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(54) **Title:** COMPOUNDS FOR TREATING PARASITIC INFECTIONS

(57) **Abstract:** Compounds and compositions comprising them are provided. The compounds and compositions are useful for inhibiting transport of heme across membranes in parasitic heme auxotrophic organisms, thereby limiting their growth or killing the parasites.



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COMPOUNDS FOR TREATING PARASITIC INFECTIONS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US patent application no. 62/157,870, filed May 6, 2015, the disclosure of which is incorporated herein by reference.

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FIELD

[0002] The present disclosure relates generally to compounds and methods for using the compounds to kill or inhibit the growth of parasites, and more specifically for killing or inhibiting growth of parasites that are heme auxotrophs.

BACKGROUND

10

[0003] Over 1.4 billion people across the globe are affected by Neglected Tropical Diseases (NTD) - a group of thirteen parasitic and bacterial infections. NTD include the soil-transmitted helminthiasis, schistosomiasis, lymphatic filariasis, onchocerciasis, and trachoma, as well as trypanosomiasis and leishmaniasis. Often, individuals are infected with multiple NTD agents simultaneously. Currently, the effectiveness of all agents used to treat parasites is diminished by four factors: **(a)** resistance develops and spreads rapidly, **(b)** parasites do not actively transport drugs into their bodies, **(c)** many drugs (eg: anthelmintics) are effective only during certain developmental stages of the parasite, and **(d)** drugs tend to act on a specific type of parasite and are ineffective in controlling other parasite populations.

15

[0004] Although these are devastating diseases of global concern, their neglected status relative to other health concerns has resulted in a limited arsenal of therapeutic compounds for their treatment [Chatelain E, et al. Drug Des Devel Ther 2011, 5:175-181]. The trypanosomatid parasites *L. major* and *L. amazonensis* are causative agents of human cutaneous leishmaniasis in the Old World and New World, respectively. Combined, these two species are responsible for an estimated 2 million new infections annually with ~350 million people living in areas of active parasite transmission. In many regions of the world, treatment of leishmaniasis still relies on toxic drugs such as pentavalent antimony, which requires high doses and lengthy courses of treatment, and alternative drugs are still costly and not widely available in endemic areas. This situation, combined with the recent increase in *Leishmania* infections in urban areas, highlights the urgent need for identification of essential pathways in these organisms that can be targeted by new drugs with lower toxicity.

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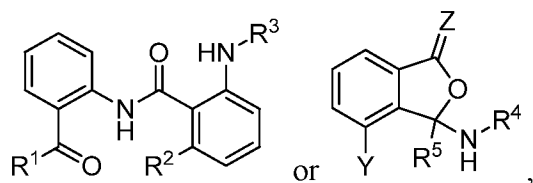
[0005] It was previously demonstrated that the free-living roundworm *C. elegans* and parasitic nematodes cannot synthesize heme, an iron-containing heterocyclic organic ring

structure, but instead acquires heme from either their environment or their host to survive and reproduce PMID: 15767563. It is generally accepted that parasites exhibit distinct adaptations that allow them to acquire nutrients unidirectionally from the host to sustain their growth and reproduction. An example of such an adaptation would be the uptake of heme which the parasite cannot produce but is synthesized by all vertebrates (hosts) via a highly conserved multi-step pathway [Hamza I, et al. Biochim Biophys Acta 2012, 1823(9):1617-1632]. This unusual requirement for external heme is also found in single-celled parasites such as *Trypanosomes* and *Leishmania* [Hamza I, et al. Biochim Biophys Acta 2012, 1823(9):1617-1632; Chang CS, et al. Mol Biochem Parasitol 1985, 16(3):267-276]. Thus, drugs that target heme transport pathways unique to the parasite and not shared by its mammalian host offers great therapeutic potential. A drug that blocks heme transport or that enters the parasite via a high affinity and essential heme uptake pathway can be a highly effective broad-spectrum therapeutic agent, since such transport mechanisms are likely to have similarities across multiple parasite species. Parasites that limit transport or become resistant to the drug will indirectly also limit heme uptake, suffering impaired growth and development, and this approach could be applied to the treatment of infections caused by a diverse group of parasites, including intestinal nematodes, lymphatic and cutaneous filarial nematodes, and the kinetoplastids *Leishmania* and *Trypanosoma*. Thus, there is an ongoing and unmet need for compounds that selectively inhibit targets that are exclusively expressed by the parasites, as opposed to their hosts, and for methods of using such compounds to limit growth or kill such parasites. The present disclosure meets this need.

SUMMARY OF THE DISCLOSURE

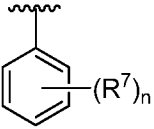
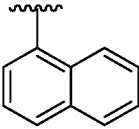
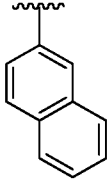
[0006] The present disclosure provides compounds and compositions comprising them. The compounds and compositions are useful for inhibiting the transport of heme across membranes in parasitic heme auxotrophic organism, and thus can be used as agents for inhibiting the growth and/or killing the parasitic heme auxotrophic organisms.

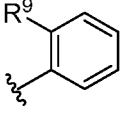
[0007] The disclosure comprises methods and compounds, and compositions comprising the compounds, where the compounds have one of the following structures:



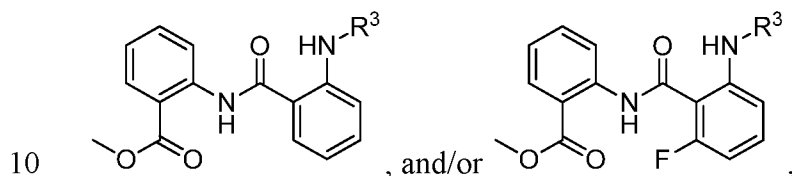
where R¹ is selected from the group consisting of -OH, -OMe, and -NHR⁶, where R⁶ is selected from the group consisting of C₁-C₅ alkyl, C₃-C₆ cycloalkyl, pyridine, and phenyl; R²

is selected from the group consisting of -H, -F, -Cl, -OMe, and -OH; R^3 is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl and pyridine; R^4 is selected from the group consisting of C₁-C₈ alkyl, C₄-C₆ cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted alkyl amine, substituted or unsubstituted naphthyl and heterocyclic; R^5 is

5 selected from the group consisting of , , and , R^7 is independently selected from the group consisting of halogen, phenyl, and alkyl; n equals 0-5,

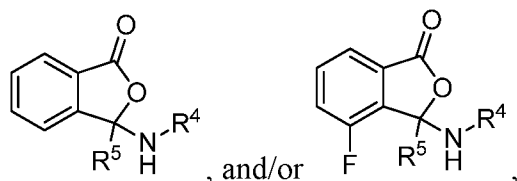
Y is -H, -F, -Cl, or -Me; and Z is O or NR⁸ where R⁸ is ; and R⁹ is selected from the group consisting of -OMe and -F.

[0008] In an embodiment, the compound has the following structures:



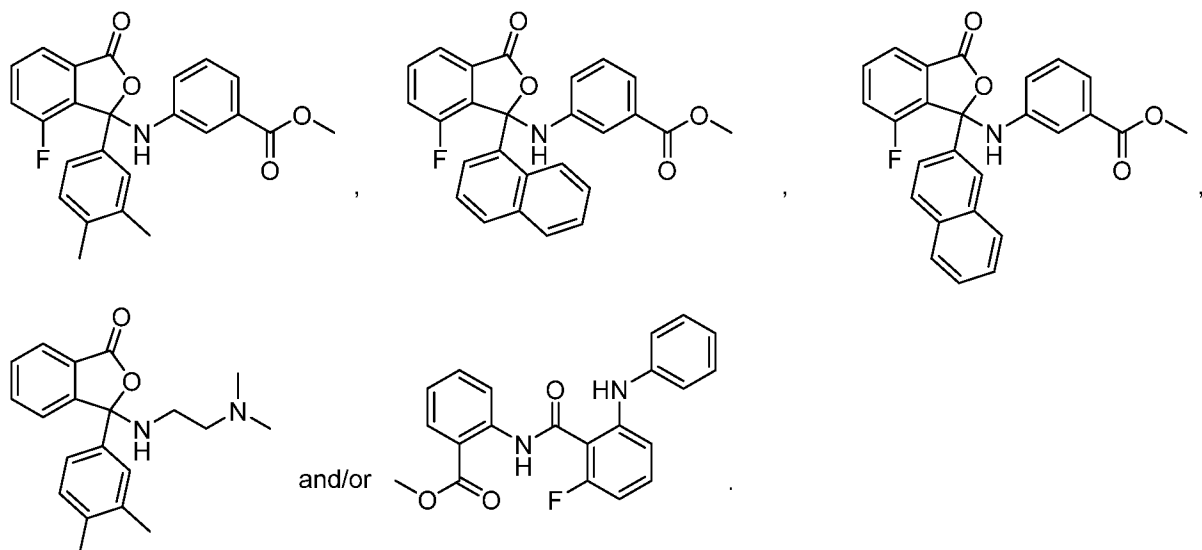
where R^3 is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl and pyridine.

[0009] In an embodiment, the compound has the following structures:



15 where R^4 is selected from the group consisting of C₁-C₈ alkyl, C₄-C₆ cycloalkyl, substituted phenyl, substituted or unsubstituted alkyl amine, naphthyl, substituted naphthyl and heterocyclic; and R^5 is selected from the group consisting of H, halogen, phenyl, and alkyl.

[0010] In an embodiment, the compound has the following structures:



[0011] In one implementation the disclosure comprises a method for inhibiting transport of heme across a membrane in a parasitic heme auxotrophic organism comprising contacting the parasitic organism with an effective amount any of the foregoing compounds.

5 In certain implementations, inhibiting the transport of heme is lethal to the parasitic heme auxotrophic organism. In non-limiting examples the parasitic heme auxotrophic organism is *Leishmania*, which can be targeted as, for example, a promastigote or an amastigote. The compositions and methods are expected to be suitable for prophylaxis and/or therapy for human and veterinary indications.

10 **[0012]** The disclosure also includes kits comprising any one or combination of the compounds described herein, and may further include printed or digitized instructions and/or an indication that compounds are for use in inhibiting the growth of or killing a parasitic heme auxotrophic organism, or for treating an individual for an infection by a parasitic heme auxotrophic organism.

15 **BRIEF DESCRIPTION OF THE DRAWINGS**

[0013] Figure 1. Compounds identified as LHR1 inhibitors decrease parasite viability. (A) Log phase axenic promastigotes were incubated in basal or heme depleted (HD) M199 media for 48 h (h = hour(s)) in the presence of 50 μ M of compounds indicated on the x-axis in 1% DMSO (V). (B) Axenic amastigotes were incubated in 5 μ M or 10 μ M of the indicated compounds in 1% DMSO for 72 h. Viability (%) was quantitated using Alamar blue compared to DMSO (V).

20 **[0014]** Figure 2. Axenic *L. amazonensis* amastigotes are sensitive to Cmpd #34 and novel derivatives. Axenic amastigotes were incubated in 5 μ M or 10 μ M of the indicated compounds in 1% DMSO (V) for 72 h. Viability was quantified using Alamar Blue.

[0015] Figure 3. Cmpd #34 and FX40 specifically inhibit LHR1, but not human HRG1, mediated heme uptake in yeast. Yeast cells expressing pGAL1-LHR1 or pGAL1-hHRG1 were incubated in growth medium containing 10 μ M compounds and GaPPIX for 42 h. Cell growth was measured by absorbance at O.D. 600 and compared to negative control (DMSO, indicated by “V”), or positive control (0.2% glucose, indicated by “Glu”). ***
5 p<0.001.

[0016] Figure 4. Inhibition of intra-macrophage *Leishmania* amastigotes. Mouse primary BMDMs were first infected with (A) *L. amazonensis* amastigotes or (B) *L. donovani* metacyclic promastigotes followed by incubation with the indicated compounds at a
10 concentration of 15 μ M or DMSO (V), which was used as a negative control, or 0.25 μ M amphotericin B (AmphoB), which was used as a positive control. Surviving intracellular amastigotes per 100 macrophages were quantified as determined by DAPI positive cells.****
p<0.0001, *** p<0.001, ** p<0.005.

[0017] Figure 5. Dose response curves (IC₅₀) of FX40 (○) and the novel derivative compound FX104 (●). (A) BMDMs infected with *L. amazonensis* amastigotes were treated
15 with various concentrations of the indicated compound for 72 h. BMDMs were lysed and released amastigotes were transformed to promastigotes for 48 h at 26 °C. IC₅₀ values were calculated by quantitation of % promastigote growth using AlamarBlue. (B) Dose response of uninfected BMDMs to compounds.

20 DETAILED DESCRIPTION

[0018] Unless defined otherwise herein, all technical and scientific terms used in this disclosure have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains.

[0019] Every numerical range given throughout this specification includes its upper
25 and lower values, as well as every narrower numerical range that falls within it, as if such narrower numerical ranges were all expressly written herein.

[0020] The present disclosure provides compounds, pharmaceutical compositions comprising the compounds, and methods of using the compounds for antagonizing one or more pathways required for the transport of heme. This therapeutic strategy was chosen
30 based at least in part on the following observations: (a) heme is utilized in multiple, critical processes; (b) heme is required in all stages of development; (c) *Leishmania* and other parasitic heme auxotrophs cannot live without heme and actively ingest it; and (d) both

humans and their parasites require heme, but the mechanisms used to fulfill this requirement differ between humans and their parasites.

[0021] In general, the disclosure is directed to killing or inhibiting the growth of parasitic organisms that acquire environmental heme, including but not necessarily limited to parasitic helminths, such as ascarids (*Ascaris*), filarias, hookworms, pinworms (*Enterobius*) and whipworms (*Trichuris trichiura*), and members of class Kinetoplastea, including but not necessarily limited to parasites of the genus *Trypanosoma*, such as *T. brucei* and *T. cruzi* and *Leishmania*, including all members of the *Leishmania* subgenuses.

[0022] In certain embodiments, the disclosure pertains to inhibiting the transport of heme across a membrane in a parasitic heme auxotrophic organism or in a heme prototroph, such as plasmodium. In embodiments, the disclosure comprises inhibiting the transport of heme across a membrane of a parasitic heme auxotrophic organism. In embodiments the organism that is present in a human or non-human animal. Thus, the disclosure includes human and veterinary therapeutic approaches. In embodiments, the parasitic heme auxotrophic organism is present in an insect or a plant. In embodiments, the parasitic heme auxotrophic organism is present in a cell. In embodiments, the disclosure comprises inhibiting the transport of heme across a membrane of a parasitic heme auxotrophic organism that is present in an insect, such as a fly, or in a mammalian cell, including but not necessarily limited to leukocytes, such as monocytes, neutrophils, macrophage, or hepatocytes. In embodiments, the infected cells are present in an organ in an individual, such as the liver or spleen or bone marrow or skin. In embodiments, the parasitic heme auxotrophic organism is an amastigote or a promastigote.

[0023] In a non-limiting aspect, the present disclosure uses *Leishmania* as a target parasitic heme auxotrophic organism to demonstrate utility of the compounds described herein. Thus, in embodiments, compounds of the disclosure are selective antagonists of one or more heme transporters in *Leishmania*. Without intending to be constrained by any particular theory, the heme transporter from *Leishmania* parasite is used as a non-limiting example of utility of the present compositions and methods according to the following rationale: (a) no satisfactory treatment available except antimonials which are toxic with serious side effects in humans; (b) single-celled protozoa that are genetically manipulatable for ectopic overexpression or gene knockouts; (c) fully sequenced and annotated genome; (d) and growth can be manipulated for pharmacologic assays in free-living (axenic), intracellular (inside macrophages) or *in vivo* (in mice/hamsters) stages.

[0024] Since previous to the present disclosure no effective drugs exist to treat leishmaniasis except antimonials which are highly toxic to humans, a cell-based, high throughput screen (HTS) of >200,000 compounds using growth assays in yeast was performed. As described below, the disclosure includes identification of several antagonists that specifically inhibit the heme transport function of the *Leishmania* LHR1 but not the corresponding human homolog (hHRG1). The identified compounds were used in part to design and synthesize the novel compounds described herein.

[0025] In embodiments, the disclosure comprises exposing *Leishmania* or other parasitic heme auxotrophs to one or more compounds of this disclosure such that is inhibited or they are killed. In certain approaches the method comprises reducing the amount of *Leishmania* promastigotes, such as in a sandfly, or comprises reducing the amount of *Leishmania* amastigotes. In one approach the disclosure includes administering to an individual in need thereof a composition comprising one or more of the compounds described herein. This is expected to result in reducing the amount of *Leishmania* organisms in the individual, or in the eradication of *Leishmania* organisms from the individual. It is contemplated that the individual can be infected with any *Leishmania* strain, and can be diagnosed with or suspected of having any type of leishmaniasis, such as cutaneous leishmaniasis, or diffuse cutaneous leishmaniasis, or visceral leishmaniasis. The individual may be a human, or a non-human mammal, including but not necessarily limited to a canine.

[0026] The compounds of this disclosure can be combined with any pharmaceutically acceptable carrier, excipient, diluent and the like to obtain pharmaceutical compositions. Compositions comprising the compounds can be administered to an individual using any suitable route, dosage and formulation. In embodiments, the compositions are administered orally, parenterally, sublingually, transdermally, transmucosally, or topically. Parenteral administration includes, but is not limited to, intravenous, intraarterial, intraperitoneal, subcutaneous, and intramuscular routes. Administration of the compounds can be performed sequentially or concurrently with any other anti-infective compositions of matter and treatment modalities. Further, the disclosure encompasses combinations of the compounds of this disclosure with known anti-infective agents. In embodiments, the compounds of this disclosure can be used in conjunction with anti-infective agents which include but are not necessarily limited to liposomal amphotericin B, pentavalent antimonials, paromomycin, including topical paromomycin, miltefosine, fluconazole, itraconazole, and combinations thereof.

[0027] The disclosure includes pharmaceutically acceptable salts of the compounds disclosed herein.

[0028] The compounds can be provided in any suitable form, including in solutions, suspensions, emulsions, or as dry material for reconstitution prior to use, or in tablets, capsules, and the like. The compounds can be formulated for rapid or sustained release. The compounds can be combined with any suitable drug delivery vehicle, including but not necessarily limited to liposomal formulations, nanoparticle formulations, or other delivery vehicles that will be apparent to those skilled in the art given the benefit of the present disclosure.

[0029] The compounds can be provided as an article of manufacture, such as a kit, that includes, for example, a closed or sealed package that contains the compounds. In certain embodiments, the package can comprise one or more closed or sealed vials, bottles, or any other suitable packaging and/or containers for the sale, or distribution, or use of the compounds. The article of manufacture can include printed information. The printed information can be provided on a label, or on a paper insert, or it can be printed on the packaging material itself. The printed information can include information that identifies the compound, the amounts and types of other active and/or inactive ingredients, and instructions for taking the composition, such as the number of doses to take over a given period of time, and/or information directed to a pharmacist and/or another health care provider, such as a physician, or a patient. In embodiments the printed information includes an indication that the contents of the article of manufacture is for use in treating a parasitic infection, examples of which are described above.

[0030] With respect to the compounds of this disclosure, the following description is pertinent. In particular, as used herein, the term “alkyl” group, unless otherwise stated, refers to branched or unbranched hydrocarbons. Examples of such alkyl groups include methyl groups, ethyl groups, propyl groups, butyl groups, isopropyl groups, sec-butyl, tert-butyl groups, and the like. For example, the alkyl group is a C₁ to C₈ alkyl group, including all integer numbers of carbons and ranges of numbers of carbons therebetween. Alkyl groups can be substituted with various other functional groups. For example, the alkyl groups can be substituted with groups such as, for example, amines (acyclic and cyclic), alcohol groups, ether groups, and halogen atoms.

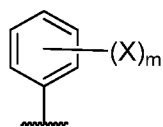
[0031] As used herein, the term “cycloalkyl” group refers to a C₄-C₆ cyclic hydrocarbon group, e.g., cyclopropyl, cyclobutyl, cyclohexyl, and cyclopentyl groups. Cycloalkyl groups can be saturated or partially unsaturated ring systems optionally

substituted with, for example, one to three groups, independently selected from the group consisting of alkyl, -NH₂, oxo (=O), phenyl, naphthyl, haloalkyl (e.g., -CF₃), halo (e.g., -F, -Cl, -Br, -I), alkoxy, and -OH groups. Additionally, alkyl substituents can be substituted with various other functional groups.

5 **[0032]** As used herein, unless otherwise indicated, “halogen” means fluorine, chlorine, bromine, and iodine, and “halo” group means fluoro, chloro, bromo and iodo.

[0033] As used herein, the term “heterocycle” or “heterocyclic” group, unless otherwise stated, refers to a cyclic compound having a ring where at least one or more of the atoms forming the ring is a heteroatom (e.g., oxygen, nitrogen, sulfur, etc.). The heterocyclic
 10 ring can be aromatic or nonaromatic, and include compounds that are saturated, partially unsaturated or fully unsaturated. Examples of such groups include azetidine, pyrrolidine, piperidine, azepane, azocane, dihydropyridinone, dihydropyridazinone, dihydrooxepinone, dihydroazepineone, furan, thiophene, oxazole, isoxazole, thiazole, oxadiazole, thiadiazole, triazolo, tetrazole, oxazoline, lactam, lactone, dihydrofuran, tetrahydrofuran, furanone,
 15 oxazolone, pyridinone, pyrimidinone, dihydropyridazine, pyranone, oxazinone, and the like. For example, the heterocyclic ring can be a 3, 4, 5, 6, 7, 8, 9, or 10 membered ring containing a number of carbon atoms ranging between 1 and 7 and a number of heteroatoms ranging between 1 and 7. The ring can be bonded to other rings to form ring systems. The heterocyclic ring can be unsubstituted or substituted with various substituents.

20 **[0034]** As used herein, unless otherwise indicated, “phenyl” group means

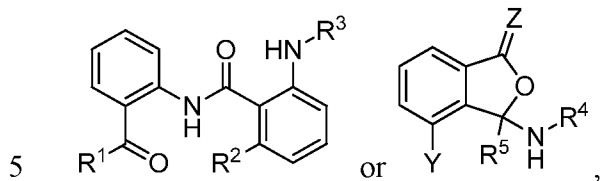


, where each X is independently a substituent on the phenyl group and m is from 0 to 5. The substituents at different occurrences can be the same or different. For example, the substituents on the phenyl group include substituted or unsubstituted C₁-C₈ alkyl, including all integer numbers of carbons and ranges of numbers of carbons therebetween,
 25 substituted or unsubstituted amino, haloalkyl (e.g., -CF₃), halo (e.g., -F, -Cl, -Br, -I), heterocyclic, substituted or unsubstituted alkoxy (e.g., -OMe), and carbonyl containing moieties such as esters and amides. In certain instances, two adjacent R groups can be connected through to form a dioxolyl group.

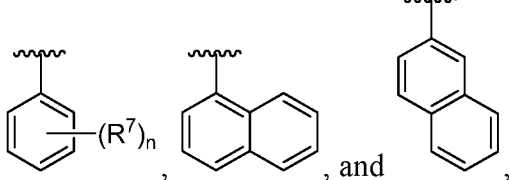
[0035] As used herein, unless otherwise stated, the term “substituents” or
 30 “substituted” refers to one or more of the following groups: alkyl groups, amide groups, amino groups, alkoxy groups, aryl groups, cycloalkyl groups, halogen atoms, alkylhalides,

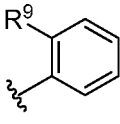
heterocyclic groups, hydroxyl groups, ketone, groups, phenyl groups, or a combination thereof.

[0036] The disclosure comprises methods and compounds, where the compounds have one of the following structures:

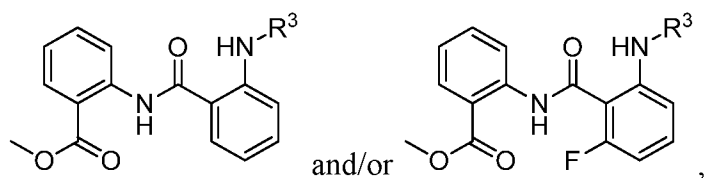


where R^1 is selected from the group consisting of -OH, -OMe, and -NHR⁶, where R^6 is selected from the group consisting of C₁-C₅ alkyl, C₃-C₆ cycloalkyl, pyridine, and phenyl; R^2 is selected from the group consisting of -H, -F, -Cl, -OMe, and -OH; R^3 is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl and pyridine; R^4 is selected from the group consisting of C₁-C₈ alkyl, C₄-C₆ cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted alkyl amine, substituted or unsubstituted naphthyl and heterocyclic; R^5 is

selected from the group consisting of , where R^7 is independently selected from the group consisting of halogen atom, phenyl, and alkyl; n

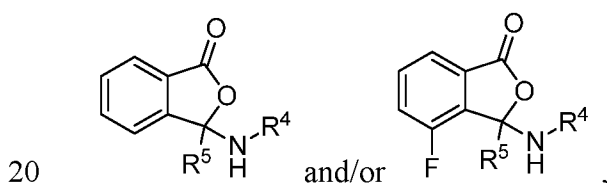
15 equals 0-5, Y is -H, -F, -Cl, or -Me; and Z is O or NR⁸, where R^8 is ; and R^9 is selected from the group consisting of -OMe and -F.

[0037] In an embodiment, the compound has the following structures:



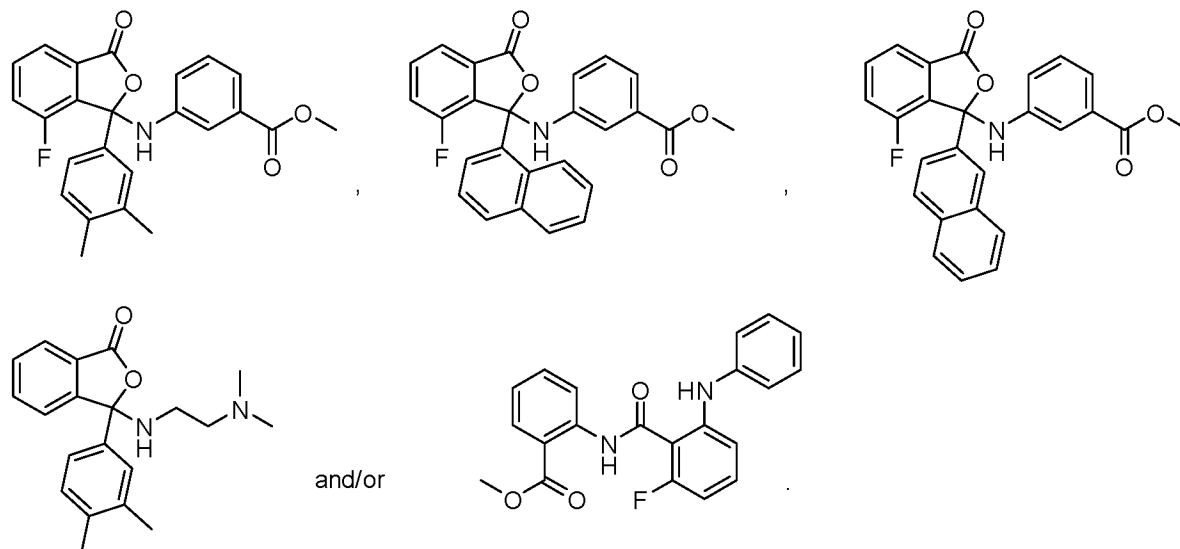
where R^3 is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl, and pyridine.

[0038] In an embodiment, the compound has the following structures:



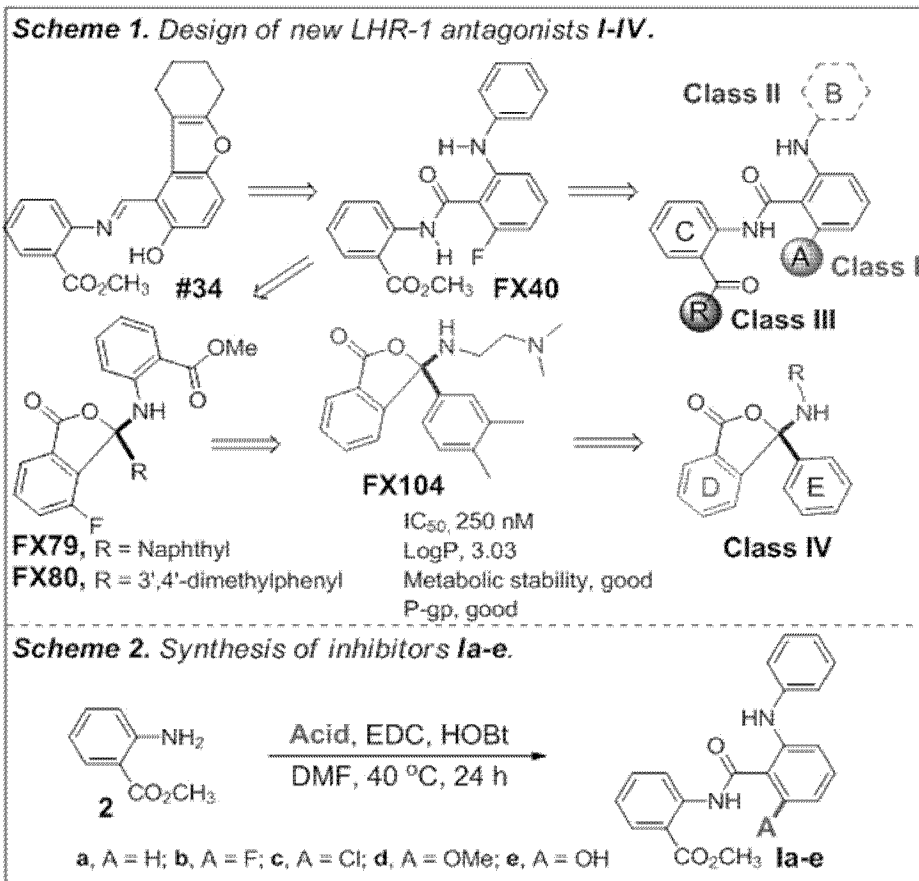
where R^4 is selected from the group consisting of C₁-C₈ alkyl, C₄-C₆ cycloalkyl, substituted phenyl, substituted or unsubstituted alkyl amine, naphthyl, substituted naphthyl and heterocyclic; and R^7 is selected from the group consisting of H, halogen, phenyl, and alkyl.

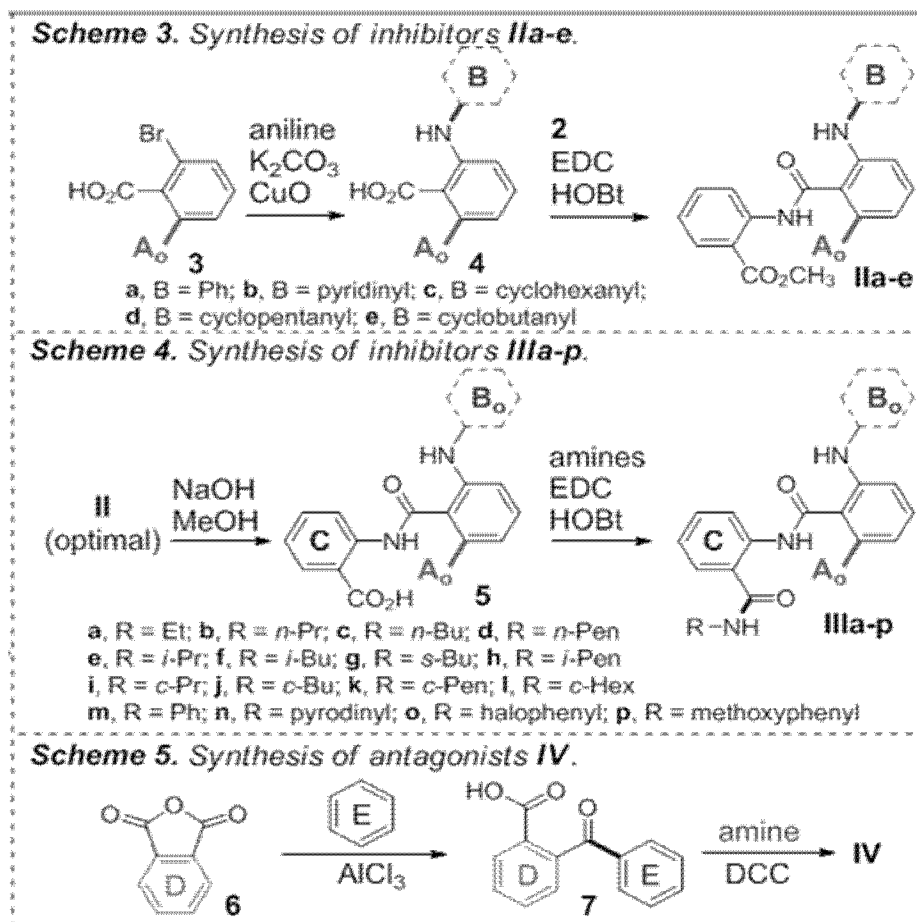
[0039] In an embodiment, the compound has the following structures:



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[0040] Without intending to be bound by any particular theory, a representative scheme is presented herein to describe the synthesis of the aforementioned molecules:





[0041] Compounds of this disclosure were designed in part using the following information and approach.

[0042] HRG-1 Heme Transporters and Heme Homeostasis: The identification of HRG-1, the first metazoan heme importer/transporter, was accomplished using *C. elegans*, a roundworm that does not synthesize its own heme. HRG-1 and its paralog HRG-4 is a four transmembrane domain permease with homologs in vertebrates. Knockdown of *hrg1* in zebrafish causes congenital malformations and profound anemia. In mammals, HRG1 is strongly expressed in macrophages of the reticuloendothelial system and specifically localizes to the phagolysosomal membranes during erythrophagocytosis (EP), a process that degrades over 5 million senescent RBCs per second. Depletion of Hrg1 in mouse macrophages causes attenuation of heme transport from the phagolysosomal compartment confirming that the long-sought heme transporter for macrophage heme-iron recycling is the mammalian homolog of *C. elegans* HRG-1.

[0043] Implications for Host-Pathogen Interactions: Like *C. elegans*, kinetoplastid parasites such as *Trypanosomes* and *Leishmania* cannot synthesize heme as they lack most of the heme synthesizing enzymes. The *Leishmania* ortholog of worm HRG-4 is designated

LHR1, and was the first parasite heme transporter to be identified. Loss of a single LHR1 allele ($LHR1^{+/-}$) causes severe growth defects while *LHR1* null mutants ($LHR1^{-/-}$) are not viable which indicates that LHR1-dependent heme transport is essential for parasite survival and that 50% reduction in LHR1 function is sufficient to significantly impair growth.

5 **[0044]** A HTS to identify small molecule antagonists of LHR1: To develop a cell-based high throughput screen (HTS) for small molecule antagonists of the LHR1, a heterologous yeast assay was developed. While *S. cerevisiae* synthesize their own heme (like humans), they lack a dedicated plasma membrane heme transporter. Correspondingly, yeast that are genetically defective in heme synthesis ($\Delta hem1$ mutant) are unable to grow unless
10 they express LHR1 or HRG1 orthologs in the presence of low amounts of supplemented heme in the growth medium. However, if wildtype yeast expressing LHR1 are grown in the presence of a toxic heme analog gallium protoporphyrin IX (GaPPIX) instead of heme in the growth medium, the yeast die because mis-incorporation of GaPPIX into the yeast hemoproteins is cytotoxic as gallium cannot undergo redox reactions like the iron within
15 heme. To exploit this assay, yeast stably-transformed with LHR1 were cultured in 384-well plates and candidate small molecule antagonists of LHR1 were identified from a chemical library of >200,000 compounds on the basis of “rescuing” yeast growth in the presence of the toxic GaPPIX as measured by optical absorbance for growth using a plate reader. The small molecule library was composed of the following compounds: 75,000 (ChemBridge Corp.);
20 100,000 (Chemical Diversity Labs); 22,000 (ComGenex); 1200 (TimTek), 1100 (Prestwick); and 450 (NIH clinical collection). The compounds in the library satisfy a relaxed set of Lipinsky’s rules with 99% having a molecular weight <550 Da.

[0045] Validation and selectivity of compounds that antagonize LHR1 but not human HRG1: The top 960 hits, defined as being >3 standard deviations from the mean, were
25 “cherry-picked” for validation and preliminary selectivity assessment (**Table 1**). From these hits, 373 displayed some activity even at 10-fold lower concentration (0.5 μ M) with the nine best leads able to fully rescue yeast growth under assay conditions. Though some of these sub- μ M leads appeared to inhibit GaPPIX uptake in counterscreens of yeast expressing HRG-1’s from other species such as *C. elegans* and human hHRG1, many were selective,
30 displaying little or no human HRG1 antagonism, ruling out the possibility that the hits simply coordinate to GaPPIX. From the 373 compounds that showed selectivity for LHR1 over hHRG1 in the yeast assays, compounds that possessed chemically labile structures, shared similar chemical scaffolds, or showed poor (high) IC_{50} values in dose-response curves in yeast were eliminated. This resulted in the identification of 54 most efficacious compounds,

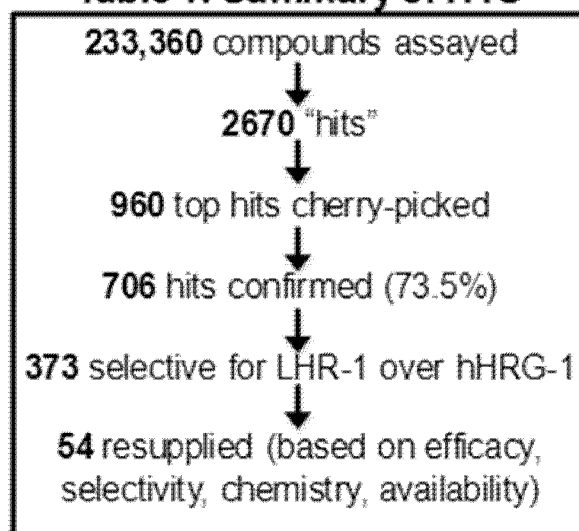
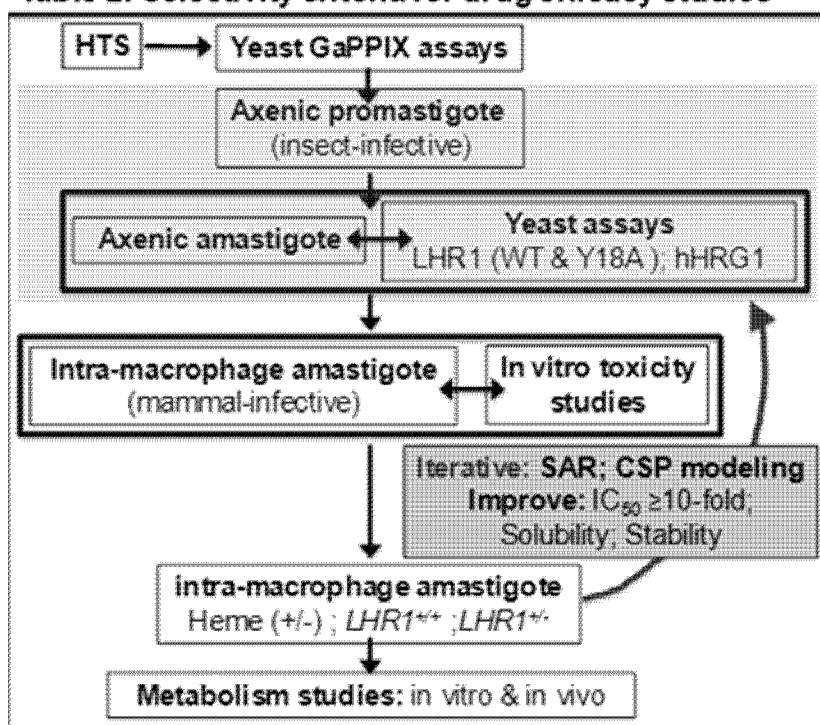
all of which were next tested in *Leishmania* grown in normal medium or in heme depleted medium which upregulates LHR1 (Fig. 1A).

[0046] Validation of compounds in *Leishmania*: Several of the initial hits are toxic to *L. amazonensis* promastigotes at 50 μ M, with enhanced toxicity observed in wildtype strains grown in heme-deficient media that upregulates LHR1, supporting the concept that the drug effects are mediated, in part, through the LHR1 transporter itself (Fig. 1A). Although some of the HTS hits appear to have metabolic and chemical liabilities, several chemical series represent potent and drug-like inhibitors. Strikingly, some of the compounds decreased *L. amazonensis* viability in axenic culture by >90% at 5 μ M and 10 μ M such as **Cmpd #34**, a 6,7,8,9-tetrahydro-2-hydroxy-1-dibenzofuranyl derivative. This compound was not identified from any other HTS that is publicly available (Fig. 1A&B).

[0047] To validate the *in vivo* efficacy of lead compounds against intracellular amastigotes in macrophages, primary bone marrow-derived macrophages (BMDMs) from mice were obtained and infected them with *L. amazonensis* amastigotes followed by incubation for 72 h with LHR1 antagonists or amphotericin B which kills intracellular *Leishmania*, as a positive control. Cells were then fixed and stained with DAPI which permits visualization of the host macrophage nuclei and parasite nuclei and kinetoplasts. Images collected with fluorescent microscopy were used to determine the ratio between total number of parasites and total number of host cells per treatment. Toxicity to host cells were measured by AlamarBlue viability assays. Both Cmpd #34 and novel antagonist FX40 killed about 50% of intracellular *L. amazonensis* amastigotes (Fig. 4A). The demonstration of this property of FX40 led to the development of FX104. (Sch.1 and Fig. 4A) The structure of FX104 is shown in Scheme 1. Without intending to be constrained by any particular theory or approach, FX104 was determined to have the best drug-like parameters with significantly lower LogP value; better solubility, absorption and metabolic stability; and decreased P-gp efflux. The results show that (a) the novel compound FX104, synthesized from the original scaffold of FX40, is over 30-fold more effective against intracellular forms of *Leishmania* (IC₅₀: 8 μ M versus 250 nM, Fig. 5A); and (b) FX104 has little or no toxicity in BMDMs even at 40-fold greater concentrations (Fig. 5B).

[0048] *L. donovani* metacyclic promastigotes were selectively enriched using a Ficoll density-step gradient. *L. donovani* metacyclic promastigotes were incubated with BMDMs at a macrophage:promastigote ratio of 1:10. After a 24 h incubation at 37 °C in 5% CO₂, non-internalized promastigotes were removed by 4 washes and test compounds were added to the cultures and incubated at 37 °C for 72 h. Intracellular parasite viability was evaluated by

DAPI staining. Consistent results obtained with *L. amazonensis*, Cmpd #34, FX40 and FX104 killed between 50-70% of intracellular parasites (**Fig. 4B**). As illustrated in Table 2, embodiments of the disclosure include iterative SAR and CSP modeling to improve the efficacy of compounds of this disclosure in intra-macrophage assays, which may be performed prior to evaluating them for metabolic stability and ADME properties. In 5 embodiments, compounds that attain an IC₅₀ value that matches or surpasses that for Amphotericin B: IC₅₀=0.05-0.1 μ M against intra-macrophage *L. amazonensis* or 0.02-0.06 μ M for intra-macrophage *L. donovani* (>90% efficacy) are provided.

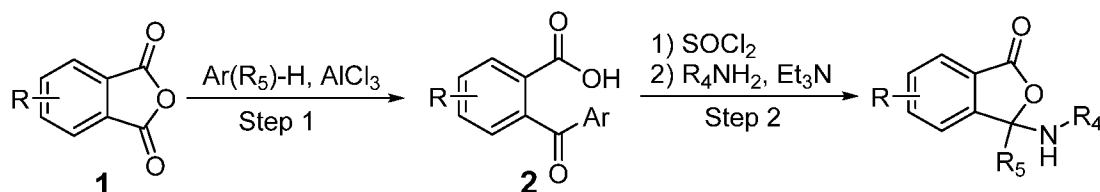
Table 1: Summary of HTS**Table 2: Selectivity criteria for drug efficacy studies**

[0049] To further assess the chemical stability of compounds of this disclosure, the compounds can be incubated with simulated gastric fluid, simulated intestinal fluid, mouse plasma, mouse liver S9 fraction as well as Caco-2 cell homogenate, and assessed as follows. After incubation for varying times, samples are removed and subsequently ice-cold

5 acetonitrile is added to precipitate cellular protein and stop enzymatic activity. The samples are centrifuged at 12,000xg at 4°C for 30 min and supernatant are collected for LC-MS/MS analysis. To determine the enzymes responsible for the metabolism of the compounds, mouse or human liver microsomes are prepared by differential centrifugation. The test compounds are incubated with microsomal preparation for varying times, along with representative
10 cytochrome P450 (CYP) inhibitors and antibodies. The metabolic reactions are stopped and the extracted samples are subjected to LC-MS/MS analysis for metabolite identification, quantitation, and metabolism kinetics. For toxicity evaluation in cells, the effect of lead compounds on the viability (MTT assay), cellular morphology (microscopy and histology), and nucleic acid stability (DNA fragmentation) can be evaluated, for example, in HeLa,
15 HepG2, and/or HEK293 cells.

[0050] The following materials and methods were employed to synthesize compounds of this disclosure as described in the Examples.

[0051] Synthesis Materials and Methods:



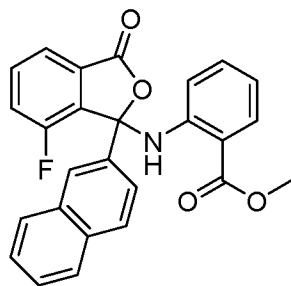
[0052] **Step 1:** To a solution of compound **1** in toluene was added $\text{Ar(R}_5\text{)-H}$ followed by AlCl_3 . The reaction mixture was allowed to stir for 24 h, and then concentrated. After removing the solvent, the crude material was purified by flash column chromatograph to get compound **2**.

[0053] **Step 2:** To a solution of compound **2** in dichloromethane was added SOCl_2 .

25 The reaction was heated under reflux for 2 h. After removing the solvent, the resulting material was redissolved in DMF, to the resulting solution was added the amino compound R_4NH_2 , followed by triethylamine. The reaction mixture was allowed to stir at room temperature overnight, and then concentrated. After removing the solvent, the crude material was purified by flash column chromatograph to get the final compounds. Examples of
30 compounds encompassed by this disclosure include but are not limited to the following:

Example 1

[0054] The following is an example of synthesis and characterization of a compound of the present disclosure. Methyl 2- (4-fluoro-1,3-dihydro-3- (naphthalen-2-yl)-1-oxoisobenzofuran-3-ylamino)benzoate



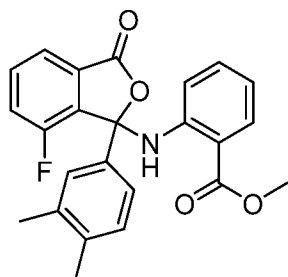
$C_{26}H_{18}FNO_4$
Mol. Wt.: 427.4

XH3027

[0055] Yield 31.0%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.51 (s, 1H), 8.12 (s, 1H), 7.96-7.94 (d, $J = 7.6$ Hz, 1H), 7.82-7.77 (m, 4H), 7.71-7.73 (d, $J = 8.4$, 1H), 7.58-7.52 (m, 1H), 7.48-7.46 (m, 2H), 7.32-7.28 (t, $J = 8.4$ Hz, 1H), 7.08-7.04 (t, $J = 8.0$ Hz, 1H), 6.79-6.76 (d, $J = 8.4$ Hz, 1H), 6.74-6.70 (t, $J = 8.0$ Hz, 1H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.3, 163.4, 52.8, 150.3, 141.4, 132.0, 130.0, 129.2, 128.6, 128.4, 128.2, 128.1, 126.6, 124.3, 123.8, 122.8, 122.1, 121.8, 121.0, 118.8, 117.8, 117.6, 117.4, 113.6, 112.5, 108.3, 90.7, 47.3. ESI-MS m/z 426.1 $[M-H]^-$.

Example 2

[0056] The following is an example of synthesis and characterization of a compound of the present disclosure. Methyl 2- (4-fluoro-1,3-dihydro-3- (3,4-dimethylphenyl)-1-oxoisobenzofuran-3-ylamino) benzoate



$C_{24}H_{20}FNO_4$
Mol. Wt.: 405.4

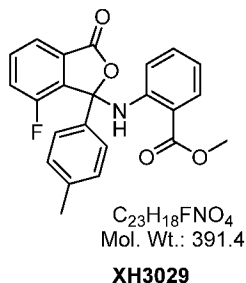
XH3028

[0057] Yield 31.8%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.37 (s, 1H), 7.95-7.93 (d, $J = 7.2$ Hz, 1H), 7.75-7.73 (d, $J = 7.6$ Hz, 1H), 7.56-7.50 (m, 1H), 7.37-7.27 (m, 3H), 7.15-7.11 (t, $J = 7.6$ Hz, 1H), 7.09-7.07 (d, $J = 7.6$ Hz, 1H), 6.75-6.71 (m, 2H), 3.87 (s, 1H), 2.20

(s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 169.0, 168.4, 155.2, 146.2, 137.9, 137.6, 134.9, 134.0, 132.8, 131.5, 130.5, 128.7, 127.2, 123.9, 122.7, 122.5, 122.2, 118.4, 117.4, 51.9, 19.9, 19.4. ESI-MS m/z 404.1 $[\text{M}-\text{H}]^-$.

Example 3

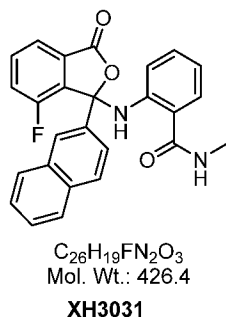
- 5 **[0058]** The following is an example of synthesis and characterization of a compound of the present disclosure. Methyl 2- (4-fluoro-1,3-dihydro-1-oxo-3-p-tolylisobenzofuran-3-ylamino)benzoate



- [0059]** Yield 32.6%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 9.40 (s, 1H), 7.97-7.95 (d, $J = 8.0$ Hz, 1H), 7.77-7.75 (d, $J = 6.8$ Hz, 1H), 7.58-7.53 (m, 1H), 7.53-7.50 (d, $J = 7.6$ Hz, 1H), 7.37-7.32 (t, $J = 7.6$ Hz, 1H), 7.17-7.13 (m, 3H), 6.75-6.72 (m, 2H), 3.89 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 169.1, 168.2, 157.6, 155.12, 146.3, 139.1, 136.8, 134.4, 133.9, 132.8, 131.4, 129.8, 128.6, 126.2, 122.5, 122.3, 122.1, 118.3, 117.3, 113.1, 95.6, 52.0, 21.2. ESI-MS m/z 390.1 $[\text{M}-\text{H}]^-$.

15 Example 4

- [0060]** The following is an example of synthesis and characterization of a compound of the present disclosure. 2- (4-fluoro-1,3-dihydro-3- (naphthalen-2-yl)-1-oxoisobenzofuran-3-ylamino)-N-methylbenzamide

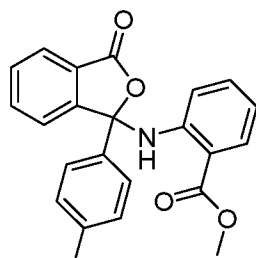


- 20 **[0061]** Yield 39.9%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 9.46 (s, 1H), 8.06 (s, 1H), 7.75-7.67 (m, 5H), 7.48-7.43 (m, 1H), 7.40-7.38 (m, 2H), 7.30-7.28 (d, $J = 7.6$ Hz, 1H), 7.22-7.20 (m, 1H), 6.95-6.91 (t, $J = 8.0$ Hz, 1H), 6.73-6.71 (d, $J = 8.8$ Hz, 1H), 6.66-6.62 (t, $J = 6.8$ Hz, 1H), 6.12 (br s, 1H), 2.92-2.91 (d, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 169.0,

168.4, 155.2, 144.2, 135.0, 133.2, 132.6, 132.0, 128.9, 128.6, 127.6, 126.9, 126.4, 123.7, 122.2, 118.4, 29.7, 26.8. ESI-MS m/z 425.1 $[M-H]^-$.

Example 5

[0062] The following is an example of synthesis and characterization of a compound of the present disclosure. Methyl 2- (1,3-dihydro-1-oxo-3-p-tolylisobenzofuran-3-ylamino)benzoate



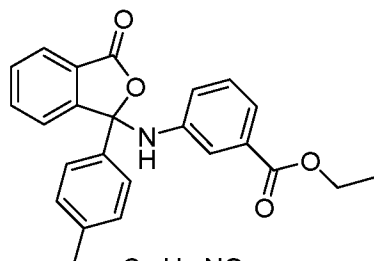
$C_{23}H_{19}NO_4$
Mol. Wt.: 373.4

XH3050

[0063] Yield 39.9%. 1H NMR ($CDCl_3$) δ (ppm) 9.20 (br s, 1H), 7.96-7.92 (t, $J = 8.8$ Hz, 2H), 7.68-7.64 (t, $J = 7.6$ Hz, 1H), 7.57-7.53 (m, 2H), 7.50-7.48 (d, $J = 7.6$ Hz, 2H), 7.16-7.14 (m, 3H), 6.83-6.81 (d, $J = 8.8$ Hz, 1H), 6.76-6.72 (t, $J = 7.6$ Hz, 1H), 3.87 (s, 3H), 2.30 (s, 3H). ^{13}C NMR ($CDCl_3$) δ (ppm) 169.5, 169.0, 151.5, 146.5, 138.8, 135.9, 135.3, 133.9, 131.2, 130.2, 130.0, 126.0, 125.5, 125.1, 122.1, 118.2, 117.5, 112.9, 91.5, 51.9, 21.1. ESI-MS m/z 372.1 $[M-H]^-$.

Example 6

[0064] The following is an example of synthesis and characterization of a compound of the present disclosure. Ethyl 3- (1,3-dihydro-1-oxo-3-p-tolylisobenzofuran-3-ylamino)benzoate



$C_{24}H_{21}NO_4$
Mol. Wt.: 387.4

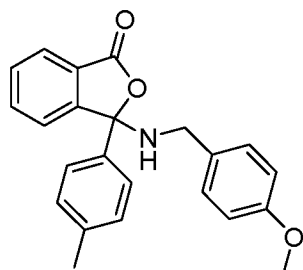
XH3051

[0065] Yield 38.2%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.91-7.89 (d, $J = 7.6$ Hz, 1H), 7.67-7.63 (t, $J = 7.6$ Hz, 1H), 7.57-7.53 (m, 4H), 7.51-7.49 (d, $J = 7.2$ Hz, 1H), 7.38 (s, 1H), 7.18-7.16 (d, $J = 8.4$ Hz, 2H), 7.13-7.09 (t, $J = 7.6$ Hz, 1H), 6.88-6.86 (d, $J = 8.0$ Hz,

1H), 4.29-4.24 (m, 2H), 2.32 (s, 3H), 1.33-1.29 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 169.2, 166.3, 150.7, 142.5, 139.0, 135.8, 134.8, 131.1, 130.3, 129.9, 128.8, 126.0, 125.6, 122.8, 122.4, 122.0, 119.3, 60.8, 21.1, 14.2. ESI-MS m/z 386.1 $[\text{M-H}]^-$.

Example 7

- 5 [0066] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (4-methoxybenzylamino)-3-p-tolylisobenzofuran-1 (3H)-one



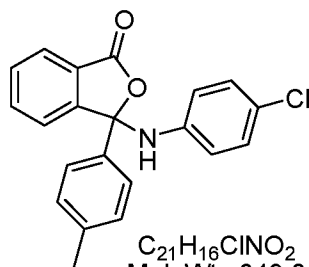
$\text{C}_{23}\text{H}_{21}\text{NO}_3$
Mol. Wt.: 359.4

XH3052

- [0067] Yield 24.3%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.63-7.62 (d, $J = 7.6$ Hz, 1H), 7.44-7.40 (t, $J = 7.2$ Hz, 1H), 7.38-7.34 (t, $J = 7.2$ Hz, 1H), 7.25-7.20 (m, 2H), 7.07-7.05 (m, 4H), 6.57-6.54 (d, $J = 8.4$ Hz, 2H), 4.49-4.46 (d, $J = 14.8$ Hz, 1H), 4.03-4.00 (d, $J = 14.8$ Hz, 1H), 3.62 (s, 3H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 167.9, 158.2, 149.4, 138.0, 135.7, 132.6, 130.3, 130.2, 129.2, 129.0, 126.3, 123.2, 122.6, 113.3, 61.7, 55.1, 42.4, 21.1. ESI-MS m/z 358.2 $[\text{M-H}]^-$.

Example 8

- 15 [0068] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (4-chlorophenylamino)-3-p-tolylisobenzofuran-1 (3H)-one



$\text{C}_{21}\text{H}_{16}\text{ClNO}_2$
Mol. Wt.: 349.8

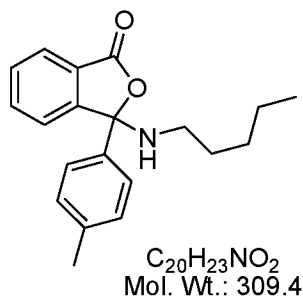
XH3053

- [0069] Yield 37.4%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.89-7.87 (d, $J = 8.0$ Hz, 1H), 7.65-7.62 (t, $J = 6.8$ Hz, 1H), 7.55-7.52 (m, 2H), 7.17-7.15 (d, $J = 7.6$ Hz, 2H), 7.04-7.01 (d, $J = 7.6$ Hz, 2H), 6.66-6.64 (d, $J = 8.4$ Hz, 2H), 2.32 (s, 3H). ^{13}C NMR (100 MHz,

CDCl_3) δ (ppm) 169.2, 14.09, 139.2, 135.5, 134.8, 130.4, 129.9, 128.8, 126.1, 125.7, 122.8, 120.0, 21.1. ESI-MS m/z 348.1 $[\text{M-H}]^-$.

Example 9

[0070] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (pentylamino)-3-p-tolylisobenzofuran-1 (3H)-one

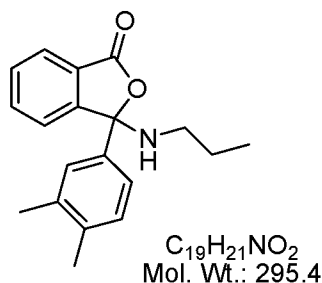


XH3055

[0071] Yield 55.4%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.70-7.68 (d, $J = 7.2$ Hz, 1H), 7.47-7.39 (m, 2H), 7.26-7.25 (m, 3H), 7.14-7.12 (d, $J = 8.0$ Hz, 2H), 3.48 (br s, 1H), 3.44-3.37 (m, 1H), 2.96-2.89 (m, 1H), 2.33 (s, 3H), 1.51-1.45 (m, 1H), 1.38-1.34 (m, 1H), 1.23-1.14 (m, 4H), 0.82-0.78 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 167.7, 149.1, 138.2, 135.6, 132.4, 130.6, 129.4, 129.1, 126.1, 123.1, 122.5, 91.4, 39.6, 29.3, 28.4, 22.2, 21.1, 13.9. ESI-MS m/z 308.2 $[\text{M-H}]^-$.

Example 10

[0072] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (3,4-dimethylphenyl)-3- (propylamino)isobenzofuran-1 (3H)-one



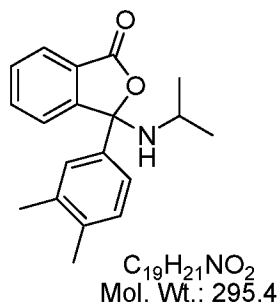
XH3057

[0073] Yield 24.7%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.59-7.57 (d, $J = 7.2$ Hz, 1H), 7.45-7.42 (t, $J = 8.0$ Hz, 1H), 7.38-7.34 (t, $J = 8.0$ Hz, 1H), 7.27-7.25 (d, $J = 7.2$ Hz, 1H), 7.13-7.07 (m, 3H), 4.12 (s, 1H), 3.37-3.30 (m, 1H), 2.90-2.82 (m, 1H), 2.24 (s, 3H), 2.22 (s, 3H), 1.51-1.47 (m, 1H), 1.40-1.34 (m, 1H), 0.79-0.75 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100

MHz, CDCl₃) δ (ppm) 167.9, 149.3, 136.8, 136.7, 136.0, 132.4, 130.4, 129.7, 129.1, 127.1, 123.6, 123.1, 122.5, 91.5, 41.3, 22.0, 19.9, 19.5. 11.7. ESI-MS m/z 294.1 [M-H]⁻.

Example 11

[0074] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (isopropylamino)-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one

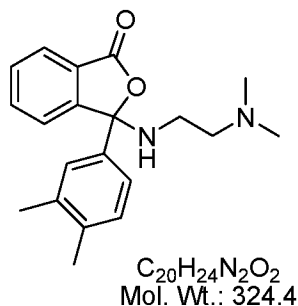


XH3058

[0075] Yield 19.6%. ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.68-7.66 (m, 1H), 7.41-7.38 (m, 2H), 7.20-7.14 (m, 3H), 7.09-7.07 (d, J = 8.0 Hz, 1H), 3.60-3.55 (m, 1H), 3.47 (br s, 1H), 2.24 (s, 3H), 2.22 (s, 3H), 1.42-1.41 (d, J = 6.8 Hz, 3H), 1.26-1.24 (d, J = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ (ppm) 167.4, 148.8, 136.7, 136.5, 136.0, 132.2, 131.6, 130.9, 130.1&129.9, 129.7&129.5, 129.3, 128.2&128.0, 127.7, 123.8, 122.9, 122.4, 91.9, 44.8, 22.3, 21.2, 19.8, 19.5. ESI-MS m/z 318.1 [M +Na]⁺.

Example 12

[0076] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (2- (dimethylamino)ethylamino)-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



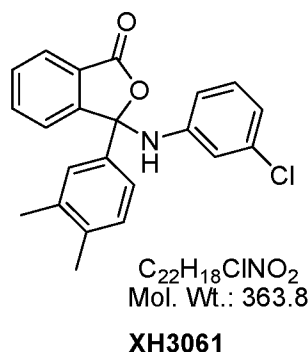
XH3060

[0077] Yield 23.6%. ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.81-7.80 (d, J = 6.8 Hz, 1H), 7.44-7.38 (m, 2H), 7.22-7.19 (m, 2H), 7.11-7.09 (m, 2H), 4.14-4.11 (d, J = 14.0 Hz, 1H), 2.82-2.73 (m, 2H), 2.28-2.18 (m, 14H). ¹³C NMR (100 MHz, CDCl₃) δ (ppm) 168.2,

150.1, 137.8, 136.8, 136.6, 132.6, 129.9, 128.6, 127.4, 126.3, 123.9, 123.3, 122.9, 122.2, 58.8, 44.6, 37.0, 19.9, 19.4. ESI-MS m/z 323.2 $[M-H]^-$.

Example 13

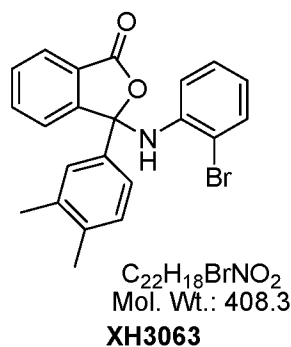
[0078] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (3-chlorophenylamino)-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



[0079] Yield 9.70%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.94-7.93 (d, J = 6.8 Hz, 1H), 7.69-7.65 (t, J = 6.8 Hz, 1H), 7.59-7.54 (m, 2H), 7.42-7.40 (m, 2H), 7.15-7.13 (d, J = 7.6 Hz, 1H), 7.02-6.98 (t, J = 8.0 Hz, 1H), 6.83-6.82 (d, J = 7.6 Hz, 1H), 6.73 (s, 1H), 6.59-6.57 (d, J = 7.2 Hz, 1H), 2.25 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.2, 143.6, 137.7, 134.8, 134.4, 130.5, 130.4, 129.8, 126.5, 126.1, 123.0, 122.7, 121.0, 118.3, 116.3, 20.0, 19.5. ESI-MS m/z 386.0 $[M + Na]^+$.

Example 14

[0080] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (2-bromophenylamino)-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one

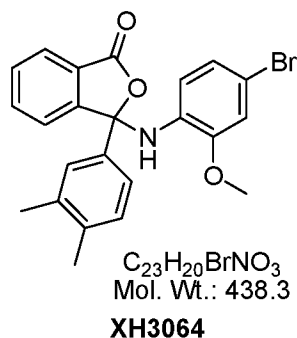


[0081] Yield 9.70%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.94-7.92 (d, J = 6.8 Hz, 1H), 7.68-7.64 (t, J = 7.6 Hz, 1H), 7.58-7.54 (m, 2H), 7.46-7.44 (d, J = 8.0 Hz, 1H), 7.38-7.36 (m, 2H), 7.12-7.10 (d, J = 8.4 Hz, 1H), 6.96-6.93 (t, J = 7.2 Hz, 1H), 6.76-6.74 (d, J =

6.8 Hz, 1H), 6.70-6.67 (t, $J = 7.6$ Hz, 1H), 2.22 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 169.3, 150.9, 140.0, 137.7, 135.8, 135.0, 132.3, 130.5, 130.4, 127.9, 126.5, 126.1, 125.5, 123.0, 122.5, 121.4, 118.5, 112.4, 20.0, 19.5. ESI-MS m/z 406.1, 408.1 $[\text{M}-\text{H}]^-$

Example 15

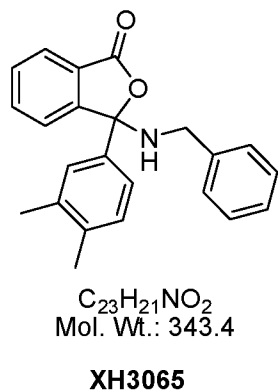
- 5 **[0082]** The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (4-bromo-2-methoxyphenylamino)-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



- [0083]** Yield 11.1%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.92-7.90 (d, $J = 8.0$ Hz, 1H), 7.66-7.63 (t, $J = 8.0$ Hz, 1H), 7.57-7.53 (m, 2H), 7.39-7.37 (m, 2H), 7.13-7.11 (d, $J = 8.4$ Hz, 1H), 6.90-6.89 (d, $J = 2.4$ Hz, 1H), 6.73-6.70 (dd, $J_1 = 8.8$ Hz, $J_2 = 1.6$ Hz, 1H), 6.37-6.34 (d, $J = 8.8$ Hz, 1H), 3.83 (s, 3H), 2.23 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 169.4, 151.0, 148.6, 137.7, 136.2, 134.8, 131.4, 130.5, 130.2, 126.5, 126.0, 125.6, 124.0, 123.8, 123.4, 123.0, 122.7, 117.5, 113.3, 112.0, 55.8, 20.0, 19.5. ESI-MS m/z 460.0, 462.0 $[\text{M} + \text{Na}]^+$.

Example 16

- [0084]** The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (benzylamino)-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



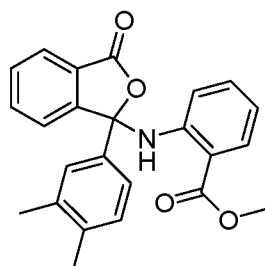
- 20 **[0085]** Yield 20.6%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.71-7.69 (d, $J = 3.6$ Hz, 1H), 7.44-7.36 (m, 2H), 7.25-7.23 (m, 1H), 7.15-7.08 (m, 5H), 7.03-7.01 (d, $J = 8.4$ Hz, 1H),

6.91 (s, 1H), 4.59-4.56 (d, $J = 15.6$ Hz, 1H), 4.08-4.04 (d, $J = 15.6$ Hz, 1H), 2.19 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 168.0, 149.2, 138.1, 136.8, 136.6, 135.5, 132.7, 130.1, 129.6, 129.3, 128.8, 128.0, 127.5, 126.9, 123.7, 123.3, 122.7, 91.7, 43.0, 19.8, 19.4. ESI-MS m/z 342.2 $[\text{M}-\text{H}]^-$.

5

Example 17

[0086] The following is an example of synthesis and characterization of a compound of the present disclosure. Methyl 2- (1,3-dihydro-1- (3,4-dimethylphenyl)-3-oxoisobenzofuran-1-ylamino)benzoate



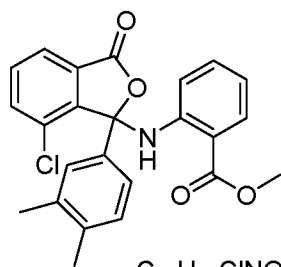
$\text{C}_{24}\text{H}_{21}\text{NO}_4$
Mol. Wt.: 387.4

XH3066

- 10 **[0087]** Yield 12.8%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 9.16 (br s, 1H), 8.00-7.91 (t, $J = 8.0$ Hz, 2H), 7.68-7.64 (t, $J = 7.6$ Hz, 1H), 7.57-7.53 (m, 2H), 7.34-7.33 (m, 2H), 7.18-7.16 (m, 1H), 7.10-7.08 (d, $J = 8.8$ Hz, 1H), 6.83-6.81 (d, $J = 8.8$ Hz, 1H), 6.75-6.72 (t, $J = 7.6$ Hz, 1H), 3.87 (s, 3H), 2.21 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 169.6, 169.0, 151.7, 146.6, 137.7, 137.4, 136.2, 135.2, 133.9, 131.2, 130.5, 130.2, 126.5, 126.0, 125.1, 122.9, 122.1, 118.1, 117.5, 112.8, 91.7, 51.9, 20.0, 19.4. ESI-MS m/z 388.1 $[\text{M}+\text{H}]^+$.
- 15

Example 18

[0088] The following is an example of synthesis and characterization of a compound of the present disclosure. Methyl-2-(4-chloro-1,3-dihydro-3-(3,4-dimethylphenyl)-1-oxoisobenzofuran-3-ylamino) benzoate



$\text{C}_{24}\text{H}_{20}\text{ClNO}_4$
Mol. Wt.: 421.9

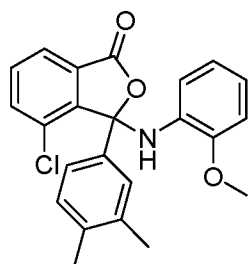
XH3069

20

[0089] Yield 17.9%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 9.44 (br s, 1H), 7.98-7.96 (d, $J = 7.2$ Hz, 1H), 7.91-7.89 (d, $J = 7.6$ Hz, 1H), 7.60-7.58 (d, $J = 7.2$ Hz, 1H), 7.54-7.51 (t, $J = 8.0$ Hz, 1H), 7.34-7.32 (d, $J = 7.6$ Hz, 1H), 7.24 (s, 1H), 7.16-7.12 (t, $J = 8.0$ Hz, 1H), 7.10-7.08 (d, $J = 8.8$ Hz, 1H), 6.77-6.73 (t, $J = 8.0$ Hz, 1H), 6.70-6.68 (d, $J = 8.4$ Hz, 1H), 3.89 (s, 3H), 2.23 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 169.0, 168.2, 146.6, 146.3, 137.8, 137.1, 136.3, 133.8, 132.9, 131.8, 131.3, 129.7, 129.3, 128.7, 127.9, 125.0, 124.6, 118.1, 117.2, 112.9, 91.9, 51.9, 20.0, 19.5. ESI-MS m/z 422.0 $[\text{M} + \text{H}]^+$.

Example 19

[0090] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (2-methoxyphenylamino)-4-chloro-3-*p*-tolylisobenzofuran-1 (3H)-one



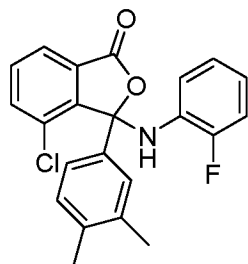
$\text{C}_{23}\text{H}_{20}\text{ClNO}_3$
Mol. Wt.: 393.9

XH3070

[0091] Yield 19.7%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.89-7.87 (d, $J = 7.2$ Hz, 1H), 7.58-7.50 (m, 2H), 7.39-7.37 (d, $J = 7.6$ Hz, 1H), 7.34 (s, 1H), 7.13-7.11 (d, $J = 8.0$ Hz, 1H), 6.84-6.76 (m, 2H), 6.61-6.57 (t, $J = 8.0$ Hz, 1H), 6.39-6.37 (d, $J = 7.6$ Hz, 1H), 3.87 (s, 3H), 2.25 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 168.1, 148.2, 146.1, 137.9, 137.0, 135.8, 134.0, 131.9, 131.7, 129.7, 129.5, 127.9, 124.7, 124.5, 120.6, 120.2, 116.2, 110.1, 55.7, 20.0, 19.5. ESI-MS m/z 394.1 $[\text{M} + \text{H}]^+$.

Example 20

[0092] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (2-fluorophenylamino)-4-chloro-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



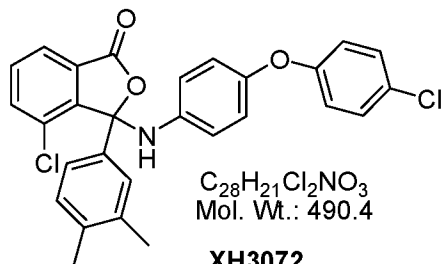
$C_{22}H_{17}ClFNO_2$
Mol. Wt.: 381.8

XH3071

[0093] Yield 29.8%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.90-7.88 (d, $J = 7.6$ Hz, 1H), 7.59-7.51 (m, 2H), 7.38-7.34 (m, 2H), 7.14-7.12 (d, $J = 8.0$ Hz, 1H), 7.05-6.99 (m, 1H), 6.79-6.75 (m, 2H), 6.55-6.51 (m, 1H), 2.25 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.8, 154.1, 151.7, 145.5, 138.2, 137.2, 136.0, 133.3, 132.0, 130.7&130.6, 129.8, 129.3, 124.6&124.6 (1C), 124.0&124.0 (1C), 121.0&120.9 (1C), 115.0, 114.8, 20.0, 19.5. ESI-MS m/z 382.0 $[M + H]^+$.

Example 21

[0094] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (4- (4-chlorophenoxy)phenylamino)-4-chloro-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



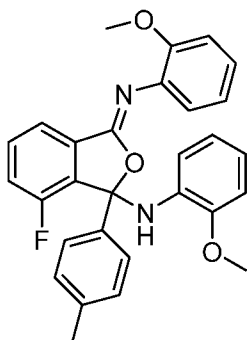
$C_{28}H_{21}Cl_2NO_3$
Mol. Wt.: 490.4

XH3072

[0095] Yield 18.2%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.77-7.75 (d, $J = 6.8$ Hz, 1H), 7.51-7.44 (m, 2H), 7.28-7.2 (m, 3H), 7.19-7.16 (d, $J = 8.8$ Hz, 2H), 7.04-6.99 (m, 3H), 6.92-6.89 (d, $J = 8.8$ Hz, 2H), 6.85-6.82 (d, $J = 8.4$ Hz, 2H), 2.20 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.9, 155.8, 144.0, 137.1, 136.4, 134.2, 133.9, 133.0, 131.3, 130.1, 129.7, 129.4, 128.8, 127.5, 123.9, 122.3, 120.4, 118.6, 19.9, 19.5. ESI-MS m/z 513.0 $[M + Na]^+$.

Example 22

[0096] The following is an example of synthesis and characterization of a compound of the present disclosure. 3,4-bis (2-methoxyphenylamino)-3-p-tolylisobenzofuran-1 (3H)-one



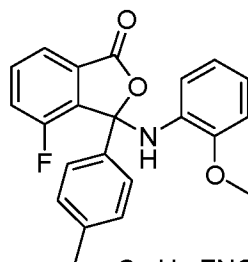
Chemical Formula: $C_{29}H_{25}FN_2O_3$
Molecular Weight: 468.53

XH3074-1

[0097] Yield 10.7%. 1H NMR (Acetone- d_6) δ (ppm) 7.79-7.77 (d, $J = 7.2$ Hz, 1H), 7.70-7.67 (m, 1H), 7.33-7.29 (t, $J = 8.4$ Hz, 2H), 7.10-7.00 (m, 5H), 6.92-6.84 (m, 3H), 6.72-6.69 (t, $J = 8.0$ Hz, 1H), 6.60-6.56 (t, $J = 8.0$ Hz, 1H), 6.40-6.38 (d, $J = 8.0$ Hz, 1H). 5.62 (br s, 1H), 3.85 (s, 3H), 3.17 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.3, 158.3, 156.3, 155.8, 148.0, 138.6, 136.3, 135.9, 134.1, 132.2, 131.6&131.5, 130.6, 129.7, 128.7, 125.6, 123.1, 121.0, 120.4, 120.0, 119.7, 119.5, 119.0, 113.8, 111.7, 109.8, 81.9, 55.8, 55.2, 21.0. ESI-MS m/z 491.1 $[M + Na]^+$.

Example 23

- 10 [0098] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (2-methoxyphenylamino)-4-fluoro-3-p-tolylisobenzofuran-1 (3H)-one



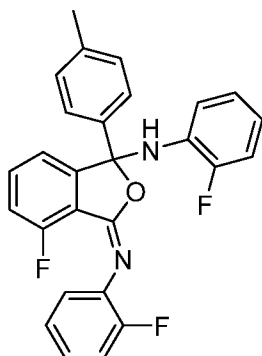
$C_{22}H_{18}FNO_3$
Mol. Wt.: 363.4

XH3074-2

- 15 [0099] Yield 31.5%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.76-7.74 (d, $J = 7.6$ Hz, 1H), 7.57-7.53 (m, 1H), 7.32-7.28 (m, 1H), 7.25-7.20 (m, 3H), 7.09-7.07 (d, $J = 8.0$ Hz, 1H), 7.00-6.98 (d, $J = 8.0$ Hz, 1H), 6.85-6.81 (t, $J = 7.6$ Hz, 1H), 6.71-6.69 (d, $J = 7.6$ Hz, 1H), 3.79 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 158.3, 156.0, 155.7, 131.9&131.9, 131.5, 130.1, 129.5&129.4, 18.8, 126.0, 121.3, 120.5, 120.3, 119.8&119.8, 56.1, 21.2. ESI-MS m/z 386.0 $[M + Na]^+$.

Example 24

[0100] The following is an example of synthesis and characterization of a compound of the present disclosure. 3,4-bis (2-fluorophenylamino)-3-p-tolylisobenzofuran-1 (3H)-one



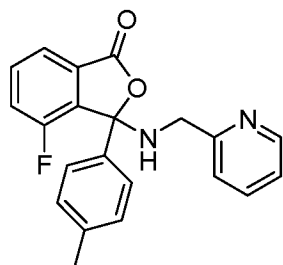
Chemical Formula: C₂₇H₁₉F₃N₂O
Molecular Weight: 444.46

XH3075

- 5 [0101] Yield 14.1%. ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.93-7.91 (d, *J* = 7.6 Hz, 1H), 7.62-7.58 (m, 1H), 7.36-7.31 (m, 1H), 7.24-7.20 (m, 2H), 7.17-7.10 (m, 5H), 7.08-7.03 (m, 2H), 6.80-6.76 (m, 2H), 6.60-6.54 (m, 1H), 5.25-5.24 (d, *J* = 4.8 Hz, 1H), 2.36 (s, 3H).
¹³C NMR (100 MHz, CDCl₃) δ (ppm) 165.1, 160.3, 158.2, 157.8, 155.6, 153.8, 151.4, 139.4, 135.4, 134.7, 132.3, 132.1, 132.1, 130.1, 130.0&130.0, 129.5, 125.1, 124.5&124.5,
10 124.3&124.3, 122.2&122.1, 120.3, 120.1&120.1, 116.5&116.3, 115.8, 114.8&114.7, 81.9, 21.1. ESI-MS *m/z* 467.1 [M + Na]⁺.

Example 25

[0102] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- ((pyridin-2-yl)methylamino)-4-fluoro-3-p-tolylisobenzofuran-1 (3H)-one



C₂₁H₁₇FN₂O₂
Mol. Wt.: 348.4

XH3076

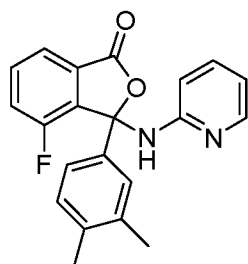
- [0103] Yield 82.9%. ¹H NMR (400 MHz, CDCl₃) δ (ppm) 9.20 (br s, 1H), 8.41-8.39 (d, *J* = 4.4 Hz, 1H), 7.69-7.65 (td, *J*₁ = 8.0 Hz, *J*₂ = 1.6 Hz, 1H), 7.59-7.57 (d, *J* = 7.6 Hz, 1H),

7.42-7.38 (m, 4H), 7.21-7.11 (m, 4H), 5.01-4.97 (d, $J = 16.4$ Hz, 1H), 4.16-4.12 (d, $J = 16.4$ Hz, 1H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 166.9, 158.2, 156.1, 155.7, 148.0, 138.4, 138.0, 136...2, 132.6, 131.3 & 131.2, 129.3, 126.3, 122.8&122.8, 120.3, 120.1, 119.2, 89.5, 44.6, 21.1. ESI-MS m/z 371.1 $[\text{M} + \text{Na}]^+$.

5

Example 26

[0104] The following is an example of synthesis and characterization of a compound of the present disclosure. 4-fluoro-3- (3,4-dimethylphenyl)-3- (pyridin-2-ylamino)isobenzofuran-1 (3H)-one



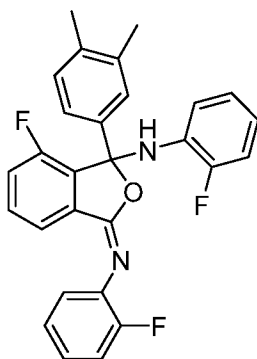
$\text{C}_{21}\text{H}_{17}\text{FN}_2\text{O}_2$
Mol. Wt.: 348.4

XH3079

- 10 **[0105]** Yield 5.52%. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 8.42-8.40 (d, $J = 7.6$ Hz, 1H), 8.15-8.14 (d, $J = 4.0$ Hz, 1H), 7.78-7.75 (m, 2H), 7.50-7.45 (m, 1H), 7.28-7.24 (m, 2H), 7.21-7.17 (t, $J = 8.4$ Hz, 1H), 7.02-6.98 (m, 2H), 2.16 (s, 6H). ^{13}C NMR (100 MHz, CDCl_3) δ (ppm) 165.9, 158.0&155.5 (1C), 151.4, 146.5, 138.6, 137.5, 136.6&136.6 (1C), 136.6&131.6 (1C), 129.6, 126.9, 123.2, 121.2 & 121.1 (1C), 120.0, 120.0, 116.5, 93.2, 19.9, 19.4. ESI-MS
- 15 m/z 371.0 $[\text{M} + \text{Na}]^+$.

Example 27

[0106] The following is an example of synthesis and characterization of a compound of the present disclosure. 3,4-bis (2-fluorophenylamino)-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



Chemical Formula: $C_{28}H_{21}F_3N_2O$

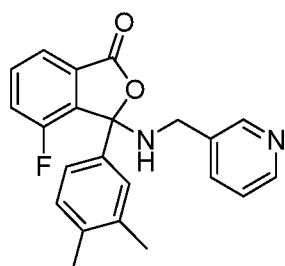
Molecular Weight: 458.48

XH3080

[0107] Yield 31.0%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.88-7.86 (d, $J = 7.2$ Hz, 1H), 7.57-7.52 (m, 1H), 7.30-7.26 (m, 1H), 7.18-7.14 (m, 2H), 7.10-6.97 (m, 6H), 6.76-6.70 (m, 2H), 6.51-6.47 (m, 1H), 5.21-5.20 (d, $J = 4.8$ Hz, 1H), 2.22 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.1, 160.3, 158.1, 157.8, 155.6, 153.8, 151.4, 138.0, 137.2, 135.7, 134.6, 132.2, 132.0, 130.0, 129.9, 129.8, 126.4, 124.5, 124.2, 122.6, 120.3, 120.1, 120.0, 116.5, 116.3, 115.8, 114.8, 114.6, 81.9, 19.9, 19.5.

Example 28

[0108] The following is an example of synthesis and characterization of a compound of the present disclosure. 3-((pyridin-3-yl)methylamino)-4-fluoro-3-(3,4-dimethylphenyl)isobenzofuran-1(3H)-one



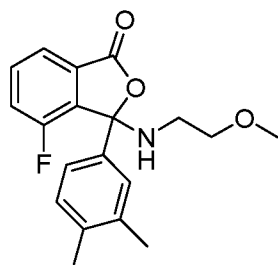
$C_{22}H_{19}FN_2O_2$
Mol. Wt.: 362.4

XH3084

[0109] Yield 68.8%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.19 (br s, 1H), 7.53-7.51 (d, $J = 6.8$ Hz, 2H), 7.45-7.38 (m, 2H), 7.22-7.20 (d, $J = 7.6$ Hz, 1H), 7.15 (s, 1H), 7.13-7.09 (t, $J = 7.6$ Hz, 2H), 6.94-6.91 (m, 1H), 4.64-4.60 (d, $J = 15.6$ Hz, 1H), 4.04-4.00 (d, $J = 15.6$ Hz, 1H), 2.24 (s, 3H), 2.19 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.6, 158.2 & 155.6 (1C,C-F), 148.2, 146.0, 137.6, 137.2, 135.3 & 135.2 (1C), 135.1, 134.2, 133.2, 131.6 & 131.5 (1C), 129.8, 127.3, 123.8, 123.2, 120.3 & 120.1 (1C), 119.1, 90.6, 40.6, 19.9, 19.5. ESI-MS m/z 363.1 $[M + H]^+$.

Example 29

[0110] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (2-methoxyethylamino)-4-fluoro-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



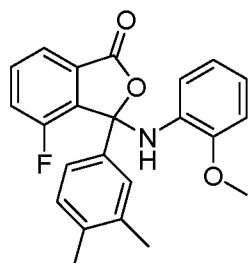
$C_{19}H_{20}FNO_3$
Mol. Wt.: 329.4

XH3087

[0111] Yield 61.4%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.64-7.62 (d, $J = 7.2$ Hz, 1H), 7.45-7.40 (m, 1H), 7.16-7.09 (m, 4H), 6.07 (br s, 1H), 4.02-3.99 (d, $J = 14.0$ Hz, 1H), 3.67-3.62 (t d, $J_1 = 10.0$ Hz, $J_2 = 1.6$ Hz, 1H), 3.38 (s, 3H), 3.01-2.93 (m, 1H), 2.23 (s, 3H), 2.22 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.9, 158.1, 155.6, 137.1 & 136.9 (1C), 135.7, 134.9 & 134.8 (1C), 132.8, 131.4 & 131.3 (1C), 129.9, 127.2, 123.8, 120.2 & 120.0 (1C), 119.2 & 119.2 (1C), 89.4, 70.9, 58.9, 39.3, 19.9, 19.5. ESI-MS m/z 352.1[M + Na] $^+$.

Example 30

[0112] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (2-methoxyphenylamino)-4-fluoro-3- (3,4-dimethylphenyl)isobenzofuran-1 (3H)-one



$C_{23}H_{20}FNO_3$
Mol. Wt.: 377.4

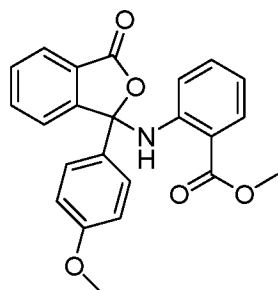
XH3088

[0113] Yield 98.8%. 1H NMR (400 MHz, $DMSO-d_6$) δ (ppm) 7.68-7.62 (m, 2H), 7.51 (br s, 1H), 7.43-7.39 (m, 1H), 7.27-7.23 (t, $J = 7.6$ Hz, 1H), 7.07-7.04 (m, 2H), 6.97-6.93 (t, $J = 7.6$ Hz, 2H), 6.90-6.86 (t, $J = 7.2$ Hz, 1H), 3.49 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, $DMSO-d_6$) δ (ppm) 164.7, 158.1, 156.2, 155.6, 136.3, 136.1, 135.5, 133.9,

132.5, 130.4, 129.6, 129.0, 127.5, 124.1, 120.6, 120.4, 120.2, 119.5, 112.6, 90.9, 55.8, 19.9, 19.5. ESI-MS m/z 400.1 $[M + Na]^+$.

Example 31

[0114] The following is an example of synthesis and characterization of a compound of the present disclosure. Methyl 2- (1,3-dihydro-1- (4-methoxyphenyl)-3-oxoisobenzofuran-1-ylamino)benzoate



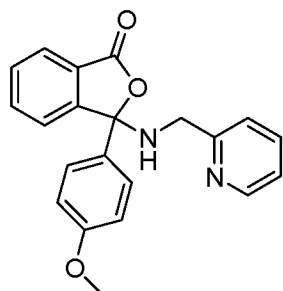
$C_{23}H_{19}NO_5$
Mol. Wt.: 389.4

XH3090

[0115] Yield 43.9%. 1H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.15 (br s, 1H), 7.93-7.89 (t, J = 8.4 Hz, 2H), 7.66-7.62 (t, J = 7.2 Hz, 1H), 7.53-7.47 (m, 4H), 7.15-7.11 (t, J = 8.0 Hz, 1H), 6.84-6.79 (m, 3H), 6.73-6.69 Hz, 1H), 3.84 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.5, 169.0, 159.9, 151.6, 146.5, 135.3, 133.9, 131.2, 130.7, 130.2, 126.9, 1326.0, 125.1, 122.1, 118.2, 117.5, 114.6, 112.9, 96.4, 55.2, 51.9. ESI-MS m/z 412.0 $[M + Na]^+$.

Example 32

[0116] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- ((pyridin-2-yl)methylamino)-3- (4-methoxyphenyl)isobenzofuran-1 (3H)-one



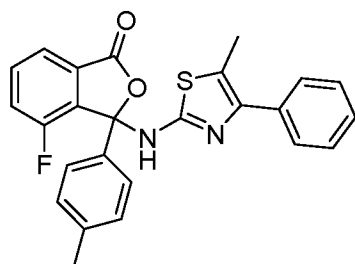
$C_{21}H_{18}N_2O_3$
Mol. Wt.: 346.4

XH3094

[0117] Yield 46.0%. ¹H NMR (400 MHz, CDCl₃) δ (ppm) 8.44-8.42 (d, *J* = 4.8 Hz, 1H), 7.79-7.77 (d, *J* = 7.2 Hz, 1H), 7.74-7.71 (t, *J* = 7.6 Hz, 1H), 7.53-7.50 (t, *J* = 7.6 Hz, 1H), 7.45-7.42 (m, 4H), 7.35-7.33 (d, *J* = 7.6 Hz, 1H), 7.24-7.22 (m, 1H), 6.89-6.88 (d, *J* = 8.4 Hz, 2H), 5.06-5.02 (d, *J* = 12.0 Hz, 1H), 4.19-4.15 (d, *J* = 12.0 Hz, 1H), 3.80 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ (ppm) 168.1, 159.6, 156.5, 147.9, 138.1, 132.9, 132.2, 129.4, 128.8, 127.8, 123.3, 122.9, 122.8, 122.6, 113.9, 90.4, 55.3, 44.6. ESI-MS *m/z* 369.0 [M + Na]⁺.

Example 33

[0118] The following is an example of synthesis and characterization of a compound of the present disclosure. 3- (5-methyl-4-phenylthiazol-2-ylamino)-4-fluoro-3-p-tolylisobenzofuran-1 (3H)-one



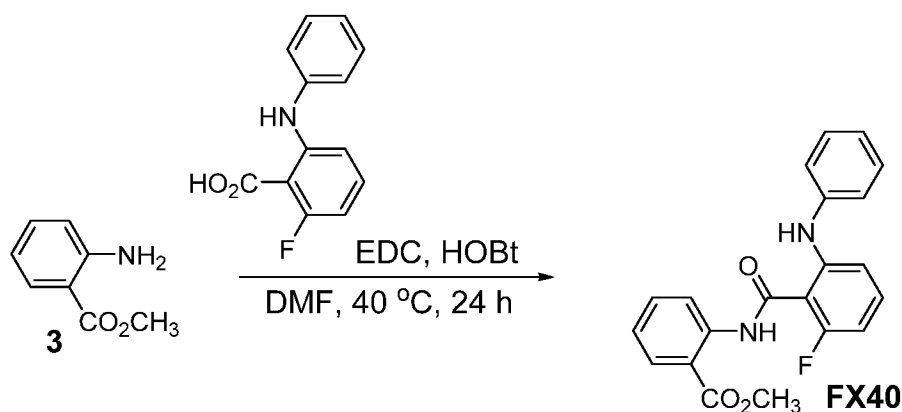
C₂₅H₁₉FN₂O₂S
Mol. Wt.: 430.5

XH10165-1

[0119] Yield 38.3%. ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.80-7.78 (d, *J* = 7.2 Hz, 1H), 7.56-7.51 (m, 1H), 7.43-7.41 (d, *J* = 8.0 Hz, 2H), 7.40-7.35 (m, 4H), 7.32-7.30 (m, 1H), 7.26-7.21 (t, *J* = 8.8 Hz, 1H), 7.16-7.14 (d, *J* = 8.0 Hz, 2H), 2.51 (s, 3H), 2.31 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ (ppm) 164.1, 158.4, 155.9, 152.0, 144.9, 138.5, 136.3, 134.4, 132.2 & 132.2 (1C), 131.3, 129.3, 128.3, 128.1, 127.5, 125.4, 122.3, 121.7, 121.5, 120.3, 92.9, 21.1, 12.3. ESI-MS *m/z* 431.0 [M + H]⁺.

Example 34

[0120] The following is an example of synthesis and characterization of a compound of the present disclosure.



[0121] To a solution of compound **3** in DMF was added 2-fluoro-6-(phenylamino)benzoic acid, followed by EDC and HOBT. The reaction mixture was allowed to sit at 40 °C for 24 h, and then concentrated. After removing the solvent, the crude material was purified by flash column chromatograph to get the final compound.

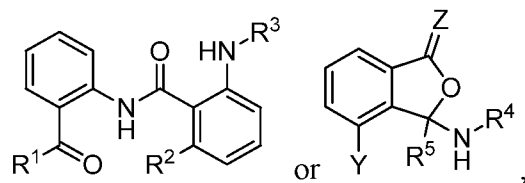
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- 35

What is claimed is:

1. A compound having the following structure:

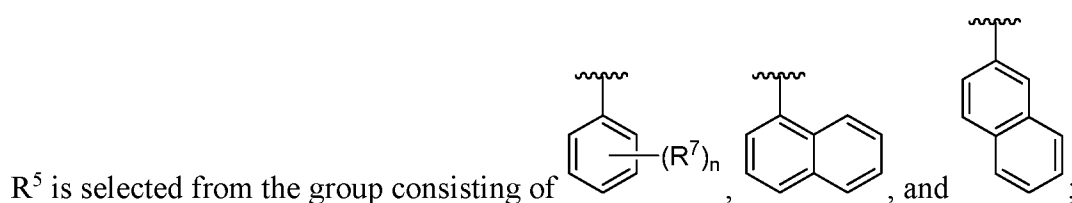


wherein R¹ is selected from the group consisting of -OH, -OMe, and -NHR⁶, wherein R⁶ is selected from the group consisting of C₁-C₅ alkyl, C₃-C₆ cycloalkyl, substituted or unsubstituted pyridine, and phenyl;

R² is selected from the group consisting of -H, -F, -Cl, -OMe, and -OH;

R³ is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl, and substituted or unsubstituted pyridine;

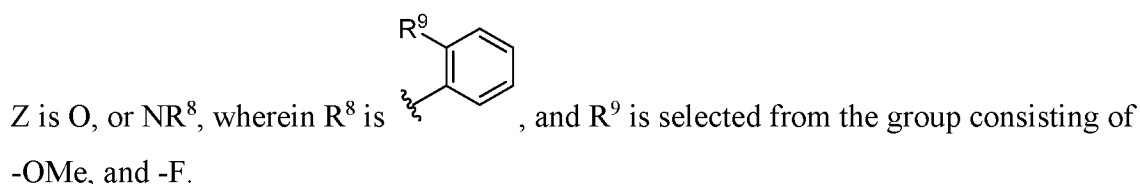
10 R⁴ is selected from the group consisting of substituted or unsubstituted C₁-C₈ alkyl, C₄-C₆ cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted alkyl amine, naphthyl, and heterocyclic;



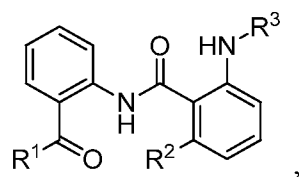
R⁷ is selected from the group consisting of halogen, phenyl, and alkyl;

15 n is 0-5;

Y is selected from the group consisting of -H, -F, -Cl, and -Me; and



20 2. The compound of claim 1, wherein the compound has the following structure:

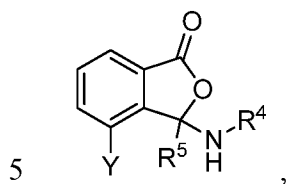


wherein R¹ is selected from the group consisting of -OH, -OMe, and -NHR⁶, wherein R⁶ is selected from the group consisting of C₁-C₅ alkyl, C₃-C₆ cycloalkyl, substituted or unsubstituted pyridine, and phenyl;

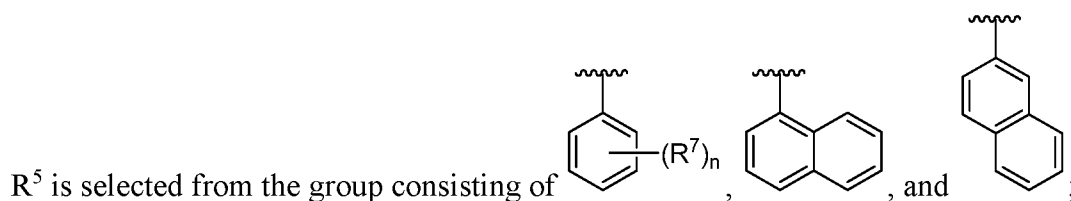
R^2 is selected from the group consisting of -H, -F, -Cl, -OMe, and -OH;

R^3 is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl and pyridine.

3. The compound of claim 1, wherein the compound has the following structure:



wherein R^4 is selected from the group consisting of C₁-C₈ alkyl, C₄-C₆ cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted alkyl amine, substituted or unsubstituted naphthyl and heterocyclic;

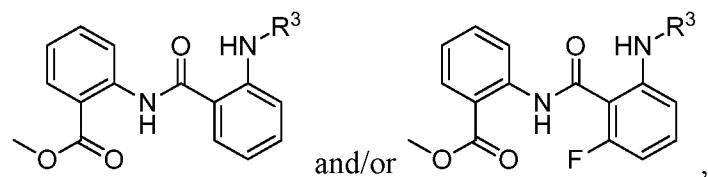


10 R^7 is selected from the group consisting of halogen, phenyl, and alkyl;

n is 0-5; and

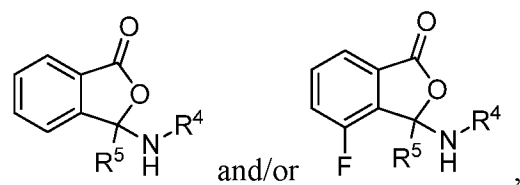
Y is selected from the group consisting of -H, -F, -Cl, and -Me.

4. The compound of any one of claims 1 or 2, wherein the compound has the following structure:

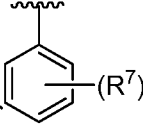
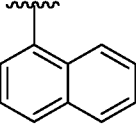
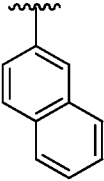


wherein R^3 is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl and pyridine.

5. The compounds of any one of claims 1 or 3, wherein the compounds have the following structures:

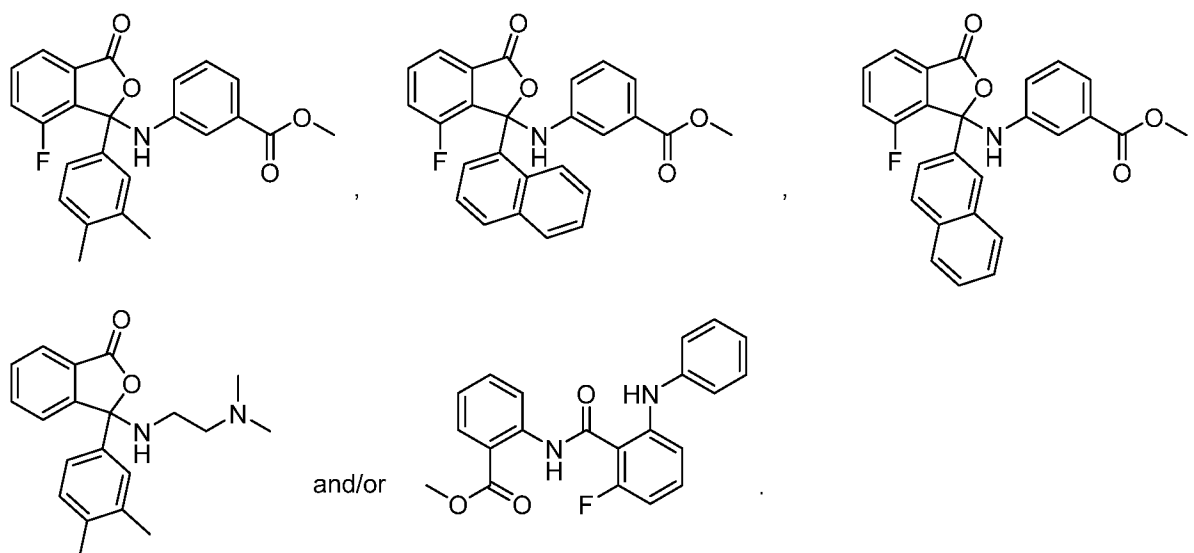


wherein R^4 is selected from the group consisting of C_1 - C_8 alkyl, C_4 - C_6 cycloalkyl, substituted or unsubstituted phenyl substituted or unsubstituted naphthyl, substituted or unsubstituted alkyl amine, and heterocyclic;

R^5 is selected from the group consisting of , , and ;

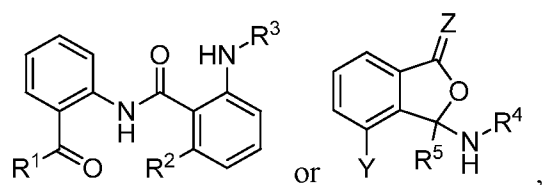
5 R^7 is selected from the group consisting of halogen, phenyl, and alkyl; and
n is 0-5.

6. The compound of claim 1, wherein the compound has the following structure:



10

7. A method for inhibiting transport of heme across a membrane in a parasitic heme auxotrophic organism comprising contacting the parasitic organism with an effective amount of compound having the following structure:



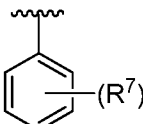
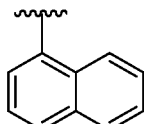
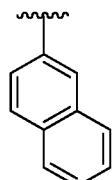
15 wherein R^1 is selected from the group consisting of -OH, -OMe, and -NHR⁶, wherein R^6 is selected from the group consisting of C_1 - C_5 alkyl, C_3 - C_6 cycloalkyl, substituted or unsubstituted pyridine, and phenyl;

R^2 is selected from the group consisting of -H, -F, -Cl, -OMe, and -OH;

R^3 is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl, and substituted or unsubstituted pyridine;

R^4 is selected from the group consisting of substituted or unsubstituted C₁-C₈ alkyl, C₄-C₆ cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted alkyl amine,

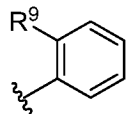
5 naphthyl, and heterocyclic;

R^5 is selected from the group consisting of , , and ;

R^7 is selected from the group consisting of halogen, phenyl, and alkyl;

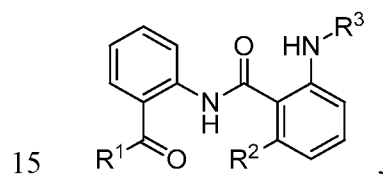
n is 0-5;

Y is selected from the group consisting of -H, -F, -Cl, and -Me; and

10 Z is O, or NR⁸ wherein R⁸ is , and R⁹ is selected from the group consisting of -OMe, and -F,

such that the transport of the heme across the membrane is inhibited or prevented.

8. The method of claim 7, wherein the compound has the following structure:

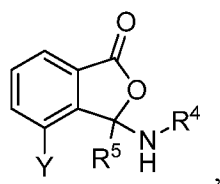


wherein R¹ is selected from the group consisting of -OH, -OMe, and -NHR₆, wherein R⁶ is selected from the group consisting of C₁-C₅ alkyl, C₃-C₆ cycloalkyl, substituted or unsubstituted pyridine, and phenyl;

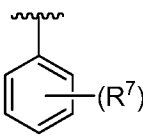
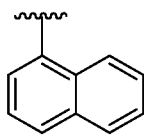
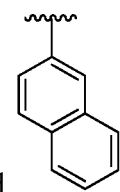
R² is selected from the group consisting of -H, -F, -Cl, -OMe, and -OH; and

20 R³ is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl and pyridine.

9. The method of claim 7, wherein the compound has the following structure:



wherein R^4 is selected from the group consisting of C_1 - C_8 alkyl, C_4 - C_6 cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted alkyl amine, substituted or unsubstituted naphthyl and heterocyclic;

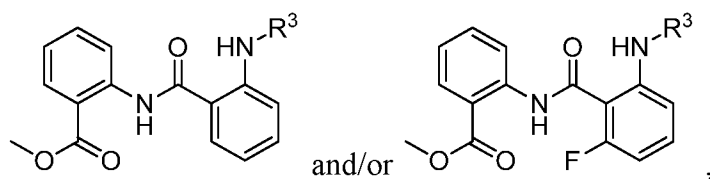
R^5 is selected from the group consisting of , , and ;

5 R^7 is selected from the group consisting of halogen, phenyl, and alkyl;

n is 0-5; and

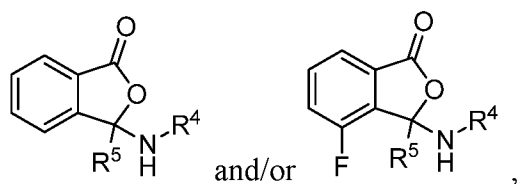
Y is selected from the group consisting of -H, -F, -Cl, and -Me.

10 10. The method of any one of claims 7 or 8, wherein the compound has the following structure:

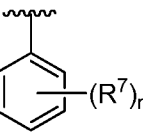
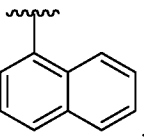
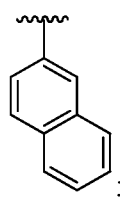


wherein R^3 is selected from the group consisting of C_4 - C_6 cycloalkyl, phenyl and pyridine.

15 11. The method of any one of claims 7 or 9, wherein the compounds have the following structures:



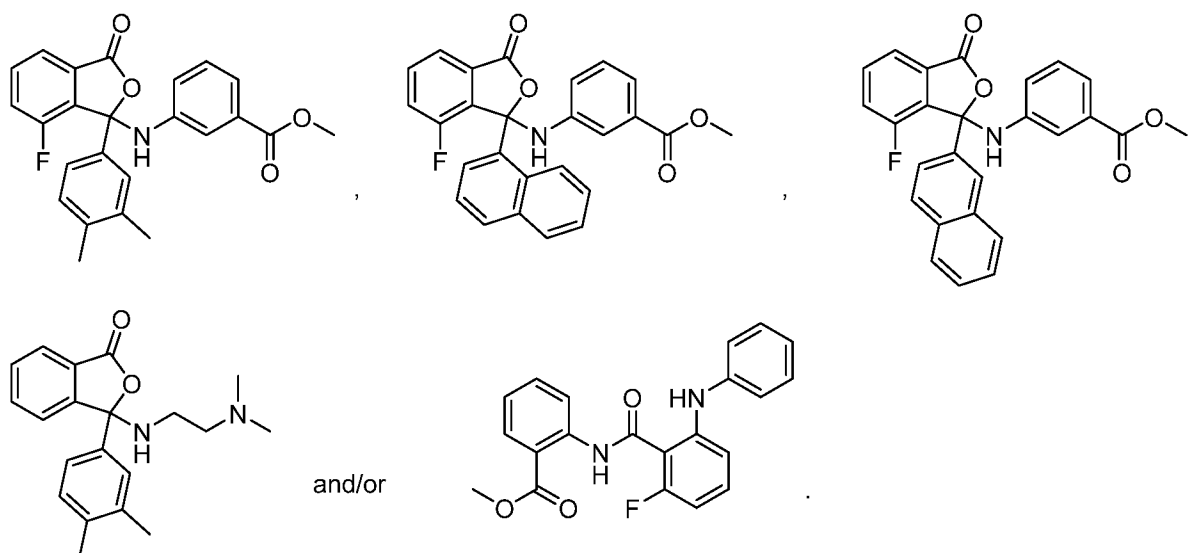
wherein R^4 is selected from the group consisting of C_1 - C_8 alkyl, C_4 - C_6 cycloalkyl, substituted or unsubstituted phenyl substituted or unsubstituted naphthyl, substituted or unsubstituted alkyl amine, and heterocyclic;

20 R^5 is selected from the group consisting of , , and ;

R^7 is selected from the group consisting of halogen, phenyl, and alkyl; and

n is 0-5.

12. The method of claim 7, wherein the compound has the following structure:



13. The method of claim 7, wherein the parasitic heme auxotrophic organism is

5 *Leishmania*.

14. The method of claim 13, wherein *Leishmania* is a promastigote or an amastigote.

15. The method of any one of claims 7, 8, 9 or 12, wherein the inhibiting the transport of
10 the heme is lethal to the parasitic heme auxotrophic organism.

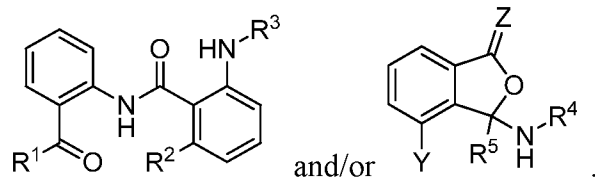
16. The method of claim 10, wherein the inhibiting the transport of the heme is lethal to the parasitic heme auxotrophic organism.

15 17. The method of claim 11, wherein the inhibiting the transport of the heme is lethal to the parasitic heme auxotrophic organism.

18. The method of claim 13, wherein the inhibiting the transport of the heme is lethal to the parasitic heme auxotrophic organism

20

19. A kit comprising a compound having the following structure:



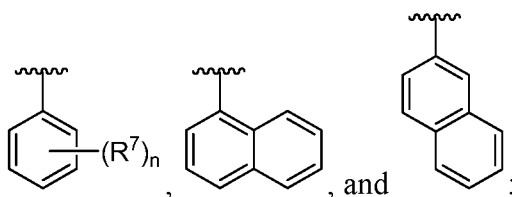
wherein R^1 is selected from the group consisting of -OH, -OMe, and -NHR⁶, wherein R^6 is selected from the group consisting of C₁-C₅ alkyl, C₃-C₆ cycloalkyl, substituted or unsubstituted pyridine, and phenyl;

5 R^2 is selected from the group consisting of -H, -F, -Cl, -OMe, and -OH;

R^3 is selected from the group consisting of C₄-C₆ cycloalkyl, phenyl, and substituted or unsubstituted pyridine;

R^4 is selected from the group consisting of substituted or unsubstituted C₁-C₈ alkyl, C₄-C₆ cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted alkyl amine,

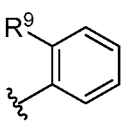
10 naphthyl, and heterocyclic;

R^5 is selected from the group consisting of , and

R^7 is selected from the group consisting of halogen, phenyl, and alkyl;

n is 0-5;

Y is selected from the group consisting of -H, -F, -Cl, and -Me; and

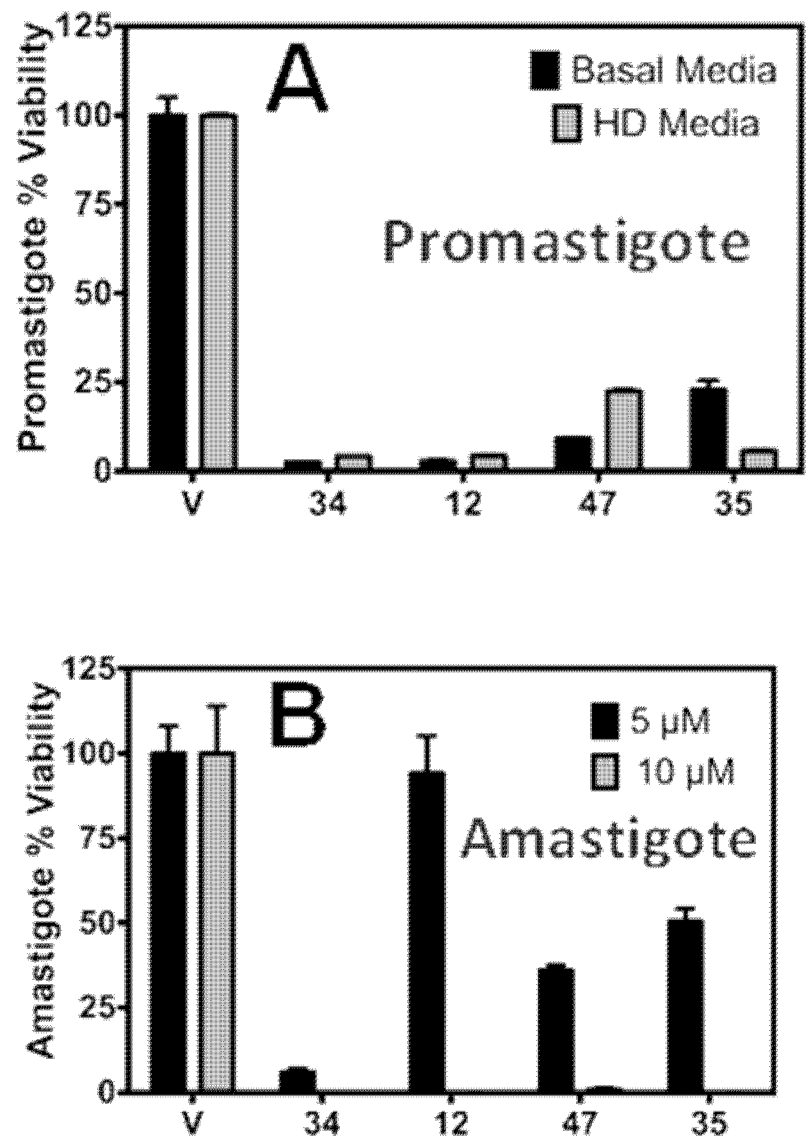
15 Z is O, or NR⁸ wherein R^8 is , and R^9 is selected from the group consisting of -OMe, and -F.

20. The kit of claim 19, further comprising instructions for using the compounds to inhibit the growth of or kill a parasitic heme auxotrophic organism.

20

21. The kit of claim 19, wherein the instructions comprise an indication that the parasitic heme auxotrophic organism is *Leishmania*.

Figure 1



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Figure 2

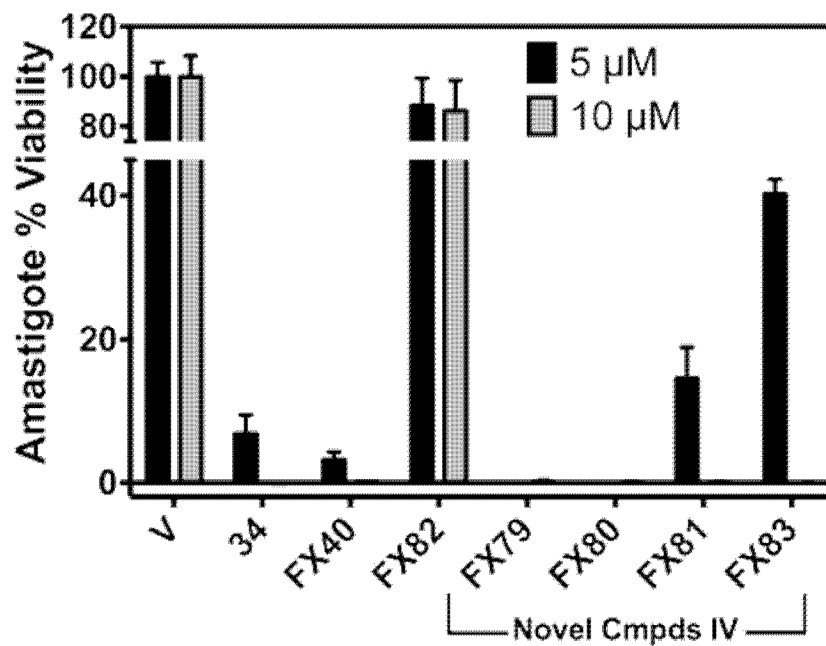
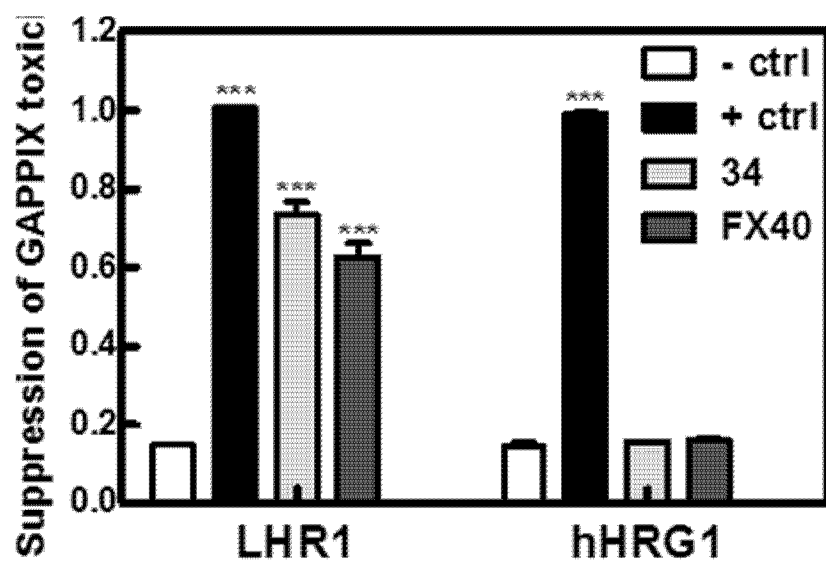


Figure 3



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Figure 4

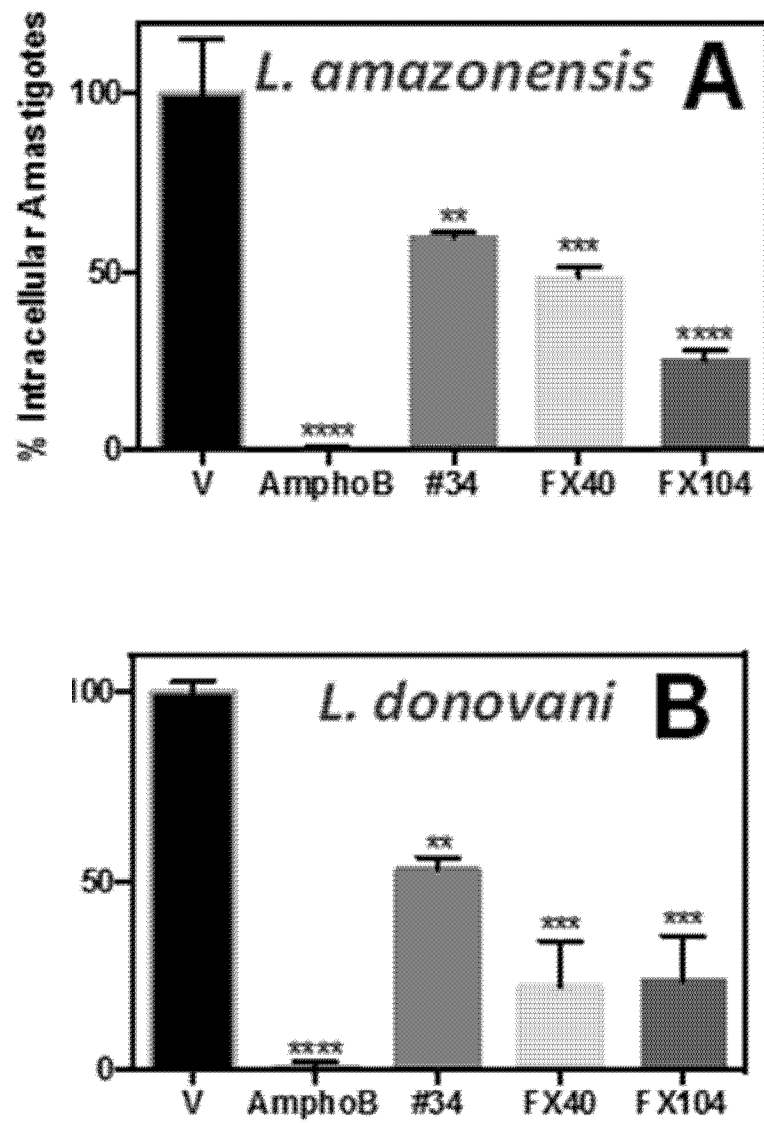
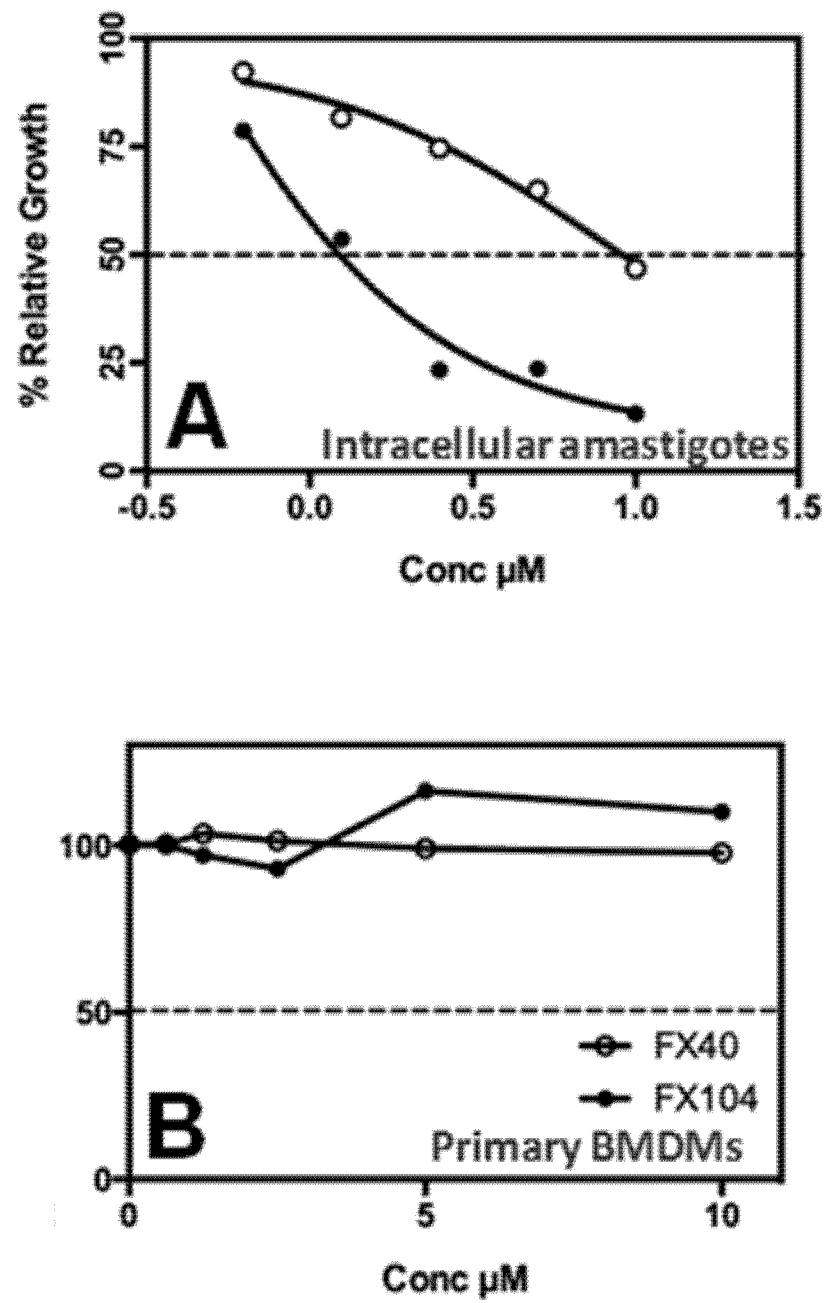


Figure 5



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/31234

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: Claims 1-6 and 19-21, directed to a compound selected from the structures of claim 1 and a kit containing the same. The compound and kit will be searched to the extent that they encompass the first species of claim 1, represented by the first structure of claim 1, wherein R1 is OH; R2 is H and R3 is C4-cycloalkyl. It is believed that claims 1-2, 4 and 19-21 read on this first named invention, and thus these claims will be searched without fee to the extent that they encompass the compound described above.

--Please see attached sheet--

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-2, 4 and 19-21

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/31234

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 37/18; C07C 237/20 (2016.01)

CPC - C07C 237/20; A01N 37/18

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)

IPC(8): A01N 37/18; C07C 237/20 (2016.01)

CPC: C07C237/20; A01N37/18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/617; 514/619; 514/621

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

PatBase, Google Scholar, PubWEST, SureChem, PubChem

benzanilide, 2-((cyclobutylamino)benzoylamino)benzoic acid, parasite, Leishmania, infection, treat, anti-parasitic, heme transport, inhibit

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2013/0210915 A1 (WADELL et al.) 15 August 2013 (15.08.2013) para [0010], [0033], [0046], [0125], [0130], [0158], [0166]-[0168], pg 22, Table 4	1-2, 4 ----- 19 ----- 20-21
Y --- A	US 2007/0099919 A1 (RANA) 03 May 2007 (03.05.2007) para [0005]-[0006]; Fig 9L; Fig 9M; para [0212], [0221]	19 ----- 20-21
A	US 2009/0305886 A1 (LANGEWALD et al.) 10 December 2009 (10.12.2009) para [0001], [0028], [0055]-[0063], Table 1; para [0143]	20-21
A	US 2005/0165068 A1 (LEPAPE) 28 July 2005 (28.07.2005) para [0069], [0075], [0079]-[0083]	20-21
A	US 2010/0062937 A1 (WADA et al.) 11 March 2010 (11.03.2010) Entire Document	1-2, 4, 19-21
A	US 3,519,678 A (FARRINGTON et al.) 07 July 1970 (07.07.1970) Entire Document	1-2, 4, 19-21
A	US 2006/0206946 A1 (HAMZA) 14 September 2006 (14.09.2006) Entire Document	1-2, 4, 19-21
A	PubChem-CID 2327258, Create Date: 15 July 2005, page 3	1-2, 4, 19-21

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"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

20 September 2016 (20.09.2016)

Date of mailing of the international search report

06 OCT 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/31234

Attachment to Box.No.III:

Applicant is invited to elect additional compounds, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched.

Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a compound having the second structure of claim 1, wherein Y is F; Z is O; R5 is phenyl substituted with (R7)_n, where n is 2 and R7 is C1-alkyl; and wherein R4 is substituted phenyl (i.e., claims 1, 3, 5, 6 and 19-21).

Group II: Claims 7-18, directed to a method for inhibiting transport of heme across a membrane in a parasitic heme auxotrophic organism comprising contacting the parasitic organism with an effective amount of a compound selected from the structures of claim 7.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I+ includes the technical feature of a unique compound, which is not required by any other invention of Group I+.

Group II includes the technical feature of a method of inhibiting transport of heme across a membrane in a parasitic heme auxotrophic organism, which is not required by Group I+.

Common technical features:

The inventions of Group I+ share the technical feature of a compound of of claim 1.

Groups I+ and II also share the technical feature of a compound having a structure of those listed in claim 1 (the same structures and description as in claim 7).

This shared technical feature, however, is not a contribution over the prior art as being anticipated by PubChem-CID 2327258 (Create Date: 15 July 2005), which discloses a compound of the first structure in claim 1, wherein R1 is OH; R2 is H and R3 is phenyl (pg 3, Fig). As said compound was known in the art at the time of the invention, this cannot be considered a special technical feature which would otherwise unify the inventions of Group I+ or those of Group I+ and II.

The inventions of Group I+ and II, thus lack unity under PCT Rule 13.