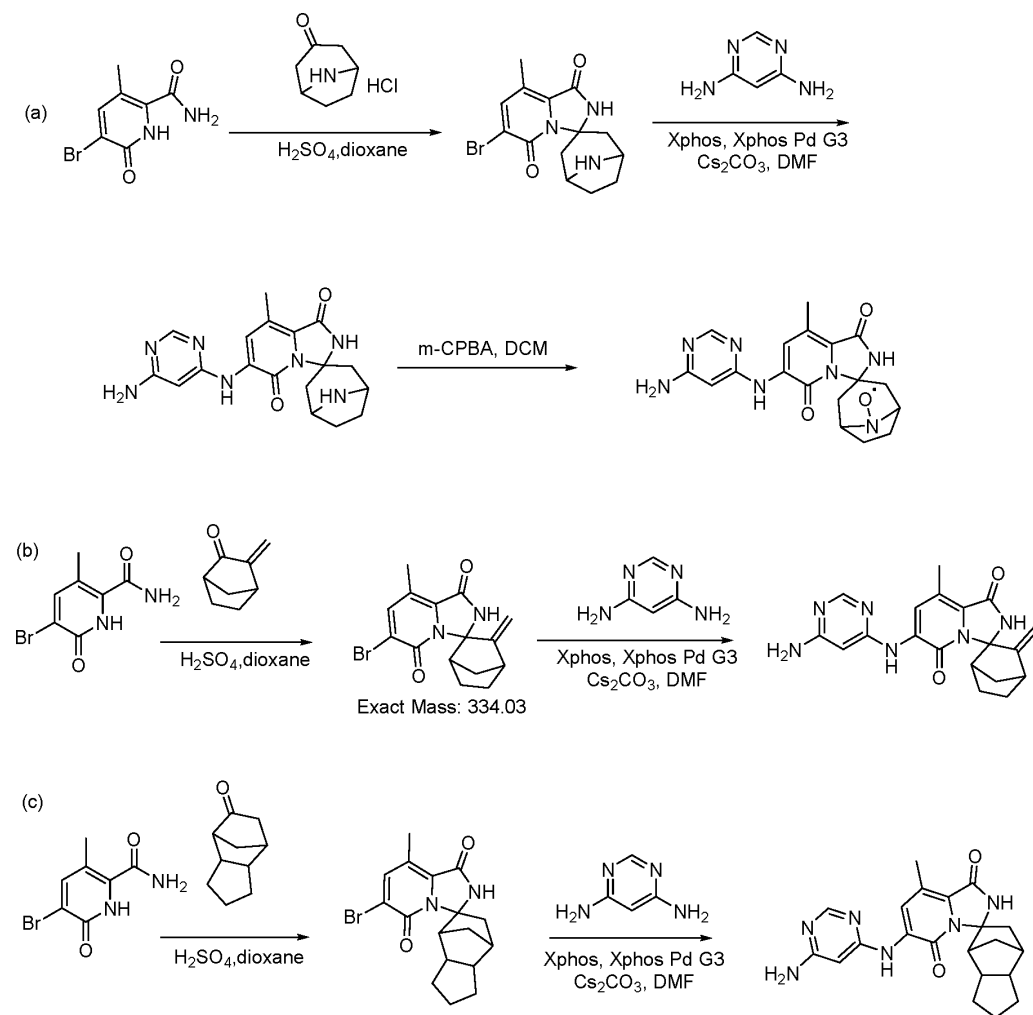
oxo-1,6-dihydropyridine-2-carboxamide or ethyl 5-bromo-3-chloro-6-oxo-1,6-dihydropyridine-2-carboxylate and 5-bromo-1,6-dihydro-3-methyl-6-oxo-2-pyridinecarboxamide following the reported procedure (Yuan, X. *et al*, *Bioorganic & Medicinal Chemistry* 27(7), 1211-1225, 2019) and substituting with corresponding polycyclic or nitroxide bearing keto compounds as shown in Scheme 1 and 2. **Scheme 1:**



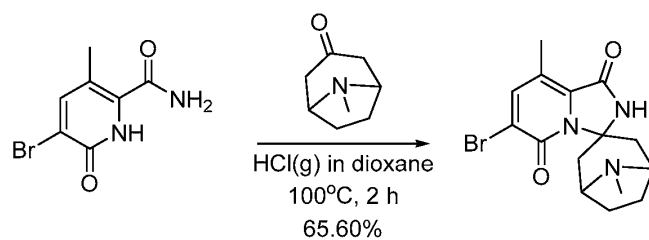
[0057] The methylene-disodium phosphate and methylene-(1-methyl-2-nitro-imidazole) substituted tertiary and quaternary salts of prodrugs are synthesized by reacting the nitrogen atom with $\text{X-CH}_2\text{-OPO}(\text{OBn})_2$ or $\text{X-CH}_2\text{OPO}(\text{OtBu})_2$ (where $\text{X} = \text{Cl}$ or Br) in presence of base such as CaCO_3 , triethylamine, DMAP or DBU in DMF, CH_2Cl_2 or THF. The corresponding dibenzyl phosphate ester will be hydrogenated using hydrogen and Pd/C in MeOH, and the di-*t*-butyl phosphate esters will be hydrolyzed with TFA or HCl in CH_2Cl_2 or THF. The phosphate ester is converted to disodium phosphate ester after treatment with sodium hydroxide in water. The hypoxia induced prodrugs are synthesized following the synthesized bay reacting with 5-chloromethyl-(1-methyl-2-nitro-imidazole) or 5-bromomethyl-

(1-methyl-2-nitro-imidazole) in presence of base such as CaCO_3 , triethylamine, DMAP or DBU in DMF, CH_2Cl_2 or THF.

Scheme 2:

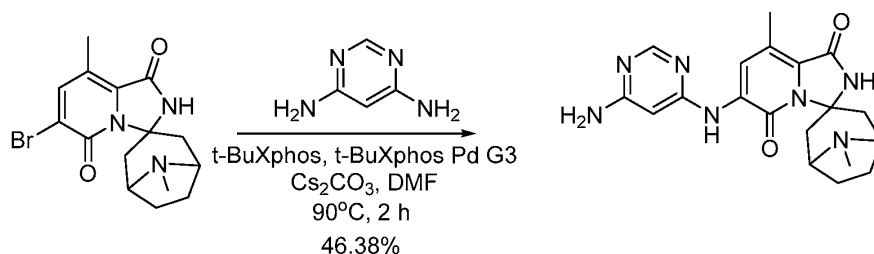


[0058] Synthesis of Li intermediate



To a stirred mixture of 5-bromo-3-methyl-6-oxo-1H-pyridine-2-carboxamide (500 mg, 2.164 mmol, 1 equiv) and tropanone (1.5 g, 10.820 mmol, 5 equiv) in dioxane (5 mL) were added 4M HCl(gas) in 1,4-dioxane (0.5 mL, 0.108 mmol, 4 mol/L) dropwise at room temperature. The resulting mixture was stirred for overnight at 100°C. The mixture was allowed to cool down to room temperature. The resulting mixture was concentrated under reduced pressure. The residue was dissolved in DMSO (5 mL). The resulting mixture was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% NH₃·H₂O), 10% to 40% gradient in 10 min; detector, UV 254 nm to afford 6'-bromo-8,8'-dimethyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (500 mg, 65.60%) as an off-white solid. LC-MS-PH-MLCL-MC-2023-05-2-1: (ES, *m/z*): [M+H]⁺ = 352.10.

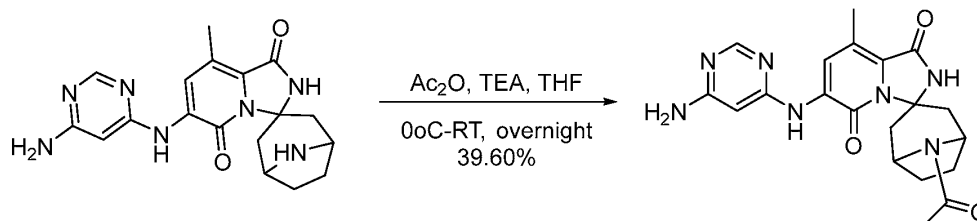
[0059] Synthesis of Ii



To a stirred mixture of 6'-bromo-8,8'-dimethyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (450 mg, 1.278 mmol, 1 equiv), pyrimidine-4,6-diamine (168.82 mg, 1.534 mmol, 1.2 equiv), t-BuXphos (54.25 mg, 0.128 mmol, 0.1 equiv) and 3rd Generation t-BuXPhos precatalyst (101.62 mg, 0.128 mmol, 0.1 equiv) in DMF (6 mL) was added Cs₂CO₃ (832.51 mg, 2.556 mmol, 2 equiv) at room temperature. The resulting mixture was stirred for overnight at 90°C under nitrogen atmosphere. The resulting mixture was filtered, the filter cake was washed with DMSO (2 x 1 mL). The resulting mixture was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% TFA), 10% to 50% gradient in 10 min; detector, UV 254 nm to obtain the crude. The crude product was purified by trituration with acetonitrile (20 mL) and washed with MeOH (2 x 10 mL). This resulted in 6'-[(6-aminopyrimidin-4-yl)amino]-8,8'-dimethyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (226 mg, 46.38%) as a white solid. LC-MS-PH-MLCL-MC-2023-05-2-0: (ES, *m/z*): [M+H]⁺ = 382.15 ¹H-NMR-PH-MLCL-MC-2023-05-2-0 (400 MHz, DMSO-*d*₆): δ 9.23 (s, 1H), 8.63 (s, 1H), 8.41 (s, 1H), 8.17 (d, *J* = 1.8 Hz, 2H), 6.51 (s,

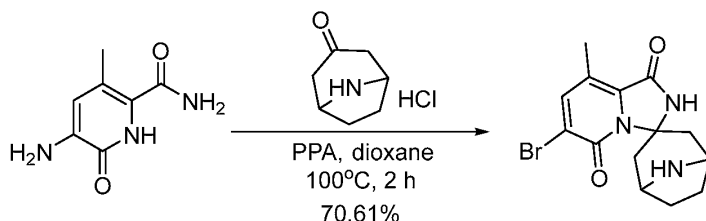
2H), 6.18 (s, 1H), 3.23 (s, 4H), 2.59 (s, 3H), 2.43 (s, 3H), 2.03 – 1.88 (m, 4H), 1.36 (d, $J = 13.4$ Hz, 2H), 1.24 (s, 1H).

[0060] Synthesis of II



To a stirred mixture of 6'-[(6-aminopyrimidin-4-yl)amino]-8'-methyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (460 mg, 1.252 mmol, 1 equiv) and TEA (633.47 mg, 6.260 mmol, 5 equiv) in THF (10 mL) was added Ac₂O (191.72 mg, 1.878 mmol, 1.5 equiv) dropwise at 0 °C. The resulting mixture was stirred for overnight at room temperature. The precipitated solids were collected by filtration and washed with DMSO (3 x 1 mL). The crude product was purified by trituration with MeOH (5 mL) to afford 8-acetyl-6'-[(6-aminopyrimidin-4-yl)amino]-8'-methyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (203 mg, 39.60%) as a white solid. LC-MS-PH-MLCL-MC-2023-05-3-0: (ES, m/z): $[M+H]^+ = 410.05$, ¹H-NMR-PH-MLCL-MC-2023-05-3-0 (400 MHz, DMSO-*d*₆): δ 9.58 (s, 1H), 8.71 (s, 1H), 8.45 (s, 1H), 8.16 (s, 1H), 6.50 (s, 2H), 6.23 (d, $J = 1.0$ Hz, 1H), 4.46 (d, $J = 81.6$ Hz, 2H), 3.36 (m, 2H), 2.54 (s, 3H), 2.42 (s, 2H), 2.07 (s, 1H), 2.04 (s, 3H), 1.90 (dd, $J = 11.6, 6.4$ Hz, 1H), 1.70 (d, $J = 13.6$ Hz, 1H), 1.59 (d, $J = 13.4$ Hz, 1H).

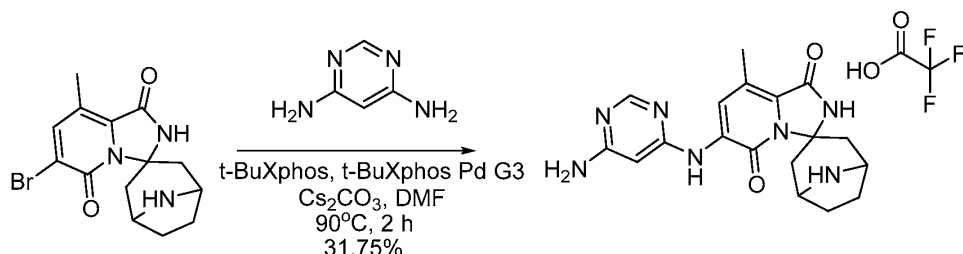
[0061] Synthesis of In intermediate



To a stirred mixture of 5-amino-3-methyl-6-oxo-1H-pyridine-2-carboxamide (700 mg, 4.187 mmol, 1 equiv) and 8-azabicyclo[3.2.1]octan-3-one hydrochloride (3.38 g, 20.935 mmol, 5 equiv) in dioxane (15 mL) was added PPA (1.5 mL). The resulting mixture was stirred for overnight at 100°C. The resulting mixture was diluted with sat. aq. NaHCO₃ (10 mL). The resulting mixture was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% NH₃·H₂O), 10% to

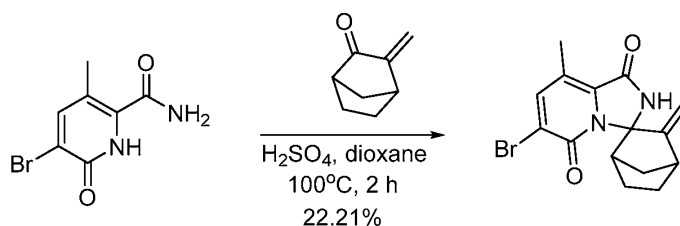
50% gradient in 10 min; detector, UV 254 nm to afford 6'-bromo-8'-methyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (1 g, 70.61%) as a yellow solid. LC-MS-PH-MLCL-MC-2023-05-4-1A: (ES, m/z): $[M+H]^+ = 338.00$.

[0062] Synthesis of In TFA salt



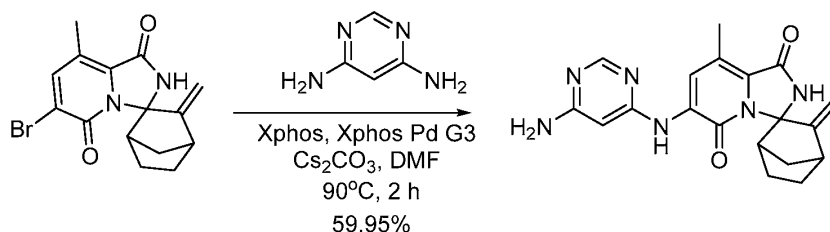
To a stirred mixture of 6'-bromo-8'-methyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (500 mg, 1.478 mmol, 1 equiv), pyrimidine-4,6-diamine (195.36 mg, 1.774 mmol, 1.2 equiv), t-BuXphos (62.78 mg, 0.148 mmol, 0.1 equiv) and 3rd Generation t-BuXPhos precatalyst (117.59 mg, 0.148 mmol, 0.1 equiv) in DMF (16 mL) was added Cs_2CO_3 (963.38 mg, 2.956 mmol, 2 equiv) at room temperature. The resulting mixture was stirred for overnight at 90°C under nitrogen atmosphere. The resulting mixture was filtered, the filter cake was washed with DMSO (1 x 1 mL). The resulting mixture was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% TFA), 10% to 50% gradient in 10 min; detector, UV 254 nm to obtain the crude. The crude product was purified by trituration with acetonitrile (20 mL) and washed with MeOH (2 x 10 mL) to afford 6'-[(6-aminopyrimidin-4-yl)amino]-8'-methyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione; trifluoroacetic acid (226 mg, 31.75%) as an off-white solid. LC-MS-PH-MLCL-MC-2023-05-4-0: (ES, m/z): $[M+H]^+ = 368.15$, $^1\text{H-NMR-PH-MLCL-MC-2023-05-4-0}$ (400 MHz, $\text{DMSO-}d_6$): δ 9.71 (s, 1H), 9.18 (s, 1H), 8.33 (d, $J = 6.2$ Hz, 2H), 8.19 (s, 1H), 8.08 (s, 1H), 6.62 (s, 2H), 6.13 (s, 1H), 4.19 (s, 2H), 3.46 (dd, $J = 15.0, 3.9$ Hz, 2H), 2.45 (d, $J = 2.1$ Hz, 3H), 2.29 (d, $J = 8.9$ Hz, 2H), 2.12 – 1.85 (m, 4H). $^{19}\text{F-NMR-PH-MLCL-MC-2023-05-4-0}$ (377 MHz, $\text{DMSO-}d_6$) δ -73.43.

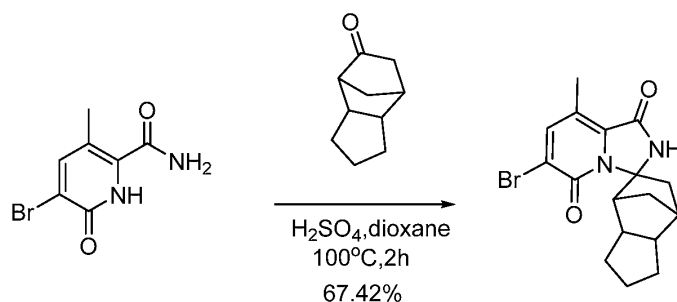
[0063] Synthesis of 1e intermediate



To a stirred mixture of 5-bromo-3-methyl-6-oxo-1H-pyridine-2-carboxamide (900 mg, 3.895 mmol, 1 equiv) and 3-methylidenebicyclo[2.2.1]heptan-2-one (951.75 mg, 7.790 mmol, 2 equiv) in dioxane (4 mL) was added conc.H₂SO₄ (382.01 mg, 3.895 mmol, 1 equiv) dropwise at room temperature. The resulting mixture was stirred for 2 h at 100°C. The mixture was allowed to cool down to room temperature. The resulting mixture was diluted by DMSO (4 mL) and purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% FA), 10% to 50% gradient in 10 min; detector, UV 254 nm to afford 6'-bromo-8'-methyl-3-methylidene-2'H-spiro[bicyclo[2.2.1]heptane-2,3'-imidazo[1,5-a]pyridine]-1',5'-dione (290 mg, 22.21%) as an off-white solid. LC-MS-PH-MLCL-MC-2023-05-5-1: (ES, *m/z*): [M+H]⁺=334.95.

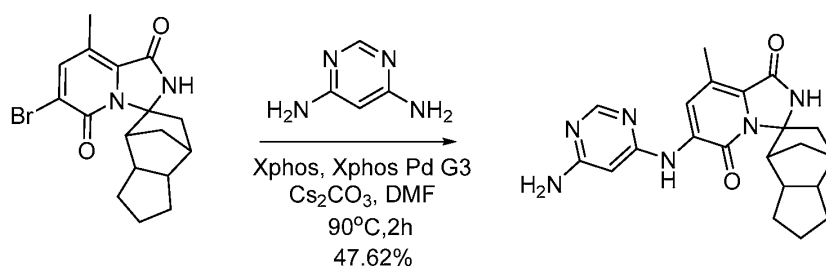
[0064] Synthesis of 1e



[0065] Synthesis of If intermediate

[0066] To a stirred mixture of 5-bromo-3-methyl-6-oxo-1H-pyridine-2-carboxamide (500 mg, 2.164 mmol, 1 equiv) and tricyclo[5.2.1.0^{2,6}]decan-8-one (1.63 g, 10.820 mmol, 5 equiv) in dioxane (10 mL) was added H₂SO₄ (106.11 mg, 1.082 mmol, 0.5 equiv) dropwise at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 2 h at 100°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was concentrated under reduced pressure. The residue was dissolved in DMSO (10 mL). The resulting mixture was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% FA), 10% to 80% gradient in 10 min; detector, UV 254 nm. This resulted in 6-bromo-8-methyl-2H-spiro[imidazo[1,5-a]pyridine-3,8'-tricyclo[5.2.1.0^{2,6}]decane]-1,5-dione (530 mg, 67.42%) as an off-white solid.

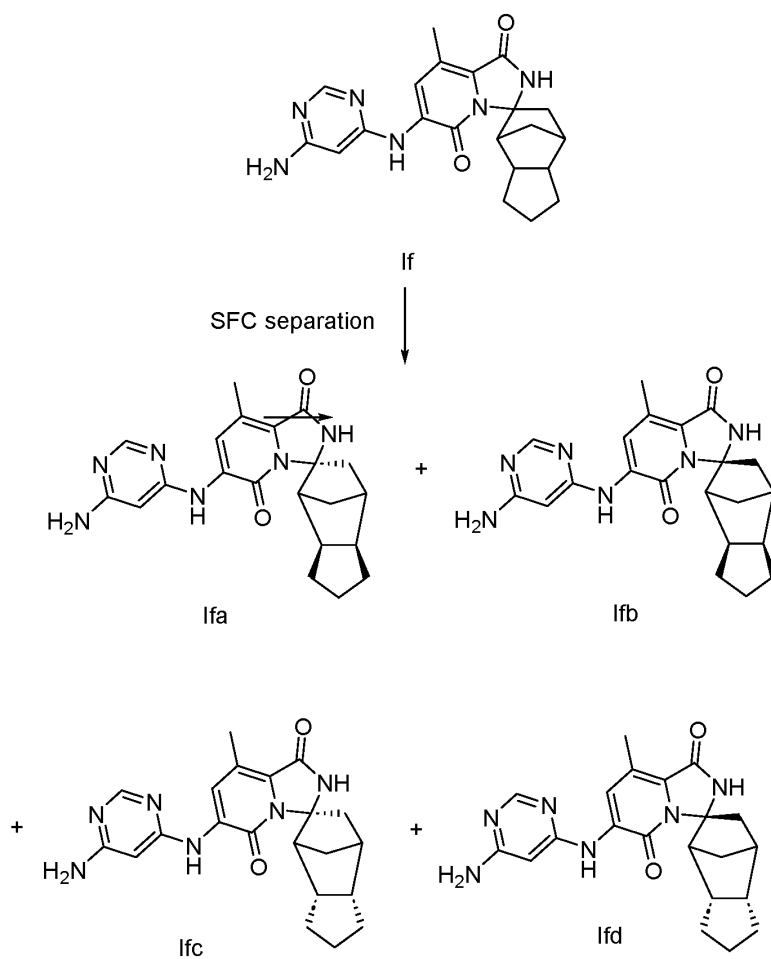
LCMS-PH-MLCL-MC-2023-05-6-1: MS (ESI) m/z 363.15[M+H].

1. Synthesis of If

To a stirred mixture of 6-bromo-8-methyl-2H-spiro[imidazo[1,5-a]pyridine-3,8'-tricyclo[5.2.1.0^{2,6}]decane]-1,5-dione (470 mg, 1.294 mmol, 1 equiv) and pyrimidine-4,6-diamine (170.98 mg, 1.553 mmol, 1.20 equiv) in DMF (10 mL) were added Cs₂CO₃ (843.13 mg, 2.588 mmol, 2.00 equiv), X-phos (61.68 mg, 0.129 mmol, 0.10 equiv) and XPhos Pd G3 (109.52 mg, 0.129 mmol, 0.10 equiv) in portions at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 2 h at 90 °C under nitrogen atmosphere.

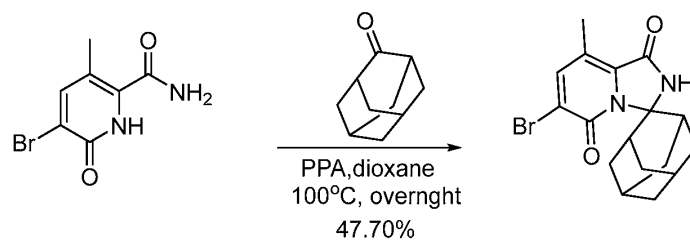
The mixture was allowed to cool down to room temperature. The resulting mixture was diluted with water (100 mL). The resulting mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 200 mL), dried over anhydrous Na₂SO₄. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% FA), 0% to 40% gradient in 20 min; detector, UV 254 nm to afford 6-[(6-aminopyrimidin-4-yl)amino]-8-methyl-2H-spiro[imidazo[1,5-a]pyridine-3,8'-tricyclo[5.2.1.0^{2,6}]decane]-1,5-dione (247 mg, 47.62%) as an off-white solid. LCMS-PH-MLCL-MC-2023-05-6-0: MS (ESI) m/z 393.10[M+H], ¹H-NMR-PH- MLCL-MC-2023-05-6-0 (400 MHz, DMSO-*d*₆): δ 9.55 (d, *J* = 39.1 Hz, 1H), 8.63 (d, *J* = 24.2 Hz, 1H), 8.37 (d, *J* = 18.4 Hz, 1H), 8.17 (d, *J* = 3.1 Hz, 1H), 6.50 (d, *J* = 5.1 Hz, 2H), 6.19 (dd, *J* = 22.8, 1.1 Hz, 1H), 3.16 (dt, *J* = 13.0, 4.4 Hz, 1H), 2.60 (dd, *J* = 16.0, 7.8 Hz, 1H), 2.54 (s, 1H), 2.42 (s, 3H), 2.20 – 2.01 (m, 2H), 1.97 – 1.57 (m, 4H), 1.51 – 1.32 (m, 2H), 1.19 – 0.82 (m, 3H).

[0067] Chiral HPLC of If indicated four peaks. The diastereomeric isomers of If (3 g) were separated by chiral super critical fluid chromatography (SFC) using mobile phase A: water/ 0.1% formic acid and mobile phase B :Acetonitrile/0.1% formic acid. See FIG. 14.



Four isomers were collected: Isomer 1 (272 mg, HPLC purity 97.1%), isomer 2 (277 mg, HPLC purity 95.7%), isomer 3 (297 mg, HPLC purity 99.5%) and isomer 4 (325 mg, HPLC purity 99.4%).

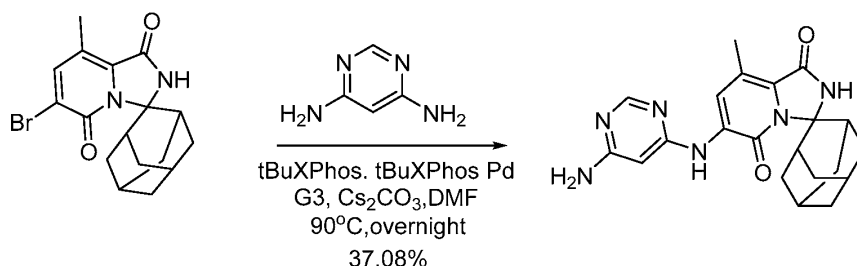
[0069] **Synthesis of Ih intermediate**



To a stirred mixture of 5-bromo-3-methyl-6-oxo-1H-pyridine-2-carboxamide (800 mg, 3.462 mmol, 1 equiv) and adamantanone (2.08 g, 13.848 mmol, 4 equiv) in dioxane (10 mL) was added polyphosphoric acid (1 mL) at room temperature under nitrogen atmosphere. The

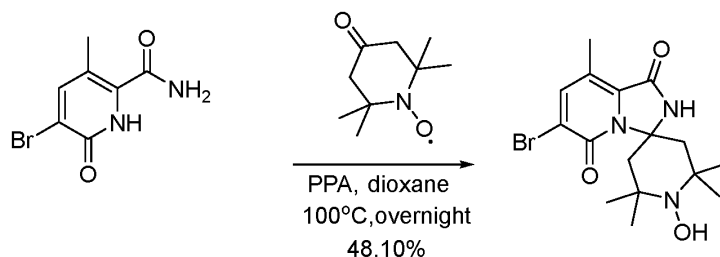
resulting mixture was stirred for overnight at 100°C under nitrogen atmosphere. The resulting mixture was concentrated under reduced pressure. The residue was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% FA), 0% to 100% gradient in 10 min; detector, UV 254 nm to afford 6'-bromo-8'-methyl-2'H-spiro[adamantane-2,3'-imidazo[1,5-a]pyridine]-1',5'-dione (600 mg, 47.70%) as a light yellow solid. LCMS- PH-MLCL-MC-2023-05-7-1: MS (ESI) m/z 365.15 [M+H+2].

[0070] Synthesis of 1h



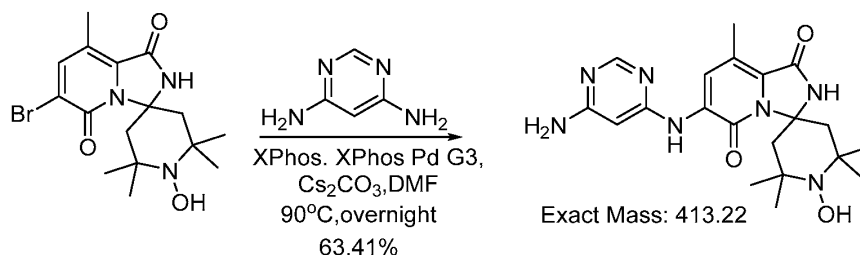
To a stirred mixture of 6'-bromo-8'-methyl-2'H-spiro[adamantane-2,3'-imidazo[1,5-a]pyridine]-1',5'-dione (500 mg, 1.376 mmol, 1 equiv) and pyrimidine-4,6-diamine (303.15 mg, 2.752 mmol, 2 equiv) in dioxane (10 mL) were added tBuXPhos Pd G3 (54.67 mg, 0.069 mmol, 0.05 equiv), di-tert-butyl({2-[2,4,6-tris(propan-2-yl)phenyl]phenyl})phosphane (58.45 mg, 0.138 mmol, 0.1 equiv), Cs₂CO₃ (896.94 mg, 2.752 mmol, 2 equiv) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at 90°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was diluted with water (20 mL) and stirred for 2 hours. The precipitated solids were collected by filtration and the solid was triturated with ACN and MeOH. The resulting solid was dried by lyophilization to afford 6'-[(6-aminopyrimidin-4-yl)amino]-8'-methyl-2'H-spiro[adamantane-2,3'-imidazo[1,5-a]pyridine]-1',5'-dione (200.3 mg, 37.08%) as a grey solid. LC-MS- PH-MLCL-MC-2023-05-7-0: MS (ESI) m/z 393.10 [M+H]⁺. ¹H NMR- PH-MLCL-MC-2023-05-7-0: (400 MHz, DMSO-d₆) δ 9.55 (s, 1H), 8.72 (s, 1H), 8.37 (s, 1H), 8.17 (s, 1H), 6.49 (s, 2H), 6.20 (d, *J* = 1.0 Hz, 1H), 3.52 (d, *J* = 12.2 Hz, 2H), 2.47 (s, 3H), 2.33 (d, *J* = 13.1 Hz, 2H), 2.05 (s, 2H), 1.88 (d, *J* = 38.5 Hz, 2H), 1.77 (d, *J* = 13.0 Hz, 4H), 1.68 (d, *J* = 12.4 Hz, 2H).

[0071] 12. Synthesis of IIa intermediate



To a stirred mixture of 5-bromo-3-methyl-6-oxo-1H-pyridine-2-carboxamide (500 mg, 2.164 mmol, 1 equiv) and 2,2,6,6-tetramethyl-4-oxopiperidin-1-yloxidanyl (1.1 g, 6.492 mmol, 3 equiv) in dioxane (10 mL) was added PPA (1 mL) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at 100°C under nitrogen atmosphere. The resulting mixture was concentrated under reduced pressure. The residue was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (10mmol/L NH_4HCO_3), 0% to 100% gradient in 10 min; detector, UV 254 nm to afford 6-bromo-1'-hydroxy-2',2',6',6',8-pentamethyl-2H-spiro[imidazo[1,5-a]pyridine-3,4'-piperidine]-1,5-dione (400 mg, 48.10%) as a light yellow solid. LCMS- PH-MLCL-MC-2023-05-8-1: MS (ESI) m/z 384.15 $[\text{M}+\text{H}]^+$

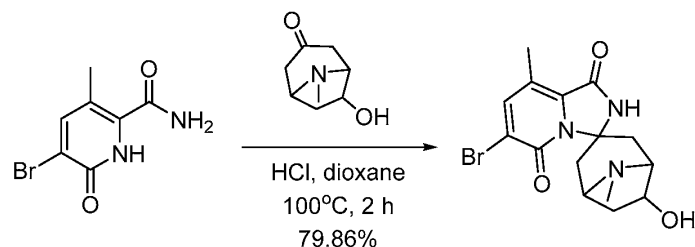
[0072] 13. Synthesis of IIa (MCL5008)



To a stirred mixture of 6-bromo-1'-hydroxy-2',2',6',6',8-pentamethyl-2H-spiro[imidazo[1,5-a]pyridine-3,4'-piperidine]-1,5-dione (300 mg, 0.781 mmol, 1 equiv) XPhos Pd G3 (33.04 mg, 0.039 mmol, 0.05 equiv) XPhos (18.61 mg, 0.039 mmol, 0.05 equiv) and pyrimidine-4,6-diamine (171.94 mg, 1.562 mmol, 2 equiv) in DMSO (5 mL) was added Cs_2CO_3 (508.73 mg, 1.562 mmol, 2 equiv) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at 90°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was filtered, the filtrate was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% FA), 0% to 100% gradient in 10 min; detector, UV 254 nm to afford 6-[(6-aminopyrimidin-4-yl)amino]-1'-hydroxy-2',2',6',6',8-pentamethyl-2H-

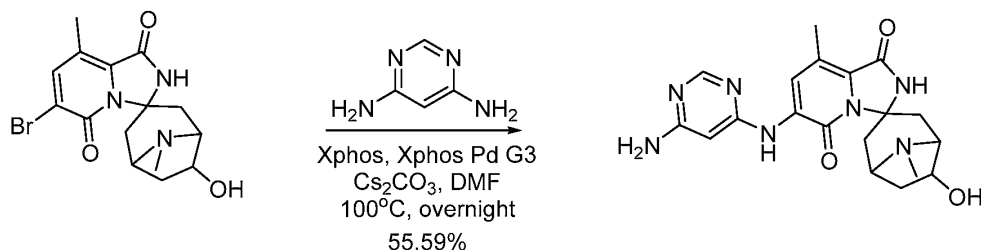
spiro[imidazo[1,5-a]pyridine-3,4'-piperidine]-1,5-dione (204.7 mg, 63.41%) as a light yellow solid. LC-MS- PH-MLCL-MC-2023-05-8-0: MS (ESI) m/z 414.20 $[M+H]^+$

[0073] Synthesis of Io intermediate



A mixture of 5-bromo-3-methyl-6-oxo-1H-pyridine-2-carboxamide (550 mg, 2.380 mmol, 1 equiv) and (1S,5S,6R)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]octan-3-one (738.88 mg, 4.760 mmol, 2 equiv) in HCl(gas) in 1,4-dioxane (10 mL, 20.087 mmol, 8.44 equiv) was stirred for 2 h at 100°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The precipitated solids were collected by filtration and washed with dioxane (3 x 5 mL). The solid was dried by lyophilization to afford 6'-bromo-7-hydroxy-8,8'-dimethyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (700 mg, 79.86%) as a light yellow solid. LCMS- PH-MLCL-MC-2023-05-9-1C: MS (ESI) m/z 369.90 $[M+H+2]$.

[0074] Synthesis of Io

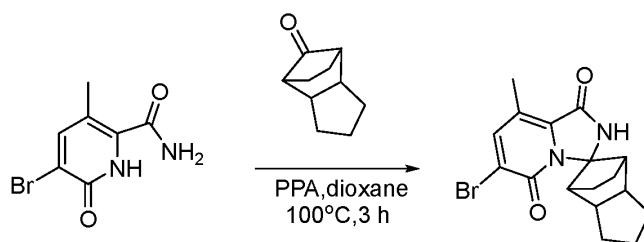


To a stirred mixture of 6'-bromo-7-hydroxy-8,8'-dimethyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (500 mg, 1.358 mmol, 1 equiv) and pyrimidine-4,6-diamine (299.05 mg, 2.716 mmol, 2 equiv) in DMSO (10 mL) was added XPhos (64.73 mg, 0.136 mmol, 0.1 equiv) and Cs_2CO_3 (1.3 g, 4.074 mmol, 3 equiv) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at 100°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was filtered, the filter cake was washed with DMSO (2 x 2 mL). The filtrate was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% FA), 0% to 100% gradient in 10 min; detector, UV 254 nm to afford 6'-[(6-aminopyrimidin-4-

yl)amino]-7-hydroxy-8,8'-dimethyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (300 mg, 55.59%) as a light yellow solid. LC-MS- PH-MLCL-MC-2023-05-9-0: MS (ESI) m/z 398.15 $[M+H]^+$

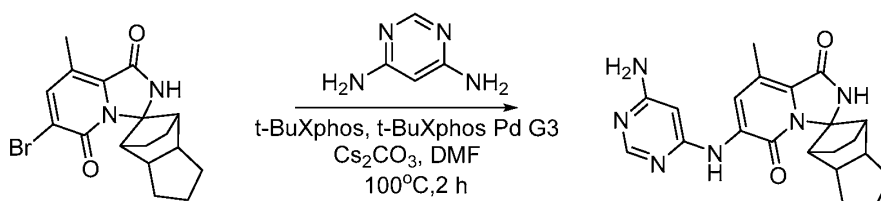
^1H NMR-PH-MLCL-MC-2023-05-9-0: (400 MHz, DMSO- d_6) δ 9.20 (s, 1H), 8.68 (s, 1H), 8.43 (s, 1H), 8.18 (d, $J = 6.0$ Hz, 2H), 6.51 (s, 2H), 6.21 (d, $J = 1.0$ Hz, 1H), 4.40 (dd, $J = 7.3, 2.9$ Hz, 1H), 3.34 (d, $J = 7.1$ Hz, 1H), 3.28 – 3.18 (m, 2H), 3.08 – 3.01 (m, 1H), 2.77 (s, 3H), 2.54 (d, $J = 2.3$ Hz, 1H), 2.43 (s, 3H), 1.79 – 1.71 (m, 1H), 1.39 (d, $J = 13.9$ Hz, 1H), 1.27 (t, $J = 13.6$ Hz, 1H).

[0075] Synthesis of Im intermediate



To a stirred mixture of 5-bromo-3-methyl-6-oxo-1H-pyridine-2-carboxamide (1 equiv) and tricyclic compound (2 equiv) in dioxane (5 mL) was added PPA (0.5 mL) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 3 h at 100°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The reaction was quenched by the addition of Water (20 mL) at room temperature. The precipitated solids were collected by filtration and washed with water (2 x 5 mL). The resulting solid was dried by lyophilization to afford 6'-bromo-8'-methyl-2'H- 1',5'-dione.

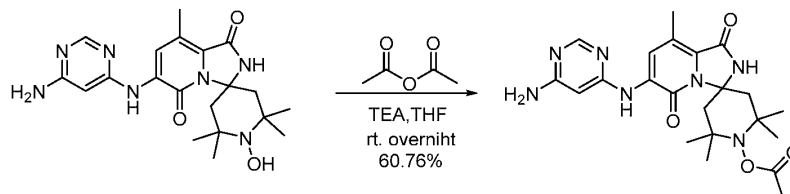
[0076] Synthesis of Im



To a stirred mixture of (6'-bromo-8'-methyl-2'H- 1',5'-dione derivative (1 equiv) and pyrimidine-4,6-diamine (2 equiv) in DMSO (10 mL) was added t-BuXPhos (0.05 equiv) 3rd Generation t-BuXPhos precatalyst (0.05 equiv) Cs_2CO_3 (2 equiv) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 2h at 100°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was filtered, the filter cake was washed with DMSO (2 x 1 mL). The filtrate was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (10mmol/L NH_4HCO_3), 0% to 100% gradient in 10

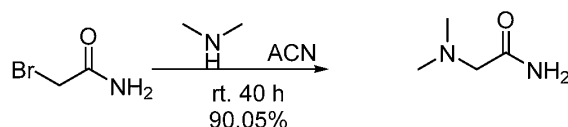
min; detector, UV 254 nm to afford crude product. The crude product was purified by trituration with MeCN (10 mL) to afford compound Im as a light yellow solid.

[0077] Synthesis of IIIa

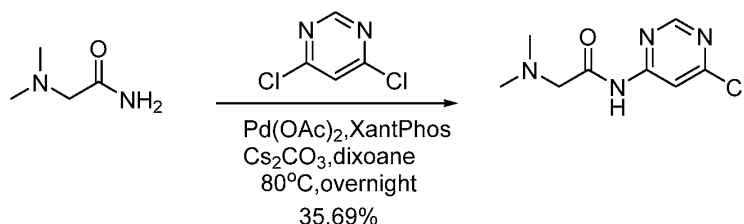


To a stirred mixture of 6-[(6-aminopyrimidin-4-yl)amino]-1'-hydroxy-2',2',6',6',8-pentamethyl-2H-spiro[imidazo[1,5-a]pyridine-3,4'-piperidine]-1,5-dione (300 mg, 0.726 mmol, 1 equiv) and TEA (1 mL) in THF (3 mL) was added acetic anhydride (222.21 mg, 2.178 mmol, 3 equiv) dropwise at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at room temperature under nitrogen atmosphere. The resulting mixture was concentrated under reduced pressure. The residue was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (10mmol/L NH_4HCO_3), 0% to 100% gradient in 10 min; detector, UV 254 nm to afford 6-[(6-aminopyrimidin-4-yl)amino]-2',2',6',6',8-pentamethyl-1,5-dioxo-2H-spiro[imidazo[1,5-a]pyridine-3,4'-piperidin]-1'-yl acetate (200.8 mg, 60.76%) as an off-white solid. LC-MS- PH-MLCL-MC-2023-05-11-0: MS (ESI) m/z 456.25 $[\text{M}+\text{H}]^+$. ^1H NMR-PH-MLCL-MC-2023-05-11-0: (400 MHz, $\text{DMSO}-d_6$) δ 9.45 (s, 1H), 8.57 (s, 1H), 8.41 (s, 1H), 8.18 (d, $J = 1.0$ Hz, 1H), 6.54 (s, 2H), 6.12 (s, 1H), 3.63 (d, $J = 13.5$ Hz, 2H), 2.45 (s, 3H), 2.09 (s, 3H), 1.64 (d, $J = 13.4$ Hz, 2H), 1.35 (s, 6H), 1.03 (s, 6H).

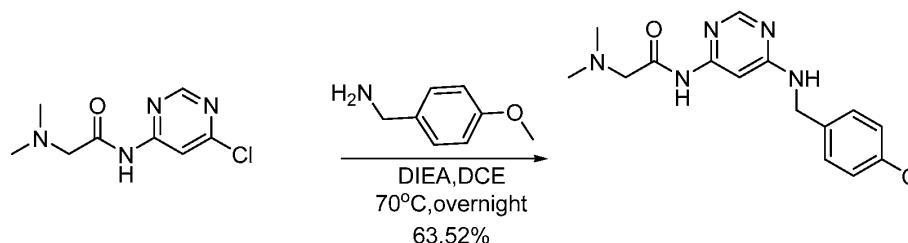
[0078] Synthesis of Is intermediate 1:



To a stirred mixture of bromoacetamide (3 g, 21.745 mmol, 1 equiv) in ACN (30.00 mL, 570.806 mmol, 26.25 equiv) was added dimethylamine (2 M in THF) (10.87 mL, 21.744 mmol, 3 equiv) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 40 h at room temperature under nitrogen atmosphere. The resulting mixture was concentrated under reduced pressure. The resulting mixture was diluted with DCM/MeOH=1/1(100 mL). It was neutralized over a carbonate resin (2.0 equiv) with gentle shaking for 20 hours. The reaction was filtered and the filtrate was evaporate to afford crude product 2-(dimethylamino)acetamide (2 g, 90.05%) as a light yellow solid. LCMS- PH-MLCL-MC-2023-05-12-1C: MS (ESI) m/z 103.05 $[\text{M}+\text{H}]$.

[0079] Synthesis of Is intermediate 2

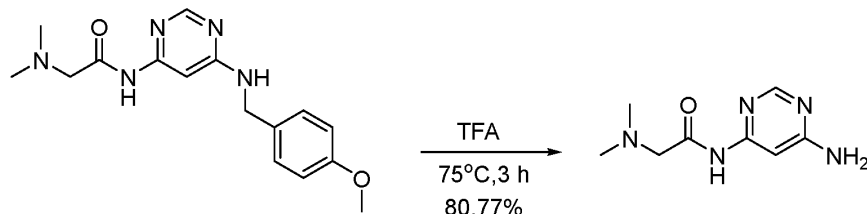
To a stirred mixture of 2-(dimethylamino)acetamide (2 g, 19.582 mmol, 1 equiv) and 4,6-dichloropyrimidine (3.50 g, 23.498 mmol, 1.2 equiv) in dioxane (40 mL) were added XantPhos (566.53 mg, 0.979 mmol, 0.05 equiv) and Cs₂CO₃ (12.76 g, 39.164 mmol, 2 equiv) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at 80°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was diluted with water (50 mL). The resulting mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over anhydrous Na₂SO₄. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE / EA (5:1) to afford N-(6-chloropyrimidin-4-yl)-2-(dimethylamino)acetamide (1.5 g, 35.69%) as a light yellow solid. LCMS-PH-MLCL-MC-2023-05-12-1A: MS (ESI) m/z 214.80 [M+H].

[0080] Synthesis of Is intermediate 3

To a stirred mixture of N-(6-chloropyrimidin-4-yl)-2-(dimethylamino)acetamide (750 mg, 3.494 mmol, 1 equiv) and benzenemethanamine, 4-methoxy- (4.8 g, 34.940 mmol, 10 equiv) in DCE (10 mL) was added DIEA (2.7 g, 20.964 mmol, 6 equiv) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at 70°C under nitrogen atmosphere. The resulting mixture was filtered, the filter cake was washed with DCE (2 x 5 mL). The filtrate was concentrated under reduced pressure. The residue was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% FA), 0% to 100% gradient in 10 min; detector, UV 254 nm to afford 2-(dimethylamino)-N-(6-((4-methoxyphenyl)methyl)amino)pyrimidin-4-ylacetamide.

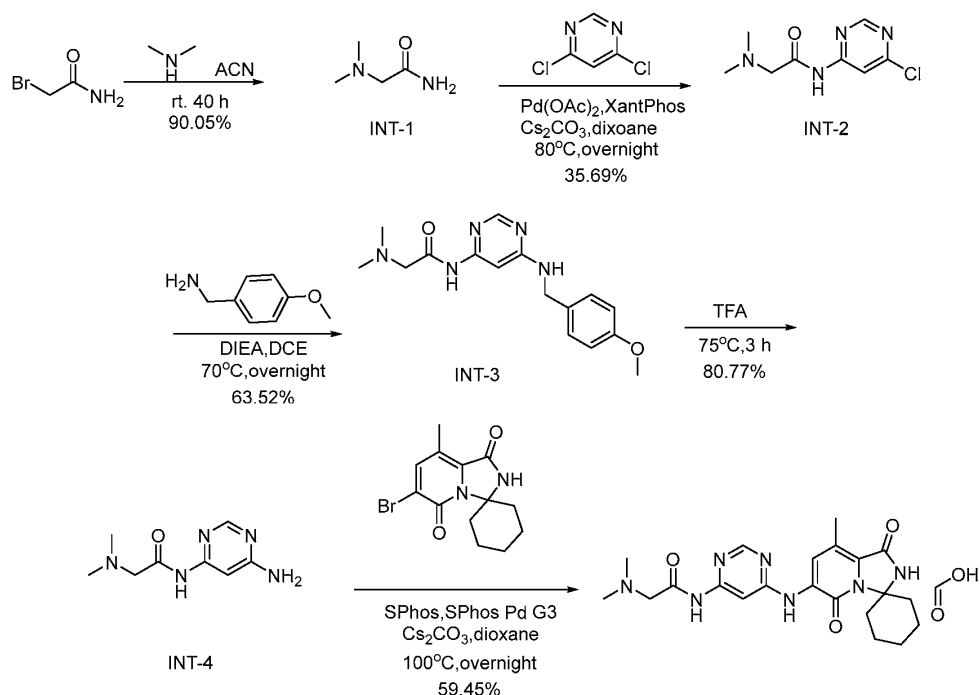
yl)acetamide (700 mg, 63.52%) as an off-white solid. LCMS-PH-MLCL-MC-2023-05-12-2C: MS (ESI) m/z 316.05 [M+H].

[0081] Synthesis of Is intermediate 4

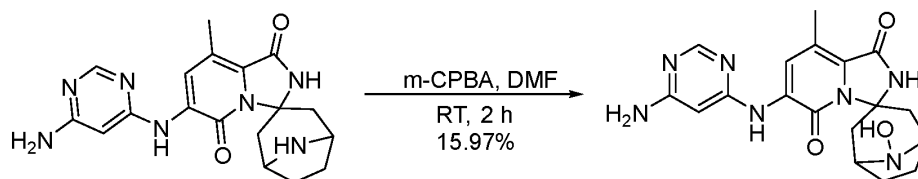


A solution of 2-(dimethylamino)-N-(6-{{[(4-methoxyphenyl)methyl]amino}pyrimidin-4-yl})acetamide (400 mg, 1.268 mmol, 1 equiv) in TFA (2 mL) was stirred for 2h at 75°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was concentrated under reduced pressure. The mixture/residue was basified to pH 9 with saturated NaHCO₃ (aq.). The resulting mixture was extracted with DCM (3 x 20 mL). The combined organic layers were dried over anhydrous Na₂SO₄. After filtration, the filtrate was concentrated under reduced pressure to afford N-(6-aminopyrimidin-4-yl)-2-(dimethylamino)acetamide (200 mg, 80.77%) as an off-white solid. LCMS-PH-MLCL-MC-2023-05-12-1: MS (ESI) m/z 196.00 [M+H].

[0082] Synthesis of Is



To a stirred mixture of N-(6-aminopyrimidin-4-yl)-2-(dimethylamino)acetamide (170 mg, 0.871 mmol, 1 equiv) and 6'-bromo-8'-methyl-2'H-spiro[cyclohexane-1,3'-imidazo[1,5-a]pyridine]-1',5'-dione (325.16 mg, 1.045 mmol, 1.2 equiv) in DMSO (1 mL) were added SPhos (17.87 mg, 0.044 mmol, 0.05 equiv), SPhos Palladacycle Gen.3 (33.97 mg, 0.044 mmol, 0.05 equiv) Cs₂CO₃ (567.44 mg, 1.742 mmol, 2 equiv) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at 100°C under nitrogen atmosphere. The mixture was allowed to cool down to room temperature. The resulting mixture was concentrated under reduced pressure. The resulting mixture was diluted with DMSO (8 mL). The residue was purified by reversed-phase flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MeCN in Water (0.1% FA), 0% to 100% gradient in 10 min; detector, UV 254 nm to afford 2-(dimethylamino)-N-(6-{8'-methyl-1',5'-dioxo-2'H-spiro[cyclohexane-1,3'-imidazo[1,5-a]pyridin]-6'-ylamino}pyrimidin-4-yl)acetamide; formic acid (244.1 mg, 59.45%) as an off-white solid. LC-MS- PH-MLCL-MC-2023-05-12-0: MS (ESI) m/z 426.20 [M+H]⁺ ¹H NMR-PH-MLCL-MC-2023-05-12-0: (400 MHz, DMSO-*d*₆) δ 10.09 (s, 1H), 9.97 (s, 1H), 9.29 (s, 1H), 8.53 (d, *J* = 1.1 Hz, 1H), 8.49 (s, 1H), 8.15 (s, 1H), 7.92 (d, *J* = 1.1 Hz, 1H), 3.17 (s, 2H), 3.00 (td, *J* = 13.1, 4.4 Hz, 2H), 2.45 (s, 3H), 2.31 (s, 6H), 1.81 – 1.56 (m, 5H), 1.45 (d, *J* = 12.3 Hz, 2H), 1.23 (s, 1H).

[0083] Synthesis of IIIh

To a stirred solution of 6'-[(6-aminopyrimidin-4-yl)amino]-8'-methyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (240 mg, 0.653 mmol, 1 equiv) in DMF (4 mL) was added m-CPBA (225.44 mg, 1.306 mmol, 2 equiv) at 0 °C. The resulting mixture was stirred for 2 hours at room temperature. The resulting mixture was purified by reverse flash chromatography with the following conditions: column, silica gel; mobile phase, MeCN in water (0.05%TFA), 10% to 50% gradient in 10 min; detector, UV 254 nm to obtain the crude. The crude product was purified by Prep-HPLC with the following conditions (Column: Sunfire prep C18 column, 30*150 mm, 5 μ m; Mobile Phase A: Water(0.05%TFA), Mobile Phase B: MeOH--HPLC; Flow rate: 60 mL/min; Gradient: 3% B to 18% B in 10 min; Wave Length: 254nm/220nm; RT1(min): 12.03) to afford 6'-[(6-aminopyrimidin-4-yl)amino]-8-hydroxy-8'-methyl-2'H-8-azaspiro[bicyclo[3.2.1]octane-3,3'-imidazo[1,5-a]pyridine]-1',5'-dione (40 mg, 15.97%) as an off white solid. LC-MS-PH-MLCL-MC-2023-05-14-0: (ES, m/z): $[M+H]^+ = 384.15$, $^1\text{H-NMR-PH-MLCL-MC-2023-05-14-0}$ (400 MHz, DMSO- d_6): δ 9.48 (s, 1H), 8.82 (s, 1H), 8.57 (s, 1H), 8.22 (s, 1H), 7.56 (s, 2H), 6.67 (s, 1H), 4.04 (s, 2H), 3.40 (d, $J = 3.9$ Hz, 2H), 2.43 (s, 3H), 2.20 (t, $J = 7.7$ Hz, 2H), 2.07 – 1.90 (m, 2H), 1.85 (d, $J = 14.3$ Hz, 2H).

EXAMPLE 2

[0084] MNK 1 and 2 Assay: The following procedures is utilized in evaluating compounds as MNK inhibitors. The ability of MNK inhibitors to inhibit activity of MNK-1 was tested using the recombinant full-length human kinase MNK-1 that is expressed in insect cells. The ability of MNK inhibitors to inhibit activity of MNK2 was tested using the recombinant full length human kinase MNK-2 that is expressed in insect cells. In the radiometric activity assay, the test compound is incubated with MNK kinase, substrate, cofactors, and radioisotope-labeled ATP, and % kinase activity determined based upon the extent of substrate phosphorylation. (Anastassiadis T, *et al.* Comprehensive assay of kinase catalytic activity reveals features of kinase inhibitor selectivity. *Nat Biotechnol.* 29(11):1039-45. doi: 10.1038/nbt.2017, Oct 30, 2011)

[0085] Reagent: Base Reaction buffer; 20 mM Hepes (pH 7.5), 10 mM MgCl₂, 1 mM EGTA, 0.01% Brij35, 0.02 mg/ml BSA, 0.1 mM Na₃VO₄, 2 mM DTT, 1% DMSO Required cofactors are added individually to each kinase reaction.

Table 6A:

Kinases	Kinase in RXN (nM)	Substrate	Substrate in Reaction (uM)
MNK1	200	MBP	20
MNK2	30	MBP	20

[0086] Reaction Procedure:

1. Prepare substrate in freshly prepared Reaction Buffer
2. Deliver any required cofactors to the substrate solution above
3. Deliver kinase into the substrate solution and gently mix
4. Deliver compounds in 100% DMSO into the kinase reaction mixture by Acoustic technology (Echo550; nanoliter range), incubate for 20 min at room temp
5. Deliver 33P-ATP into the reaction mixture to initiate the reaction
6. Incubate for 2 hours at room temperature
7. Detect kinase activity by P81 filter-binding method

[0087] IC₅₀ data for a few compounds is provided in Table 6B.

Table 6B:

Compound	MNK1 IC ₅₀ (M)	MNK2 IC ₅₀ (M)
Im	2.57E-08	1.66E-09
Ii	2.26E-08	7.62E-09
In	1.75E-08	3.41E-09
Ie (isomer 1)	2.52E-08	6.09E-10
If (isomer 1)	2.68E-08	1.59E-09
If (isomer 2)	3.96E-08	3.09E-09
Ik	3.49E-08	1.10E-09
IIa	4.16E-08	3.11E-09
Io	3.05E-08	2.90E-09
Ip (R1 = H)/eFT508	2.90E-08	8.06E-10
Staurosporine	8.32E-08	1.35E-08

EXAMPLE 3

[0088] Blood-brain Barrier Permeability: Permeability of the blood-brain barrier to various MNK inhibitors provided herein and tomivosertib (eFT508) as a comparison was

evaluated using an *in vitro* drug and metabolism pharmacokinetic (DMPK) study. The study was conducted in Madin Darby Canine Kidney (MDCK) cells that express the MDR1 gene (ABCB1) that encodes for the efflux protein, P-gp. MDCK-MDR1 is a stable-transfected cell line originating from MDCK cells, with over-expression of human MDR1 gene. Because MDCK-MDR1 permeability correlates well with brain exposure it is often utilized as predictor of blood-brain-barrier penetration. In the assay, test compounds were evaluated for Papp A-B and Papp B-A. Net flux ratio between the two directional transports was calculated as shown in Table 7 and 8.

[0089] Preparation of MDCKII-MDR1 Cells

- 1) 50 μ L and 25 mL of cell culture medium were added to each well of the Transwell insert and reservoir, respectively. And then the HTS transwell plates were incubated at 37 °C, 5% CO₂ for 1 hour before cell seeding.
- 2) MDCKII-MDR1 cells were diluted to 1.56x10⁶ cells/mL with culture medium and 50 μ L of cell suspension were dispensed into the filter well of the 96-well HTS Transwell plate. Cells were cultivated for 4-8 days in a cell culture incubator at 37 °C, 5% CO₂, 95% relative humidity. Cell culture medium was replaced every other day, beginning no later than 24 hours after initial plating.

[0090] Preparation of Stock Solutions

[0091] 10 mM stock solutions of test compounds were prepared in DMSO. The stock solutions of positive controls were prepared in DMSO at the concentration of 10 mM. Metoprolol and Digoxin were used as control compounds in this assay.

[0092] 3. Assessment of Cell Monolayer Integrity

- 1) Medium was removed from the reservoir and each Transwell insert and replaced with prewarmed fresh culture medium.
- 2) Transepithelial electrical resistance (TEER) across the monolayer was measured using Millicell Epithelial Volt-Ohm measuring system (Millipore, USA).
- 3) The Plate was returned to the incubator once the measurement was done.

[0093] The TEER value was calculated according to the following equation:
TEER measurement (ohms) x Area of membrane (cm²) = TEER value (ohm•cm²)
TEER value should be greater than 42 ohm•cm², which indicates the well-qualified MDCKII-MDR1 monolayer.

[0094] Assay Procedures

- 1) The MDCKII-MDR1 plate was removed from the incubator and washed twice with pre-warmed HBSS (10 mM HEPES, pH 7.4), and then incubated at 37 °C for 30 minutes.
- 2) The stock solutions of the control compounds and test compounds were diluted in DMSO to get 200 µM solutions and then diluted with HBSS (10 mM HEPES, pH 7.4) to get 1 µM working solutions. The final concentration of DMSO in the incubation system was 0.5%.
- 3) To determine the rate of drug transport in the apical to basolateral direction. Add 125 µL of the working solution to the Transwell insert (apical compartment), and transfer 50 µL sample immediately from the apical compartment to 200 µL of acetonitrile containing IS (100 nM alprazolam, 200 nM caffeine, 200 nM labetalol and 100 nM tolbutamide) in a new 96-well plate as the initial donor sample (A-B). Vortex at 1000 rpm for 10 minutes. Fill the wells in the receiver plate (basolateral compartment) with 235 µL of transport buffer
- 4) To determine the rate of drug transport in the basolateral to apical direction. Add 285 µL of the working solution to the receiver plate wells (basolateral compartment), and transfer 50 µL sample immediately from the basolateral compartment to 200 µL of acetonitrile containing IS (100 nM alprazolam, 200 nM caffeine, 200 nM labetalol and 100 nM tolbutamide) in a new 96-well plate as the initial donor sample (B-A). Vortex at 1000 rpm for 10 minutes. Fill the Transwell insert (apical compartment) with 75 µL of transport buffer. The apical to basolateral direction and the basolateral to apical direction need to be done at the same time.
- 5) The plates were incubated at 37 °C for 2 hours.
- 6) At the end of the incubation, 50 µL samples from donor sides (apical compartment for Ap→Bl flux, and basolateral compartment for Bl→Ap) and receiver sides (basolateral compartment for Ap→Bl flux, and apical compartment for Bl→Ap) were transferred to wells of a new 96-well plate, followed by the addition of 4 volume of acetonitrile containing IS (100 nM alprazolam, 200 nM caffeine, 200 nM labetalol and 100 nM tolbutamide). Samples were Vortexed for 10 minutes and then centrifuged at 3,220 g for 40 minutes. An aliquot of 100 µL of the supernatant was mixed with an appropriate volume of ultra-pure water before LC-MS/MS analysis.
- 7) To determine the Lucifer Yellow leakage after 2-hour transport period, stock solution of Lucifer yellow was prepared in DMSO and diluted with HBSS (10 mM HEPES, pH 7.4) to reach the final concentration of 100 µM. 100 µL of the Lucifer

yellow solution was added to each Transwell insert (apical compartment), followed by filling the wells in the receiver plate (basolateral compartment) with 300 μ L of HBSS (10 mM HEPES, pH 7.4). The plates were Incubated at 37 °C for 30 mins. 80 μ L samples were removed directly from the apical and basolateral wells (using the basolateral access holes) and transferred to wells of new 96 wells plates. The Lucifer Yellow fluorescence (to monitor monolayer integrity) signal was measured in a fluorescence plate reader at 480 nM excitation and 530 nM emission.

[0095] Data Analysis

Apparent permeability (Papp) can be calculated for drug transport assays using the following equation:

Where: Papp is apparent permeability (cm/s $\times 10^{-6}$)

VA is the volume (in mL) in the acceptor well

Area is the surface area of the membrane (0.143 cm² for Transwell-96 Well

Permeable Supports)

Time is the total transport time in seconds.

Efflux ratio can be determined using the following equation:

$$\text{Efflux Ratio} = \frac{\text{Papp (B - A)}}{\text{Papp (A - B)}}$$

[0096] Where Papp (B-A) indicates the apparent permeability coefficient in basolateral to apical direction, and Papp (A-B) indicates the apparent permeability coefficient in apical to basolateral direction.

[0097] Mass balance (% recovery) can be determined using the following equation:

$$\text{Recovery \%} = \frac{[\text{drug}]_{\text{acceptor}} \times V_a + [\text{drug}]_{\text{donor}} \times V_d}{[\text{drug}]_{\text{initial donor}} \times V_d}$$

where VA is the volume (in mL) in the acceptor well (0.235 mL for A→B flux, and 0.075 mL for B→A), VD is the volume (in mL) in the donor well (0.075 mL for A→B flux, and 0.235 mL for B→A)

[0098] Lucifer yellow leakage of monolayer can be calculated using the following equation:

$$\text{LY Leakage} = \left[\frac{\text{Iacceptor} \times 0.3}{(\text{Iacceptor} \times 0.3 + \text{Idonor} \times 0.1)} \right] \times 100\%$$

where Iacceptor is the fluorescence intensity in the acceptor well (0.3 mL), and Idonor is the fluorescence intensity in the donor well (0.1 mL) and expressed as % leakage. Lucifer yellow percentage amount transported values should be less than 1.5 %. However, if the Papp determined in that transwell is qualitatively similar to that determined in the replicate transwells, based upon the scientific judgement of the responsible scientist, then the monolayer is considered acceptable.

Table 7. Permeability results of test compounds and control compounds in MDCKII-MDR1 cell line

Compound ID	Papp (A-B) (10 ⁻⁶ , cm/s)	Papp (B-A) (10 ⁻⁶ , cm/s)	Efflux Ratio	Recovery (%) A-B
Metoprolol	28.63	26.69	0.93	109.08
Digoxin	0.51	11.91	23.91	100.88
eFT508	21.76	30.34	1.39	102.75
Ila	5.29	26.48	5.06	93.66
Metoprolol	25.96	26.79	1.03	106.83
Digoxin	0.53	12.29	23.07	98.40
In	0.17	0.48	2.78	98.01

Table 8. The assessment of MDCKII-MDR1 cell monolayer integrity

Compound ID	TEER A-B (Ω × cm ²)	TEER B-A (Ω × cm ²)	LY Leakage A-B (%)	LY Leakage B-A (%)
Metoprolol	78.86	84.16	0.32	0.49
Digoxin	77.86	83.80	0.36	0.60
eFT508	80.08	85.51	0.42	0.60
Ila	80.08	81.37	0.47	0.37
Metoprolol	78.15	83.23	0.18	0.16
Digoxin	78.65	82.65	0.17	0.17
In	85.16	86.94	0.17	0.16

EXAMPLE 4

[0099] Permeability Determination of MNK inhibitors by BBB-PAMPA Method (Table 9).

1) The stock solutions of positive controls were prepared in DMSO or acetonitrile at the concentration of 10 mM. Testosterone and methotrexate were used as control compounds in this assay.

2) Prepare a stock solution of compound in DMSO at the concentration of 10 mM, and further dilute with PBS (1× PBS, pH 7.4). The final concentration of the test compound is 10 μM.

[00100] 2. Assay Procedures:

- 1) Prepare a 2 % solution (w/v) of porcine brain lipid in dodecane, then sonicate the mixture to ensure a complete dissolution.
- 2) Carefully pipette 6 μL of the lecithin/dodecane mixture into each acceptor plate well (top compartment), avoiding pipette tip contact with the membrane.
- 3) Immediately after the application of the artificial membrane (within 10 minutes), add 300 μL of PBS (1× PBS, pH 7.4) solution to each well of the acceptor plate. Add 300 μL of drug-containing solutions to each well of the donor plate (bottom compartment) in triplicate.
- 4) Slowly and carefully place the acceptor plate into the donor plate, making sure the underside of the membrane is in contact with the drug-containing solutions in all wells.
- 5) Replace the plate lid and incubate at 25°C, 60 rpm for 16 hours.
- 6) After incubation, aliquots of 50 μL from each well of acceptor and donor plate are transferred into a 96-well plate. Add 450 μL of methanol (containing IS: 100 nM Alprazolam, 200 nM Caffeine, 100 nM Tolbutamide) into each well.
- 7) Cover with plate lid. Vortex at 750 rpm for 100 seconds. Samples were centrifuged at 3,220 g for 20 minutes. Determine the compound concentrations by LC/MS/MS.

[00101] 3. Data Analysis

[00102] The effective permeability (Pe), in units of centimeter per second, can be calculated using the following equation:

$$\text{Log Pe} = \text{Log} \left\{ C \times \left[-\text{Ln} \left(1 - \frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{equilibrium}}} \right) \right] \right\}$$

Compounds which have a -Log Pe < 6 are classified as high permeability and compounds with a -Log Pe > 6 are classified as low permeability.

Table 9. The effective permeability coefficients (-Log Pe) of test compounds and control compounds

Compound ID	-Log Pe	Recovery%
Methotrexate*	>8.89	<96.80
Testosterone	4.43	90.77

eFT508	5.29	102.98
In	>9.03	<100.40
Ila	5.36	35.00

EXAMPLE 5

[00103] Efficacy of MNK inhibitor in combination with tirzepatide and retatrutide in high-fat diet induced and genetic model of obesity. Experimental Design Animals employed in all procedures were 18-week-old male C57BL/6J wild-type (WT) mice. All mice were individually housed in plastic ‘tub’ cages with a stainless-steel grid lid and wood shavings scattered on the floor. The vivarium was maintained at 23°C on a 12-h light/12-h dark cycle with lights off at 0700 hours. The mice have access to pelleted Rodent Diet with 60% of calories from fat. Deionized water and food was available.

[00104] The study was conducted on 8 mice for each experimental condition. Body weight was measured weekly. The MNK inhibitor, MCL-5000/eFT508 (10 mg/kg) formulated in HPCD was administered by oral gavage every day for 3 weeks, following the standard operating procedure. Tirzepatide and Retatrutide were administered SQ (Q3D).

[00105] After 3 weeks, all groups undergone *in vivo* evaluation of the major physiological parameters of obesity. Subsequently, tissues were examined for biochemical and immunohistochemical evidence of inflammation, oxidative and nitrosative stress, and PARP activation. After 3 weeks, 4 animals from each group will be sampled to obtain skeletal muscle and visceral white adipose tissue for RT-PCR and immunoblotting analysis; the tissues will be processed and frozen for the analyses. The effects of MNK inhibitors MCL5000 and MCL5008 versus or in combination with Tirzepatide or retatrutide for prevention of weight gain, increase in adipocyte mitochondrial content, decrease in fat mass, and improved endocrinology (leptin secretion), blood lipid profile, and glucose homeostasis are displayed in FIG. 2 - 7 and FIG. 12 - 13. Animals lacking either or both MNK genes were protected against high-fat diet induced weight gain and cardiometabolic complications. MNK1 and MNK2 phosphorylate the conserved site S209 of eIF4E protein following activation by ERK and p38. eIF4E phosphorylation by MNK increases translation of a subset of mRNAs that contribute to diet-induced sarcopenic obesity. MCL5008 selectively blocks the phosphorylation of eIF4E in fat tissue by targeted inhibition of MNK1/2, and reverses weight gain in older animals with diet induced obesity.

[00106] Efficacy of MCL5008 in age-related sarcopenic obesity and weight loss effects in both young and aged mice with diet induced obesity. Young mice were treated with a

daily oral gavage of MCL5008 in comparison or combination with an approved GLP-1 receptor. After 21 days of treatment in younger mice (32 weeks old on day 1), MCL5008 reduced body weight through the specific loss of fat mass, leaving lean and muscle mass intact relative to vehicle treated controls. Additionally, MCL5008 significantly enhanced fat loss from Tirzepatide with greater preservation of lean mass relative to Tirzepatide alone.

[00107] In aged mice (52 weeks at day 1), extended treatment with MCL5008 for 118 days led to a 14% reduction in body weight that was also fat-specific with complete preservation of lean and muscle mass relative to vehicle controls (FIGs. 9, 10 and 13)). H&E staining demonstrated that MCL5008 decreased the size of fat cells in abdominal and omental adipose tissue and improved liver steatosis in aged mice with obesity (FIG. 11).

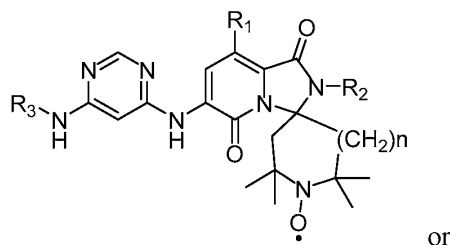
[00108] Protein levels of eIF4E and p-eIF4E in adipose were significantly elevated in older mice with obesity. Knockdown of eIF4E protein leads to increased fatty acid oxidation and energy expenditure, and the only kinases that phosphorylate eIF4E are mitogen-activated protein kinase interacting kinases, MNK1 and MNK2. In preclinical studies, MCL5008 decreased the expression of eIF4E and selectively blocked phosphorylation of eIF4E in adipose tissue in vivo via inhibition of MNK1/2. Phosphorylation of eIF4E in adipose promotes lipogenesis and decreases mitochondrial content, whereas blocking phosphorylation with MCL5008 promoted mitochondrial biogenesis in white adipose, improved fat cell endocrinology, and decreased fat cell size. Taken together, these findings demonstrate that eIF4E phosphorylation is enhanced in adipose in obesity, and blocking EIF4E phosphorylation with MCL5008 promotes weight maintenance in aged mice with obesity to improve overall health (FIG. 9).

[00109] Weight regain is a common problem in obesity therapy including for patients that discontinue GLP-1 treatment. As a model of this phenomenon, mice that were treated with tirzepatide (10 nmol/kg, SC, Q3D) regained all weight lost within 2 weeks after stopping treatment. In contrast, mice from the same cohort that received MCL5008 as a follow-on therapy after tirzepatide treatment maintained all weight lost throughout the follow-on treatment period (FIG. 8).

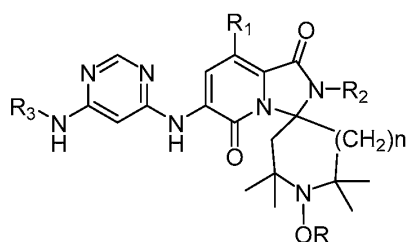
[00110] While example embodiments have been particularly shown and described, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the embodiments encompassed by the appended claims.

CLAIMS

1. A dual acting nitroxide substituted compound selected from Formula II or III,



II



III

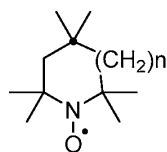
or enantiomers, epimers, diastereomers, racemates, or pharmaceutically acceptable salts thereof,

wherein,

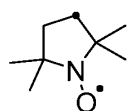
R1 is selected from F, Cl, Br, I, Me, Et, OMe, OCF₃, NH₂ or OH

R2, R3 and R4 are independently selected from H, CH₂OCOCH₃, CH₂OPO(ONa)₂, COCH₂NMe₂, COCH₂CH₂COOEt, COCH₂CH₂COOH, amide chain linked to the amino acids such as glycine, valine, lysine, arginine, tyrosine, leucine, alanine, cysteine, serine, tryptophan or proline

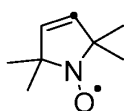
n is 0 to 3



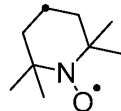
is selected from the following nitroxide rings:



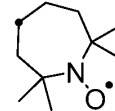
3-oxo-proxyl
derivative



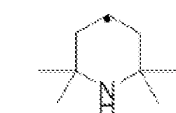
3-oxo-proxyl
derivative



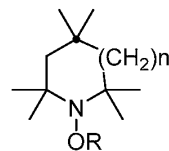
4-oxo-TEMPO
derivative



4-oxo-TEMAO/
azepane derivative or

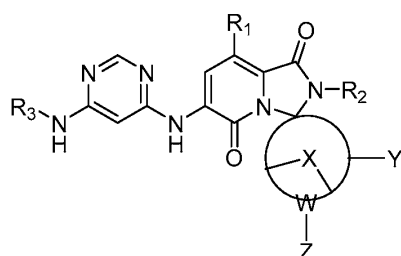


or



where R is selected from H, COCH₃, and amino acids.

2. A compound of Formula I



I

or enantiomers, epimers, diastereomers, racemates, or pharmaceutically acceptable salts thereof,

wherein:

R₁ is selected from F, Cl, Br, I, Me, Et, OMe, OCF₃, NH₂ or OH

R₂ and R₃ are independently selected from H, CH₂OCOCH₃, CH₂OPO(ONa)₂, COCH₂NMe₂, COCH₂CH₂COOEt, COCH₂CH₂COOH, amide chain linked to the amino acids such as glycine, valine, lysine, arginine, tyrosine, leucine, alanine, cysteine, serine, tryptophan or proline

W is selected from N or N-Me

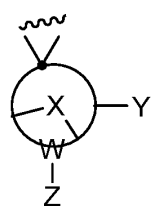
X is selected from N, NR, NR₂, O, CH₂, CH₂CH₂ or CMe₂, where R is H, Me, Et,

Y is selected from H, =CH₂, Me, F or combination of Me and OH or forming C₃-C₆ rings (e.g., cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl),

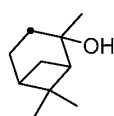
Z is selected from O• (O radical), OH, OCOCH₃, OR₂ or OR₃ or OR₄

n is 0 to 3

or wherein



is selected from the following polycyclic rings:



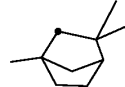
R/R/R -pinanone
derivative



tropinone
derivative



camphor
derivative



enchone
derivative



methylene-2-nor
bornanone derivative



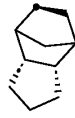
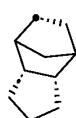
tricyclic-deca
derivative



tricyclic-dec-en
derivative

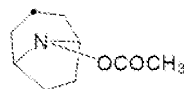


2-adamantanone
derivative



hexahydro-1H-4,7-methano
inden-5(6H)-one derivatives

or



tropinone and tetramethylpiperidine derivatives

3. The compound of claim 1 or claim 2 for use in the treatment of cancer, Alzheimer's disease, autism, fragile X syndrome, obesity and fat cell associated diseases such as liposarcoma, MASH, MAFLD, sarcopenic obesity and polycystic ovary syndrome, and a method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain.

4. A method of treating cancer, Alzheimer's disease, autism, fragile X syndrome, obesity and fat cell associated diseases such as liposarcoma, MASH, MAFLD, sarcopenic obesity and polycystic ovary syndrome by administering the compound of claim 1 or claim 2.

5. A method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain, by administering the compound of claim 1 or claim 2.
6. The method of claim 4 or claim 5, further comprising administering one or more other small molecule pain therapeutics.
7. The method of claim 6, wherein the small molecule pain therapeutics is salicylates, such as Aspirin (acetylsalicylic acid), Diflunisal and Salsalate, Propionic acid derivatives (Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen), Acetic acid derivatives, (Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Nabumetone), Enolic acid (Oxicam) derivatives (Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lomoxicam, Isoxicam), Fenamic acid derivatives or "Fenamates" (Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid), Selective COX-2 inhibitors (Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib), Sulphonanilides such as Nimesulide, and a range of other compounds (Licofelone, Lysine clonixinate, Hyperfbrin, Figwort), and any combinations thereof.
8. The method of claim 4 or claim 5, further comprising administering the compound of Formula I, II or III in combination with one or more drugs for obesity, and/or sarcopenic obesity such as Phentermine (ADIPEX-P®, LOMAIRA®, SUPRENZA®), Benzphetamine (DIDREX®, REGIMEX®), Diethylpropion (DEPLETITE 2®, RADTUE®, TENUATE®), Phendimetazine (BONTRIL®, MELFIAT®), Bupropion-naltrexone (CONTRAVE®), Lisdexamfetamine dimesylate (VYVANSE®), Cellulose and citric acid (PLENITY®), Liraglutide (SAXENDA®), Retatrutide, Exenatide, Albiglutide, Dulaglutide, Lixisenatide, Tirzepatide (Mounjaro/Zepbound), Danuglipron, Survodutide, RG6640, RG6641, RG6652, PF-06954522, HS-10535, Orphorglipron, Taspoglutide HU6, ECC5004, Pemvidutide, Mazdutide, Oxytocin and Semaglutide (WEGOVY®) in combination with Phentermine-topiramate (QSYMIA®) or SGLT2 inhibitors - glucagon-like-1 receptor agonists or Orlistat (XENICAL®, ALLI®).
9. The method of claim 5, wherein the cancer is selected from multiple myeloma, leukemia, alveolar rhabdomyosarcoma, melanoma, lymphoma, astrocytoma, biphasic synovial sarcoma, bladder carcinoma, bone cancer, breast cancer, cecum adenocarcinoma, cervical cancer, cns cancer, colon cancer, colorectal cancer, duodenal adenocarcinoma, embryonal rhabdomyosarcoma, endometrial cancer, epithelioid sarcoma, fibrosarcoma,

gastric cancer, signet ring cell gastric adenocarcinoma, gestational choriocarcinoma, glioblastoma, hereditary thyroid gland medullary carcinoma, hypopharyngeal squamous cell carcinoma, invasive ductal carcinoma, liposarcoma, lung cancer, neuroblastoma, osteosarcoma, ovarian cancer, uterine cancer, pancreatic cancer, papillary renal cell carcinoma, prostate cancer, rectal adenocarcinoma, medulloblastoma, renal cancer, testicular embryonal carcinoma, or tongue squamous cell carcinoma.

10. The method of claim 9, wherein that the method induces or enhances an anti-cancer response to a chemotherapeutic, the chemotherapeutic comprising a B-Raf inhibitor, a MEK inhibitor, a VEGF inhibitor, a VEGFR inhibitor, a tyrosine kinase inhibitor, an anti-mitotic agent, or any combination thereof.

11. The method of any one of claims 4-10, wherein the compound is administered by a route selected from transdermal, topical, oral, buccal, sublingual, intra venous, intramuscular, vaginal, rectal, nasal or follicular.

12. The compound of any one of claims 1-3, further comprising a pharmaceutical excipient.

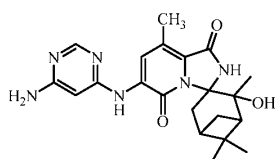
13. A dosage form suitable for administration to a mammal comprising the compound of any one of claims 1-3.

14. The compound of any one of claims 1-3, wherein the compounds is selected from Ia-Iu, IIa – IIu and IIIa – IIIu or an enantiomer, diastereomer, racemate, or a pharmaceutically acceptable salt thereof.

15. The compound of claim 14, for use in the treatment of cancer, Alzheimer's disease, autism, fragile X syndrome, obesity and fat cell associated diseases such as liposarcoma, MASH, MAFLD, sarcopenic obesity and polycystic ovary syndrome.

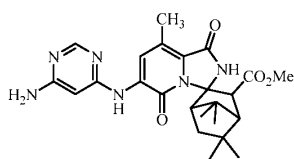
16. The compound of claim 14, for use in a method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain.

17. The compound of any one of claims 1-3, wherein the compound is selected from



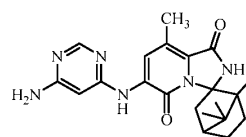
(R/R/R -pinanone derivative))

Ia,



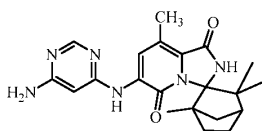
(R/R/R -kitopinic acid derivative))

Ib,



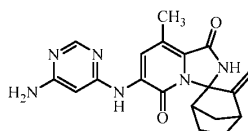
(R-camphor derivative)

Ic,



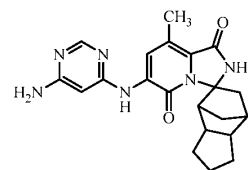
(fenchone derivative)

Id,



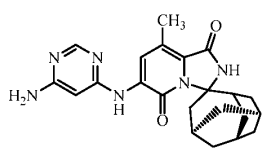
(methylene-2-norbornanone)

Ie,



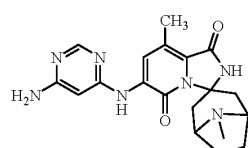
(hexahydro-1H-4,7-methanoinden-5(6H)-one derivative)

Ifa-d,



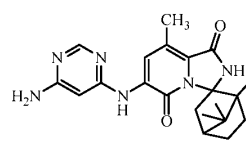
(2-adamantanone derivative)

Ih,



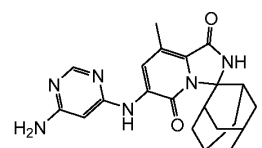
(tropinone derivative)

Ii,

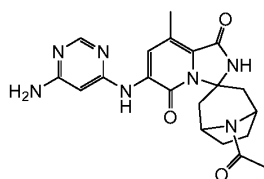


(S-camphor derivative)

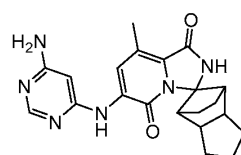
Ij,



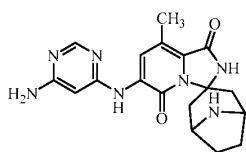
Ik,



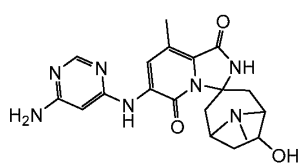
Il,



Im,



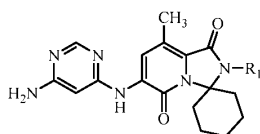
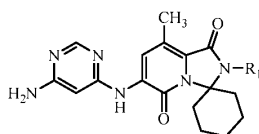
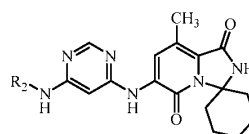
(tropinone derivative)



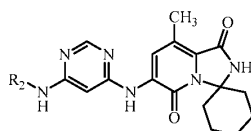
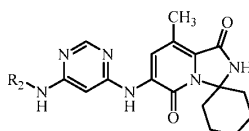
Io,

In,

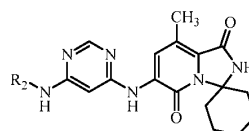
18. The compound of any one of claims 1-3, wherein the compound is selected from

(R1 = CH₂OPO(ONa)₂)**Ih,**(R1 = CH₂OCOC(=O)CH₃)**Ii,**

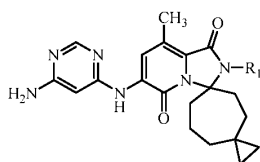
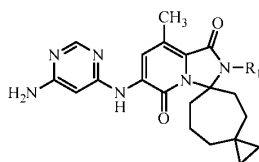
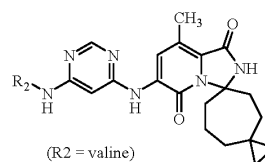
(R2 = valine)

Ij,(R2 = COCH₂NMe₂)**Ik,**

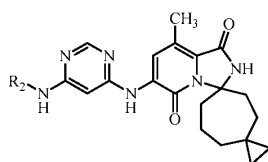
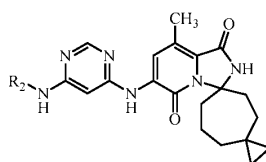
(R2 = leucine)

Il,

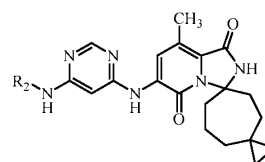
(R2 = arginine)

Im,(R1 = CH₂OPO(ONa)₂)**In,**(R1 = CH₂OCOC(=O)CH₃)**Io,**

(R2 = valine)

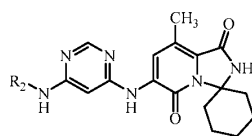
Ip,(R2 = COCH₂NMe₂)**Iq,**

(R2 = leucine)

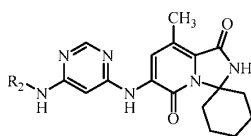
Ir,

(R2 = arginine)

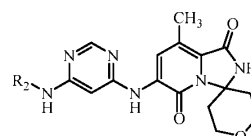
Is,



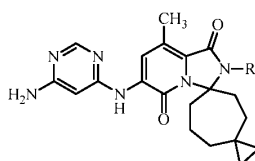
(R2=leucine)

It,

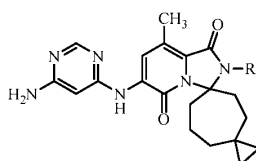
(R2=arginine)

Iu,

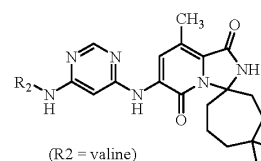
(R2=leucine or arginine or valine)

Iu1-Iu3,

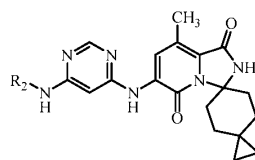
(R1 = CH2OPO(ONa)2)

Iv,

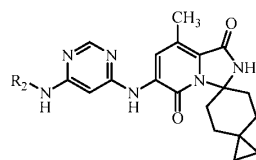
(R1 = CH2OCOCH3)

Iw,

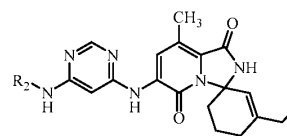
(R2 = valine)

Ix,

(R2=COCH2NMe2)

Iy,

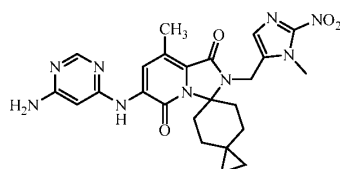
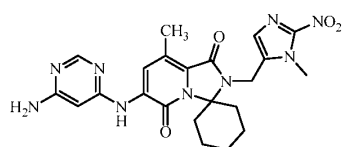
(R2=valine)

Iz or

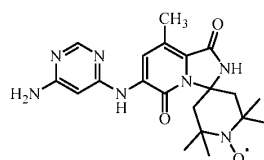
(R2=valine or arginine or leucine)

It3-5.

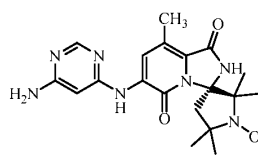
19. The compound of any one of claims 1-3, wherein the compound is selected from

**It1 and It2.**

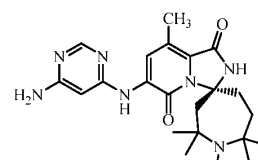
20. The compound of any one of claims 1-3, wherein the compound is selected from



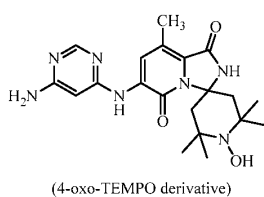
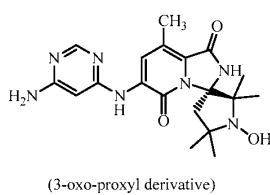
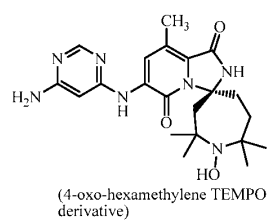
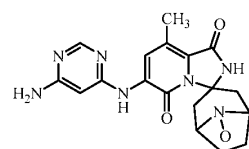
(4-oxo-TEMPO derivative)



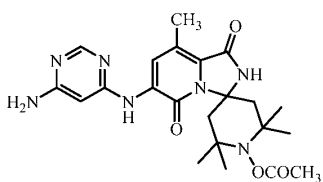
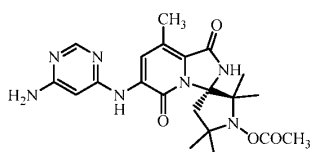
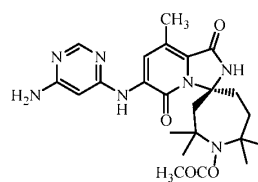
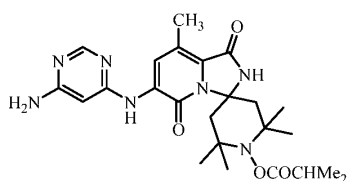
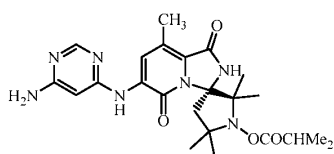
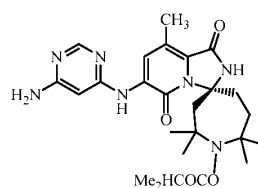
(3-oxo-proxyl derivative)



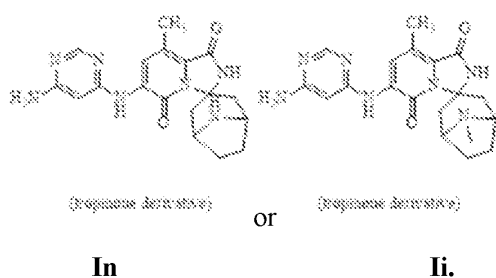
(4-oxo-hexamethylene TEMPO derivative)

IIa,**IIb,****IIc,****II d,****IIe,****II f or****II g.**

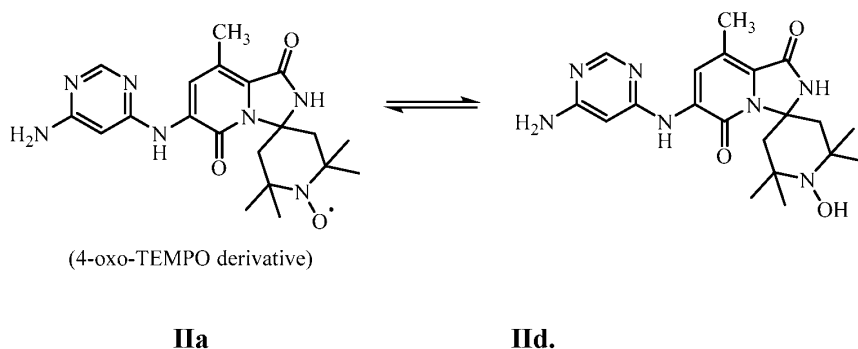
21. The compound of any one of claims 1-3, wherein the compound is selected from

**IIIa,****IIIb,****IIIc,****IIId,****IIIe or****III f.**

22. The compound of any one of claims 1-3, wherein the compound is selected from



23. The compound of any one of claims 1-3, wherein the compound is selected from



24. The compound of any one of claims 17-23, for use in the treatment of cancer, Alzheimer's disease, autism, fragile X-syndrome (FXS), obesity and fat cell associated diseases such as liposarcoma, MASH, MAFLD, sarcopenic obesity and polycystic ovary syndrome.

25. The compound of any one of claims 17-23, for use in a method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain.

26. A method of treating cancer, Alzheimer's disease, autism, fragile X-syndrome (FXS), obesity and fat cell associated diseases such as liposarcoma, MASH, MAFLD, sarcopenic obesity and polycystic ovary syndrome by administering the compound of any one of claims 17-23.

27. A method for treating, preventing, or mitigating the effects of a migraine or symptoms related to a migraine and a nerve pain, by administering the compound of any one of claims 17-23.

28. The method of claim 26 or claim 27, further comprising administering one or more other small molecule pain therapeutics.

29. The method of claim 28, wherein the small molecule pain therapeutics is salicylates, such as Aspirin (acetylsalicylic acid), Diflunisal and Salsalate, Propionic acid derivatives (Ibuprofen, Dexibuprofen, Naproxen, Fenoprofen, Ketoprofen, Dexketoprofen, Flurbiprofen, Oxaprozin, Loxoprofen), Acetic acid derivatives, (Indomethacin, Tolmetin, Sulindac, Etodolac, Ketorolac, Diclofenac, Nabumetone), Enolic acid (Oxicam) derivatives (Piroxicam, Meloxicam, Tenoxicam, Droxicam, Lomoxicam, Isoxicam), Fenamic acid derivatives or "Fenamates" (Mefenamic acid, Meclofenamic acid, Flufenamic acid, Tolfenamic acid), Selective COX-2 inhibitors (Celecoxib, Rofecoxib, Valdecoxib, Parecoxib, Lumiracoxib, Etoricoxib, Firocoxib), Sulphonanilides such as Nimesulide, and a range of other compounds (Licofelone, Lysine clonixinate, Hyperfbrin, Figwort), or any combinations thereof.

30. The method of claim 26 or 27, further comprising further administering an anti-obesity drugs, and or an anti-sarcopenic obesity such as Phentermine (ADIPEX-P®, LOMAIRA®, SUPRENZA®), Benzphetamine (DIDREX®, REGIMEX®), Diethylpropion (DEPLETITE 2®, RADTUE®, TENUATE®), Phendimetazine (BONTRIL®, MELFIAT®), Bupropion-naltrexone (CONTRAVE®), Lisdexamfetamine dimesylate (VYVANSE®), Cellulose and citric acid (PLENITY®), Liraglutide (SAXENDA®), Retatrutide, Exenatide, Albiglutide, Dulaglutide, Lixisenatide, Tirzepatide (Mounjaro/Zepbound), Danuglipron, PF-06954522 Orphorglipron, Taspoglutide HU6, ECC5004, Pemvidutide, Mazdutide, Oxytocin and Semaglutide (WEGOVY®), in combination with Phentermine-topiramate (ZSYMIA®) or SGLT2 inhibitors - glucagon-like-1 receptor agonists or Orlistat (XENICAL®, ALLI®).

31. The method of claim 26, wherein the cancer is selected from multiple myeloma, leukemia, alveolar rhabdomyosarcoma, melanoma, lymphoma, astrocytoma, biphasic synovial sarcoma, bladder carcinoma, bone cancer, breast cancer, cecum adenocarcinoma, cervical cancer, cns cancer, colon cancer, colorectal cancer, duodenal adenocarcinoma, embryonal rhabdomyosarcoma, endometrial cancer, epithelioid sarcoma, fibrosarcoma, gastric cancer, signet ring cell gastric adenocarcinoma, gestational choriocarcinoma, glioblastoma, hereditary thyroid gland medullary carcinoma, hypopharyngeal squamous cell carcinoma, invasive ductal carcinoma, liposarcoma, lung cancer, neuroblastoma, osteosarcoma, ovarian cancer, uterine cancer, pancreatic cancer, papillary renal cell carcinoma, prostate cancer, rectal adenocarcinoma, medulloblastoma, renal cancer, testicular embryonal carcinoma, or tongue squamous cell carcinoma.

32. The method of claim 31, wherein that the method induces or enhances an anti-cancer response to a chemotherapeutic, the chemotherapeutic comprising a B-Raf inhibitor, a MEK inhibitor, a VEGF inhibitor, a VEGFR inhibitor, a tyrosine kinase inhibitor, an anti-mitotic agent, or any combination thereof.

33. The method of any one of claims 26-32, wherein the compound is administered by a route selected from transdermal, topical, oral, buccal, sublingual, intra venous, intramuscular, vaginal, rectal, nasal or follicular.

34. The method of any one of claims 26-33, wherein the compound enhances fat mass loss while preserving lean mass in sarcopenia in combination with Phentermine (ADIPEX-P®, LOMAIRA®, SUPRENZA®), Benzphetamine (DIDREX®, REGIMEX®), Diethylpropion (DEPLETITE 2®, RADTUE®, TENUATE®), Phendimetazine (BONTRIL®, MELFIAT®), Bupropion-naltrexone (CONTRAVE®), Lisdexamfetamine dimesylate (VYVANSE®), Cellulose and citric acid (PLENITY®), Liraglutide (SAXENDA®), Retatrutide, Exenatide, Albiglutide, Dulaglutide, Lixisenatide, Tirzepatide (Mounjaro/Zepbound), Danuglipron, PF-06954522, Orphoglipron, Taspoglutide HU6, ECC5004, Pemvidutide, Mazdutide, Oxytocin and Semaglutide (WEGOVY®) in combination with Phentermine-topiramate (QSYMIA®) or SGLT2 inhibitors - glucagon-like-1 receptor agonists or Orlistat (XENICAL®, ALLI®).