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(54) Title: INHIBITORS OF PTPMT1

(57) Abstract: Disclosed herein are compounds and methods for treating a disease or disorder characterized by mitochondrial phosphatase activity.

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INHIBITORS OF PTPMT1

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under CA208642, and CA016042 awarded by the National Institutes of Health. The government has certain rights in the invention.

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 63/527,899, filed July 20, 2023; the entire contents of which are incorporated herein by reference.

BACKGROUND

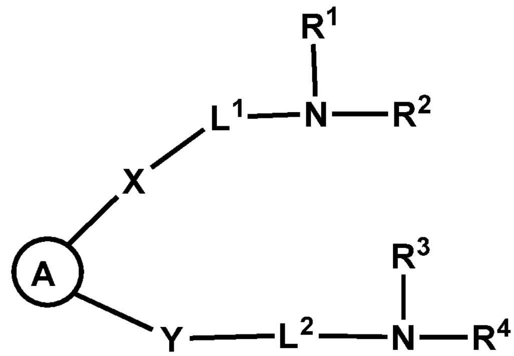
Cancer was the second leading cause of death, after heart disease, in the United States in 2022. In 2022, there were 609,360 cancer deaths; 287,270 were among females and 322,090 among males. Cancer arises from the transformation of normal cells into tumor cells in a multi-stage process that generally progresses from a pre-cancerous lesion to a malignant tumor.

Mitochondria, the "powerhouses" of cells, play a crucial role in the metabolism and energy production of cancer cells and have attracted considerable attention as targets for the development of novel anticancer agents because they have a central role in the resistance of malignant cells to regulated cell death induction by treatment. Modifications in the mitochondrial metabolic status influence multiple intracellular pathways and contribute to the acquisition of malignant traits. As such, mitochondrial function is fundamental for tumor progression, and an understanding of the pathways allowing tumors to take advantage of altered mitochondrial function to increase their growth rate and invasiveness will be crucial to establish effective therapeutic strategies.

Accordingly, there is an ongoing need to identify novel compounds for the treatment of disease or disorder associated with this deadly disease.

SUMMARY OF THE INVENTION

In one aspect, the present disclosure provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof,



Formula (I)

wherein:

A is alkyl, cycloalkyl, or heterocyclyl;

X is absent, NH or $–(CH_2)_{n1}NH–$;

Y is absent, NH or $–(CH_2)_{n2}NH–$;

n1 and n2 are each independently 1, 2, or 3;

L^1 and L^2 are each independently -C(=NH)NHC(=NH)- or -C(=NH)-; and

each R^1, R^2, R^3, and R^4 is independently selected from hydrogen, alkyl, alkoxy, cyano, cycloalkyl, (cycloalkyl)alkyl, heterocyclyl, (heterocyclyl)alkyl, aryl, aralkyl, heteroaryl, and heteroaralkyl; or R^1 and R^2 can combine to form a heterocyclyl; or R^3 and R^4 can combine to form a heterocyclyl.

In another aspect, the present disclosure provides a pharmaceutical composition comprising a compound disclosed herein, or a pharmaceutically acceptable thereof; and a pharmaceutically acceptable excipient.

In yet another aspect, the present disclosure provides methods of treating a disease or disorder characterized by mitochondrial phosphatase activity.

DESCRIPTION OF THE DRAWINGS

FIGs. 1A-1G show that Alexidine induces cell death in LKB1 and EGFR mutant lung tumor cells. **FIG. 1A** shows IC_{50} curves of Kras and Kras;Lkb1-/- lung tumors cells treated with ADHC. **FIG. 1B** shows bar graphs of JC-1 staining of A549 isogenic cell lines untreated or treated with alexidine (ADHC 1μM). **FIG. 1C** shows bar graphs of seahorse analysis on the RH2 (LKB1-/-) human lung tumor cell line measuring oxygen consumption rate (OCR). **FIG. 1D** shows bar graphs of seahorse analysis on the RH2 (LKB1-/-) human lung tumor cell line measuring extracellular acidification rate (ECAR). **FIG. 1E** shows immunoblot of Kras and

KL MEFs untreated or treated with ADHC (1μM) and probed for the indicated antibodies. **FIG. 1F** shows viability of a panel of LKB1 wildtype (WT) or mutant (MUT) human lung tumor cell lines. **FIG. 1G** shows viability of a panel of EGFR mutant LUAD cell line H1975 treated with vehicle (Veh) or ADHC (4μM).

FIGs. 2A-2D show inhibition of the mitochondrial phosphatase PTPMT1 chemosensitizes tumor cells. **FIG. 2A** shows schematic diagram of cardiolipin biosynthesis pathway. PA, phosphatidic acid; CDS, CDP-DAG synthase; PGS, PGP synthase; CLS, cardiolipin synthase; TAZ, cardiolipin remodeling enzyme, Tafazzin. **FIG. 2B** shows immunoblot of scr vs PTPMT1 KD human lung tumor cells. Blots are probed with the indicated antibodies. **FIG. 2C** shows cell viability in chemotherapy resistant shRNA scramble (scr) or PTPMT1 knockdown (KD) lung tumor cells. Cells treated with vehicle, 10uM cisplatin (CIS) or 100nM topotecan for 72 hours. **FIG. 2D** shows immunoblots of scr vs. shPTPMT1 cells treated vehicle or 10μM cisplatin for 72 hours and probed with the indicated antibodies.

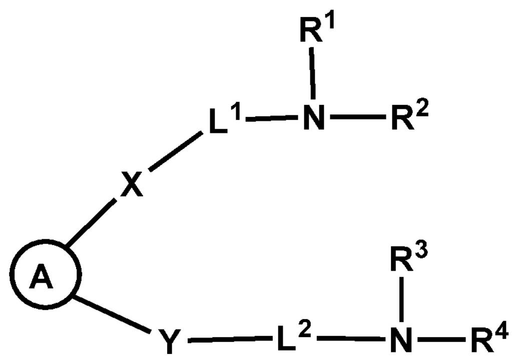
FIGs. 3A-3E show that JSW compounds remodel mitochondrial cristae and induce cell death in therapy resistant lung tumor cells. **FIG. 3A** shows a model depicting reorganization of the cristae from closed conformation (left) to an open conformation (right) in response to therapy. **FIG. 3B** shows immunoblots of NSCLC cells treated with vehicle or increasing doses of ADHC. Cell lysates were probed for the indicated antibodies. **FIG. 3C** shows immunoblots of NSCLC cells treated with vehicle, ADHC or JSW derivatives 02, 06 or 08. Cell lysates were probed for the indicated antibodies. **FIG. 3D** shows cell viability graphs of H1975 NSCLC cells treated with vehicle (veh) or increasing doses of ADHC. **FIG. 3E** shows cell viability graphs of H1975 NSCLC cells treated with vehicle (veh) or increasing doses of JSW002.

DETAILED DESCRIPTION OF THE INVENTION

The use of novel derivatives of the dibiguanide alexidine dihydrochloride as an anti-cancer drug through selectively remodeling of mitochondrial networks in tumor cells is proposed. Alexidine is an inhibitor of the mitochondrial phosphatase PTPMT1 that is required for cardiolipin synthesis and maintenance of mitochondrial structure and function. Upon inhibition of PTPMT1 activity, tumor cells re-organize their mitochondria into highly fragmented networks and become sensitive to therapeutic agents. Through modulation of mitochondrial networks, it has been discovered that PTPMT1 inhibitors function to induce tumor cell death.

Novel alexidine derivatives denoted as JSW compounds which act as modulators of mitochondrial networks that are described as mitochondrial perturbagens for use as cytotoxic and anti-cancer therapeutic sensitizing agents are designed and synthesized. These novel compounds sensitize tumors to and/or synergize with therapies that include, but are not limited to, cytotoxic chemotherapy (DNA damaging agents), mitocans (mitochondrial targeted agents) as well as antibody drug conjugates (ADCs) for the treatment of malignancies.

In one aspect, the present disclosure provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof,



Formula (I)

wherein:

A is alkyl, cycloalkyl, or heterocyclyl;

X is absent, NH or $–(CH_2)_{n1}NH$-;

Y is absent, NH or $–(CH_2)_{n2}NH$-;

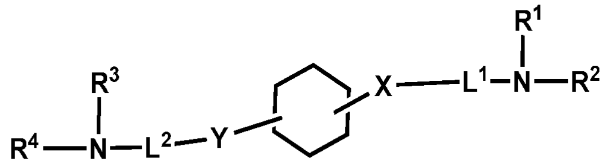
n1 and n2 are each independently 1, 2, or 3;

L^1 and L^2 are each independently -C(=NH)NHC(=NH)-, -C(=NH)-; and

each R^1 , R^2 , R^3 , R^4 is independently selected from hydrogen, alkyl, alkoxy, cyano, cycloalkyl, (cycloalkyl)alkyl, heterocyclyl, (heterocyclyl)alkyl aryl, aralkyl, heteroaryl, and heteroaralkyl; or R^1 and R^2 can combine to form a heterocyclyl; or R^3 and R^4 can combine to form a heterocyclyl.

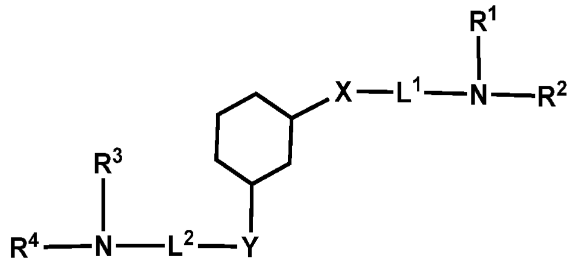
In certain especially preferred embodiments, A is cycloalkyl.

In certain embodiments, the compound or a pharmaceutically acceptable salt thereof has a structure represented by Formula (Ia),



(Ia).

In certain embodiments, the compound or a pharmaceutically acceptable salt thereof has a structure represented by Formula (Ia-1),



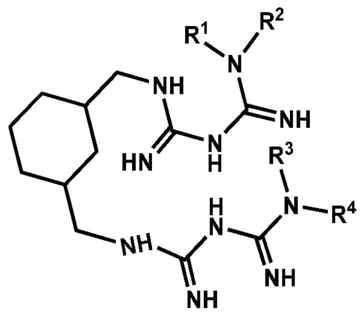
(Ia-1).

In some preferred embodiments, X is NH. In other embodiments, X is $(CH_2)_{n1}NH$. In further embodiments, n1 is 1. In some preferred embodiments, Y is NH. In other embodiments, Y is $(CH_2)_{n2}NH$. In further embodiments, n2 is 1. In certain preferred embodiments, L^1 is-C(=NH)NHC(=NH)-. In some preferred embodiments, L^2 is -C(=NH)NHC(=NH)-.

In certain embodiments, R^1 is hydrogen, alkyl (*e.g.*, hexyl, octyl, isobutyl, or ethylhexyl), alkoxy (*e.g.*, methoxyethyl or methoxypropyl), cycloalkyl (*e.g.*, cyclohexyl), (cycloalkyl)alkyl (*e.g.*, cyclohexylmethyl), heterocyclyl, aryl (*e.g.*, phenyl), aralkyl (*e.g.*, benzyl or phenylbutyl), heteroaryl, or heteroaralkyl (*e.g.*, furylmethyl). In some preferred embodiments, R^1 is ethylhexyl. In certain embodiments, R^2 is hydrogen, alkyl (*e.g.*, hexyl, octyl, isobutyl, or ethylhexyl), alkoxy (*e.g.*, methoxyethyl or methoxypropyl), cycloalkyl (*e.g.*, cyclohexyl), (cycloalkyl)alkyl (*e.g.*, cyclohexylmethyl), heterocyclyl, aryl (*e.g.*, phenyl), aralkyl (*e.g.*, benzyl or phenylbutyl), heteroaryl, or heteroaralkyl (*e.g.*, furylmethyl). In some preferred embodiments, R^2 is ethylhexyl. In certain embodiments, R^3 is hydrogen, alkyl (*e.g.*, hexyl, octyl, isobutyl, or ethylhexyl), alkoxy (*e.g.*, methoxyethyl or methoxypropyl), cycloalkyl (*e.g.*, cyclohexyl), (cycloalkyl)alkyl (*e.g.*, cyclohexylmethyl), heterocyclyl, aryl (*e.g.*, phenyl), aralkyl (*e.g.*, benzyl or phenylbutyl), heteroaryl, or heteroaralkyl (*e.g.*, furylmethyl). In some preferred embodiments, R^3 is ethylhexyl. In certain embodiments, R^4 is hydrogen, alkyl (*e.g.*, hexyl, octyl, isobutyl, or ethylhexyl), alkoxy (*e.g.*, methoxyethyl or methoxypropyl), cycloalkyl (*e.g.*, cyclohexyl), (cycloalkyl)alkyl (*e.g.*, cyclohexylmethyl), heterocyclyl, aryl (*e.g.*, phenyl), aralkyl (*e.g.*, benzyl or phenylbutyl), heteroaryl, or heteroaralkyl (*e.g.*, furylmethyl). In some preferred embodiments, R^4 is hydrogen.

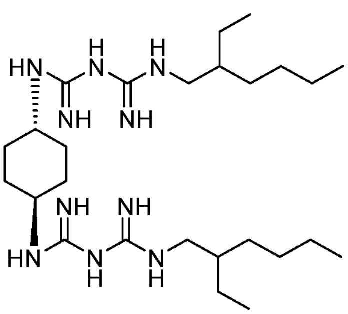
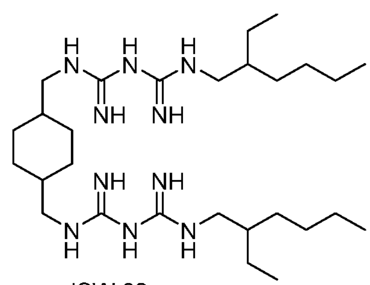
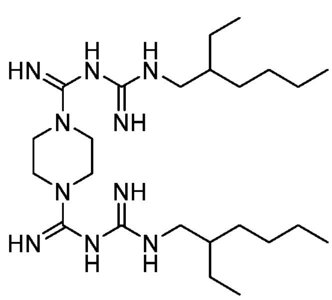
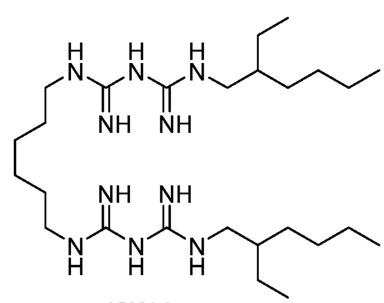
In certain embodiments, the aryl, aralkyl, heteroaryl, heteroaralkyl is optionally substituted by one or more substituents independently selected from alkyl, halogen, alkoxy, cyano, haloalkoxy, and haloalkyl.

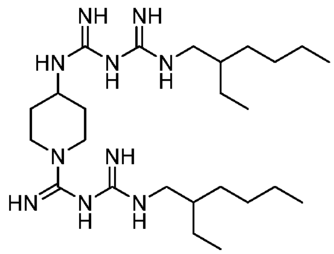
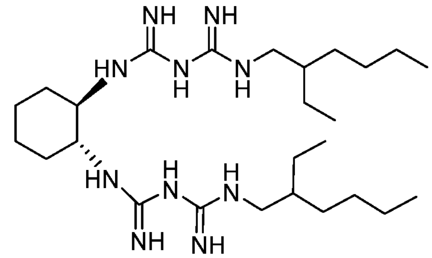
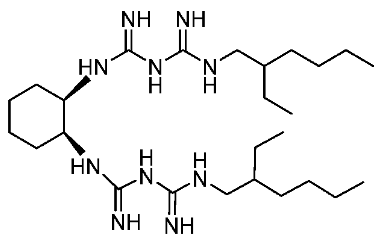
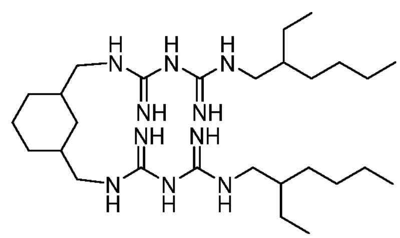
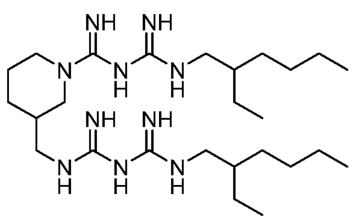
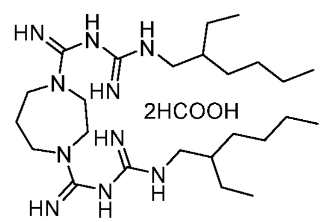
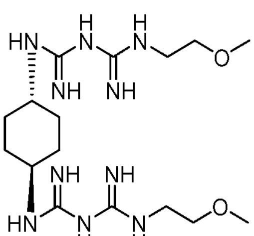
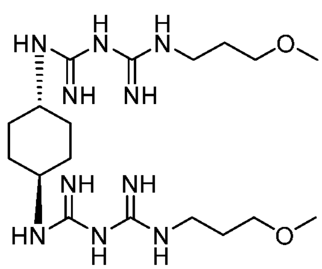
In certain embodiments, the compound or a pharmaceutically acceptable salt thereof has a structure represented by Formula (Ia-2),

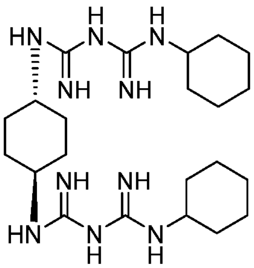
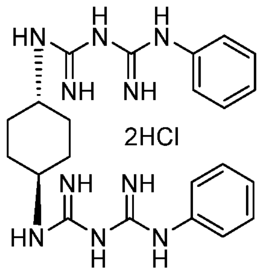
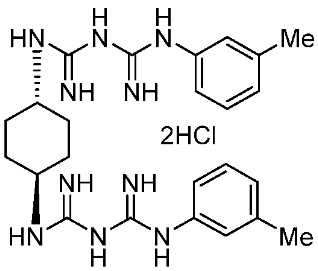
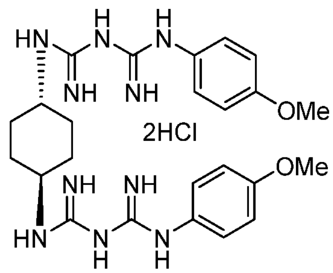
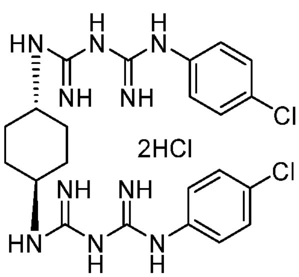
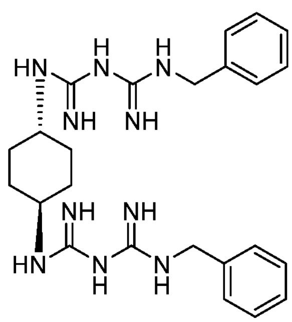
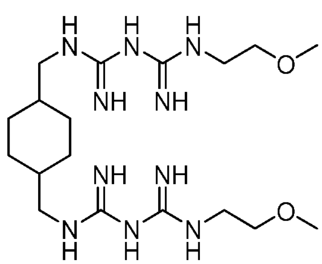
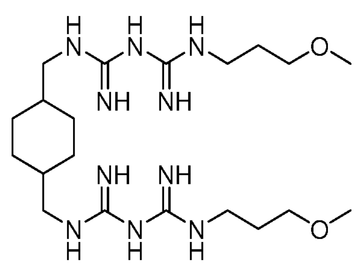


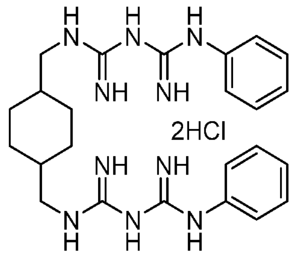
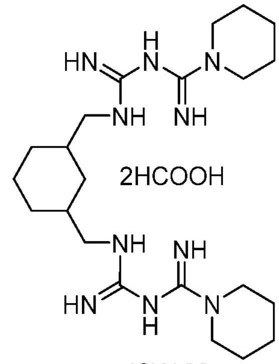
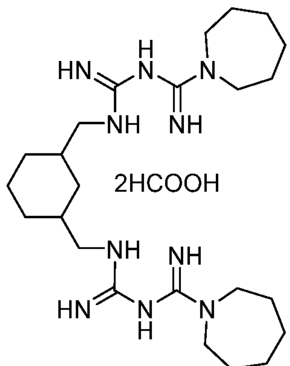
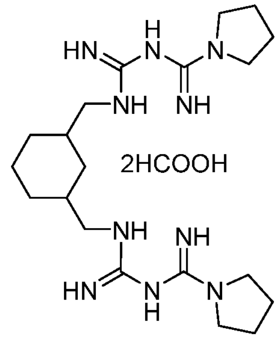
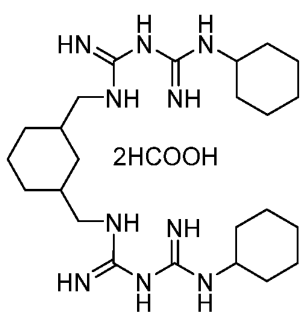
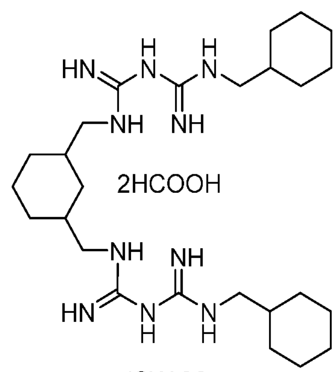
Formula (Ia-2).

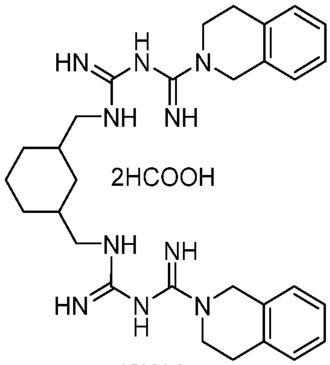
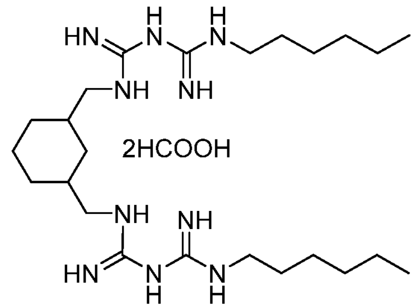
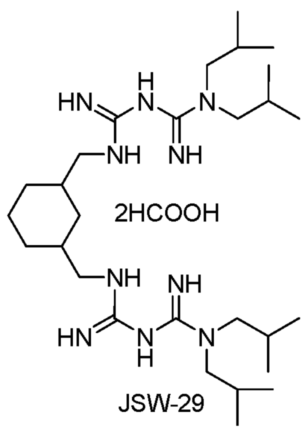
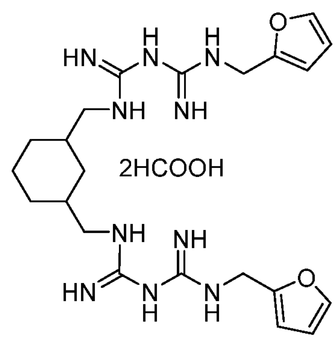
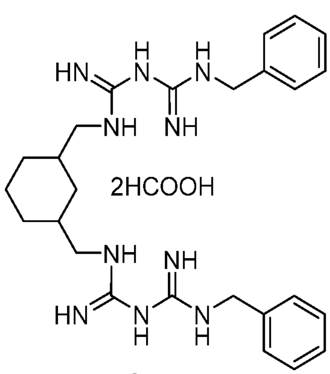
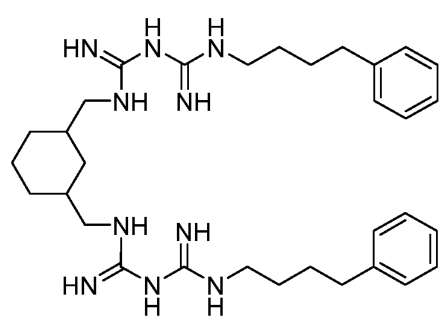
In certain embodiments, the compound or a pharmaceutically acceptable salt thereof, is selected from the following table:

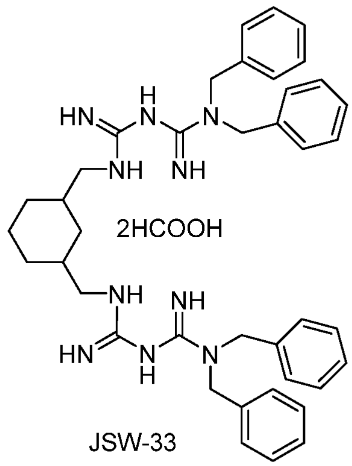
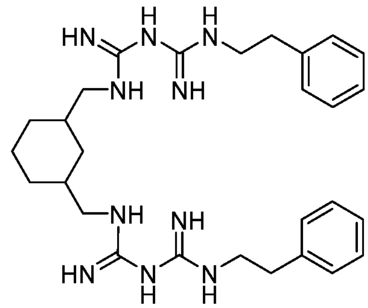
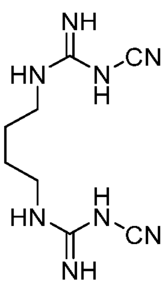
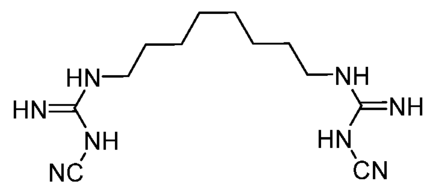
 JSW-01	 JSW-02
 JSW-03	 JSW-04

 JSW-05	 JSW-06
 JSW-07	 JSW-08
 JSW-09	 JSW-10
 JSW-11	 JSW-12

 JSW-13	 JSW-14
 JSW-15	 JSW-16
 JSW-17	 JSW-18
 JSW-19	 JSW-20

 JSW-21	 JSW-22
 JSW-23	 JSW-24
 JSW-25	 JSW-26

 JSW-27	 JSW-28
 JSW-29	 JSW-30
 JSW-31	 JSW-32

 JSW-33	 JSW-34
 JSW-m-10	 JSW-m-11

In yet another aspect, the present disclosure provides methods of treating a disease or disorder characterized by mitochondrial phosphatase activity in a subject in need thereof, comprising administering a compound disclosed herein to the subject.

In yet another aspect, the present disclosure provides methods of treating cancer in a subject in need thereof, comprising administering a compound disclosed herein to the subject.

Pharmaceutical Compositions

The compositions and methods of the present invention may be utilized to treat an individual in need thereof.

In certain embodiments, the individual is a mammal such as a human, or a non-human mammal. When administered to an animal, such as a human, the composition or the compound is preferably administered as a pharmaceutical composition comprising, for example, a compound of the invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil, or injectable organic esters. In preferred embodiments, when such pharmaceutical compositions are for human administration, particularly for invasive routes of administration (i.e., routes, such as injection or implantation, that circumvent transport or diffusion through an epithelial barrier), the aqueous solution is pyrogen-free, or substantially pyrogen-free. The excipients can be chosen, for example, to effect delayed release of an agent or to selectively target one or more cells, tissues or organs. The pharmaceutical composition can be in dosage unit form such as tablet, capsule (including sprinkle capsule and gelatin capsule), granule, lyophile for reconstitution, powder, solution, syrup, suppository, injection or the like. The composition can also be present in a transdermal delivery system, *e.g.*, a skin patch. The composition can also be present in a solution suitable for topical administration, such as a lotion, cream, or ointment.

A pharmaceutically acceptable carrier can contain physiologically acceptable agents that act, for example, to stabilize, increase solubility or to increase the absorption of a compound such as a compound of the invention. Such physiologically acceptable agents include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. The choice of a pharmaceutically acceptable carrier, including a physiologically acceptable agent, depends, for example, on the route of administration of the composition. The preparation or pharmaceutical composition can be a selfemulsifying drug delivery system or a selfmicroemulsifying drug delivery system. The pharmaceutical composition (preparation) also can be a liposome or other polymer matrix, which can have incorporated therein, for example, a compound of the invention. Liposomes, for example, which comprise phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

The phrase "pharmaceutically acceptable" is art-recognized, and is employed herein to refer to those compounds, materials, compositions, excipients, adjuvants, polymers and other materials, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

A pharmaceutical composition (preparation) can be administered to a subject by any of a number of routes of administration including, for example, orally (for example, drenches as in aqueous or non-aqueous solutions or suspensions, tablets, capsules (including sprinkle capsules and gelatin capsules), boluses, powders, granules, pastes for application to the tongue); absorption through the oral mucosa (*e.g.*, sublingually); subcutaneously; transdermally (for example as a patch applied to the skin); and topically (for example, as a cream, ointment or spray applied to the skin). The compound may also be formulated for inhalation. In certain embodiments, a compound may be simply dissolved or suspended in sterile water. Details of appropriate routes of administration and compositions suitable for same can be found in, for example, U.S. Pat. Nos. 6,110,973, 5,763,493, 5,731,000, 5,541,231, 5,427,798, 5,358,970 and 4,172,896, as well as in patents cited therein.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Methods of preparing these formulations or compositions include the step of bringing into association an active compound, such as a compound of the invention, with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules (including sprinkle capsules and gelatin capsules), cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), lyophile, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. Compositions or compounds may also be administered as a bolus, electuary or paste.

To prepare solid dosage forms for oral administration (capsules (including sprinkle capsules and gelatin capsules), tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; (10) complexing agents, such as, modified and unmodified cyclodextrins; and (11) coloring agents. In the case of capsules (including sprinkle capsules and gelatin capsules), tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions, such as dragees, capsules (including sprinkle capsules and gelatin capsules), pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms useful for oral administration include pharmaceutically acceptable emulsions, lyophiles for reconstitution, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, cyclodextrins and derivatives thereof, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an active compound, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the active compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion. Pharmaceutical compositions suitable for parenteral administration comprise one or more active compounds in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissue.

For use in the methods of this invention, active compounds can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Methods of introduction may also be provided by rechargeable or biodegradable devices. Various slow release polymeric devices have been developed and tested *in vivo* in recent years for the controlled delivery of drugs, including proteinaceous biopharmaceuticals. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of a compound at a particular target site.

Actual dosage levels of the active ingredients in the pharmaceutical compositions may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound or combination of compounds employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound(s) being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound(s) employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the therapeutically effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the pharmaceutical composition or compound at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. By “therapeutically effective amount” is meant the concentration of a compound that is sufficient to elicit the desired therapeutic effect. It is generally understood that the effective amount of the compound will vary according to the weight, sex, age, and medical history of the subject. Other factors which influence the effective amount may include, but are not limited to, the severity of the patient's condition, the disorder being treated, the stability of the compound, and, if desired, another type of therapeutic agent being administered with the compound of the invention. A larger total dose can be delivered by multiple administrations of the agent. Methods to determine efficacy and dosage are known to those skilled in the art (Isselbacher et al. (1996) Harrison’s Principles of Internal Medicine 13 ed., 1814-1882, herein incorporated by reference).

In general, a suitable daily dose of an active compound used in the compositions and methods of the invention will be that amount of the compound that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

If desired, the effective daily dose of the active compound may be administered as one, two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In certain embodiments of the present invention, the active compound may be administered two or three times daily. In preferred embodiments, the active compound will be administered once daily.

The patient receiving this treatment is any animal in need, including primates, in particular humans; and other mammals such as equines, cattle, swine, sheep, cats, and dogs; poultry; and pets in general.

In certain embodiments, compounds of the invention may be used alone or conjointly administered with another type of therapeutic agent.

The term "pharmaceutically acceptable acid addition salt" as used herein means any non-toxic organic or inorganic salt of any base compounds represented by Formula I. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono or di-acid salts can be formed, and such salts may exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of compounds of Formula I are more soluble in water and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of the appropriate salt will be known to one skilled in the art. Other non-pharmaceutically acceptable salts, *e.g.*, oxalates, may be used, for example, in the isolation of compounds of Formula I for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

The term "pharmaceutically acceptable basic addition salt" as used herein means any non-toxic organic or inorganic base addition salt of any acid compounds represented by Formula I or any of their intermediates. Illustrative inorganic bases which form suitable salts include lithium, sodium, potassium, calcium, magnesium, or barium hydroxide. Illustrative organic bases which form suitable salts include aliphatic, alicyclic, or aromatic organic amines such as methylamine, trimethylamine and picoline or ammonia. The selection of the appropriate salt will be known to a person skilled in the art.

The present disclosure includes the use of pharmaceutically acceptable salts of compounds of the invention in the compositions and methods of the present invention. In certain embodiments, contemplated salts of the invention include, but are not limited to, alkyl, dialkyl, trialkyl or tetra-alkyl ammonium salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, L-arginine, benenthamine, benzathine, betaine, calcium hydroxide, choline, deanol, diethanolamine, diethylamine, 2-(diethylamino)ethanol, ethanolamine, ethylenediamine, N-methylglucamine, hydrabamine, 1H-imidazole, lithium, L-lysine, magnesium, 4-(2-hydroxyethyl)morpholine, piperazine, potassium, 1-(2-hydroxyethyl)pyrrolidine, sodium, triethanolamine, tromethamine, and zinc salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, Na, Ca, K, Mg, Zn or other metal salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, 1-hydroxy-2-naphthoic acid, 2,2-dichloroacetic acid, 2-hydroxyethanesulfonic acid, 2-oxoglutaric acid, 4-acetamidobenzoic acid, 4-aminosalicylic acid, acetic acid, adipic acid, l-ascorbic acid, l-aspartic acid, benzenesulfonic acid, benzoic acid, (+)-camphoric acid, (+)-camphor-10-sulfonic acid, capric acid (decanoic acid), caproic acid (hexanoic acid), caprylic acid (octanoic acid), carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, d-glucoheptonic acid, d-gluconic acid, d-glucuronic acid, glutamic acid, glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, hydrobromic acid, hydrochloric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, l-malic acid, malonic acid, mandelic acid, methanesulfonic acid , naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, nicotinic acid, nitric acid, oleic acid, oxalic acid, palmitic acid, pamoic acid, phosphoric acid, proprionic acid, l-pyroglutamic acid, salicylic acid, sebacic acid, stearic acid, succinic acid, sulfuric acid, l-tartaric acid, thiocyanic acid, p-toluenesulfonic acid, trifluoroacetic acid, and undecylenic acid acid salts.

The pharmaceutically acceptable acid addition salts can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Definitions

Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, cell and tissue culture, molecular biology, cell and cancer biology, neurobiology, neurochemistry, virology, immunology, microbiology, pharmacology, genetics and protein and nucleic acid chemistry, described herein, are those well known and commonly used in the art.

The methods and techniques of the present disclosure are generally performed, unless otherwise indicated, according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout this specification. See, *e.g.* “Principles of Neural Science”, McGraw-Hill Medical, New York, N.Y. (2000); Motulsky, “Intuitive Biostatistics”, Oxford University Press, Inc. (1995); Lodish et al., “Molecular Cell Biology, 4th ed.”, W. H. Freeman & Co., New York (2000); Griffiths et al., “Introduction to Genetic Analysis, 7th ed.”, W. H. Freeman & Co., N.Y. (1999); and Gilbert et al., “Developmental Biology, 6th ed.”, Sinauer Associates, Inc., Sunderland, MA (2000).

Chemistry terms used herein, unless otherwise defined herein, are used according to conventional usage in the art, as exemplified by “The McGraw-Hill Dictionary of Chemical Terms”, Parker S., Ed., McGraw-Hill, San Francisco, C.A. (1985).

All of the above, and any other publications, patents and published patent applications referred to in this application are specifically incorporated by reference herein. In case of conflict, the present specification, including its specific definitions, will control.

The term "agent" is used herein to denote a chemical compound (such as an organic or inorganic compound, a mixture of chemical compounds), a biological macromolecule (such as a nucleic acid, an antibody, including parts thereof as well as humanized, chimeric and human antibodies and monoclonal antibodies, a protein or portion thereof, *e.g.*, a peptide, a lipid, a carbohydrate), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. Agents include, for example, agents whose structure is known, and those whose structure is not known.

A "patient," "subject," or "individual" are used interchangeably and refer to either a human or a non-human animal. These terms include mammals, such as humans, primates, livestock animals (including bovines, porcines, etc.), companion animals (*e.g.*, canines, felines, etc.) and rodents (*e.g.*, mice and rats).

"Administering" or "administration of" a substance, a compound or an agent to a subject can be carried out using one of a variety of methods known to those skilled in the art. For example, a compound or an agent can be administered, intravenously, arterially, intradermally, intramuscularly, intraperitoneally, subcutaneously, ocularly, sublingually, orally (by ingestion), intranasally (by inhalation), intraspinally, intracerebrally, and transdermally (by absorption, *e.g.*, through a skin duct). A compound or agent can also appropriately be introduced by rechargeable or biodegradable polymeric devices or other devices, *e.g.*, patches and pumps, or formulations, which provide for the extended, slow or controlled release of the compound or agent. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

Appropriate methods of administering a substance, a compound or an agent to a subject will also depend, for example, on the age and/or the physical condition of the subject and the chemical and biological properties of the compound or agent (*e.g.*, solubility, digestibility, bioavailability, stability and toxicity). In some embodiments, a compound or an agent is administered orally, *e.g.*, to a subject by ingestion. In some embodiments, the orally administered compound or agent is in an extended release or slow release formulation, or administered using a device for such slow or extended release.

As used herein, the phrase "conjoint administration" refers to any form of administration of two or more different therapeutic agents such that the second agent is administered while the previously administered therapeutic agent is still effective in the body (*e.g.*, the two agents are simultaneously effective in the patient, which may include synergistic effects of the two agents). For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic agents.

A "therapeutically effective amount" or a "therapeutically effective dose" of a drug or agent is an amount of a drug or an agent that, when administered to a subject will have the intended therapeutic effect. The full therapeutic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a therapeutically effective amount may be administered in one or more administrations. The precise effective amount needed for a subject will depend upon, for example, the subject's size, health and age, and the nature and extent of the condition being treated, such as cancer or MDS. The skilled worker can readily determine the effective amount for a given situation by routine experimentation.

As used herein, the terms "optional" or "optionally" mean that the subsequently described event or circumstance may occur or may not occur, and that the description includes instances where the event or circumstance occurs as well as instances in which it does not. For example, "optionally substituted alkyl" refers to the alkyl may be substituted as well as where the alkyl is not substituted.

It is understood that substituents and substitution patterns on the compounds of the present invention can be selected by one of ordinary skill in the art to result chemically stable compounds which can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials. If a substituent is itself substituted with more than one group, it is understood that these multiple groups may be on the same carbon or on different carbons, so long as a stable structure results.

As used herein, the term "optionally substituted" refers to the replacement of one to six hydrogen radicals in a given structure with the radical of a specified substituent including, but not limited to: hydroxyl, hydroxyalkyl, alkoxy, halogen, alkyl, nitro, silyl, acyl, acyloxy, aryl, cycloalkyl, heterocyclyl, amino, aminoalkyl, cyano, haloalkyl, haloalkoxy, -OCO-CH_2-O-alkyl, -OP(O)(O-alkyl)$_2$ or –CH_2-OP(O)(O-alkyl)$_2$. Preferably, "optionally substituted" refers to the replacement of one to four hydrogen radicals in a given structure with the substituents mentioned above. More preferably, one to three hydrogen radicals are replaced by the substituents as mentioned above. It is understood that the substituent can be further substituted.

As used herein, the term "alkyl" refers to saturated aliphatic groups, including but not limited to C_1-C_{10} straight-chain alkyl groups or C_1-C_{10} branched-chain alkyl groups.

Preferably, the "alkyl" group refers to C_1-C_6 straight-chain alkyl groups or C_1-C_6 branched-chain alkyl groups. Most preferably, the "alkyl" group refers to C_1-C_4 straight-chain alkyl groups or C_1-C_4 branched-chain alkyl groups. Examples of "alkyl" include, but are not limited to, methyl, ethyl, 1-propyl, 2-propyl, n-butyl, sec-butyl, tert-butyl, 1-pentyl, 2-pentyl, 3-pentyl, neo-pentyl, 1-hexyl, 2-hexyl, 3-hexyl, 1-heptyl, 2-heptyl, 3-heptyl, 4-heptyl, 1-octyl, 2-octyl, 3-octyl or 4-octyl and the like. The "alkyl" group may be optionally substituted.

The term "acyl" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-.

The term "acylamino" is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbylC(O)NH-.

The term "acyloxy" is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)O-, preferably alkylC(O)O-.

The term "alkoxy" refers to an alkyl group having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term "alkoxyalkyl" refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term "alkyl" refers to saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl-substituted cycloalkyl groups, and cycloalkyl-substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (*e.g.*, C_{1-30} for straight chains, C_{3-30} for branched chains), and more preferably 20 or fewer.

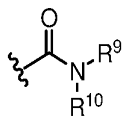
Moreover, the term "alkyl" as used throughout the specification, examples, and claims is intended to include both unsubstituted and substituted alkyl groups, the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc.

The term "C_{x-y}" or "C_x-C_y", when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. C_0alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. A C_{1-6}alkyl group, for example, contains from one to six carbon atoms in the chain.

The term "alkylamino", as used herein, refers to an amino group substituted with at least one alkyl group.

The term "alkylthio", as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

The term "amido", as used herein, refers to a group



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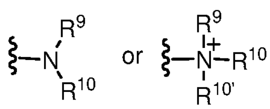
wherein R^9 and R^{10} each independently represent a hydrogen or hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, *e.g.*, a moiety that can be represented by



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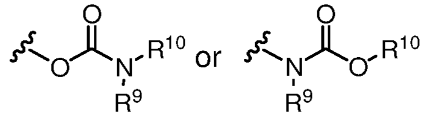
wherein R^9, R^{10}, and $R^{10'}$ each independently represent a hydrogen or a hydrocarbyl group, or R^9 and R^{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "aminoalkyl", as used herein, refers to an alkyl group substituted with an amino group.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group.

The term "aryl" as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The term "carbamate" is art-recognized and refers to a group



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wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group.

The term “carbocyclylalkyl”, as used herein, refers to an alkyl group substituted with a carbocycle group.

The term “carbocycle” includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic carbocycle may be selected from saturated, unsaturated and aromatic rings. Carbocycle includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term “fused carbocycle” refers to a bicyclic carbocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused carbocycle may be selected from saturated, unsaturated and aromatic rings. In exemplary embodiments, an aromatic ring, *e.g.*, phenyl, may be fused to a saturated or unsaturated ring, *e.g.*, cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary “carbocycles” include cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene and adamantane. Exemplary fused carbocycles include decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]octane, 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene. “Carbocycles” may be substituted at any one or more positions capable of bearing a hydrogen atom.

The term “carbocyclylalkyl”, as used herein, refers to an alkyl group substituted with a carbocycle group.

The term “carbonate” is art-recognized and refers to a group $-OCO_2-$.

The term “carboxy”, as used herein, refers to a group represented by the formula $-CO_2H$.

The term “cycloalkyl” includes substituted or unsubstituted non-aromatic single ring structures, preferably 4- to 8-membered rings, more preferably 4- to 6-membered rings. The term “cycloalkyl” also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is cycloalkyl and the substituent (*e.g.*, R^{100}) is attached to the cycloalkyl ring, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine, denzodioxane, tetrahydroquinoline, and the like.

The term “ester”, as used herein, refers to a group $-C(O)OR^9$ wherein R^9 represents a hydrocarbyl group.

The term “ether”, as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include “alkoxyalkyl” groups, which may be represented by the general formula alkyl-O-alkyl.

The terms “halo” and “halogen” as used herein means halogen and includes chloro, fluoro, bromo, and iodo.

The terms “hetaralkyl” and “heteroaralkyl”, as used herein, refers to an alkyl group substituted with a hetaryl group.

The terms “heteroaryl” and “hetaryl” include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heteroaryl” and “hetaryl” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

The term “heteroatom” as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The term “heterocyclylalkyl”, as used herein, refers to an alkyl group substituted with a heterocycle group.

The terms “heterocyclyl”, “heterocycle”, and “heterocyclic” refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms “heterocyclyl” and “heterocyclic” also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, *e.g.*, the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The term "hydrocarbyl", as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and even trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocycle, alkyl, alkenyl, alkynyl, and combinations thereof.

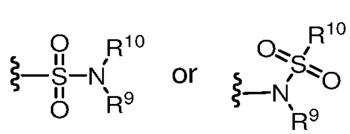
The term "hydroxyalkyl", as used herein, refers to an alkyl group substituted with a hydroxy group.

The term "lower" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer atoms in the substituent, preferably six or fewer. A "lower alkyl", for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

The terms "polycyclyl", "polycycle", and "polycyclic" refer to two or more rings (*e.g.*, cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, *e.g.*, the rings are "fused rings". Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

The term "sulfate" is art-recognized and refers to the group $-OSO_3H$, or a pharmaceutically acceptable salt thereof.

The term "sulfonamido" is art-recognized and refers to the group represented by the general formulae



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl.

The term "sulfoxide" is art-recognized and refers to the group–S(O)-.

The term “sulfonate” is art-recognized and refers to the group SO_3H, or a pharmaceutically acceptable salt thereof.

The term “sulfone” is art-recognized and refers to the group $–S(O)_2-$.

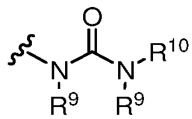
The term “substituted” refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that “substitution” or “substituted with” includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *e.g.*, which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate.

The term “thioalkyl”, as used herein, refers to an alkyl group substituted with a thiol group.

The term “thioester”, as used herein, refers to a group $-C(O)SR^9$ or $–SC(O)R^9$ wherein R^9 represents a hydrocarbyl.

The term “thioether”, as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term “urea” is art-recognized and may be represented by the general formula



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl.

The term “modulate” as used herein includes the inhibition or suppression of a function or activity (such as cell proliferation) as well as the enhancement of a function or activity.

“Pharmaceutically acceptable salt” or “salt” is used herein to refer to an acid addition salt or a basic addition salt which is suitable for or compatible with the treatment of patients.

Many of the compounds useful in the methods and compositions of this disclosure have at least one stereogenic center in their structure. This stereogenic center may be present in a R or a S configuration, said R and S notation is used in correspondence with the rules described in Pure Appl. Chem. (1976), 45, 11-30. The disclosure contemplates all stereoisomeric forms such as enantiomeric and diastereoisomeric forms of the compounds, salts, prodrugs or mixtures thereof (including all possible mixtures of stereoisomers). See, *e.g.*, WO 01/062726.

Furthermore, certain compounds which contain alkenyl groups may exist as Z (zusammen) or E (entgegen) isomers. In each instance, the disclosure includes both mixture and separate individual isomers.

“Prodrug” or “pharmaceutically acceptable prodrug” refers to a compound that is metabolized, for example hydrolyzed or oxidized, in the host after administration to form the compound of the present disclosure (*e.g.*, compounds of formula I). Typical examples of prodrugs include compounds that have biologically labile or cleavable (protecting) groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, or dephosphorylated to produce the active compound. Examples of prodrugs using ester or phosphoramidate as biologically labile or cleavable (protecting) groups are disclosed in U.S. Patents 6,875,751, 7,585,851, and 7,964,580, the disclosures of which are incorporated herein by reference. The prodrugs of this disclosure are metabolized to produce a compound of Formula I. The present disclosure includes within its scope, prodrugs of the compounds described herein. Conventional procedures for the selection and preparation of suitable prodrugs are described, for example, in “Design of Prodrugs” Ed. H. Bundgaard, Elsevier, 1985.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filter, diluent, excipient, solvent or encapsulating material useful for formulating a drug for medicinal or therapeutic use.

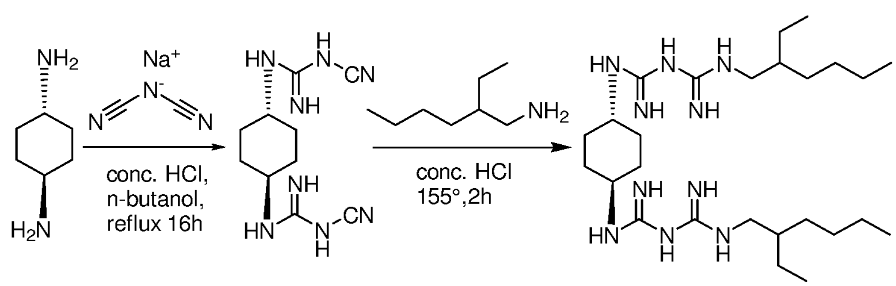
The term "Log of solubility", "LogS" or "logS" as used herein is used in the art to quantify the aqueous solubility of a compound. The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. A low solubility often goes along with a poor absorption. LogS value is a unit stripped logarithm (base 10) of the solubility measured in mol/liter.

EXAMPLES

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention and are not intended to limit the invention.

Example 1: Synthesis of Exemplary Compounds of the Disclosure

Experimental procedure for the preparation of JSW-01

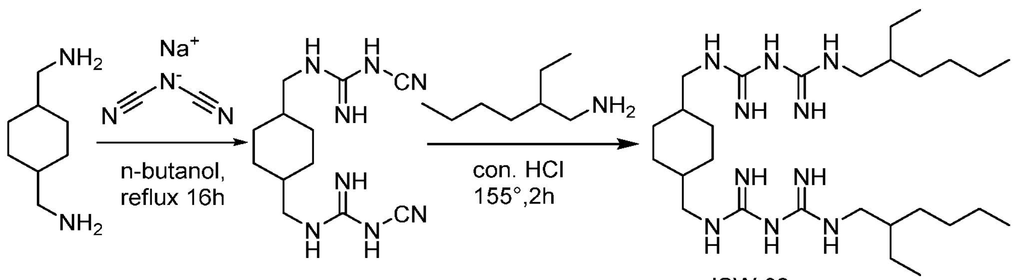


JSW-m-01 JSW-01

trans-N,N'''-1,4-cyclohexanediylbis(N'-cyanoguanidine) (JSW-m-01). To a solution of trans-cyclohexane-1,4-diamine (5 mmol) in n-butanol (10 mL) was added conc. aqueous hydrochloric acid (10 mmol). Then sodium dicyanamide (10 mmol) was added to the system. The mixture was refluxed for 16h. The suspension was cooled to room temperature and filtered. Washed with distilled water before drying under vacuum to give white solid (42% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.73-6.48 (m, 6H), 3.37 (br, 2H), 1.76-1.74 (m, 2H), 0.86-0.81 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ160.86, 118.75, 49.35, 31.28. HRMS m/z: $[M+H]^+$ calcd. for $C_{10}H_{17}N_8^+$: 249.1576, found 249.1592.

trans-N,N'-1,4-cyclohexanediylbis(5-[2-ethylhexyl]biguanide) (JSW- 01). To *Trans-N,N'''-1,4-cyclo hexanediylbis(N'-cyanoguanidine)* (**JSW-m-01**, 1.13 mmol) was added 2-ethylhexan-1-amine (2.26 mmol).Then concentrated HCl (2.26 mmol) was added. The mixture was thoroughly mixed and heated to 155°C for 2h. After completion of the reaction, the crude product was washed with water, ethanol, successively to give white solid (31% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54-7.44 (m, 4H), 6.85 -6.70 (m, 6H), 3.41 -3.38 (m, 2H), 3.00 (br, 4H), 1.85 (br, 4H), 1.37 -1.19 (m, 22H), 0.83 -0.81 (m, 12H).^{13}C NMR (100 MHz, DMSO-d_6) δ 159.53, 49.50, 49.49, 43.76, 31.23, 30.70, 28.80, 24.01, 22.96, 14.46, 11.14. HRMS m/z: [M+H]$^+$ calcd. for $C_{26}H_{55}N_{10}^+$: 507.4611, found 507.4777.

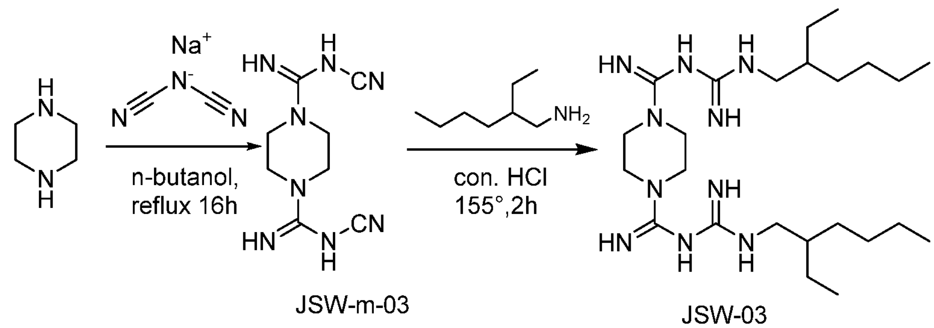
Experimental procedure for the preparation of JSW-02



N-cyano-N'-[4-((3-cyanoguanidino)methyl)cyclohexyl]-methyl-guanidine (JSW-m-02). Following the procedure of preparation of **JSW-m-01. JSW-m-02** was prepared from *piperazine* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (45% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.76-6.50 (m, 6H), 2.94-2.84 (m, 4H), 1.64 (br, 3H), 1.32 (br, 4H), 0.79 (br, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.75, 118.86, 47.22, 37.79, 35.25, 29.97, 26.08. HRMS m/z: [M+H]$^+$ calcd. for $C_{12}H_{21}N_8^+$: 277.1889, found 277.1913.

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[2-ethylhexyl]biguanide) (JSW-02). Following the procedure of preparation of **JSW-01. JSW-02** was prepared from ***JSW-m-02*** (1.13 mmol) and 2-ethyl hexan-1-amine (2.26 mmol) to give white solid (41% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (br, 1H), 7.56 (br, 3H), 6.83 (br, 6H), 3.00 -2.90 (m, 7H), 2.62 (br, 1H), 1.71-1.68 (m, 4H), 1.57-1.18 (m, 21H), 0.83-0.76 (m, 15H).^{13}C NMR (100 MHz, DMSO-d_6) δ 159.06, 47.42, 43.79, 41.94, 37.31, 30.68, 30.19, 28.80, 23.98, 22.97, 14.45, 11.15. HRMS m/z: [M+H]$^+$ calcd. for $C_{28}H_{59}N_{10}^+$: 535.5032, found 535.4924.

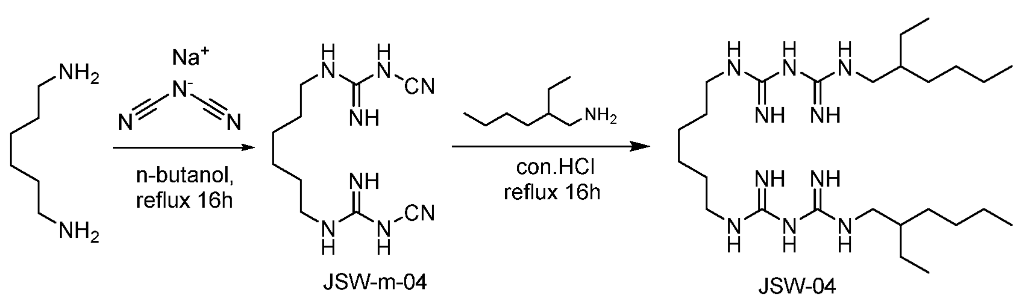
Experimental procedure for the preparation of JSW-03



N[1],N[4]-dicyano-1,4-piperazinedicarboximidamide (JSW-m-03). Following the procedure of preparation of JSW-m-01. JSW-m-03 was prepared from *piperazine* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (45% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.16 (s, 4H), 3.41 (8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.59, 118.45, 44.05. HRMS m/z: $[M+H]^+$ calcd. for $C_8H_{13}N_8^+$: 221.1263, found 221.1281.

N[1],N[4]-bis(N-(2-ethylhexyl)carbamimidoyl)piperazine-1,4-bis(carboximidamide)(JSW-03). Following the procedure of preparation of JSW-01. JSW-03 was prepared from *JSW-m-03* (1.13 mmol) and 2-ethylhexan-1-amine (2.26 mmol) to give light yellow solid (35% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (br, 3H), 7.75 (br, 1H), 7.53 -6.79 (m, 4H), 3.69 -3.38 (m, 7H), 3.12 -2.86 (m, 3H), 2.62 (br, 2H), 1.54-1.19 (m, 18H), 0.83-0.76 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.94, 44.32, 41.95, 37.33, 29.95, 28.31, 23.22, 22.79, 14.38, 10.64. HRMS m/z: $[M+H]^+$ calcd. for $C_{24}H_{51}N_{10}^+$: 479.4298, found 479.3828.

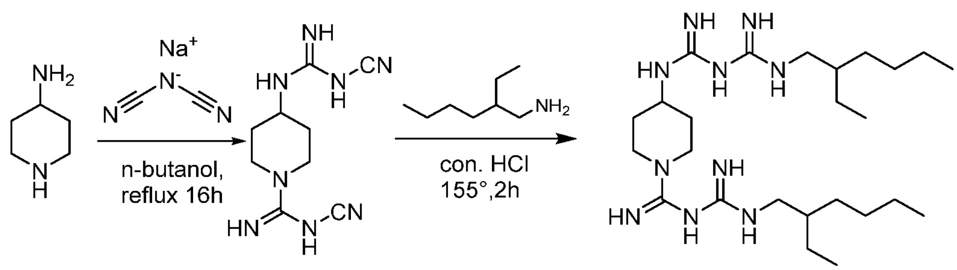
Experimental procedure for the preparation of JSW-04



N,N'''-1,6-hexanediylbis[N'-cyano]-guanidine (JSW-m-04). Following the procedure of preparation of JSW-m-01. JSW-m-04 was prepared from *hexane-1,6-diamine* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (45% isolated yield). The resulting spectroscopic data was matched with that of Gräber M, Hell M, Gröst C, et al. *Angew Chem Int Ed Engl.* 2013;52(16):4487-4491.

1,1'-Hexamethylene-bis(5-[2-ethylhexyl]biguanide) (Alexidine, JSW-04). Following the procedure of preparation of JSW-01. JSW-04 was prepared from *JSW-m-04* (1.13 mmol) and 2-ethylhexan-1-amine (2.26 mmol) to give white solid (45% isolated yield). The resulting spectroscopic data was matched with that of Gräber M, Hell M, Gröst C, et al. *Angew Chem Int Ed Engl.* 2013;52(16):4487-4491.

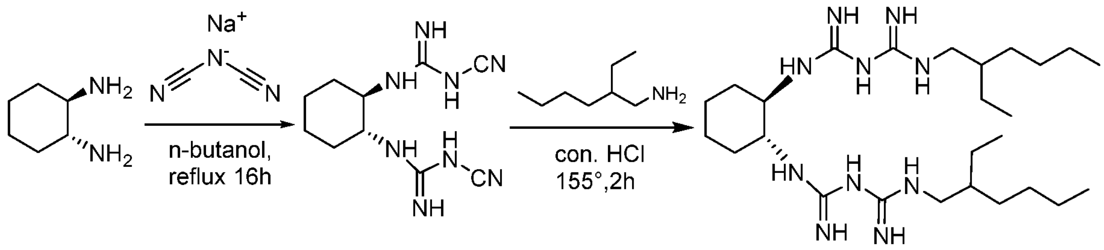
Experimental procedure for the preparation of JSW-05



N-cyano-4-(3-cyanoguanidino)piperidine-1-carboximidamide (JSW-m-05). Following the procedure of preparation of JSW-m-01. JSW-m-05 was prepared from *piperidin-4-amine* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (40% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.07-6.64 (m, 5H), 3.91-3.59 (m, 3H), 2.86-2.46 (m, 2H), 1.70 (br, 2H), 1.22-0.90 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 160.87, 118.73, 47.90, 43.82, 31.56. HRMS m/z: $[M+H]^+$ calcd. for $C_9H_{15}N_8^+$: 235.1420, found 235.1462.

N-(N-(2-ethylhexyl)carbamimidoyl)-4-(3-(N-(2-ethylhexyl)carbamimidoyl)guanidino)piperidine-1-carboximidamide (JSW-05). Following the procedure of preparation of JSW-01. JSW-05 was prepared from *JSW-m-05* (1.13 mmol) and 2-ethylhexan-1-amine (2.26 mmol)to give light yellow solid (29% isolated yield).^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.02 (br, 4H), 7.60 -7.21 (m, 5H), 3.91-3.68 (m, 1H), 3.11 -2.87 (m, 5H), 2.63-2.60 (m, 3H), 2.04-1.18 (m, 22H), 0.83-0.78 (m, 12H).^{13}C NMR (100 MHz, DMSO-d$_6$) δ 156.57, 45.03, 43.24, 41.96, 37.31, 30.72, 29.95, 28.31, 23.22, 22.79, 14.39, 10.64. HRMS m/z: $[M+H]^+$ calcd. for $C_{25}H_{53}N_{10}^+$: 493.4455, found 493.3963.

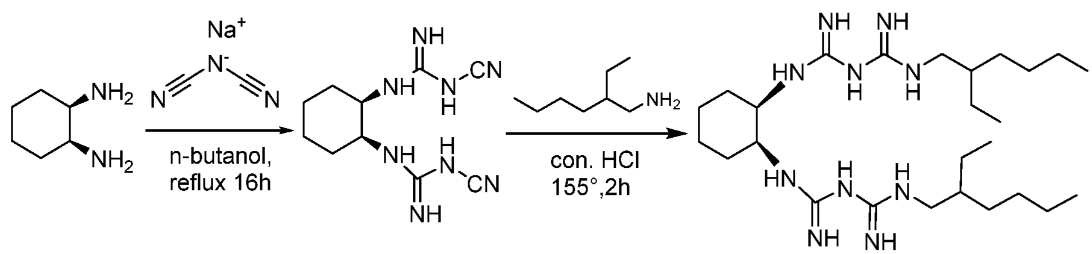
Experimental procedure for the preparation of JSW-06



trans-N,N'''-1,2-cyclohexanediylbis(N'-cyanoguanidine) (JSW-m-06). Following the procedure of preparation of JSW-m-01. JSW-m-06 was prepared from *trans-cyclohexane-1, 2-diamine* (5 mmol) and sodium dicyanamide (10 mmol) to give light yellow oil (30% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 6.60 (br, 6H), 3.74 (br, 2H), 1.48-1.27 (m, 8H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 161.41, 118.60, 50.13, 35.14, 28.61. HRMS m/z: $[M+H]^+$ calcd. for $C_{10}H_{17}N_8^+$: 249.1578, found 249.1596.

trans-N,N'-1,2-cyclohexanediylbis(5-[2-ethylhexyl]biguanide) (JSW-06). Following the procedure of preparation of JSW-01. JSW-06 was prepared from JSW-m-06 (1.13 mmol) and *2-ethylhexan-1-amine* (2.26 mmol) to give light yellow solid (23% isolated yield).^{1}H NMR (400 MHz, MeOD) δ 8.52 (br, 4H), 3.78-3.36 (m, 4H), 3.14 -3.05 (m, 2H), 2.16-2.08 (m, 1H), 1.91-1.69 (m, 3H), 1.56-1.19 (m, 22H), 0.92-0.89 (m, 12H).^{13}C NMR (100 MHz, MeOD) δ 159.37, 54.63, 42.95, 38.55, 35.62, 33.45, 29.09, 28.65, 23.69, 22.73, 13.06, 9.92. HRMS m/z: $[M+H]^+$ calcd. for $C_{26}H_{55}N_{10}^+$: 507.4611, found 507.4641.

Experimental procedure for the preparation of JSW-07

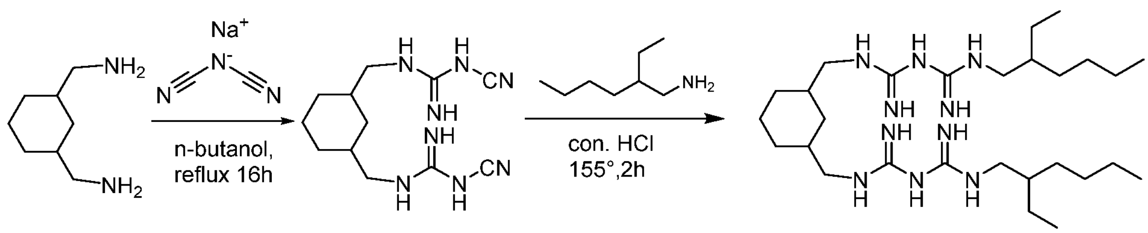


cis-N,N'''-1,2-cyclohexanediylbis(N'-cyanoguanidine) (1). Following the procedure of preparation of JSW-m-01. Compound 1 was prepared from *cis-cyclohexane-1,2-diamine* (5 mmol) and sodium dicyanamide (10 mmol) to give light yellow oil (34% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 6.61 (br, 6H), 3.73 (br, 2H), 1.47-1.26 (m, 8H). ^{13}C NMR (100

MHz, DMSO-d_6) δ 161.31, 117.59, 50.13, 35.23, 28.66. HRMS m/z: $[M+H]^+$ calcd. for $C_{10}H_{17}N_8^+$: 249.1578, found 249.1589.

cis-N,N'-1,2-cyclohexanediylbis(5-[2-ethylhexyl]biguanide) (JSW-07). Following the procedure of preparation of JSW-01. JSW-06 was prepared from JSW-m-07 (1.13 mmol) and *2-ethylhexan-1-amine* (2.26 mmol) to give light yellow solid (29% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 (br, 6H), 6.94 (4H), 3.24-3.08 (m, 6H), 2.04 (br, 1H), 1.64-1.42 (m, 5H), 1.36-1.14 (m, 20H), 0.82-0.80 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.20, 56.22, 47.10, 37.78, 30.33, 28.50, 24.11, 24.05, 22.28, 19.34, 14.41, 10.94. HRMS m/z: $[M+H]^+$ calcd. for $C_{26}H_{55}N_{10}^+$: 507.4611, found 507.4643.

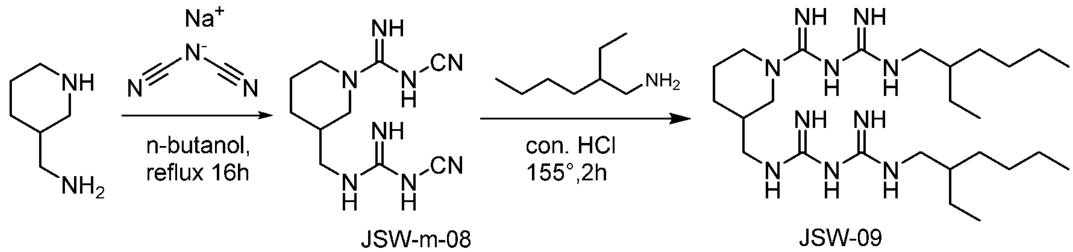
Experimental procedure for the preparation of JSW-08



N-cyano-N'-[3-((3-cyanoguanidino)methyl)cyclohexyl]-methyl-guanidine (2). Following the procedure of preparation of JSW-m-01. Compound 2 was prepared from *cyclohexane-1,3-diyldimethanamine* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (39% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29-6.72 (m, 6H), 3.05-2.86 (m, 4H), 1.66 (br, 4H), 1.36-0.99 (m, 5H), 0.80-0.48 (m, 3H). HRMS m/z: $[M+H]^+$ calcd. For $C_{12}H_{21}N_8^+$: 277.1889, found 277.1921.

N,N'-[1,3-cyclohexanediylbis(methylene)]-bis(5-[2-ethylhexyl]biguanide) (JSW-08). Following the procedure of preparation of JSW-01. JSW-08 was prepared from compound 2 (1.13 mmol) and *2-ethylhexan-1-amine* (2.26 mmol) to give white solid (22% isolated yield). ^{1}H NMR (400 MHz, MeOD) δ 8.44 (br, 2H), 3.16-3.07 (m, 8H), 1.80 (br, 4H), 1.56-1.29 (24H), 0.92-0.87 (m, 12H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.11, 49.81, 47.56, 37.83, 36.60, 35.01, 30.71, 28.80, 25.45, 23.96, 23.01, 17.97, 14.46, 11.11. HRMS m/z: $[M+H]^+$ calcd. for $C_{28}H_{59}N_{10}^+$: 535.4924, found 535.4942.

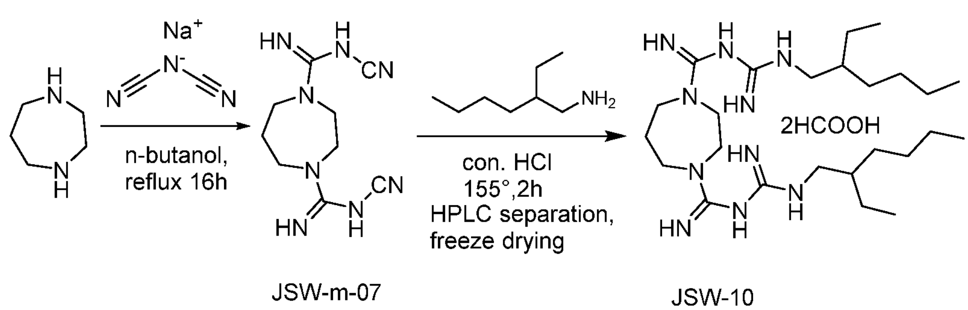
Experimental procedure for the preparation of JSW-09



N-cyano-3-((3-cyanoguanidino)methyl)piperidine-1-carboximidamide (*JSW-m-08*). Following the procedure of preparation of JSW-m-01. JSW-m-08 was prepared from *piperidin-3-ylmethanamine* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (37% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.73-7.62 (m, 1H), 7.43-7.31 (m, 2H), 6.94-6.79 (m, 2H), 3.16-3.08 (m, 3H), 2.67-2.62 (m, 2H), 1.87-1.61 (m, 4H), 1.33-0.99 (m, 2H). HRMS m/z: [M+H]$^+$ calcd. for $C_{10}H_{17}N_8^+$: 249.1576, found 249.1592.

N-(N-(2-ethylhexyl)carbamimidoyl)-3-((3-(N-(2-ethylhexyl)carbamimidoyl)guanidino)methyl) piperidine-1-carboximidamide (JSW-09). Following the procedure of preparation of JSW-01. JSW-09 was prepared from compound 3 (1.13 mmol) and *2-ethylhexan-1-amine* (2.26 mmol) to give light yellow solid (22% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.83-7.75 (m, 1H), 7.42-7.26 (m, 4H), 7.01 (br, 3H), 6.39 (br, 1H),3.14-3.04 (m, 9H), 2.65 (br, 1H), 1.78-1.01 (m, 23H), 0.84-0.77 (m, 12H).^{13}C NMR (126 MHz, DMSO-d$_6$) δ 156.27, 156.21, 156.05, 44.61, 44.08, 43.50, 42.07, 38.12, 37.41, 30.90, 30.61, 29.93, 28.67, 23.92, 23.00, 14.44, 14.40, 10.64. HRMS m/z: [M+H]$^+$ calcd. for $C_{26}H_{55}N_{10}^+$: 507.4611, found 507.4631.

Experimental procedure for the preparation of JSW-10

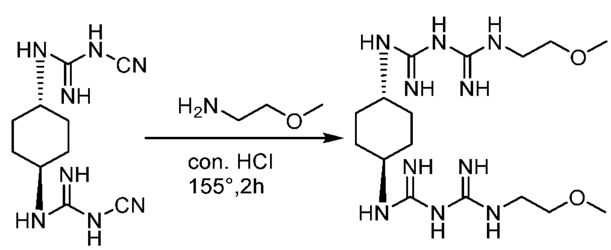


N1,N4-dicyano-1,4-diazepane-1,4-bis(carboximidamide) (*JSW-m-07*). Following the procedure of preparation of JSW-m-01. JSW-m-07 was prepared from *1,4-diazepane* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (40% isolated yield). ^{1}H NMR

(400 MHz, DMSO-d_6) δ 7.04 (br, 4H), 3.39-3.00 (m, 8H), 1.69-1.63 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.39, 118.55, 47.98, 45.70, 25.95. HRMS m/z: $[M+H]^+$ calcd. for $C_9H_{15}N_8^+$: 235.1420, found 235.1432.

N1,N4-bis(N-(2-ethylhexyl)carbamimidoyl)-1,4-diazepane-1,4-bis(carboximidamide) diformate (JSW-10). Following the procedure of preparation of JSW-01. JSW-10 was prepared from JSW-m-07 (1.13 mmol) and *2-ethylhexan-1-amine* (2.26 mmol). The residue was purified by HPLC (0.1% Formic acid in water, 0.1% Formic acid in MeCN), removed part of solvent *in vacuo*. Then the left solvent was removed under freeze-drying conditions to give white solid JSW-10 (30% isolated yield). ^{1}H NMR (500 MHz, D_2O) δ 8.33 (br, 2H), 3.70 (t, J = 5.0 Hz, 2H), 3.58 (t, J = 5.0 Hz, 2H), 3.52 (t, J = 5.0 Hz, 4H), 3.04 (d, J = 5.0 Hz, 2H), 2.83-2.81 (m, 2H), 1.82-1.77 (m, 2H), 1.55-1.50 (m, 2H),1.39 (br, 1H), 1.30-1.14 (m, 16H), 0.78-0.72 (m, 12H).^{13}C NMR (126 MHz, D_2O) δ 171.01, 156.22, 47.00, 42.35, 36.90, 29.89, 29.36, 27.63, 23.50, 22.72, 22.12, 13.21, 9.44. HRMS m/z: $[M-2HCOOH+H]^+$ calcd. for $C_{25}H_{53}N_{10}^+$: 493.4455, found 493.4513.

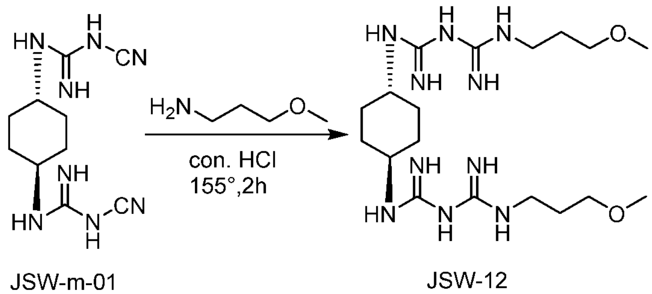
Experimental procedure for the preparation of JSW-11



JSW-m-01 JSW-11

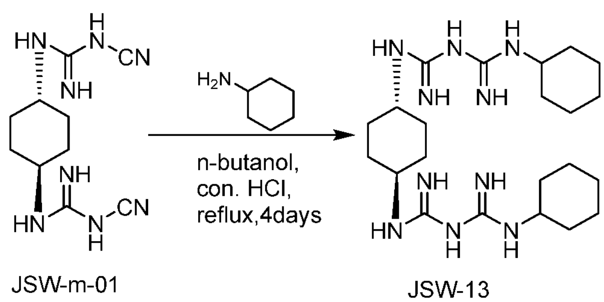
trans-N,N'-1,4-cyclohexanediylbis(5-[2-methoxyethanyl]biguanide) (JSW-11). Following the procedure of preparation of JSW-01. JSW-11 was prepared from JSW-m-01 (1.13 mmol) and 2-methoxyethan-1-amine (2.26 mmol) to give white solid (41% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60-6.67 (m, 10H), 3.48 (t, J = 4.0 Hz, 1H), 3.37-3.34 (m, 4H), 3.26-3.19 (m, 10H), 2.92-2.89 (m, 1H), 1.87-1.84 (m, 4H), 1.23-1.19 (m, 4H).^{13}C NMR (126 MHz, DMSO-d_6) δ159.32, 68.54, 58.45, 49.53, 38.81, 31.08.HRMS m/z: $[M+H]^+$ calcd. for $C_{16}H_{35}N_{10}O_2^+$: 399.2944, found 399.2937.

Experimental procedure for the preparation of JSW-12



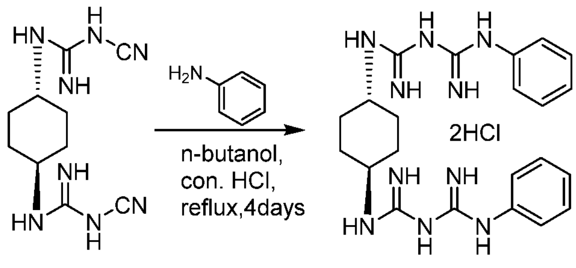
trans-N,N'-1,4-cyclohexanediylbis(5-[3-methoxypropanyl]biguanide) (JSW-12). Following the procedure of preparation of JSW-01. JSW-11 was prepared from JSW-m-01 (1.13 mmol) and *3-methoxy propan-1-amine* (2.26 mmol) to give white solid (45% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45-7.22 (m, 4H), 6.93-6.57 (m, 6H), 3.48-3.44 (m, 2H), 3.18-3.3.07 (m, 13H), 1.85-1.62 (m, 8H), 1.20(br, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.20, 72.70, 56.22, 47.10, 37.78, 30.33, 28.50. HRMS m/z: $[M+H]^+$ calcd. for $C_{18}H_{39}N_{10}O_2^+$: 427.3257, found 427.3318.

Experimental procedure for the preparation of JSW-13



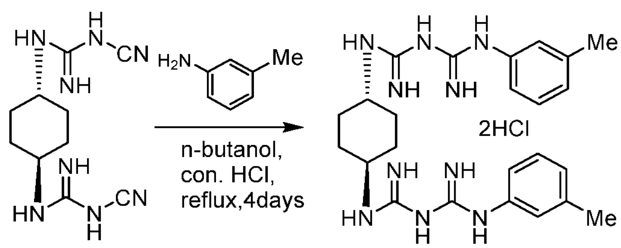
trans-N,N'-1,4-cyclohexanediylbis(5-cyclohexanyl-biguanide) (JSW-13). To a solution of *trans-N,N'''-1,4-cyclo hexanediylbis(N'-cyanoguanidine)* (JSW-m-01, 1.13mmol) in n-butanol (7 mL) was added cyclohexanamine (2.26 mmol).Then concentrated HCl (2.26mmol) was added. The mixture was thoroughly mixed and refluxed for 4days. After cooled to room temperature, the crude product was washed with water, ethanol, successively to give white solid (29% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.53-6.53 (m, 10H), 3.41-3.36 (m, 4H), 1.86-1.75 (m, 7H), 1.65-1.48 (m, 7H), 1.35-0.98 (m, 14H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.15, 49.69, 47.96, 35.15, 33.81, 25.80, 24.80. HRMS m/z: $[M+H]^+$ calcd. for $C_{22}H_{43}N_{10}^+$: 447.3672, found 447.3740.

Experimental procedure for the preparation of JSW-14



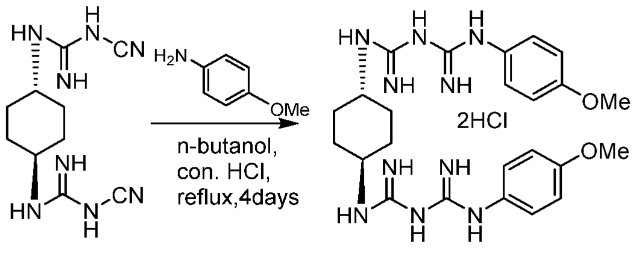
trans-N,N'-1,4-cyclohexanediylbis(5-phenyl-biguanide) dihydrochloride (JSW-14). Following the procedure of preparation of JSW-13. JSW-14 was prepared from JSW-m-01 (1.13 mmol) and aniline (2.26 mmol) to give white solid (40% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (br, 1H), 9.54 (br, 1H), 8.01-7.67 (m, 4H), 7.34-7.22 (m, 11H), 6.99 (br, 3H), 6.74 (br, 2H), 3.24 (m, 2H), 1.85 (m, 4H), 1.24 (br, 4H).^{13}C NMR (100 MHz, DMSO-d_6) δ 159.12, 135.13, 129.07, 121.23, 120.99, 30.68. HRMS m/z: $[M\text{-}2HCl+H]^+$ calcd. for $C_{22}H_{31}N_{10}^+$: 435.2733, found 435.2765.

Experimental procedure for the preparation of JSW-15



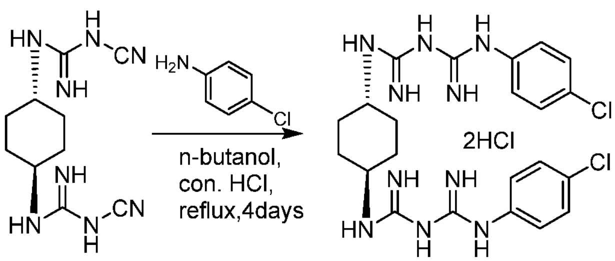
trans-N,N'-1,4-cyclohexanediylbis(5-[3-methylphenyl]biguanide) dihydrochloride (JSW-15). Following the procedure of preparation of JSW-13. JSW-15 was prepared from JSW-m-01 (1.13 mmol) and m-toluidine (2.26 mmol) to give white solid (44% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (br, 1H), 9.40 (br, 1H), 7.87-7.63 (m, 3H), 7.25-7.00 (m, 10H), 6.85-6.71 (m, 4H), 6.33-6.27 (m, 1H), 3.41-3.37 (m, 1H), 3.21-3.20 (m, 1H), 2.22 (s, 6H), 1.86-1.84 (m, 4H), 1.24 (br, 4H).^{13}C NMR (126 MHz, DMSO-d_6) δ 159.82, 149.01, 138.34, 128.95, 118.88, 117.01, 111.64, 49.92, 30.72, 21.61. HRMS m/z: $[M\text{-}2HCl+H]^+$ calcd. for $C_{24}H_{35}N_{10}^+$: 463.3046, found 463.3140.

Experimental procedure for the preparation of JSW-16



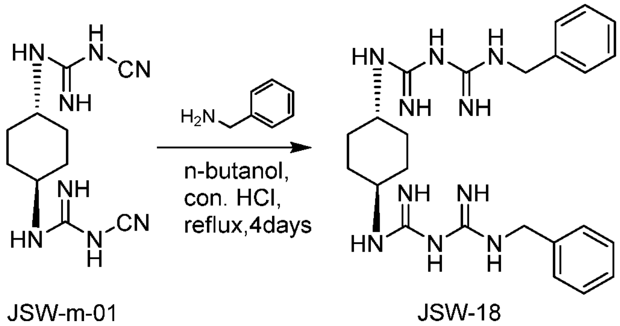
trans-N,N'-1,4-cyclohexanediylbis(5-[4-methoxyphenyl]biguanide) dihydrochloride (JSW-16). Following the procedure of preparation of JSW-13. JSW-16 was prepared from JSW-m-01 (1.13 mmol) and *4-methoxyaniline* (2.26 mmol) to give white solid (39% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (br, 1H), 9.23 (br, 1H), 7.74-7.54 (m, 2H), 7.29 (d, J = 8.0 Hz, 1H), 7.21-7.18 (m, 4H), 7.05-6.81 (m, 9H), 6.64 (br, 1H), 3.68 (s, 6H), 3.24-3.20 (m, 2H), 1.83-1.81 (m, 4H), 1.21 (br, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.66, 133.62, 124.01, 120.09, 114.34, 55.70, 49.84, 31.17. HRMS m/z: $[M-2HCl+H]^+$ calcd. for $C_{24}H_{35}N_{10}O_2^+$: 495.2944, found 495.3035.

Experimental procedure for the preparation of JSW-17



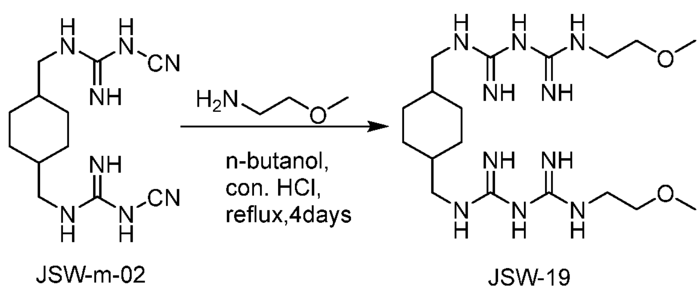
trans-N,N'-1,4-cyclohexanediylbis(5-[4-chlorophenyl]biguanide) dihydrochloride (JSW-17). Following the procedure of preparation of JSW-13. JSW-17 was prepared from JSW-m-01 (1.13 mmol) and *4-chloroaniline* (2.26 mmol) to give white solid (43% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (br, 1H), 9.74 (br, 1H), 8.00-7.75 (m, 3H), 7.45-7.17 (m, 12H), 6.76-6.75 (m, 2H), 3.23-3.20 (m, 2H), 1.85 (br, 4H), 1.25 (br, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.00, 138.17, 129.11, 128.95, 122.18, 46.85, 30.60. HRMS m/z: $[M-2HCl+H]^+$ calcd. for $C_{22}H_{29}N_{10}Cl_2^+$: 503.1954, found 503.1930.

Experimental procedure for the preparation of JSW-18



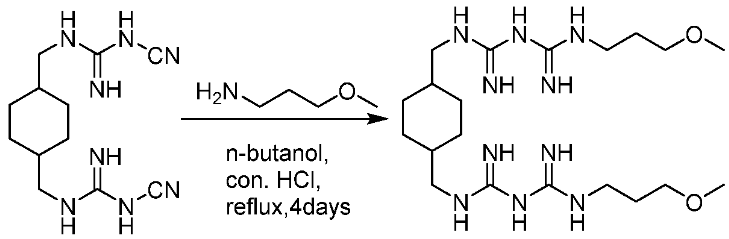
trans-N,N'-1,4-cyclohexanediylbis(5-phenylmethyl-biguanide) (JSW-18). Following the procedure of preparation of JSW-13. JSW-18 was prepared from JSW-m-01 (1.13 mmol) and phenylmethanamine (2.26 mmol) to give white solid (45% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.83-7.51 (m, 4H), 7.33-7.21 (m, 11H), 6.87 (br, 6H), 4.30-4.28 (d, *J* = 8.0 Hz, 4H), 3.30-3.20 (m, 2H), 1.77 (br, 4H), 1.22-1.12 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 160.32, 144.71, 128.81, 127.72, 127.47, 48.10, 44.68, 31.17. HRMS m/z: $[M+H]^+$ calcd. for $C_{24}H_{35}N_{10}^+$: 463.3046, found 463.3079.

Experimental procedure for the preparation of JSW-19



N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[2-methoxyethanyl]biguanide) (JSW-19). Following the procedure of preparation of JSW-13. JSW-19 was prepared from JSW-m-02 (1.13 mmol) and *2-methoxyethan-1-amine* (2.26 mmol) to give white solid (40% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.62-7.29 (m, 4H), 6.87 (br, 6H), 3.35 (br, 4H), 3.22 (s, 6H), 3.01-2.91 (m, 4H), 1.71-1.69 (m, 4H), 1.34 (br, 2H), 0.86-0.81 (m, 4H).^{13}C NMR (126 MHz, DMSO-d$_6$) δ 160.08, 70.93, 58.44, 47.54, 38.08, 30.15. HRMS m/z: $[M+H]^+$ calcd. for $C_{18}H_{39}N_{10}O_2^+$: 427.3257, found 427.3316.

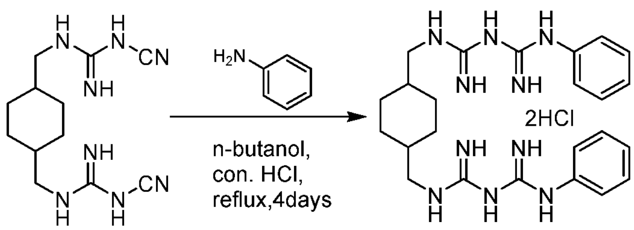
Experimental procedure for the preparation of JSW-20



JSW-m-02 JSW-20

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[3-methoxypropanyl]biguanide) (JSW-20). Following the procedure of preparation of JSW-13. JSW-20 was prepared from JSW-m-02 (1.13 mmol) and *3-methoxypropan-1-amine* (2.26 mmol) to give white solid (47% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (br, 4H), 6.82 (br, 6H), 3.18-2.91 (m, 18H), 1.70-1.63 (m, 8H), 1.34 (br, 2H), 0.83 (br, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.21, 69.95, 58.40, 47.44, 38.13, 37.96, 30.17, 29.42. HRMS m/z: $[M+H]^+$ calcd. for $C_{20}H_{43}N_{10}O_2^+$: 455.3570, found 455.3715.

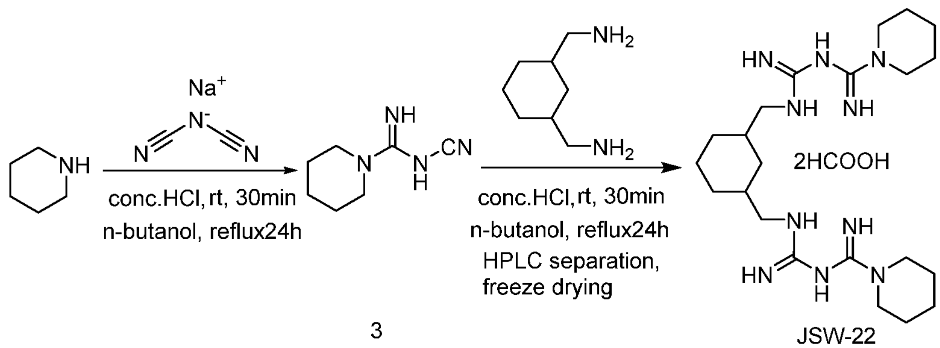
Experimental procedure for the preparation of JSW-21



JSW-m-02 JSW-21

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-phenylbiguanide) dihydrochloride (JSW-21). Following the procedure of preparation of JSW-13. JSW-21 was prepared from JSW-m-02 (1.13 mmol) and aniline (2.26 mmol) to give white solid (37% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (br, 1H), 9.45 (br, 1H), 7.94-7.57 (m, 4H), 7.34-6.99 (m, 15H), 6.73 (br, 1H), 2.92 (br, 4H), 1.70 (br, 4H), 1.36 (br, 2H), 0.85-0.80 (m, 4H).^{13}C NMR (126 MHz, DMSO-d_6) δ 160.88, 139.12, 129.09, 123.89, 121.12, 47.64, 38.11, 30.09. HRMS m/z: $[M+H]^+$ calcd. for $C_{24}H_{35}N_{10}^+$: 463.3046, found 463.3040.

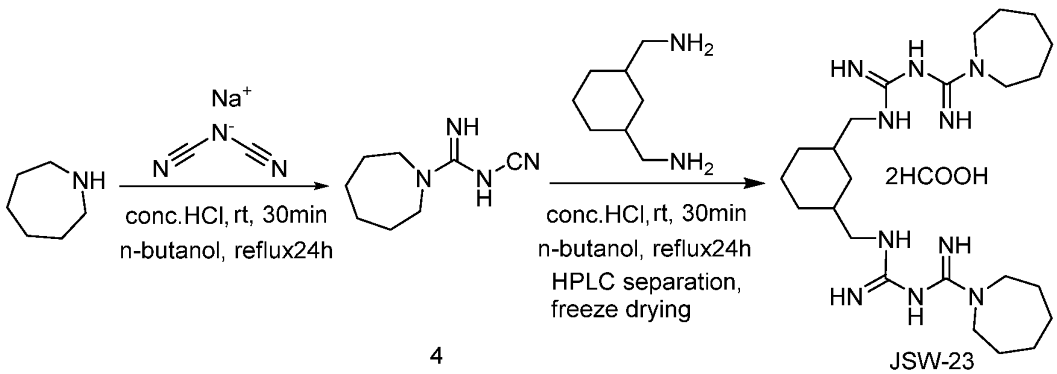
Experimental procedure for the preparation of JSW-22



***N*-cyanopiperidine-1-carboximidamide (3).** To a solution of piperidine (23.5 mmol) in n-butanol (8 mL) was added concentrated HCl (23.5 mmol) and stirred at 0°C for 30min. Sodium dicyanamide (25.8 mmol) was added to the system, and the resulting reaction mixture was refluxed for 24h. After cooled to room temperature, the mixture was filtered. And the filtrate was then concentrated *in vacuo*. Distilled water (2.5 mL) was added to the concentrate and stirred at room temperature for 30min. the solid was filtered, and dried under reduced pressure to give a white solid (60% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 6.96-6.94 (m, 2H), 3.36-3.33 (m, 4H), 1.52-1.48 (m, 2H), 1.42-1.38 (m, 4H). HRMS m/z: $[M+H]^+$ calcd. for $C_7H_{13}N_4^+$: 153.1140, found 153.1154.

***N,N''*-(((cyclohexane-1,3-diylbis(methylene))bis(azanediyl))bis(iminomethylene))bis(piperidine-1-carboximidamide) diformate (JSW-22).** Concentrated HCl (1.94 mmol) was added to a solution of cyclohexane-1,3-diyldimethanamine (0.97 mmol) in n-butanol (10 mL) and stirred at room temperature for 30min. *N*-cyanopiperidine-1-carboximidamide (3, 3.88 mmol) was added to the reaction mixture and refluxed for 24h. the mixture was concentrated *in vacuo*. Using water and EtOH to wash it if needed. The residue was purified by HPLC (0.1% Formic acid in water, 0.1% Formic acid in MeCN), removed part of solvent *in vacuo*. Then the left solvent was removed under freeze-drying conditions to give white solid JSW-22 (39% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42(s, 2H), 7.17-7.10(m, 8H), 3.42-3.38(m, 8H), 2.91-2.86(m, 4H), 1.79-1.37(m, 18H), 1.15-1.11(m, 1H), 0.78-0.69(m, 2H), 0.51-0.42(m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 166.47, 158.73, 47.66, 46.16, 37.88, 35.11, 30.76, 25.58, 24.19, 20.84. HRMS m/z: $[M-2HCOOH+H]^+$ calcd. for $C_{22}H_{43}N_{10}^+$: 447.3672, found 447.3675.

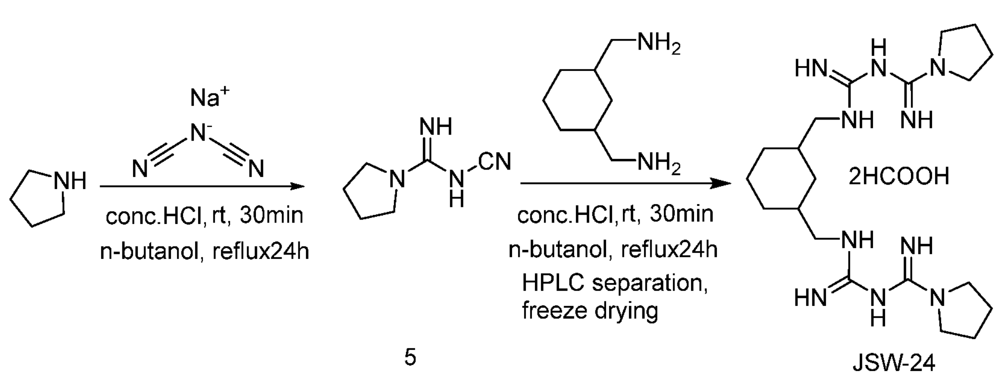
Experimental procedure for the preparation of JSW-23



N-cyanoazepane-1-carboximidamide (4). Following the procedure of preparation of Compound 3. Compound 4 was prepared from azepane (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white solid (61% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 6.84 (br, 2H), 3.37-3.34 (m, 4H), 1.58-1.53 (m, 4H), 1.44-1.40 (m, 4H). HRMS m/z: $[M+H]^+$ calcd. for $C_8H_{15}N_4^+$: 167.1297, found 167.1281.

N,N''-(((cyclohexane-1,3-diylbis(methylene))bis(azanediyl))bis(iminomethylene))bis(azepane-1-carboximidamide) diformate (JSW-23). Following the procedure of preparation of JSW-22. JSW-23 was prepared from *cyclohexane-1,3-diyldimethanamine* (0.97 mmol) and *N-cyanoazepane-1-carboximidamide* (4, 3.88 mmol) to give white solid (27% isolated yield).^{1}H NMR (500 MHz, DMSO-d_6) δ 8.44 (s, 2H), 7.09-6.93 (m, 8H), 3.42-3.39 (m, 8H), 2.91-2.90 (m, 4H), 1.79-1.62 (m, 11H), 1.49-1.12 (m, 12H), 0.86-0.72 (m, 2H), 0.53-0.48 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 166.52, 158.08, 48.04, 45.04, 35.10, 30.76, 27.69, 26.69, 25.57, 14.10. HRMS m/z: $[M-2HCOOH+H]^+$ calcd. for $C_{24}H_{47}N_{10}^+$: 475.3985, found 475.3975.

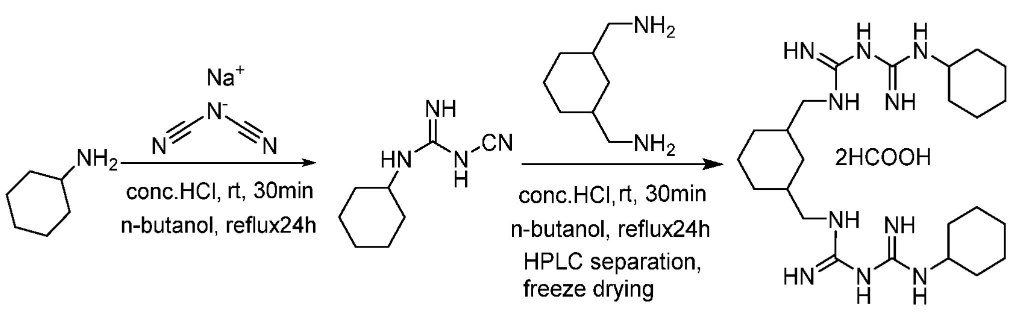
Experimental procedure for the preparation of JSW-24



N-cyanopyrrolidine-1-carboximidamide (5). Following the procedure of preparation of Compound 3. Compound 5 was prepared from pyrrolidine (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white solid (55% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 6.82 (br, 2H), 3.23-3.20 (m, 4H), 1.77 (br, 4H). HRMS m/z: $[M+H]^+$ calcd. for $C_6H_{11}N_4^+$: 139.0984, found 139.0978.

N,N''-(((cyclohexane-1,3-diylbis(methylene))bis(azanediyl))bis(iminomethylene))bis(pyrrolidine-1-carboximidamide) diformate (JSW-24). Following the procedure of preparation of JSW-22. JSW-24 was prepared from *cyclohexane-1,3-diyldimethanamine* (0.97 mmol) and *N-cyanopyrrolidine-1-carboximidamide* (5, 3.88 mmol) to give white solid (37% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 2H), 8.24 (br, 1H), 7.04-6.92 (m, 7H), 3.28-3.24 (m, 8H), 2.91-2.89 (m, 4H), 1.82-1.62 (m, 12H), 1.40-1.12 (m, 3H), 0.76-0.72 (m, 2H), 0.48-0.45 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 166.71, 159.35, 47.60, 37.83, 35.07, 30.75, 25.54, 25.29, 20.84. HRMS m/z: $[M-2HCOOH+H]^+$ calcd. for $C_{20}H_{39}N_{10}^+$: 419.3359, found 419.3440.

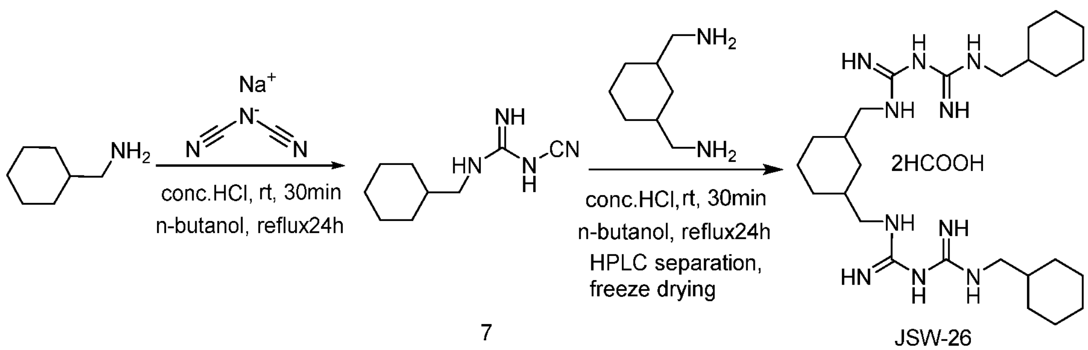
Experimental procedure for the preparation of JSW-25



N-cyano-N'-cyclohexylguanidine (6). Following the procedure of preparation of Compound 3. Compound 6 was prepared from cyclohexanamine (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white solid (68% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 6.71-6.48 (m, 2H), 3.41-3.38 (m, 1H), 1.78-1.46 (m, 4H), 1.24-1.01 (m, 4H). HRMS m/z: $[M+H]^+$ calcd. for $C_8H_{15}N_4^+$: 167.1297, found 167.1315.

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-cyclohexylbiguanide) diformate (JSW-25). Following the procedure of preparation of JSW-22. JSW-25 was prepared from *cyclohexane-1,3-diyldimethanamine* (0.97 mmol) and *N-cyano-N'-cyclohexylguanidine* (6, 3.88 mmol) to give white solid (41% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.40 (s, 2H), 7.64-7.00 (m, 10H), 3.54-3.52 (m, 2H), 3.03-2.79 (m, 4H), 1.77-1.62 (m, 11H), 1.51-1.39 (m, 4H), 1.21-1.06 (m, 12H), 0.78-0.69 (m, 2H), 0.52-0.43 (m, 1H).^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.06, 159.00, 50.19, 47.63, 35.04, 32.88, 30.75, 25.60, 25.36, 24.97, 20.72.HRMS m/z: [M-2HCOOH+H]$^+$ calcd. for $C_{24}H_{47}N_{10}^+$: 475.3985, found 475.3991.

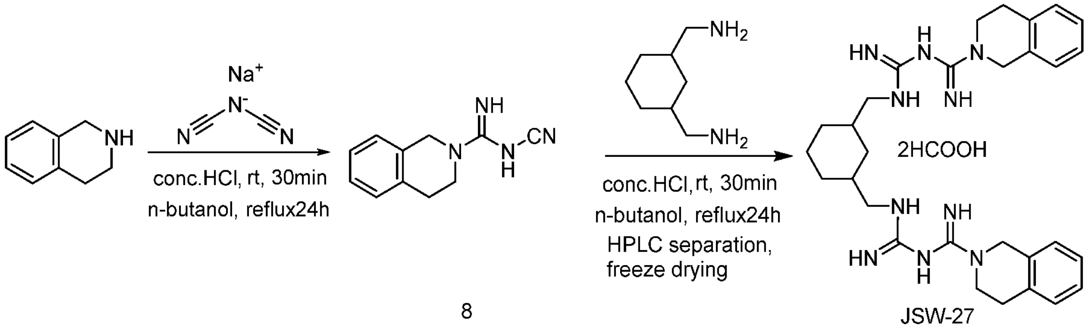
Experimental procedure for the preparation of JSW-26



N-cyano-N'-[1-cyclohexylmethyl]guanidine(7). Following the procedure of preparation of Compound 3. Compound 7 was prepared from *cyclohexylmethanamine* (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white solid (60% isolated yield).^{1}H NMR (400 MHz, DMSO-d$_6$) δ 7.83 (br, 2H), 2.60-2.55 (m, 2H), 1.70-1.49 (m, 6H), 1.17-1.10 (m, 3H), 0.89-0.85 (m, 2H). HRMS m/z: [M+H]$^+$ calcd. for $C_9H_{17}N_4^+$: 181.1453, found 181.1471.

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[1-cyclohexylmethyl]biguanide)) diformate (JSW-26). Following the procedure of preparation of JSW-22. JSW-26 was prepared from *cyclohexane-1,3-diyldimethanamine* (0.97 mmol) and *N-cyano-N'-[1-cyclohexylmethyl]guanidine* (7, 3.88 mmol) to give white solid (46% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.42 (s, 2H), 8.25 (br, 2H), 7.08 (s, 8H), 3.01-2.87 (m, 8H), 1.76-1.58 (m, 13H), 1.38 (br, 5H), 1.17-1.05 (m, 7H), 0.86-0.68 (m, 6H), 0.50-0.41 (m, 1H).^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.16, 159.07, 47.56, 47.45, 37.92, 35.02, 30.78, 29.40, 26.52, 25.88, 25.50, 20.76. HRMS m/z: [M-2HCOOH+H]$^+$ calcd. for $C_{26}H_{51}N_{10}^+$: 503.4298, found 503.4308.

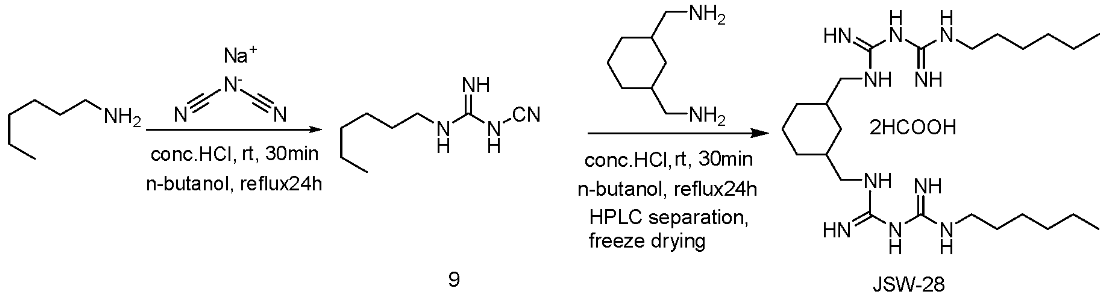
Experimental procedure for the preparation of JSW-27



N-cyano-3,4-dihydro-2(1H)-Isoquinolinecarboximidamide (8). Following the procedure of preparation of Compound 3. Compound 8 was prepared from *1,2,3,4-tetrahydroisoquinoline* (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white solid (57% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.16-7.08 (m, 6H), 4.54 (s, 2H), 3.60-3.57 (m, 2H), 2.78-2.75 (m, 2H). HRMS m/z: $[M+H]^+$ calcd. for $C_{11}H_{13}N_4^+$: 201.1140, found 201.1162.

N,N''-(((cyclohexane-1,3-diylbis(methylene))bis(azanediyl))bis(iminomethylene))bis(3,4-dihydroiso quinoline-2(1H)-carboximidamide) diformate (JSW-27). Following the procedure of preparation of JSW-22. JSW-27 was prepared from cyclohexane-1,3-diyldimethanamine (0.97 mmol) and *N-cyano-3,4-dihydro-2(1H)-Isoquinolinecarboximidamide* (8, 3.88 mmol) to give white solid (40% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 2H), 7.26-7.08 (m, 16H), 4.56 (br, 4H), 3.62-3.58 (m, 4H), 2.92-2.90 (m, 3H), 2.83-2.80 (m, 5H), 1.77-1.66 (m, 3H), 1.47-1.31 (m, 3H), 1.15-1.12 (m, 1H), 0.75-0.72 (m, 2H), 0.47-0.44 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 166.52, 158.08, 135.10, 125.77, 124.65, 123.12, 120.06, 116.71, 48.04, 47.59, 45.04, 35.10, 30.76, 27.69, 26.69, 25.57.HRMS m/z: $[M-2HCOOH+H]^+$ calcd. for $C_{30}H_{43}N_{10}^+$: 543.3672, found 543.3694.

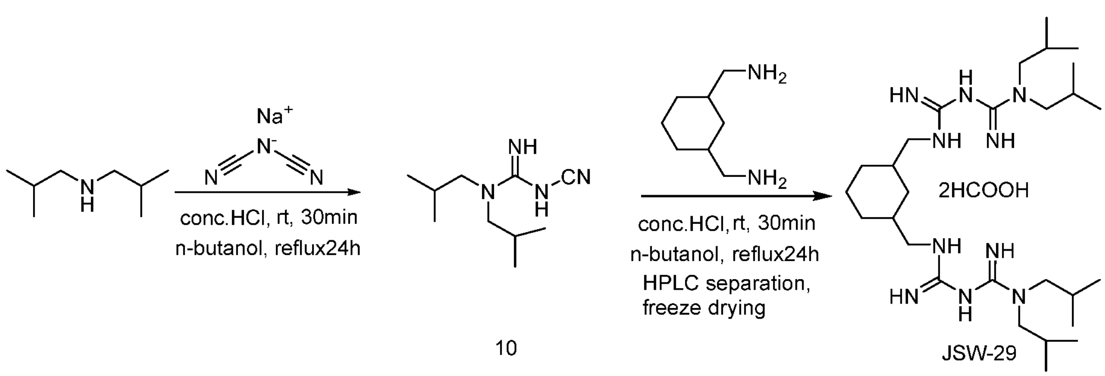
Experimental procedure for the preparation of JSW-28



N-cyano-N'-hexyl-guanidine (9). Following the procedure of preparation of Compound 3. Compound 9 was prepared from hexan-1-amine (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give colorless oil (47% isolated yield).^{1}H NMR (400 MHz, DMSO-d$_6$) δ 6.69-6.51 (m, 3H), 3.00-2.93 (m, 2H), 1.38-1.32 (m, 2H), 1.26-1.17 (m, 6H), 0.83-0.80 (m, 3H). HRMS m/z: $[M+H]^+$ calcd. for $C_8H_{17}N_4^+$: 169.1453, found 169.1461.

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[1-hexanyl]biguanide) diformate (JSW-28). Following the procedure of preparation of JSW-22. JSW-28 was prepared from *cyclohexane-1, 3-diyldimethan amine* (0.97 mmol) and *N-cyano-N'-hexyl-guanidine* (9, 3.88 mmol) to give white solid (37% isolated yield). ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.42 (s, 2H), 8.22 (br, 2H), 7.08 (br, 6H), 3.04-2.89 (m, 8H), 1.77-1.63 (m, 3H), 1.41-1.11 (m, 20H), 0.84-0.69 (m, 8H), 0.51-0.42 (m, 1H).^{13}C NMR (126 MHz, DMSO-d$_6$) δ 167.01, 159.13, 47.56, 41.25, 35.02, 31.47, 30.73, 29.49, 26.55, 26.47, 25.51, 22.55, 14.40. HRMS m/z: $[M-2HCOOH+H]^+$ calcd. for $C_{24}H_{51}N_{10}^+$: 479.4298, found 479.4312.

Experimental procedure for the preparation of JSW-29

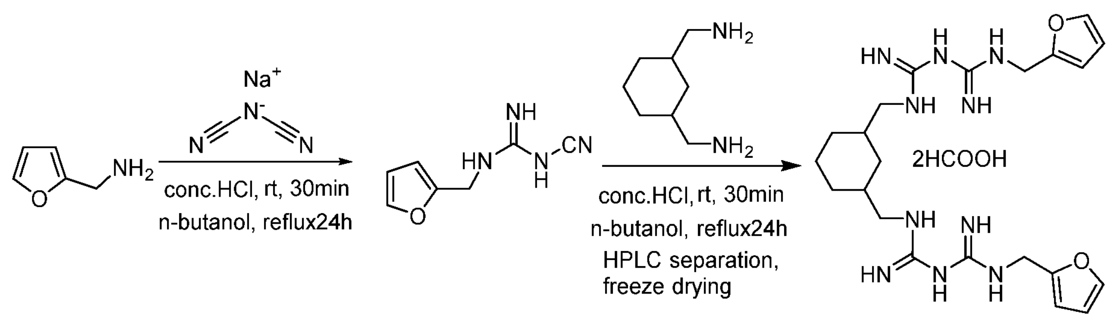


N'-cyano-N,N-bis(2-methylpropyl)-guanidine (10). Following the procedure of preparation of Compound 3. Compound 10 was prepared from diisobutylamine (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give light yellow oil (45% isolated yield).^{1}H NMR

(400 MHz, DMSO-d_6) δ 6.86-6.83 (m, 2H), 3.07-3.05 (d, J = 8.0Hz, 4H), 0.82-0.76 (m, 12H). HRMS m/z: $[M+H]^+$ calcd. for $C_{10}H_{21}N_4^+$: 197.1766, found 197.1758.

N,N''-[1,3-cyclohexanediylbis(methylene)]bis[N',N'-bis(isobutyl)biguanide diformate (JSW-29). Following the procedure of preparation of JSW-22. JSW-29 was prepared from cyclohexane-1,3-diyldimethan amine (0.97 mmol) and *N'-cyano-N,N-bis(2-methylpropyl)-guanidine* (10, 3.88 mmol) to give white solid (33% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 2H), 7.08 (br, 6H), 3.16-3.10 (m, 8H), 2.91-2.77 (m, 4H), 1.95-1.88 (m, 4H), 1.76-1.64 (m, 3H), 1.45-1.31 (m, 2H), 1.16-1.09 (m, 1H), 0.88-0.84 (m, 1H), 0.83-0.77 (m, 24H), 0.74-0.71 (m, 2H), 0.47-0.41 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.72, 158.43, 55.65, 47.46, 40.48, 35.09, 30.82, 27.14, 25.58, 20.22, 18.98. HRMS m/z: $[M-2HCOOH+H]^+$ calcd. for $C_{28}H_{59}N_{10}^+$: 535.4924, found 535.5008.

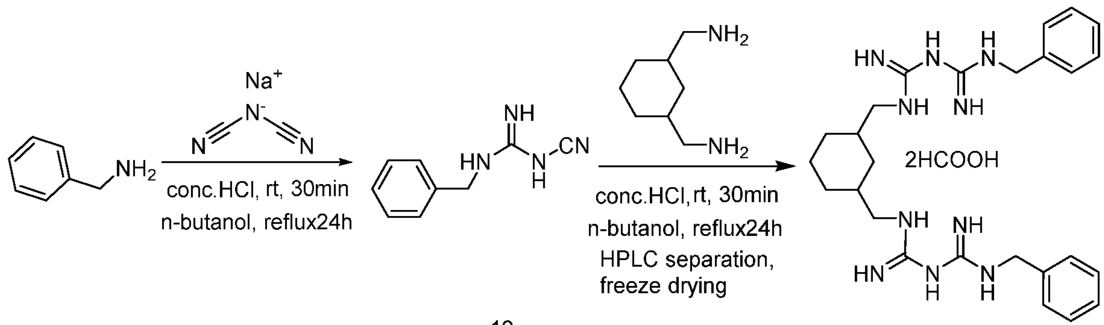
Experimental procedure for the preparation of JSW-30



N-cyano-N'-[furan-2-ylmethyl]guanidine (11). Following the procedure of preparation of Compound 3. Compound 11 was prepared from *furan-2-ylmethanamine* (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give yellow oil (43% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55-7.54 (m, 1H), 7.20 (br, 1H), 6.74 (br, 2H), 6.36-6.34 (m, 1H), 6.23-6.22 (m, 1H), 4.22 (d, J = 4.0Hz, 2H). HRMS m/z: $[M+H]^+$ calcd. for $C_7H_9N_4O^+$: 165.0776, found 165.0790.

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[furan-2-ylmethanyl]biguanide) diformate (JSW-30). Following the procedure of preparation of JSW-22. JSW-30 *was prepared from cyclohexane-1, 3-diyldimethan amine (0.97 mmol) and N-cyano-N'-[furan-2-ylmethyl]guanidine (*11, 3.88 mmol) to give white solid (31% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.32 (s, 2H), 8.17-8.06 (m, 2H), 7.53 (br, 2H), 7.23-7.04 (m, 6H), 6.36-6.34 (m, 2H), 6.23-6.17 (m, 2H), 4.25 (br, 4H), 3.01-2.74 (m, 4H), 1.71-1.57(m, 4H), 1.34 (br, 2H), 1.13-1.06 (m, 1H), 0.74-0.66 (m, 2H), 0.47-0.38(m, 1H).[13]C NMR (126 MHz, DMSO-d_6) δ 166.04, 159.26, 152.63, 142.58, 110.96, 107.35, 47.58, 38.14, 34.80, 30.57, 25.42, 20.66. HRMS m/z: [M-2HCOOH+H]$^+$ calcd. for $C_{22}H_{35}N_{10}O_2^+$: 471.2944, found 471.2957.

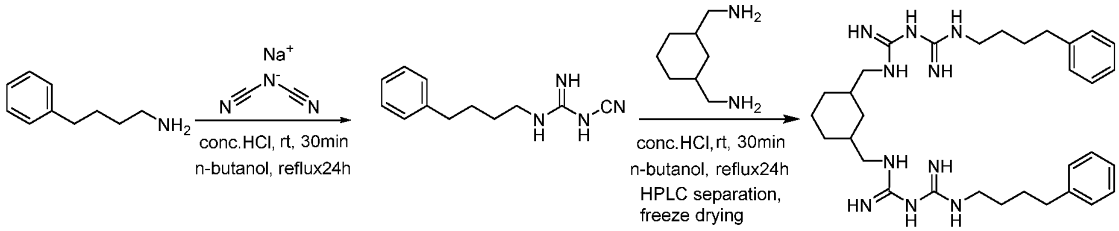
Experimental procedure for the preparation of JSW-31



N-cyano-N'-[phenylmethanyl]guanidine (12). Following the procedure of preparation of Compound 3. Compound 12 was prepared from phenylmethanamine (23.5mmol) and sodium dicyanamide (25.8mmol) to give white oil (62% isolated yield).[1]H NMR (400 MHz, DMSO-d_6) δ 7.32-7.28 (m, 3H), 7.23-7.21 (m, 3H), 6.75 (br, 2H), 4.25 (d, J = 4.0 Hz, 2H). HRMS m/z: [M+H]$^+$ calcd. for $C_9H_{11}N_4^+$: 175.0984, found 175.0978.

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[phenylmethanyl]biguanide) diformate (JSW-31). Following the procedure of preparation of JSW-22. JSW-31 *was prepared from cyclohexane-1,3-diyldimethan amine (0.97 mmol) and N-cyano-N'-[phenylmethanyl]guanidine, (*12, 3.88 mmol) to give white solid (42% isolated yield). [1]H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 2H), 7.30-7.17 (m, 18H), 4.26 (d, J = 4.0 Hz, 2H), 2.81-2.76 (m, 4H), 1.64-1.52 (m, 4H), 1.27-1.05 (m, 5H), 0.65-0.55 (m, 2H), 0.38-0.27 (m, 1H).[13]C NMR (126 MHz, DMSO-d_6) δ 167.12, 159.27, 138.28, 128.70, 127.56, 127.25, 44.56, 38.39, 34.72, 30.53, 25.41, 20.66. HRMS m/z: [M-2HCOOH+H]$^+$ calcd. for $C_{26}H_{39}N_{10}^+$: 491.3359, found 451.3480.

Experimental procedure for the preparation of JSW-32

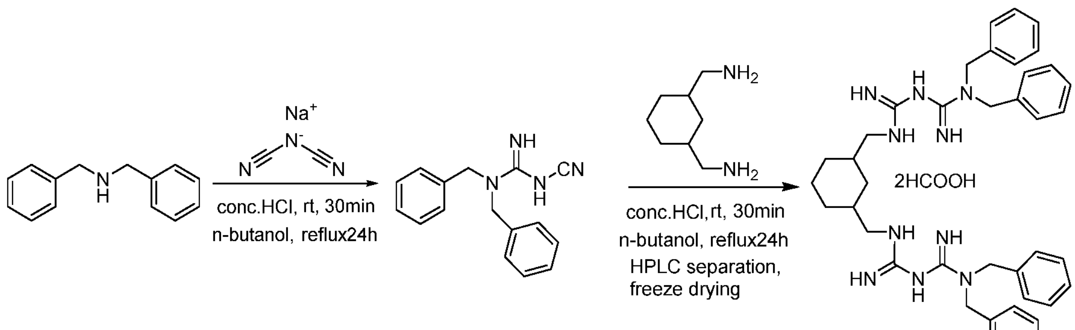


13 JSW-32

N-cyano-N'-[4-phenylbutanyl]guanidine (13). Following the procedure of preparation of Compound 3. Compound 13 was prepared from *4-phenylbutan-1-amine* (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white oil (52% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.25-7.21 (m, 2H), 7.16-7.10 (m, 3H), 6.74-6.54 (m, 3H), 3.05-3.00 (m, 2H), 2.53 (t, J = 8.0 Hz, 2H), 1.52-1.46 (m, 2H), 1.42-1.36 (m, 2H). HRMS m/z: $[M+H]^+$ calcd. for $C_{12}H_{17}N_4^+$: 217.1453, found 217.1471.

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[4-phenylbutanyl]biguanide) diformate (JSW-32). Following the procedure of preparation of JSW-22. JSW-32 *was prepared from cyclohexane-1,3-diyldimethan amine (0.97 mmol) and N-cyano-N'-[4-phenylbutanyl]guanidine (*12, 3.88 mmol). The residue was purified by HPLC (water and MeCN), removed part of solvent *in vacuo*. Then the left solvent was removed under freeze-drying conditions to give white solid (45% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.40 (br, 3H), 7.25-7.21 (m, 4H), 7.16-7.10 (m, 7H), 6.78 (br, 6H), 3.09-3.04 (m, 4H), 2.91-2.89 (m, 2H), 2.53 (t, J = 8.0 Hz, 4H), 1.71-1.62 (m, 4H), 1.58-1.50 (m, 4H), 1.45-1.33 (m, 7H), 1.15-1.09 (m, 1H), 0.81-0.71 (m, 3H), 0.53-0.44 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 158.91, 142.48, 128.75, 128.73, 126.18, 47.66, 41.09, 37.73, 35.26, 34.92, 30.57, 28.70, 25.41, 20.20. HRMS m/z: $[M+H]^+$ calcd. for $C_{32}H_{51}N_{10}^+$: 575.4298, found 575.4311.

Experimental procedure for the preparation of JSW-33



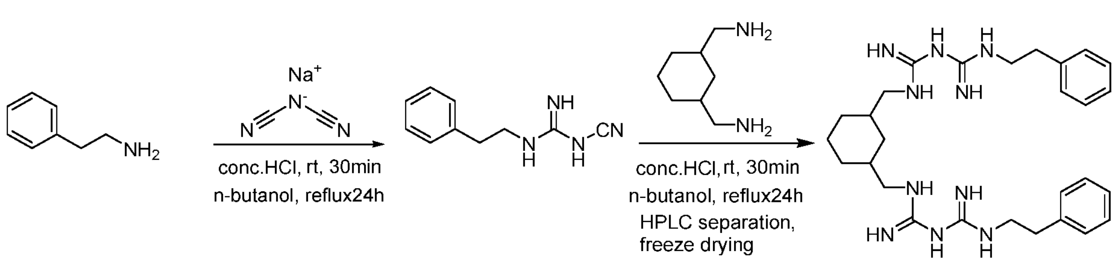
14

JSW-33

N'-cyano-N,N-bis(phenylmethyl)-guanidine (14). Following the procedure of preparation of Compound 3. Compound 14 was prepared from dibenzylamine (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white oil (62% isolated yield).[1]H NMR (400 MHz, DMSO-d_6) δ 7.34-7.22 (m, 8H), 7.18-7.15 (m, 4H), 4.50 (s, 4H). HRMS m/z: $[M+H]^+$ calcd. for $C_{16}H_{17}N_4^+$: 265.1453, found 265.1471.

N,N''-[1,3-cyclohexanediylbis(methylene)]bis[N',N'-bis(phenylmethyl)biguanide diformate (JSW-33). Following the procedure of preparation of JSW-22. JSW-33 *was prepared from cyclohexane-1,3-diyldimethan amine (0.97 mmol) and N'-cyano-N,N-bis(phenylmethyl)-guanidine (*14, 3.88 mmol) to give white solid (41% isolated yield). [1]H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 2H), 7.32-7.21 (m, 28H), 4.49 (s, 8H), 2.88-2.73 (m, 4H), 1.68-1.51 (m, 4H), 1.32-1.19 (m, 2H), 1.07-0.99 (m, 1H), 0.69-0.56 (m, 2H), 0.40-0.28 (m, 1H).[13]C NMR (126 MHz, DMSO-d_6) δ 167.00, 159.64, 137.36, 128.96, 128.94, 127.72, 50.38, 47.75, 30.59, 25.44, 20.70, 19.08. HRMS m/z: $[M-2HCOOH+H]^+$ calcd. for $C_{40}H_{51}N_{10}^+$: 671.4298, found 671.4429.

Experimental procedure for the preparation of JSW-34



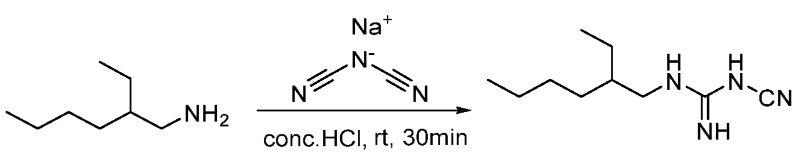
15

JSW-34

N-cyano-N'-[2-phenylethanyl]guanidine (15). Following the procedure of preparation of Compound 3. Compound 15 was prepared from *2-phenylethan-1-amine* (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white solid (57% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28-7.24 (m, 2H), 7.19-7.14 (m, 3H), 6.73-6.61 (m, 3H), 3.28-3.23 (m, 2H), 2.69 (t, J = 8.0 Hz, 2H). HRMS m/z: $[M+H]^+$ calcd. for $C_{10}H_{13}N_4^+$: 189.1140, found 189.1162.

N,N'-[1,4-cyclohexanediylbis(methylene)]-bis(5-[2-phenylethanyl]biguanide) diformate (JSW-34). Following the procedure of preparation of JSW-22. JSW-34 *was prepared from cyclohexane-1,3-diyldimethan amine (0.97 mmol) and N-cyano-N'-[2-phenylethanyl]guanidine (*15, 3.88 mmol). The residue was purified by HPLC (water and MeCN), removed part of solvent *in vacuo*. Then the left solvent was removed under freeze-drying conditions to give white solid (41% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.40 (br, 2H), 7.28-7.14 (m, 12H), 6.82 (br, 6H), 3.29-3.26 (m, 4H), 2.92 (br, 2H), 2.74-2.71 (m, 4H), 1.72-1.61 (m, 4H), 1.45-1.35 (m, 2H), 1.17-1.07 (m, 1H), 0.80-0.71 (m, 2H), 0.57-0.48 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 159.37, 139.55, 129.15, 128.84, 126.70, 47.61, 42.91, 37.75, 35.60, 34.90, 30.57, 25.39. HRMS m/z: $[M+H]^+$ calcd. for $C_{28}H_{43}N_{10}^+$: 519.3672, found 519.3694.

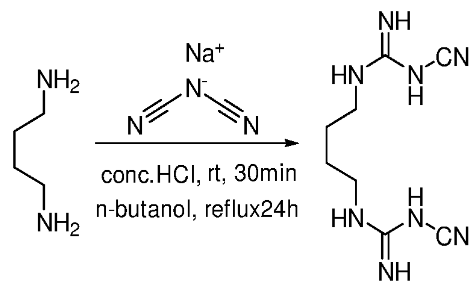
Experimental procedure for the preparation of JSW-m-09



JSW-m-09

N-(2-ethylhexyl)carbamimidoyl cyanide (JSW-m-09). Following the procedure of preparation of Compound 3. JSW-m-09 was prepared from 2-ethylhexan-1-amine (23.5 mmol) and sodium dicyanamide (25.8 mmol) to give white oil (57% isolated yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 (br, 1H), 6.93-6.74 (m, 2H), 3.01-2.89 (m, 2H), 1.32-1.16 (m, 9H), 0.84-0.75 (m, 6H). HRMS m/z: $[M+H]^+$ calcd. for $C_{10}H_{21}N_4^+$: 197.1766, found 197.1758.

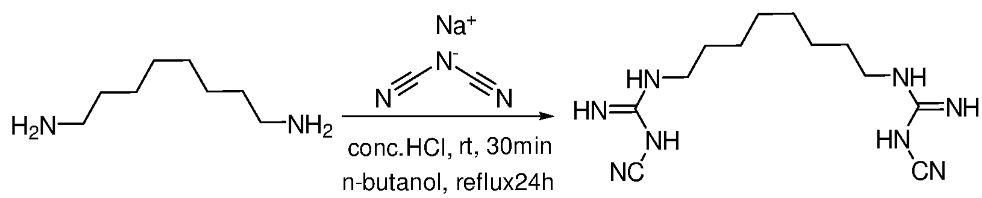
Experimental procedure for the preparation of JSW-m-10



JSW-m-10

N,N'''-1,4-butanediylbis[N'-cyano]-guanidine (JSW-m-10). Following the procedure of preparation of JSW-m-01. JSW-m-10 was prepared from *butane-1,4-diamine* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (50% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31-6.67 (m, 6H), 2.99 (br, 4H), 1.34 (br, 4H). HRMS m/z: $[M+H]^+$ calcd. for $C_8H_{15}N_8^+$: 223.1420, found 223.1442.

Experimental procedure for the preparation of JSW-m-11



JSW-m-11

N,N'''-1,8-octanediylbis[N'-cyano]-guanidine (JSW-m-11). Following the procedure of preparation of JSW-m-01. JSW-m-11 was prepared from *octane-1,8-diamine* (5 mmol) and sodium dicyanamide (10 mmol) to give white solid (48% isolated yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.81-6.55 (m, 6H), 3.00-2.95 (m, 4H), 1.37-1.33 (m, 4H), 1.20 (br, 8H). HRMS m/z: $[M+H]^+$ calcd. for $C_{12}H_{23}N_8^+$: 279.2046, found 279.2062.

Example 2: <u>Exemplary Biological Activity of Compounds of the Disclosure</u>

<u>Screen of OXPHOS inhibitors identified Alexidine induces synthetic lethality in tumors.</u>

A screen of OXPHOS inhibitors identified ADHC as a selective and cytotoxic agent with an IC_{50} of 500nM (**Fig. 1A**) that selectively induced loss of ΔΨ in KRAS/LKB1 mutant isogenic lung tumors cells but not those expressing WT LKB1 (**Fig. 1B**). ADHC is a selective inhibitor of PTPMT11 that has been shown to induce apoptosis in cancer cells but not in transformed cells and reduce tumor cell seeding in xenografts models. This work shows that ADHC potently inhibits OXPHOS in human LKB1-/- lung tumor cells (**Fig. 1C**) while inducing an increase in glucose metabolism (**Fig. 1D**). ADHC induced energetic stress and mitochondrial stress in Kras lung tumor cells as shown by elevated P-AMPKα and heat shock protein Grp78. ADHC selectively induced apoptosis in Kras/Lkb1-/- (KLluc) but not Kras (Kluc) cells expressing wt Lkb1 (**Fig. 1E**). Lastly, viability in a panel of LKB1MUT (n=7 lines), LKB1WT (n=4 lines) and the EGFRMUT human lung tumor cell line H1975 treated with ADHC was measured, and it was found that LKB1MUT and EGFRMUT cells were more sensitive than WT ones (**Fig. 1F** and **Fig. 1G**).

Inhibition of PTPMT1 induces mitochondrial remodeling and therapeutic-sensitization of tumor cells.

A small molecule screen identified that the bisbiguanide Alexidine (ADHC), an inhibitor of protein tyrosine phosphatase mitochondrion 1 (PTPMT1) selectively induced cell death in LKB1-/- lung tumor cells. The protein tyrosine phosphatase mitochondrion 1 (PTPMT1) is a mitochondrial localized phosphatase that regulates cardiolipin synthesis (**Fig. 2A**). PTPMT1 inhibition induced downregulation of the Optic Atrophy 1 (OPA1), a central regulator of mitochondrial fusion and cristae junction integrity and modest decrease in Mitofusin 1 (MFN1) protein while protein expression levels for the mitochondrial fission regulator dynamin-related protein 1 (DRP1), remained unaltered (**Fig. 2B**). PTPMT1 KD significantly increased tumor cell death and apoptosis in chemotherapy-resistant lung cancer lines (**Figs. 2C-D**).

JSW compounds induce remodeling of the mitochondrial cristae and induced cell death in cancer cells.

During the course of this work, mitochondrial networks with open cristae are identified to be more sensitive to therapy than those with closed cristae (**Fig. 3A**). Importantly, the long form of OPA1, denoted L-OPA1 is required for maintenance of the cristae junction in a closed state and the prevention of apoptosis. Loss of L-OPA1 and enrichment for the short form of OPA1 (S-OPA1) leads to opening of the cristae junction and pro-apoptotic phenotype. It has been identified that treatment of chemotherapy resistant H1975 lung tumor cells with either ADHC and the JSW002 compound led to a dramatic reduction in L-OPA1 and opening of the cristae junction (**Figs. 3C-D**). Cell viability assays demonstrated that JSW002 induced significant cell death in chemotherapy resistant H1975 cells as much lower concentrations than ADHC suggesting that JSW002 is an equal or slightly more potent analog of Alexidine (**Figs. 3D-E**).

INCORPORATION BY REFERENCE

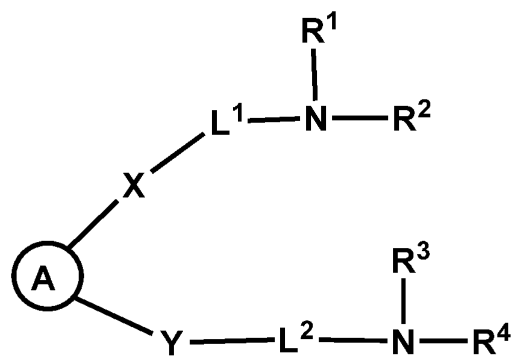
All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

EQUIVALENTS

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

We claim:

1. A compound of Formula (I) or a pharmaceutically acceptable salt thereof,



Formula (I)

wherein:

A is alkyl, cycloalkyl, or heterocyclyl;

X is absent, NH or $–(CH_2)_{n1}NH-$;

Y is absent, NH or $–(CH_2)_{n2}NH-$;

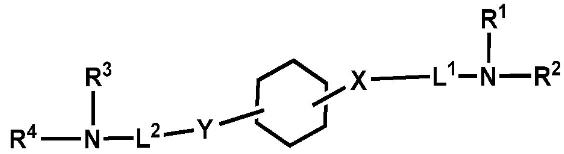
n1 and n2 are each independently 1, 2, or 3;

L^1 and L^2 are each independently -C(=NH)NHC(=NH)-, -C(=NH)-; and

each R^1, R^2, R^3, R^4 is independently selected from hydrogen, alkyl, alkoxy, cyano, cycloalkyl, (cycloalkyl)alkyl, heterocyclyl, (heterocyclyl)alkyl aryl, aralkyl, heteroaryl, and heteroaralkyl; or R^1 and R^2 can combine to form a heterocyclyl; or R^3 and R^4 can combine to form a heterocyclyl.

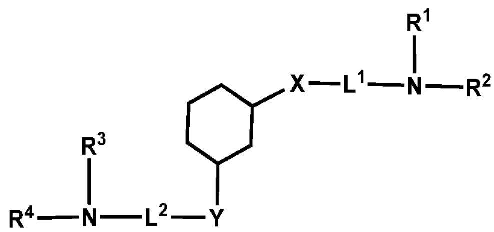
2. The compound of claim 1, wherein A is cycloalkyl.

3. The compound of claim 1 or 2, having a structure represented by Formula (Ia):



(Ia).

4. The compound of claim 3, having a structure represented by Formula (Ia-1):



(Ia-1).

5. The compound of any one of claims 1-4, wherein X is NH.

6. The compound of any one of claims 1-4, wherein X is $(CH_2)_{n1}NH$.

7. The compound of claim 6, wherein n1 is 1.

8. The compound of any one of claims 1-7, wherein Y is NH.

9. The compound of any one of claims 1-7, wherein Y is $(CH_2)_{n2}NH$.

10. The compound of claim 9, wherein n2 is 1.

11. The compound of any one of claims 1-10, wherein L^1 is-C(=NH)NHC(=NH)-.

12. The compound of any one of claims 1-11, wherein L^2 is -C(=NH)NHC(=NH)-.

13. The compound of any one of claims 1-12, wherein R^1 is hydrogen, alkyl (*e.g.*, hexyl, octyl, isobutyl, or ethylhexyl), alkoxy (*e.g.*, methoxyethyl or methoxypropyl), cycloalkyl (*e.g.*, cyclohexyl), (cycloalkyl)alkyl (*e.g.*, cyclohexylmethyl), heterocyclyl, aryl (*e.g.*, phenyl), aralkyl (*e.g.*, benzyl or phenylbutyl), heteroaryl, or heteroaralkyl (*e.g.*, furylmethyl).

14. The compound of claim 13, wherein R^1 is ethylhexyl.

15. The compound of any one of claims 1-14, wherein R^2 is hydrogen, alkyl (*e.g.*, hexyl, octyl, isobutyl, or ethylhexyl), alkoxy (*e.g.*, methoxyethyl or methoxypropyl), cycloalkyl (*e.g.*, cyclohexyl), (cycloalkyl)alkyl (*e.g.*, cyclohexylmethyl), heterocyclyl, aryl (*e.g.*, phenyl), aralkyl (*e.g.*, benzyl or phenylbutyl), heteroaryl, or heteroaralkyl (*e.g.*, furylmethyl).

16. The compound of claim 15, wherein R^2 is ethylhexyl.

17. The compound of any one of claims 1-6, wherein R^3 is hydrogen, alkyl (*e.g.*, hexyl, octyl, isobutyl, or ethylhexyl), alkoxy (*e.g.*, methoxyethyl or methoxypropyl), cycloalkyl (*e.g.*, cyclohexyl), (cycloalkyl)alkyl (*e.g.*, cyclohexylmethyl), heterocyclyl, aryl (*e.g.*, phenyl), aralkyl (*e.g.*, benzyl or phenylbutyl), heteroaryl, or heteroaralkyl (*e.g.*, furylmethyl).

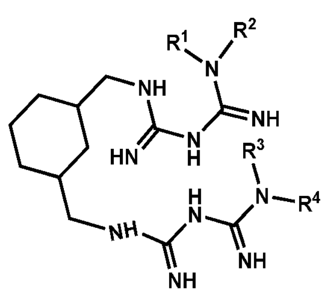
18. The compound of claim 17, wherein R^3 is ethylhexyl.

19. The compound of any one of claims 1-18, wherein R^4 is hydrogen, alkyl (*e.g.*, hexyl, octyl, isobutyl, or ethylhexyl), alkoxy (*e.g.*, methoxyethyl or methoxypropyl), cycloalkyl (*e.g.*, cyclohexyl), (cycloalkyl)alkyl (*e.g.*, cyclohexylmethyl), heterocyclyl, aryl (*e.g.*, phenyl), aralkyl (*e.g.*, benzyl or phenylbutyl), heteroaryl, or heteroaralkyl (*e.g.*, furylmethyl).

20. The compound of claim 19, wherein R^4 is hydrogen.

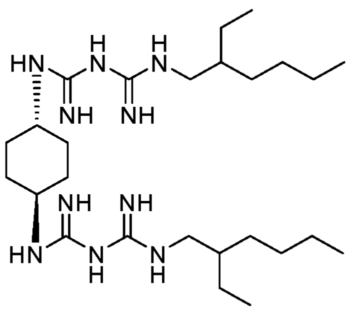
21. The compound of any one of claims 1-20, wherein the aryl, aralkyl, heteroaryl, heteroaralkyl is optionally substituted by one or more substituents independently selected from alkyl, halogen, alkoxy, cyano, haloalkoxy, and haloalkyl.

22. The compound of any one of claims 1-21, having a structure of Formula (Ia-2):

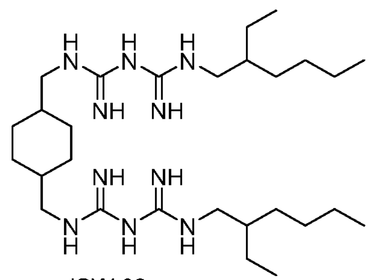


Formula (Ia-2).

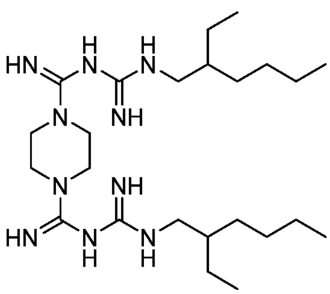
23. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein the compound is selected from:



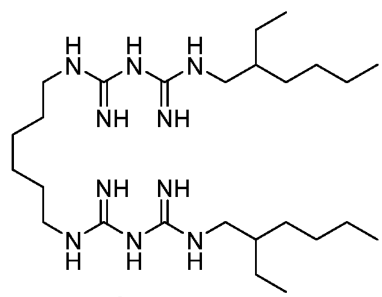
JSW-01



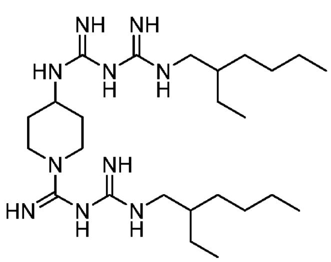
JSW-02



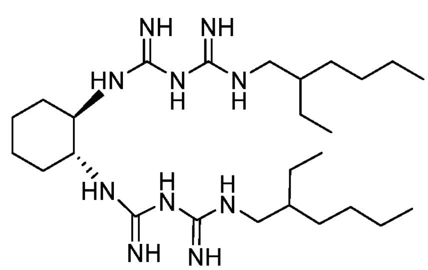
JSW-03



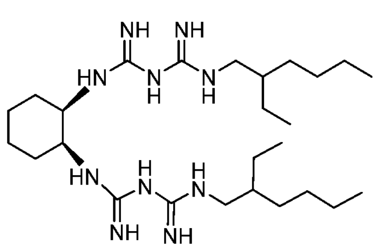
JSW-04



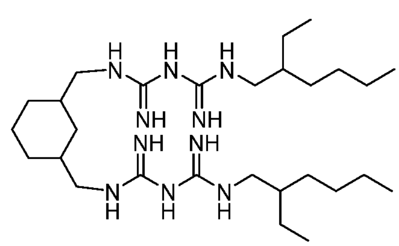
JSW-05



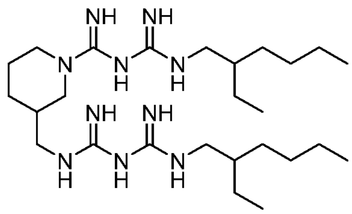
JSW-06



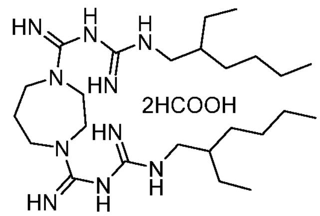
JSW-07



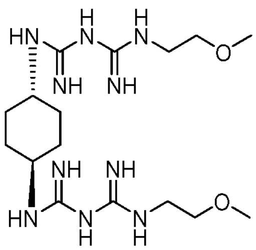
JSW-08



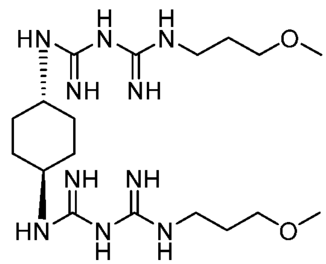
JSW-09



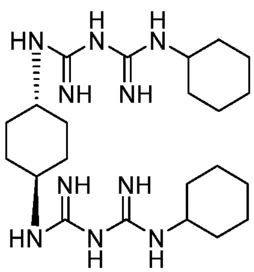
JSW-10



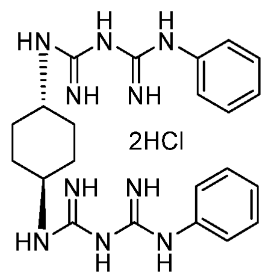
JSW-11



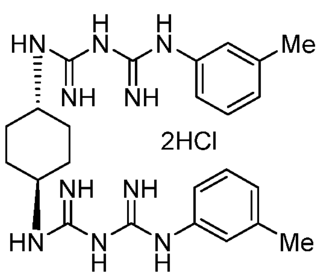
JSW-12



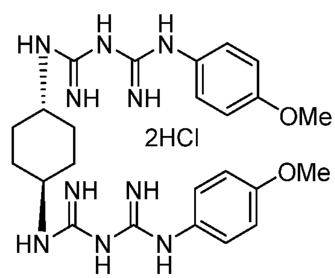
JSW-13



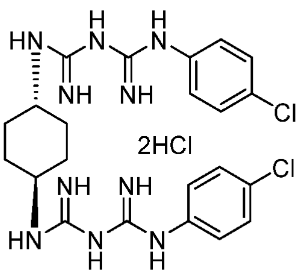
JSW-14



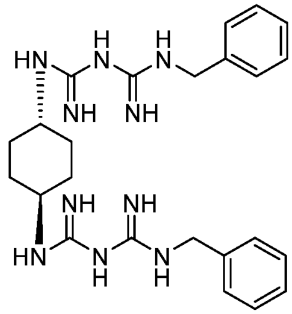
JSW-15



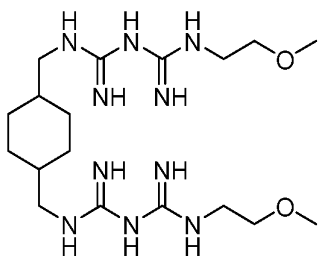
JSW-16



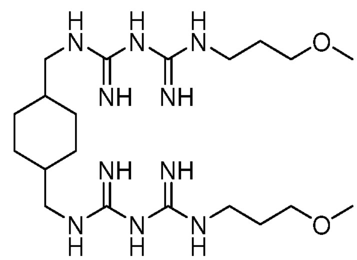
JSW-17



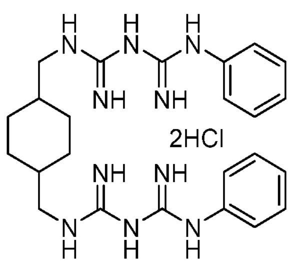
JSW-18



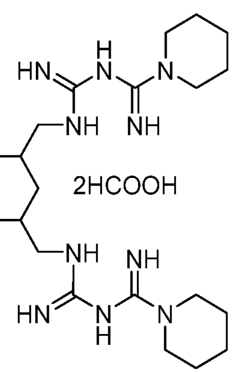
JSW-19



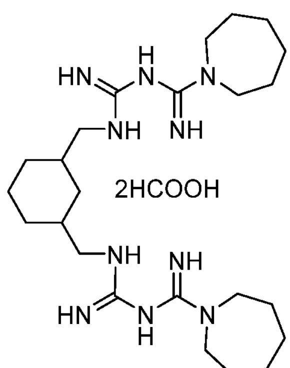
JSW-20



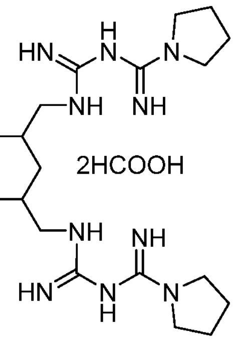
JSW-21



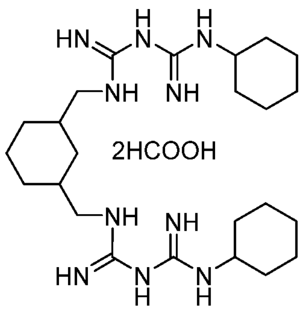
JSW-22



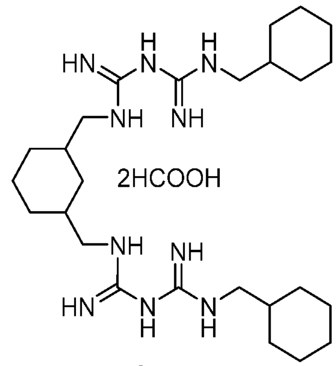
JSW-23



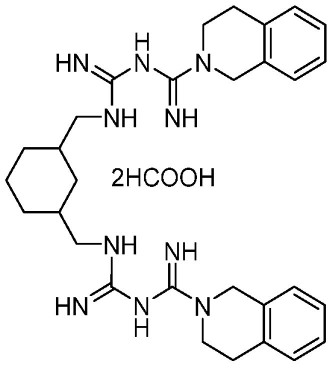
JSW-24



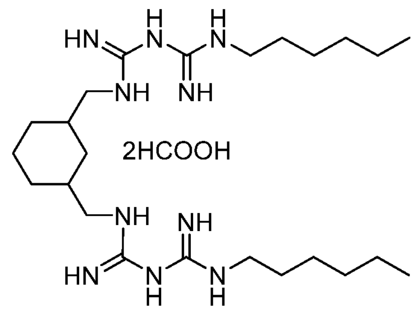
JSW-25



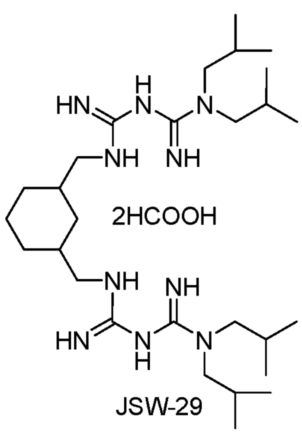
JSW-26



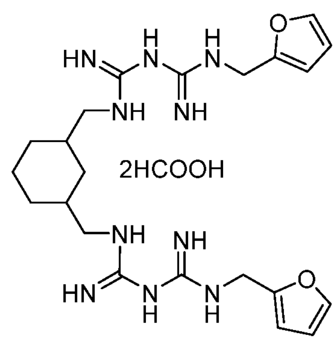
JSW-27



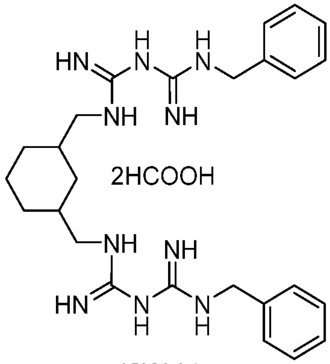
JSW-28



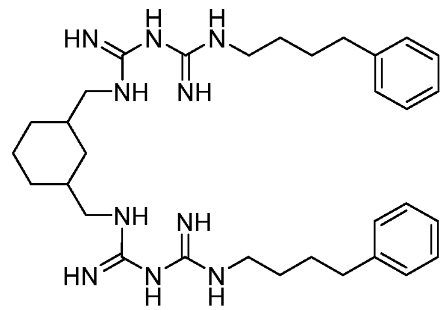
JSW-29



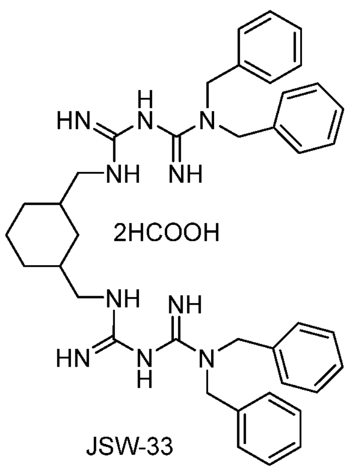
JSW-30



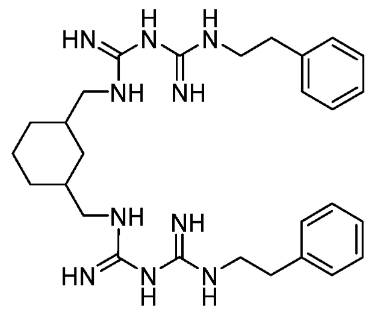
JSW-31



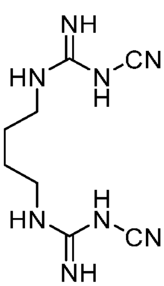
JSW-32



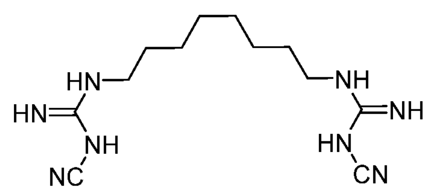
JSW-33



JSW-34



JSW-m-10 and



JSW-m-11 .

24. A pharmaceutical composition, comprising the compound of any one of claims 1-23, or a pharmaceutically acceptable salt thereof; and a pharmacetically accepted excipient.

25. A method of treating a disease or disorder characterized by mitochondrial phosphatase activity in a subject in need thereof, comprising administering a compound of any one of claims 1-23, or a pharmaceutically acceptable salt thereof, to the subject.

26. The method of claim 25, wherein the disease or disorder is further characterized by inhibition of PTPMT1 activity in a tumor cell.

27. The method of claim 26, wherein the method remodels mitochondrial networks in a tumor cell.

28. The method of any one of claims 25-27, wherein the method induces tumor cell death.

29. The method of claim 26, wherein the disease or disorder is cancer.

30. A method of treating cancer in a subject in need thereof, comprising administering a compound of any one of claims 1-23 to the subject.

31. The method of claim 29 or 30, wherein the cancer is lung cancer.

FIG. 1A

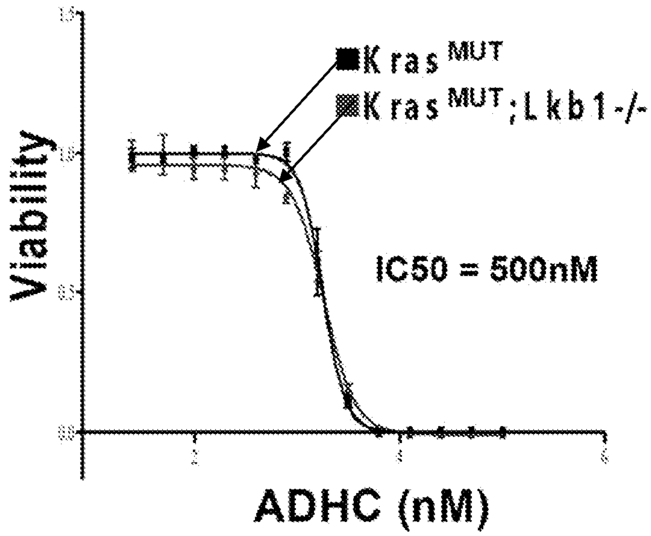

Kras MUT
Kras MUT;Lkb1-/-
Viability
IC50 = 500nM
ADHC (nM)

FIG. 1B

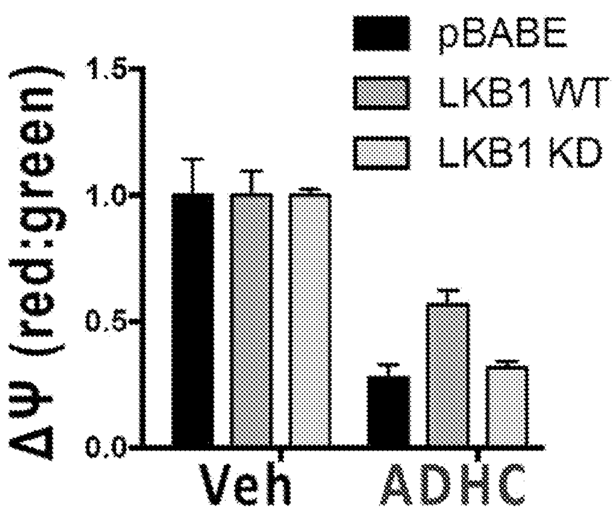

pBABE
LKB1 WT
LKB1 KD
ΔΨ (red:green)
1.5
1.0
0.5
0.0
Veh
ADHC

FIG. 1C

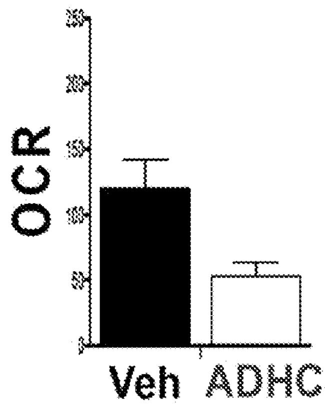


FIG. 1D

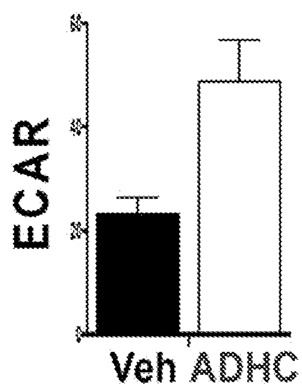


FIG. 1E

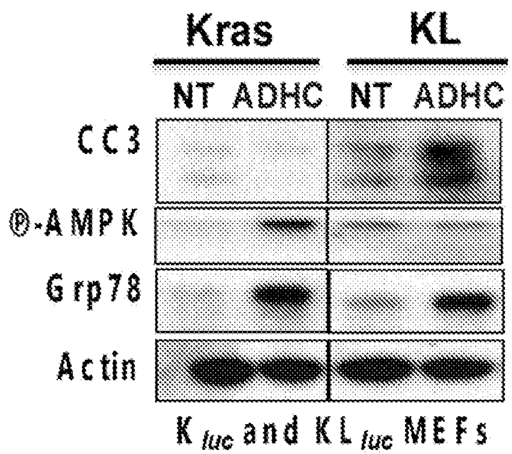

Kras
KL
NT ADHC NT ADHC
CC3
℗-AMPK
Grp78
Actin
K luc and KL luc MEFs

FIG. 1F

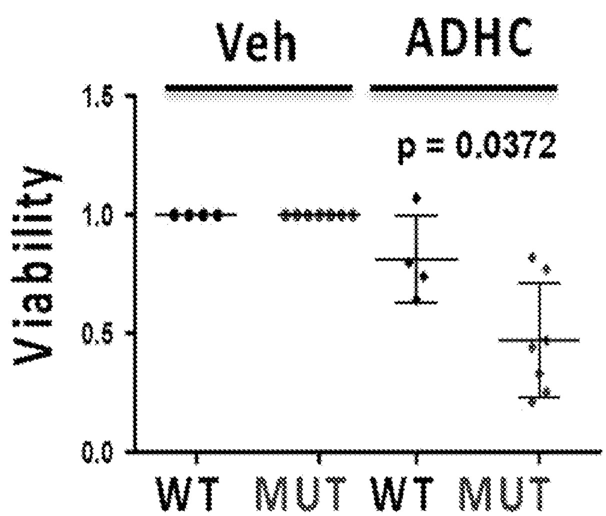

Veh
ADHC
p = 0.0372
Viability
1.5
1.0
0.5
0.0
WT MUT WT MUT

FIG. 1G

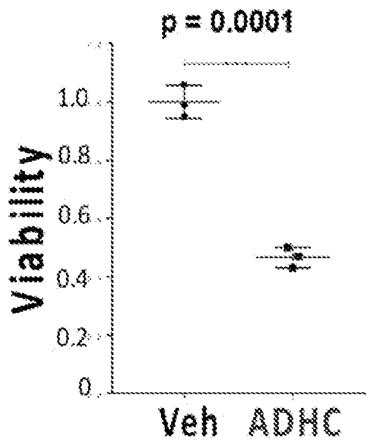

p = 0.0001
Viability
1.0
0.8
0.6
0.4
0.2
0
Veh
ADHC

FIG. 2A

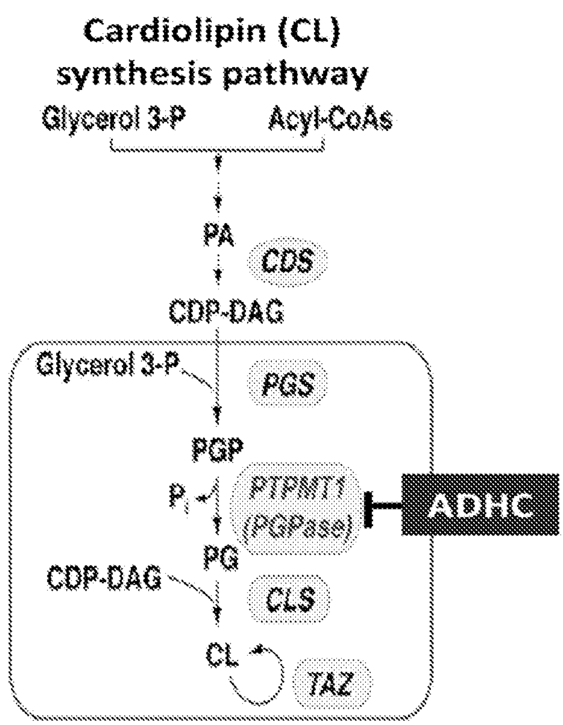

Cardiolipin (CL)
synthesis pathway
Glycerol 3-P
Acyl-CoAs
PA
CDS
CDP-DAG
Glycerol 3-P
PGS
PGP
P
PTPMT1
(PGPase)
ADHC
PG
CDP-DAG
CLS
CL
TAZ

FIG. 2B

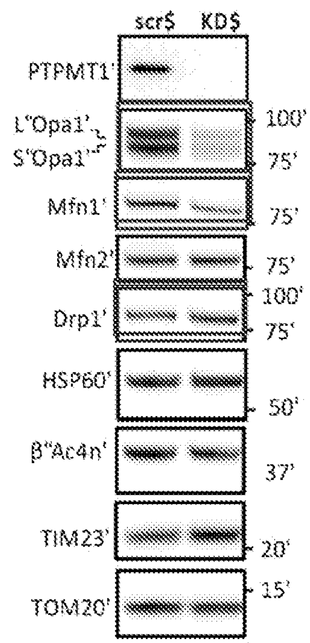

scr$ KD$
PTPMT1'
L"Opa1'
S"Opa1'
100'
75'
Mfn1'
75'
Mfn2'
75'
Drp1'
100'
75'
HSP60'
50'
β"Ac4n'
37'
TIM23'
20'
TOM20'
15'

FIG. 2C

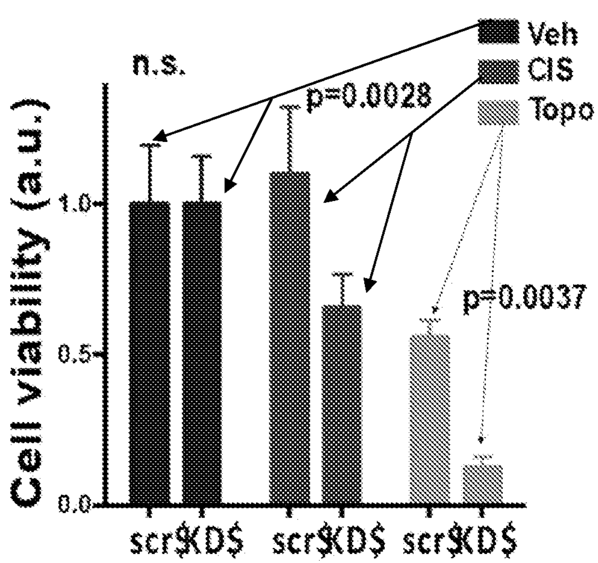

Veh
CIS
Topo
n.s.
p=0.0028
p=0.0037
Cell viability (a.u.)
1.0
0.5
0.0
scr$ KD$ scr$ KD$ scr$ KD$

FIG. 2D

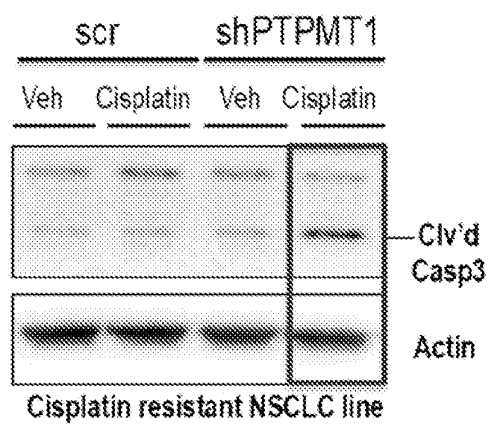

scr
shPTPMT1
Veh
Cisplatin
Veh
Cisplatin
Clv'd Casp3
Actin
Cisplatin resistant NSCLC line

FIG. 3A

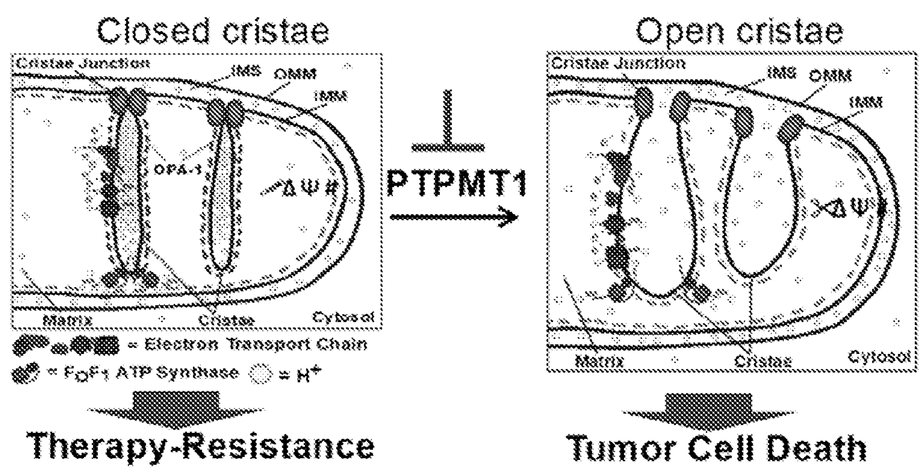

Closed cristae
Open cristae
Cristae Junction
IMS
OMM
IMM
OPA-1
Matrix
Cristae
Cytosol
= Electron Transport Chain
= FoF1 ATP Synthase
= H+
PTPMT1
Therapy-Resistance
Tumor Cell Death

FIG. 3B

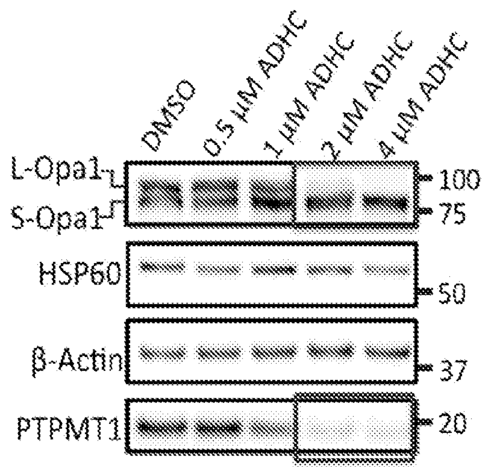

DMSO
0.5 µM ADHC
1 µM ADHC
2 µM ADHC
4 µM ADHC
L-Opa1
S-Opa1
HSP60
β-Actin
PTPMT1
100
75
50
37
20

FIG. 3C

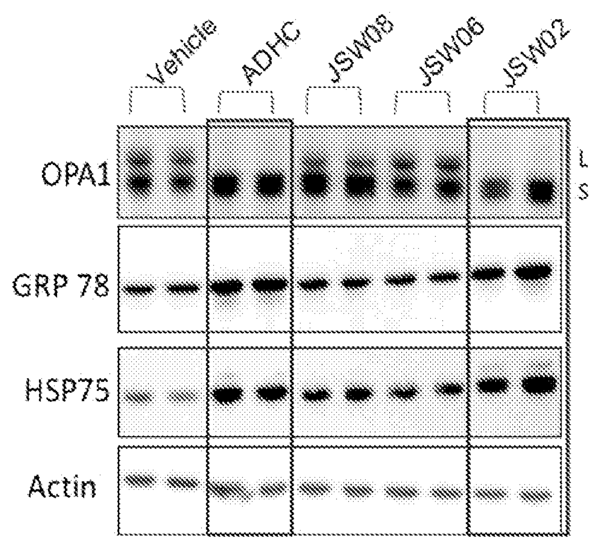

Vehicle
ADHC
JSW08
JSW06
JSW02
OPA1
GRP 78
HSP75
Actin

FIG. 3D

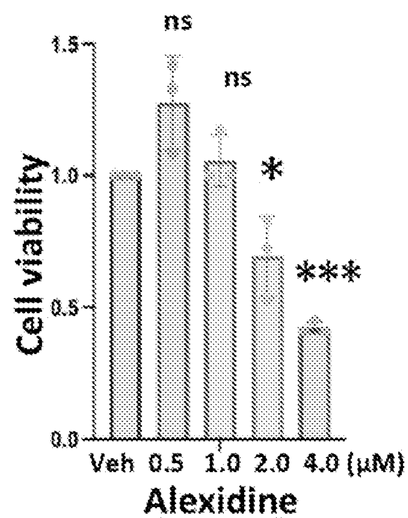
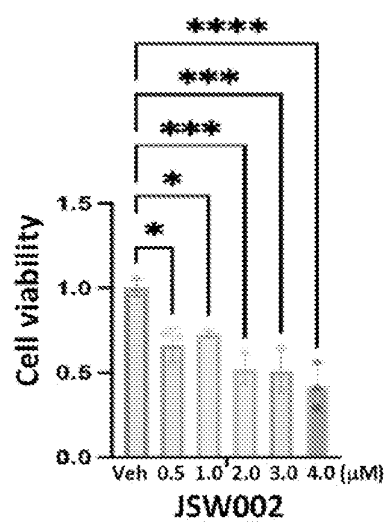

ns
ns
*
***
1.5
1.0
0.5
0.0
Cell viability
Veh 0.5 1.0 2.0 4.0 (μM)
Alexidine

FIG. 3E


****
***
***
*
*
1.5
1.0
0.5
0.0
Cell viability
Veh 0.5 1.0 2.0 3.0 4.0 (μM)
JSW002

A. CLASSIFICATION OF SUBJECT MATTER

C07C 211/10(2024.01)i; ***C07C 211/16***(2024.01)i; ***C07C 211/34***(2024.01)i; ***C07C 211/46***(2024.01)i; ***C07D 213/40***(2024.01)i; ***C07D 213/74***(2024.01)i; ***C07D 213/75***(2024.01)i; ***C07C 279/08***(2024.01)i; ***C07C 279/26***(2024.01)i; ***C07D 405/12***(2024.01)i; ***C07D 207/20***(2024.01)i; ***C07D 401/12***(2024.01)i; ***C07D 295/135***(2024.01)i; ***C07D 295/215***(2024.01)i; ***C07D 307/52***(2024.01)i; ***C07D 295/195***(2024.01)i; ***A61K 31/4965***(2024.01)i; ***A61K 31/5513***(2024.01)i; ***A61K 31/4453***(2024.01)i; ***A61K 31/444***(2024.01)i; ***A61K 31/4025***(2024.01)i; ***A61K 31/472***(2024.01)i; ***A61K 31/341***(2024.01)i; ***A61K 31/155***(2024.01)i; ***A61P 35/00***(2024.01)i

CPC:C07C 211/10; C07C 211/16; C07C 211/34; C07C 211/46; C07D 213/40; C07D 213/74; C07D 213/75; C07C 279/08; C07C 279/26; C07D 405/12; C07D 207/20; C07D 401/12; C07D 295/135; C07D 295/215; C07D 307/52; C07D 295/195; A61K 31/4965; A61K 31/5513; A61K 31/4453; A61K 31/444; A61K 31/4025; A61K 31/472; A61K 31/341; A61K 31/155; A61P 35/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07C 211/10; C07C 211/16; C07C 211/34; C07C 211/46; C07D 213/40; C07D 213/74; C07D 213/75; C07C 279/08; C07C 279/26; C07D 405/12; C07D 207/20; C07D 401/12; C07D 295/135; C07D 295/215; C07D 307/52; C07D 295/195; A61K 31/4965; A61K 31/5513; A61K 31/4453; A61K 31/444; A61K 31/4025; A61K 31/472; A61K 31/341; A61K 31/155; A61P 35/00

CPC:C07C 211/10; C07C 211/16; C07C 211/34; C07C 211/46; C07D 213/40; C07D 213/74; C07D 213/75; C07C 279/08; C07C 279/26; C07D 405/12; C07D 207/20; C07D 401/12; C07D 295/135; C07D 295/215; C07D 307/52; C07D 295/195; A61K 31/4965; A61K 31/5513; A61K 31/4453; A61K 31/444; A61K 31/4025; A61K 31/472; A61K 31/341; A61K 31/155; A61P 35/00

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

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

Databases consulted: Google Patents, Google Scholar, REAXYS Search terms used: alexidine, mitochondrial phosphatase PTPMT1-mediated disease or disorder, two biguanidyl radicals.

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
09 September 2024	**09 September 2024**

Name and mailing address of the ISA/IL	Authorized officer
Israel Patent Office **Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel** **Israel** Telephone No. **972-73-3927136** Email: **pctoffice@justice.gov.il**	**VOLKOV Karina** Telephone No.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	WO 2022106505 A1 (INST CURIE[FR]; INST NAT SANTE RECH MED[FR]; CENTRE NAT RECH SCIENT[FR]; UNIV PARIS SCIENCES ET LETTRES[FR]) 27 May 2022 (2022-05-27)	
X	page 84 line 3 to page 85 line 16; page 86 line 6 to page 89 line 5; see p.52 line 15; compounds LCC-8; LCC-9; LCC-10; LCC-12; LCC-8,3; LCC-10,2; LCC-10,3; LCC-10,5; LCC-12,2; LCC-12,3; LCC-12,4; LCC-12,6 (p. 77-78); third compound on page 13 line 5; third and sixth compounds on page 39. Furthermore, 1,8-bis(cyanoguanidino)octane (p.101 line 28)	1-31
Y	formula I	1-31
	WO 2019233982 A1 (INST CURIE[FR]; INST NAT SANTE RECH MED[FR]; CENTRE NAT RECH SCIENT[FR]) 12 December 2019 (2019-12-12)	
X	claim 14, p. 19 lines 13-16, Formula I	1-31
Y	Formula I	1-31
	Graber, Martin, et al. "Oral disinfectants inhibit protein–protein interactions mediated by the anti-apoptotic protein bcl-xl and induce apoptosis in human oral tumor cells." Angewandte Chemie International Edition 52.16 (2013): 4487-4491.19 March 2013. https://doi.org/10.1002/anie.201208889. (2013/03/19)	
X	compounds 1-3 Table 1	1,5-21,23-30
	El Mai, Mounir, et al. "A novel screen for expression regulators of the Telomeric protein TRF2 identified small molecules that impair TRF2 dependent immunosuppression and tumor growth." Cancers 13.12 (2021): 2998.15 June 2021. https://doi.org/10.3390/cancers13122998. (2021/06/15)	
X	alexidine	1,5-20,23-30
	CN 114671830 A (XIAN PETROLEUM UNIV) 28 June 2022 (2022-06-28)	
X	[0003],[0010]-[0011]	1,11-13,15,17,19,21,24-30
	WO 0217916 A1 (SHETTY B VITHAL[US]) 07 March 2002 (2002-03-07)	
X	formulae I and II ,claim 1,compound 6 on page 56, compound 1 on page 57, compound 2 on page 63.	1-3,5-13,15,17,19,20,24
	Warner, Victor D., et al. "Quantitative structure-activity relationships for biguanides, carbamimidates, and bisbiguanides as inhibitors of Streptococcus mutans No. 6715." Journal of Medicinal Chemistry 22.4 (1979): 359-366.1 April 1979. https://doi.org/10.1021/jm00190a006. (1979/04/01)	
X	formula III, see Table II compounds 24, 26 and 37.	1,5-21,23,24
	Loesche, Anne, et al. "Repurposing N, N'-bis-(arylamidino)-1, 4 piperazinedicarboxamidines : An unexpected class of potent inhibitors of cholinesterases." European Journal of Medicinal Chemistry 125 (2017): 430-434.19 September 2016. https://doi.org/10.1016/j.ejmech.2016.09.051. (2016/09/19)	
X	PD, Fig. 1	1,11-13,15,17,19-21,24
	EP 3222614 A2 (IMMUNOMET THERAPEUTICS INC[US]) 27 September 2017 (2017-09-27)	
Y	Formula 1	1-31
	Knippler, Christina M., et al. "Bisbiguanide analogs induce mitochondrial stress to inhibit lung cancer cell invasion." Iscience 27.4 (2024). 28 March 2024. DOI: 10.1016/j.isci.2024.109591 (2024/03/28)	
P,X	the whole document	1-31

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2024/038793

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2022106505	A1	27 May 2022	WO	2022106505	A1	27 May 2022
				EP	4247352	A1	27 September 2023
				JP	2023550402	A	01 December 2023
				US	2023416196	A1	28 December 2023
WO	2019233982	A1	12 December 2019	WO	2019233982	A1	12 December 2019
				EP	3802522	A1	14 April 2021
				JP	2021525777	A	27 September 2021
				JP	7358403	B2	10 October 2023
				JP	2024001077	A	09 January 2024
				US	2021230105	A1	29 July 2021
CN	114671830	A	28 June 2022	CN	114671830	A	28 June 2022
WO	0217916	A1	07 March 2002	WO	0217916	A1	07 March 2002
				AR	035196	A1	05 May 2004
				AU	8660401	A	13 March 2002
				EP	1406619	A1	14 April 2004
				EP	1406619	A4	02 February 2005
				US	2004132993	A1	08 July 2004
				US	6699989	B1	02 March 2004
EP	3222614	A2	27 September 2017	EP	3222614	A2	27 September 2017
				EP	3222614	A4	17 October 2018
				EP	3222614	B1	29 July 2020
				CA	2973193	A1	26 May 2016
				CN	107108536	A	29 August 2017
				HK	1244488	A1	10 August 2018
				JP	2018502146	A	25 January 2018
				JP	6677739	B2	08 April 2020
				JP	2020114831	A	30 July 2020
				JP	7026716	B2	28 February 2022
				KR	20160060512	A	30 May 2016
				KR	102216701	B1	18 February 2021
				US	2018044283	A1	15 February 2018
				US	10626085	B2	21 April 2020
				US	2020277255	A1	03 September 2020
				US	11572341	B2	07 February 2023
				WO	2016080810	A2	26 May 2016
				WO	2016080810	A3	13 October 2016