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Synthesis and Antileishmanial Evaluation of Arylimidamide-Azole Hybrids Containing a Phenoxyalkyl Linker

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Abstract

Due to the limitations of existing medications, there is a critical need for new drugs to treat visceral leishmaniasis. Since arylimidamides and antifungal azoles both show oral activity in murine visceral leishmaniasis models, a molecular hybridization approach was employed where arylimidamide and azole groups were separated by phenoxyalkyl linkers in an attempt to capitalize on the favorable antileishmanial properties of both series. Among the target compounds synthesized, greater antileishmanial potency against intracellular *Leishmania donovani* was observed as the linker length increased from two to eight carbons and when an imidazole ring was employed as the terminal group compared to a 1,2,4-triazole group. Compound **24c** (*N*-(4-((8-(1H-imidazol-1-yl)octyl)oxy)-2-isopropoxyphenyl) picolinimidamide) displayed activity against *L. donovani* intracellular amastigotes with an IC_{50} value of 0.53 μM. When tested in a murine visceral leishmaniasis model, compound **24c** at a dose of 75 mg/kg/day p.o. for five consecutive days resulted in a modest 33% decrease in liver parasitemia compared to the control group, indicating that further optimization of these molecules is needed. While potent hybrid compounds bearing an imidazole terminal group were also strong inhibitors of recombinant CYP51 from *L. donovani* as assessed by a fluorescence-based assay, additional targets are likely to play an important role in the antileishmanial action of these compounds.

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DEDICATION

This manuscript is dedicated to Dr. Jonathan Vennerstrom in honor of his significant contributions to the field of antimalarial drug discovery and development.

AUTHOR CONTRIBUTIONS

A.A., M.Z.W., and K.A.W conceived and designed the experiments. A.A., A.C.J., E.M.Z., X.L., H.L.M., Y.K., M.F., and Y.J. performed the experiments. A.A., A.C.J., E.M.Z., C.L., X.L., H.L.M., Y.K., J.L., C.A.M., M.F., Y.J., M.Z.H., and K.A.W. analyzed the results. A.A., M.Z.W., and K.A.W. wrote and revised the manuscript. All authors approved the final version of the manuscript.

SUPPORTING INFORMATION

This information is available free of charge on the ACS Publications website: ^{1}H and ^{13}C NMR spectra for all target compounds.

Keywords

Leishmaniasis; azole antifungal; arylimidamide; CYP51; molecular hybridization

Leishmaniasis continues to be an important public health problem. According to recent World Health Organization estimates, approximately 30,000 and over one million new cases of visceral leishmaniasis and cutaneous leishmaniasis occur each year, respectively, and over 1 billion people live in areas where leishmaniasis occurs.[1] The visceral form of leishmaniasis is the most serious; without drug treatment, symptomatic visceral leishmaniasis is usually fatal. Treatment of visceral leishmaniasis generally involves the use of pentavalent antimonials, amphotericin B, paromomycin, miltefosine, or combinations of two of these drugs. Pentavalent antimonials suffer from cardiotoxicity and the loss of efficacy on the Indian subcontinent.[2] Amphotericin B is expensive when given as a liposomal formulation and nephrotoxic when administered as the more economical deoxycholate formulation; this drug is more effective for treating visceral leishmaniasis in India than in East Africa,[3] although higher doses of amphotericin B are effective against East African visceral leishmaniasis.[4] Like the antimonials and amphotericin B, paromomycin is given by injection (for three weeks in the case of this aminoglycoside). As with amphotericin B, paromomycin is more effective in treating visceral leishmaniasis on the Indian subcontinent than in East Africa.[5] Miltefosine has the advantage of being the only oral drug of the four listed above, but it is relatively expensive, is teratogenic, and its efficacy has decreased for the treatment of visceral disease in recent years.[2] Given the weaknesses of the current antileishmanial drugs, the need for new, effective oral treatments is clear.

The potency of arylimidamides (AIAs) against *Leishmania* has been known for some time. Initially termed reversed amidines, AIAs displayed potent activity against *Leishmania donovani*[6, 7] and *Leishmania* species responsible for cutaneous disease.[7, 8] Such compounds contain pyridyl imidamide groups at both ends of the molecule and are thus referred to as bis-AIAs. Mono-AIAs (which contain only one pyridyl imidamide group) were less potent than bis-AIAs, but several mono-AIAs nevertheless showed submicromolar antileishmanial activity in vitro.[9] The most promising AIA identified to date, the bis-AIA **1** (DB766), also displayed good in vivo activity in rodent models of visceral leishmaniasis when given orally, but >90% inhibition of liver parasitemia was not observed at achievable doses.[10, 11] Orally available azole-containing antifungal drugs have also shown antileishmanial activity[12, 13] and have occasionally been used in the clinic against leishmaniasis, with varying success.[14–16] Although the target of the AIAs has not been determined, azole antifungal drugs are thought to act on sterol-14α-demethylase (CYP51), a critical enzyme in the sterol biosynthesis pathway in both fungi and kinetoplastid parasites such as *Leishmania*.[17] Inhibition of this target is believed to occur through coordination of the active site heme group of the enzyme by an imidazole or triazole nitrogen lone pair, while the remainder of the molecule makes important contacts within the substrate binding channel of CYP51.[17]

We demonstrated that oral combinations of **1** with posaconazole displayed moderately synergistic activity in a murine model of visceral leishmaniasis as defined by the combination index method, while combinations of **1** with ketoconazole were less effective in this model.[11] It was striking that posaconazole and ketoconazole, which were over two orders of magnitude less potent in our hands against intracellular *L. donovani* in vitro compared to **1**, displayed comparable, if not superior, efficacy to **1** in the murine visceral leishmaniasis model.[11] In addition, *L. donovani* axenic amastigotes which became resistant to compound **1** due to prolonged pressure with **1** in culture exhibited hypersensitivity to posaconazole and ketoconazole.[18] Based on these observations above, as well as the desire to take advantage of polypharmacology,[19] the synthesis of AIA-azole hybrid compounds displaying features of mono-AIAs together with features of azole-containing antifungal drugs was undertaken. Molecular hybridization is a strategy in which two or more structural components possessing distinct pharmacologic activities are incorporated in one molecule to attack different targets.[20] This strategy has been used widely in infectious disease drug discovery in an attempt to address resistance issues,[21–24] to improve pharmacokinetic properties,[24] and even to combat different infections using the same molecule.[25] Structures of the frontrunner bis-AIA **1**, the mono-AIA **2** (DB2002), the azole antifungals posaconazole (**3**) and ketoconazole (**4**), and the scaffold of AIA-azole hybrid compounds are shown in Figure 1. The synthesis and evaluation of an initial series of such hybrid compounds containing a phenoxyalkyl linker against *L. donovani* is described here.

RESULTS

The synthesis of AIA azole hybrids **9a-l** is shown in Scheme 1. According to a previously published procedure,[26] reaction of dibromo alkanes of different chain lengths with **5a-c** in dry acetonitrile under basic conditions provided **6a-i**. Similarly, reaction of **6a-i** with imidazole or 1,2,4-triazole under the same basic conditions afforded compounds **7a-l**. Tin chloride-mediated reduction of the nitro group yielded **8a-l**. Compounds **8a-l** rapidly changed within less than an hour to dark gummy materials, possibly due to auto-oxidation upon exposure to air. This is reminiscent of our earlier observation regarding the auto-oxidation of 1-benzyl-2,2,4-trimethyl-1,2-dihydroquinolin-6-ols,[27] which contain a similar structural motif. Arylamines **8a-l** were thus used without further purification to yield target compounds **9a-l** through reaction with the pyridyl thioimidate salt under the general conditions reported previously.[7] Since the resultant AIA azole hybrids were stable as free bases, permitting satisfactory elemental analysis and testing in biological assays, conversion to the salt form as mentioned in Reid et al.[7] was not necessary.

The method for preparing AIA-azole hybrids possessing additional terminal groups is shown in Scheme 2. Compound **9m** contains a pyrrole group rather than an imidazole or triazole substitution at the azole terminus while a phenyl group is present in compound **9n** instead of a pyridine at the imidamide end. The route used to synthesize these molecules is similar to the one shown in Scheme 1 except that the installation of the pyrrole ring in target compound **9m** required the use of a stronger base (KOH) along with the more polar DMF as a solvent to facilitate the reaction.[28] In addition, the reduction of nitroaromatic intermediate **7m** to the corresponding aniline **8m** could be accomplished with either tin(II)chloride

dehydrate or Zn metal/ammonium chloride. Target compound **9n** was obtained using the phenyl thioimidate salt rather than the pyridyl thioimidate salt.

Synthesis of hybrid target compounds **17a-c** containing alkoxy substitutions *meta* to the imidamide group is shown in Scheme 3. MOMCl protection of 4-nitrocatechol (**10**) was carried out according to a previous method[29] followed by reaction of methyl, ethyl, or isopropyl iodide with the protected nitrophenol **11** under basic conditions, providing compounds **12a-c**. Deprotection under acidic conditions followed by reaction of the resulting phenols **13a-c** with dibromooctane under basic conditions in dry acetonitrile provided the intermediate monobromo derivatives **14a-c**, which were transformed to **15a-c** by treatment with imidazole in the presence of potassium carbonate. Nitro reduction followed by reaction of the resulting arylamines **16a-c** with the pyridyl thioimidate salt as described above yielded target compounds **17a-c**.

Scheme 4 shows the preparation of AIA azole target compounds **24a-c** bearing alkoxy groups *ortho* to the imidamide moiety. Installation of the alkyl groups through reaction of alkyl iodides with 5-fluoro-2-nitrophenol (**18**) followed by replacement of fluorine via nucleophilic aromatic substitution using sodium hydroxide as reported by Lebold and Kerr[30] yielded nitrophenols **20a-c**. Conversion of **20a-c** to the desired target compounds **24a-c** followed the general synthetic strategy described above.

Synthesis of the AIA azole hybrid lacking a phenoxy linker (**28**) is illustrated in Scheme 5. Compound **26** was prepared through reaction of potassium phthalimide (**25**) with 1,8-dibromooctane. Reaction of **26** with imidazole under basic conditions followed by subjecting the intermediate to Gabriel synthesis conditions provided the primary amine **27** according to a previously published procedure.[31] Reaction of **27** with the pyridyl thioimidate salt under the conditions described earlier yielded target compound **28** in low yield.

Structure-activity and structure-toxicity relationships.

In designing the hybrid target compounds, the effect of three factors was examined: 1) chain length, 2) identity of the terminal groups, and 3) substitution of the phenoxy linker. Data from the evaluation of the potency of the hybrid compounds against intracellular *L. donovani* and their effect against two mammalian cell lines is shown in Table 1. Increasing the length of the alkyl chain improved the antileishmanial potency but also increased activity against both J774 macrophages and HepG2 cells. The best balance of potency and toxicity in this series was achieved with an octyl chain; compound **9h** possessed an IC_{50} value of 1.0 μM against *L. donovani* intracellular amastigotes and IC_{50} values of 11 μM and 12 μM against J774 macrophages and HepG2 cells, respectively. Increasing the chain length to ten carbons (compound **9l**) did not improve the antileishmanial activity but decreased the selectivity against both the macrophage and hepatocarcinoma cell lines. Compounds containing imidazole as the azole terminal group showed greater antileishmanial potency than the corresponding triazoles. For the compounds bearing four carbon alkyl linkers, imidazole **9d** was at least five-fold more potent against intracellular *L. donovani* than triazole **9e**. In the eight carbon linker series, replacement of imidazole with triazole led to a >10-fold decrease in potency when **9h** is compared with **9k** (a precise IC_{50} value

could not be calculated for compound **9k** in the intracellular *L. donovani* assay because of toxicity to the host cells at higher concentrations, but ~50% inhibition of intracellular parasitemia was observed for **9k** at a concentration of 12.5 μM as indicated in Table 1). Little effect on antileishmanial potency and mammalian cell toxicity was observed when the imidazole ring was replaced with pyrrole (compare **9h** to **9m**). Regarding the arylimidamide moiety, replacement of the 2-pyridyl group with a phenyl moiety (compound **9n**) resulted in a 22-fold increase in potency against J774 cells, a 2.4-fold increase in HepG2 activity, and no increase in antileishmanial potency compared to **9h**. In terms of substitutions on the phenoxy portion of the linker, placement of chlorine atoms either *meta* (**9i**) or *ortho* (**9j**) to the imidamide end did not increase antileishmanial potency but may have decreased antileishmanial selectivity compared to **9h**. Minor effects on antileishmanial potency and selectivity were observed upon substitution of the phenoxy ring with alkoxy groups. The most favorable substitutions of those tested appear to be ethoxy and isopropoxy groups *ortho* to the imidamide (compounds **24b** and **24c**, respectively). These changes resulted in slight increases in antileishmanial potency, similar selectivity for intracellular *L. donovani* vs. J774 macrophages compared to **9h**, and slight improvements in selectivity for intracellular parasites vs. HepG2 cells compared to **9h**. For example, compound **24c** displayed sub-micromolar potency against *L. donovani* with selectivity indexes (IC_{50} vs. mammalian cell line/IC_{50} vs. *L. donovani*) of 13 and 23 for intracellular parasites compared to J774 macrophages and HepG2 cells, respectively. Notably, the removal of the phenoxy linker (compound **28**) resulted in a total loss of activity against *L. donovani* and HepG2 cells. The higher hydrophilicity of compound **28** and the presumed higher pK_a value of its imidamide moiety may contribute to its lack of antileishmanial activity.

Binding to L. donovani CYP51.

Purified CYP51 showed good purity with a single major band in both SDS-PAGE and Western blot analyses (Figure 2A) and exhibited a characteristic absorbance at 450 nm (the active P450 form) upon dithionite reduction and carbon monoxide (CO)-binding (Figure 2B). The P450 form of the protein was stabilized by lanosterol as evidenced by a reduction in the inactive P420 form (Figure 2B),[32] likely due to its binding at the active site. When titrated with **24c** or **9h**, CYP51 exhibited typical Type II binding spectra (Figure 2C and 2D) with an absorbance maximum at 431 nm and a minimum at 412–413 nm, indicating direct binding to the active site of CYP51. A Type II binding with CYP protein shows an absorption maximum above the 415–418 nm Soret band range (red shifted Soret band for low spin Fe^{3+}), and is indicative of inhibitory ligand binding as the ligand replaces the water molecule to form the sixth coordination with the heme iron, stabilizes low spin Fe^{3+}, and prevents substrate binding.[33]

Inhibition of L. donovani CYP51.

A fluorescence-based inhibition assay was developed for the *L. donovani* CYP51 using 7-benzyloxy-4-trifluoromethylcoumarin (BFC) as substrate, which was readily converted via oxidative O-debenzylation to its fluorescent metabolite 7-hydroxy-4-trifluoromethylcoumarin (HFC, excitation 410 nm, emission 538 nm) by the purified CYP51 in the presence of cumene hydroperoxide (CuOOH) as a cofactor (Figure 3A). CuOOH has

previously been shown to enable CYP-catalyzed reactions in the absence of CYP natural cofactors (NADPH:P450 oxidoreductase and NADPH).[34]

The azole antifungal drugs posaconazole, ketoconazole and fluconazole exhibited strong-to-moderate inhibition of the *L. donovani* CYP51 with IC_{50} values (mean ± S.E.M. of triplicate determinations) of 0.059 ± 0.001, 0.048 ± 0.004, and 0.96 ± 0.03 μM, respectively. Table 1 also shows the inhibition of CYP51-mediated conversion of BFC to HFC by the hybrid compounds. A representative inhibition curve is shown for **24c** (Figure 3B). Generally consistent with observations regarding antiparasitic potency, compounds possessing longer linkers display greater inhibition of CYP51. In the imidazole series, inhibitory activity increases as chain length increases from 3 carbons (**9c**) through 10 carbons (**9l**). Hybrids bearing an imidazole ring at the "azole end" of the molecule have higher potency than those containing the triazole ring. This is clearly illustrated by comparing four carbon linker containing imidazole **9d** and triazole **9e** (CYP51 IC_{50} values of 6.4 μM and 89 μM, respectively) and eight carbon linker containing imidazole **9h** and triazole **9k** (CYP51 IC_{50} values of 0.36 μM and 1.9 μM, respectively). The importance of the imidazole ring in conveying CYP51 inhibition is further demonstrated through comparison of the pyrrole terminal compound **9m** and the corresponding imidazole terminal compound **9h**. Lacking nitrogen at position 3 of the heterocycle, compound **9m** displays an IC_{50} value of 14 μM against CYP51 as opposed to an IC_{50} value of 0.36 μM for corresponding imidazole compound **9h** as mentioned earlier. This observation for **9m** represents a departure from the antiparasitic SAR described above. Within the series of imidazole-containing phenoxyalkyl hybrids bearing eight carbon linkers, the substitution on the phenoxyalkyl ring and the opposite terminal group have relatively little influence on CYP51 inhibition, as phenoxyalkyl substituted compounds **17a-c** and **24a-c** along with phenyl terminal compound **9n** show CYP51 IC_{50} values similar to that of **9h**. It should be noted, however, that Cl substituted compounds **9i** and **9j** are about 2-fold more potent as inhibitors of *L. donovani* CYP51 compared to **9h**.

Inhibition of human CYP3A4.

CYP3A enzymes are critical in drug metabolism, being expressed in the liver, intestine, and other tissues.[35] Since several azole drugs are potent inhibitors of CYP3A4, the inhibition of recombinant human CYP3A4 by hybrid compounds **9h** and **24c** was evaluated using BFC as a substrate.[36] Compounds **9h** and **24c** were found to be strong inhibitors of recombinant human CYP3A4, displaying IC_{50} values of 0.047 ± 0.003 μM and 0.080 ± 0.009 μM, respectively. For comparison, azole antifungal drugs ketoconazole, posaconazole, and fluconazole exhibited IC_{50} values of 0.0070 ± 0.0003 μM, 0.15 ± 0.01 μM, and 18 ± 2 μM, respectively. Fluconazole is known to be a relatively weak inhibitor of CYP3A4.[37]

Microsomal stability.

Selected hybrid compounds were also evaluated for their in vitro microsomal stability (Table 2). These compounds were incubated with either human or mouse liver microsomes at a substrate concentration of 3 μM; disappearance of the compound of interest was measured over time by LC-MS or LC-UV detection. All compounds exhibited $t_{1/2} > 50$ min. Compounds **9l** (bearing a ten carbon linker) and **24c** (containing an eight carbon linker

and an isopropoxy substitution *ortho* to the imidamide) were less stable in the presence of both human and mouse liver microsomes, possessing $t_{1/2}$ of 52–68 min under these conditions. Compound **9h** (containing an eight carbon linker and an unsubstituted phenoxy ring) displayed $t_{1/2}$ = 70 min in the presence of mouse liver microsomes but was not degraded by human liver microsomes over a period of one hour. For the rest of the hybrid compounds tested, 80% or more remained unchanged in the presence of either human or mouse liver microsomes after a 60 min incubation. Metabolic stability data for AIAs **1** and **2** are provided for comparison.

In vivo evaluation of compound 24c.

Since **24c** displayed a $t_{1/2}$ of >60 minutes in the presence of mouse liver microsomes and was the most potent hybrid compound tested in the phenoxyalkyl hybrid series, it was evaluated for antileishmanial efficacy in vivo. When **24c** was given by oral gavage at a dose of 100 mg/kg/day for five days to a pair of female 6–8 week old BALB/c mice, no weight loss, signs of overt toxicity, or obvious effects on the GI tract, liver, spleen, or kidneys were observed upon necropsy performed one day after administration of the final dose. Oral doses of **24c** at 75 mg/kg/day × 5 and 37.5 mg/kg/day × 5 were then administered to *L. donovani*-infected female BALB/c mice starting at one week post infection (these doses were chosen based on compound availability). Liver parasitemia was then determined three days after the final dose (Figure 4). In animals treated with 75 mg/kg **24c**, 33 ± 7% (mean ± S.E.M.) reduction in liver parasitemia was observed, while the liver parasite burden was reduced by 17 ± 5% in those animals given **24c** at 37.5 mg/kg. Consistent with previous studies in this murine visceral leihsmaniasis model, oral treatment with miltefosine at 10 mg/kg/day × 5 led to a 95 ± 2% reduction of liver infection.[7, 11]

DISCUSSION

Molecular hybridization is a powerful approach that has been widely used in the search for promising antileishmanial and antitrypanosomal candidates.[38–41] The design and synthesis of hybrid compounds displaying structural features of both antifungal azoles and AIAs may be a promising strategy toward the discovery of new antileishmanial leads because members of both classes of compounds display in vivo antileishmanial activity when given orally. Although they are not FDA approved for antileishmanial treatment, antifungal azoles are considered for off-label use against leishmaniasis,[42] while AIAs have shown oral activity in rodent models of visceral leishmaniasis.[9, 10, 43] It is widely accepted that antifungal azoles exert their activity against fungi by inhibiting CYP51, the 14α-demethylase enzyme crucial for sterol biosynthesis; this enzyme is also inhibited by azoles displaying antileishmanial activity.[44] However, it has been speculated that the development of new antileishmanial therapies targeting sterol biosynthesis may require the discovery of more powerful inhibitors, drug combinations, or molecular hybridization approaches.[17, 45] While the precise mechanism(s) of action of AIAs remain(s) unknown, *L. donovani* half knockout parasites for CYP5122A1, a protein involved in *Leishmania* ergosterol biosynthesis,[46] were less susceptible to **1** than wild type parasites but were more susceptible to ketoconazole.[18] Work with these hybrid compounds may uncover additional insights regarding *Leishmania* sterol metabolism that could aid antileishmanial drug design.

In terms of their potency against intracellular *L. donovani*, the hybrid compounds reported here are comparable to the mono-AIAs.[9] The most potent compounds reported thus far from each group exhibit high nanomolar IC_{50} values against these intracellular parasites and show toxicity to J774 macrophages at concentrations near 10 μM. There are caveats to this comparison; the mono-AIAs appear to be slightly more selective for *Leishmania* and the intracellular *L. donovani* assays reported in the two studies were performed by different methods. Nonetheless, considering their comparable in vitro biological activities given the available data, it is appropriate to ask whether the hybrid target compounds reported here are simply a subset of the mono-AIAs. Although the target(s) of the AIAs remain unknown, the interactions of active compounds from both series with *L. donovani* CYP51 are discussed below.

Azole antifungals disrupt sterol biosynthesis in *Leishmania*.[47] Fluconazole inhibits CYP51, the 14α-demethylase involved in sterol biosynthesis, from *Leishmania* as shown by Hargrove et al.[48] and as demonstrated in the present work. Imidazole-containing compounds VNI, VFV, and their analogs also inhibit *Leishmania* CYP51[44, 49] and display in vitro and in vivo antileishmanial effects.[44] Molecules consisting of an imidazole group and an aromatic substituent connected by a hydrocarbon chain were identified by high throughput screening as strong binders to *T. cruzi* CYP51 and also displayed submicromolar potency against intracellular *T. cruzi* amastigotes.[50] Most of the hybrid compounds reported here that display single-digit micromolar or greater potency against intracellular *L. donovani* also inhibit *Leishmania* CYP51 (Table 1); imidazole containing hybrid compounds are more active against both *Leishmania* CYP51 and against intracellular parasites than the corresponding triazole compounds. While these data show the ability of selected hybrid compounds to bind to this parasite enzyme involved in sterol biosynthesis, they do not clearly demonstrate that CYP51 is the target for these compounds. Given that the bis-AIA **1** and the related mono-AIA **2** are weak inhibitors of *L. donovani* CYP51 at concentrations 5–10 times greater than their IC_{50} against intracellular *L. donovani* (M.Z. Wang, unpublished observations), CYP51 inhibition does not appear to be required in order for molecules possessing an arylimidamide group to display potent antileishmanial activity. In addition, the pyrrole containing compound **9m** is only slightly less potent against intracellular *L. donovani* than the corresponding imidazole **9h**, yet it is 39-fold less active as a CYP51 inhibitor. The protein CYP5122A1 also has a role in ergosterol synthesis in *Leishmania donovani*, although its precise function is currently unknown.[46] Given the range of inhibitors that have been identified for *T. cruzi* CYP51, it may also be possible to target CYPs in *Leishmania* with a variety of inhibitors. In an attempt to test the hypothesis mentioned above that improved inhibitors of *Leishmania* sterol biosynthesis may result in the development of new therapeutics, the identification of new CYP450 inhibitors in *Leishmania* and the characterization of their effects on this parasite is an area of active investigation in our laboratories.

Like ketoconazole and posaconazole, two hybrid compounds (**9h** and **24c**) are shown to be potent inhibitors of human CYP3A4, which poses a risk for drug-drug interactions with molecules that are metabolized by human CYP3A4. It was reported that human CYP51 is resistant to inhibition by azole antifungals, including posaconazole, ketoconazole

and fluconazole.[51] As such, in this first report on AIA-azole hybrid compounds, these agents were not evaluated for inhibition of human CYP51. Future studies should consider the inhibition of a wider panel of human CYPs by the AIA-azole hybrid compounds, especially those CYPs commonly involved in drug metabolism, to prioritize hits for further optimization and development.

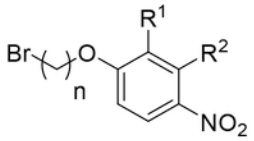
The AIA-azole hybrid agents described here hold promise as antileishmanials based on their strong activity against intracellular parasites (Table 1) and the opportunity they present to explore a wide range of chemical space in the design of new derivatives to improve potency and drug-like properties. The data shown in Table 2 also indicate that, when compared to AIAs **1** and **2**, the inclusion of the azole group in the hybrid molecules does not have a clear negative effect on metabolic stability. Lead compound **24c** did not display signs of toxicity when administered orally to BALB/c mice at doses up to 100 mg/kg, although the in vivo efficacy of this compound in a murine model of visceral leishmaniasis was modest (Figure 4). Work is in progress to improve the antileishmanial properties of the AIA-azole hybrids, to describe their interactions with potential target molecules, and to examine their influence on sterol metabolism in *Leishmania*.

METHODS

General Chemistry Methods.

Reactions were carried out and monitored and compounds were purified according to the techniques described earlier[52] unless noted otherwise. Commercial solvents and reagents were purchased from Sigma-Aldrich, VWR, Fisher Scientific, or Combi-Blocks and were used without further purification. NMR spectra were recorded on Bruker AV300 or DRX400 or Avance III HD Ascend 700 MHz spectrometers at 300 °K unless noted and were calibrated using the residual undeuterated solvent peak ($CDCl_3$: δ 7.26 ppm for ^{1}H NMR, 77.16 ppm for ^{13}C NMR; DMSO-d_6: δ 2.50 ppm for ^{1}H NMR, 39.52 ppm for ^{13}C NMR).[53] Proton (^{1}H) and carbon (^{13}C) NMR data are reported as outlined previously.[52] High resolution mass spectra (HRMS) and melting points were obtained as described earlier.[52]

Synthesis of 4-nitrophenoxy alkyl bromides (6a-i).



6a-i

6a: n=2, R^1=R^2=H
6b: n=3, R^1=R^2=H
6c: n=4, R^1=R^2=H
6d: n=5, R^1=R^2=H
6e: n=6, R^1=R^2=H
6f: n=8, R^1=R^2=H
6g: n=8, R^1=Cl, R^2=H
6h: n=8, R^1=H, R^2=Cl
6i: n=10, R^1=R^2=H

To a solution of 4-nitrophenols **5a-c** (2.9–14.4 mmol) in dry acetonitrile (20 mL) was added 2 equivalents of K_2CO_3 (5.8–28.8 mmol) and 5 equivalents of dibromoalkane (14.4–71.8 mmol). The mixture was stirred at 80 °C overnight according to a previously published

procedure.[26, 54] After completion of the reaction, the suspension was filtered and the filtrate was concentrated under reduced pressure. The crude product was subjected to silica gel chromatography employing ethyl acetate/hexanes 1:10 as the eluent to afford the product in 62–93% yield.

1-(2-Bromoethoxy)-4-nitrobenzene (6a).[54]

White powder, 2.2 g, yield 62% starting from 2.0 g 4-nitrophenol (**5a**, 14.4 mmol).

1-(3-Bromopropoxy)-4-nitrobenzene (6b).[54]

White powder, 3.0 g, yield 80% starting from 2.0 g 4-nitrophenol (**5a**, 14.4 mmol).

1-(4-Bromobutoxy)-4-nitrobenzene (6c).[54]

White powder, 2.5 g, yield 63% starting from 2.0 g 4-nitrophenol (**5a**, 14.4 mmol).

1-(5-Bromopentoxy)-4-nitrobenzene (6d).[54]

White powder, 3.2 g, yield 77% starting from 2.0 g 4-nitrophenol (**5a**, 14.4 mmol).

1-(6-Bromohexoxy)-4-nitrobenzene (6e).[54]

Yellow oil, 3.30 g, yield 76% starting from 2.0 g 4-nitrophenol (**5a**, 14.4 mmol).

1-((8-Bromooctyl)oxy)-4-nitrobenzene (6f).[54]

White powder, 1.10 g, yield 92% starting from 0.5 g 4-nitrophenol (**5a**, 3.60 mmol).

1-((8-Bromooctyl)oxy)-2-chloro-4-nitrobenzene (6g).

Yellow oil, 0.98 g, yield 93% starting from 0.5 g 2-chloro-4-nitrophenol (**5b**, 2.88 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31–1.56 (m, 8H), 1.80–1.94 (m, 4H), 3.40 (t, J = 6.8 Hz, 2H), 4.13 (t, J = 6.4 Hz, 2H), 6.96 (d, J = 9.1 Hz, 1H), 8.13 (dd, J = 9.1, 2.7 Hz, 1H), 8.28 (d, J = 2.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 28.2, 28.7, 28.9, 29.2, 32.9, 34.0, 70.0, 111.8, 123.6, 124.1, 126.2, 141.1, 159.9.

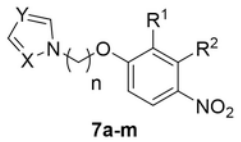
4-((8-Bromooctyl)oxy)-2-chloro-1-nitrobenzene (6h).

Yellow oil, 0.97 g, yield 92% starting from 0.5 g 3-chloro-4-nitrophenol (**5c**, 2.88 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30–1.52 (m, 8H), 1.75–1.92 (m, 4H), 3.41 (t, J = 6.7 Hz, 2H), 4.02 (t, J = 6.4 Hz, 2H), 6.85 (dd, J = 9.1, 2.6 Hz, 1H), 6.99 (d, J = 2.6 Hz, 1H), 7.99 (d, J = 9.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 28.2, 28.7, 29.0, 29.2, 32.9, 34.0, 69.3, 113.5, 117.4, 128.2, 129.8, 140.6, 162.7.

1-(10-Bromodecyoxy)-4-nitrobenzene (6i).[54]

White powder, 1.0 g, yield 78%, starting from 0.5 g 4-nitrophenol (**5a**, 3.60 mmol).

Synthesis of 4-nitrophenoxy alkyl azoles (7a-l) and pyrrole 7m.



7a-m

7a: n=2, X=CH, Y=N, R^1=R^2=H
7b: n=2, X=Y=N, R^1=R^2=H
7c: n=3, X=CH, Y=N, R^1=R^2=H
7d: n=4, X=CH, Y=N, R^1=R^2=H
7e: n=4, X=Y=N, R^1=R^2=H
7f: n=5, X=CH, Y=N, R^1=R^2=H
7g: n=6, X=CH, Y=N, R^1=R^2=H
7h: n=8, X=CH, Y=N, R^1=R^2=H
7i: n=8, X=CH, Y=N, R^1=Cl, R^2=H
7j: n=8, X=CH, Y=N, R^1=H, R^2=Cl
7k: n=8, X=N, Y=N, R^1=R^2=H
7l: n=10, X=CH, Y=N, R^1=R^2=H
7m: n=8, X=Y=CH, R^1=R^2=H

Synthesis of 7a-l.

To a solution of **6a-i** (1.40–5.5 mmol) in dry acetonitrile (20 mL) was added 2 equivalents K_2CO_3 (2.80–11.00 mmol) and 2 equivalents of imidazole or triazole (2.80–11.00 mmol) and the mixture was stirred at 80 °C overnight. After the reaction was completed, the suspension was filtered and the filtrate was concentrated under reduced pressure. The crude product was subjected to silica gel chromatography employing DCM/MeOH 20:1 as the eluent to afford the product in 51–86% yield.

1-(2-(4-Nitrophenoxy)ethyl)-1*H*-imidazole (7a).

White powder, 0.72 g, yield 76% starting from 1.00 g **6a** (4.06 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.28–4.33 (m, 2H), 4.37–4.43 (m, 2H), 6.93 (d, *J* = 9.3 Hz, 2H), 7.03 (t, 1.2 Hz, 1H), 7.08 (s, 1H), 7.60 (s, 1H), 8.20 (d, *J* = 9.3 Hz, 2H).

1-(2-(4-Nitrophenoxy)ethyl)-1*H*-1,2,4-triazole (7b).

White powder, 0.86 g, yield 75% starting from 1.20 g **6a** (4.87 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.44 (t, *J* = 4.9 Hz, 2H), 4.62 (t, *J* = 5.0 Hz, 2H), 6.91 (d, *J* = 9.2 Hz, 2H), 7.96 (s, 1H), 8.14–8.23 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 49.0, 66.4, 114.6, 126.1, 142.3, 144.1, 152.6, 162.8.

1-(3-(4-Nitrophenoxy)propyl)-1*H*-imidazole (7c).[55]

White powder, 1.0 g, yield 78% starting from 1.35 g **6b** (5.19 mmol).

1-(4-(4-Nitrophenoxy)butyl)-1*H*-imidazole (7d).[55]

White powder, 0.73 g, yield 51% starting from 1.5 g **6c** (5.47 mmol).

1-(4-(4-Nitrophenoxy)butyl)-1*H*-1,2,4-triazole (7e).[55]

White powder, 1.10 g, yield 76% starting from 1.5 g **6c** (5.47 mmol).

1-(5-(4-Nitrophenoxy)pentyl)-1*H*-imidazole (7f).

White powder, 0.96 g, yield 67% starting from 1.5 g **6d** (5.20 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42–1.54 (m, 2H), 1.78–1.92 (m, 4H), 3.97 (t, *J* = 7.0 Hz, 2H), 4.02 (t, *J* = 6.1 Hz,

2H), 6.88–6.95 (m, 3H), 7.05 (s, 1H), 7.46 (s, 1H), 8.17 (d, J = 9.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.3, 28.6, 30.9, 46.9, 68.4, 114.5, 118.8, 126.0, 129.7, 137.2, 141.6, 164.0.

1-(6-(4-Nitrophenoxy)hexyl)-1*H*-imidazole (7g).

White powder, 1.10 g, yield 72% starting from 1.6 g **6e** (5.29 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30–1.42 (m, 2H), 1.44–1.55 (m, 2H), 1.75–1.86 (m, 4H), 3.94 (t, J = 7.0 Hz, 2H), 4.01 (t, J = 6.3 Hz, 2H), 6.88–6.94 (m, 3H), 7.04 (s, 1H), 7.45 (s, 1H), 8.18 (d, J = 9.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6, 26.4, 28.9, 31.1, 47.0, 68.6, 114.5, 118.8, 126.0, 129.6, 137.2, 141.6, 164.2.

1-(8-(4-Nitrophenoxy)octyl)-1*H*-imidazole (7h).

Off-white powder, 0.41 g, yield 86% starting from 0.5 g **6f** (1.51 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25–1.50 (m, 8H), 1.72–1.85 (m, 4H), 3.93 (t, J = 7.1 Hz, 2H), 4.03 (t, J = 6.4 Hz, 2H), 6.88–6.96 (m, 3H), 7.05 (s, 1H), 7.45 (s, 1H), 8.19 (d, J = 9.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 26.6, 29.0, 29.1, 29.2, 31.2, 47.1, 68.9, 114.5, 118.9, 126.0, 129.6, 137.2, 141.5, 164.3.

1-(8-(2-Chloro-4-nitrophenoxy)octyl)-1*H*-imidazole (7i).

Yellow oil, 0.41 g, yield 60%, starting from 0.71 g **6f** (1.94 mmol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.41 (m, 6H), 1.43–1.53 (m, 2H), 1.72–1.81 (m, 2H), 1.81–1.90 (m, 2H), 3.92 (t, J = 7.1 Hz, 2H), 4.11 (t, J = 6.4 Hz, 2H), 6.89 (s, 1H), 6.95 (d, J = 9.1 Hz, 1H), 7.04 (s, 1H), 7.44 (s, 1H), 8.12 (dd, J = 9.1, 2.7 Hz, 1H), 8.27 (d, J = 2.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.9, 26.6, 28.8, 29.0, 29.1, 31.1, 47.1, 69.9, 111.8, 118.8, 123.5, 124.1, 126.1, 129.5, 137.2, 141.1, 159.8.

1-(8-(3-Chloro-4-nitrophenoxy)octyl)-1*H*-imidazole (7j).

Synthesis of **7j** followed the general synthesis procedure except for the use of 1.2 equivalents of imidazole (115 mg, 1.69 mmol) and 1.2 equivalents of potassium carbonate (231 mg, 1.66 mmol) with gentle reflux overnight. Yellow oil, 0.24 g, yield 49% starting from 0.51 g **6f** (1.39 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22–1.48 (m, 8H), 1.71–1.83 (m, 4H), 3.92 (t, J = 7.1 Hz, 2H), 4.00 (t, J = 6.4 Hz, 2H), 6.83 (dd, J = 9.2, 2.6 Hz, 1H), 6.88 (s, 1H), 6.98 (d, J = 2.6 Hz, 1H), 7.03 (s, 1H), 7.44 (s, 1H), 7.97 (d, J = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 26.6, 28.9, 29.1, 29.2, 31.1, 47.1, 69.2, 113.5, 117.3, 118.8, 128.1, 129.5, 129.8, 137.2, 140.6, 162.6.

1-(8-(4-Nitrophenoxy)octyl)-1*H*-1,2,4-triazole (7k).

White powder, 0.41 g, yield 70% starting from 0.6 g **6f** (1.81 mmol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.49 (m, 8H), 1.75–1.84 (m, 2H), 1.85–1.94 (m, 2H), 4.03 (t, J = 6.4 Hz, 2H), 4.16 (t, J = 7.1 Hz, 2H), 6.92 (d, J = 9.2 Hz, 2H), 7.93 (s, 1H), 8.04 (s, 1H), 8.18 (d, J = 9.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.9, 26.5, 29.0, (overlapped 2Cs), 29.2, 29.9, 49.8, 68.9, 114.5, 126.0, 141.5, 142.9, 152.1, 164.3.

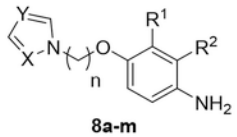
1-(10-(4-Nitrophenoxy)decyl)-1*H*-imidazole (7l).

Off-white powder, 0.32 g, yield 65%, starting from 0.5 g **6i** (1.40 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22–1.50 (m, 12H), 1.71–1.86 (m, 4H), 3.91 (t, *J* = 7.1 Hz, 2H), 4.03 (t, *J* = 6.5 Hz, 2H), 6.88–6.95 (m, 3H), 7.04 (s, 1H), 7.45 (s, 1H), 8.18 (d, *J* = 9.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 26.6, 29.1, 29.2, 29.35, 29.45, 29.49, 31.2, 47.1, 69.0, 114.5, 118.9, 126.0, 129.5, 137.2, 141.5, 164.3.

Synthesis of 1-(8-(4-nitrophenoxy)octyl)-1*H*-pyrrole (7m).

Compound **7m** was synthesized according to a previously published procedure.[28] To a solution of pyrrole (0.21 g, 3.13 mmol) in DMF (5 mL) was added 1.14 equivalent of potassium hydroxide (0.20 g, 3.56 mmol) and the suspension was stirred at rt for 15 min. 1.16 equivalent of **6f** (1.2 g, 3.56 mmol) was added and the mixture was stirred at 80 °C for 16h. After the reaction was complete, the mixture was quenched with water, extracted with ethyl acetate, and dried over sodium sulfate. The organic layer was evaporated under reduced pressure and purified by column chromatography using hexanes/DCM (5:1) as the eluent to yield the product as a yellow oil, 0.35 g, 35%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25–1.52 (m, 8H), 1.72–1.87 (m, 4H), 3.87 (t, *J* = 7.1 Hz, 2H), 4.04 (t, *J* = 6.5 Hz, 2H), 6.14 (t, *J* = 2.1 Hz, 2H), 6.65 (t, *J* = 2.1 Hz, 2H), 6.94 (d, *J* = 9.3 Hz, 2H), 8.19 (d, *J* = 9.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 26.8, 29.0, 29.2, 29.3, 31.6, 49.7, 68.9, 107.9, 114.5, 120.6, 126.0, 141.5, 164.3.

Synthesis of 4-aminophenoxy alkyl azoles (8a-l) and pyrrole 8m.



8a-m

8a: n=2, X=CH, Y=N, R^1=R^2=H
8b: n=2, X=Y=N, R^1=R^2=H
8c: n=3, X=CH, Y=N, R^1=R^2=H
8d: n=4, X=CH, Y=N, R^1=R^2=H
8e: n=4, X=Y=N, R^1=R^2=H
8f: n=5, X=CH, Y=N, R^1=R^2=H
8g: n=6, X=CH, Y=N, R^1=R^2=H
8h: n=8, X=CH, Y=N, R^1=R^2=H
8i: n=8, X=CH, Y=N, R^1=Cl, R^2=H
8j: n=8, X=CH, Y=N, R^1=H, R^2=Cl
8k: n=8, X=N, Y=N, R^1=R^2=H
8l: n=10, X=CH, Y=N, R^1=R^2=H
8m: n=8, X=Y=CH, R^1=R^2=H

To a solution of **7a-m** (0.60–1.70 mmol) in ethyl acetate (20 mL) was added 5 equivalents of tin(II) chloride dihydrate (3.0–8.5 mmol) and the mixture was refluxed for 8–12 h. After the reaction was complete, the suspension was made basic with 1N NaOH solution (pH = 11) and extracted with ethyl acetate (30 mL × 3). The organic layer was dried over sodium sulfate and filtered. The filtrate was evaporated under reduced pressure yielding the product in 81–99% yield which was taken directly to the next step without further purification. For this reason, only ^{1}H NMR spectra were obtained for **8a-l**. Since the compounds were crude, ^{1}H NMR spectra for the anilines reported in this manuscript often contain solvent peaks such as DCM and ethyl acetate along with traces of starting material in some cases (occasionally resulting in slightly higher than expected integration for the alkyl protons, particularly the most upfield alkyl multiplet).

4-(2-(1*H*-imidazol-1-yl)ethoxy)aniline (8a).

Yellow powder, 0.10 g, yield 82% starting from 0.14 g **7a** (0.60 mmol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.08 (t, J = 5.2 Hz, 2H), 4.27 (t, J = 5.2 Hz, 2H), 4.62 (brs, $ArNH_2$), 6.49 (d, J = 8.8 Hz, 2H), 6.63 (d, J = 8.8 Hz, 2H), 6.88 (s, 1H), 7.21 (s, 1H), 7.65 (s, 1H).

4-(2-(1*H*-1,2,4-triazol-1-yl)ethoxy)aniline (8b).

Yellow powder, 0.28 g, yield 81% starting from 0.40 g **7b** (1.70 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.26–3.65 (brs, $ArNH_2$), 4.24 (t, J = 5.0 Hz, 2H), 4.50 (t, J = 5.0 Hz, 2H), 6.60 (d, J = 9.0 Hz, 2H), 6.67 (d, J = 9.3 Hz, 2H), 7.94 (s, 1H), 8.20 (s, 1H).

4-(3-(1*H*-imidazol-1-yl)propoxy)aniline (8c).

Brown oil, 0.26 g, yield 99% starting from 0.30 g **7c** (1.21 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.12–2.23 (m, 2H), 2.86–3.93 (brs, $ArNH_2$), 3.83 (t, J = 5.7 Hz, 2H), 4.17 (t, J = 6.8 Hz, 2H), 6.63 (d, J = 8.8 Hz, 2H), 6.72 (d, J = 8.9 Hz, 2H), 6.91 (s, 1H), 7.05 (s, 1H), 7.48 (s, 1H).

4-(4-(1*H*-imidazol-1-yl)butoxy)aniline (8d).

Buff powder, 0.25 g, yield 95% starting from 0.3 g **7d** (1.14 mmol); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.67–1.78 (m, 2H), 1.91–2.02 (m, 2H), 3.09–3.46 (brs, $ArNH_2$), 3.88 (t, J = 6.0 Hz, 2H), 4.00 (t, J = 7.1 Hz, 2H), 6.62 (d, J = 8.9 Hz, 2H), 6.71 (d, J = 8.9 Hz, 2H), 6.92 (s, 1H), 7.05 (s, 1H), 7.47 (s, 1H).

4-(4-(1*H*-1,2,4-triazol-1-yl)butoxy)aniline (8e).

Buff powder, 0.32 g, yield 91% starting from 0.40 g **7e** (1.52 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.68–1.79 (m, 2H), 2.02–2.13 (m, 2H), 3.16–3.69 (brs, $ArNH_2$), 3.91 (t, J = 6.0 Hz, 2H), 4.24 (t, J = 7.1 Hz, 2H), 6.62 (d, J = 8.9 Hz, 2H), 6.71 (d, J = 8.9 Hz, 2H), 7.93 (s, 1H), 8.06 (s, 1H).

4-((5-(1*H*-imidazole-1-yl)pentyl)oxy)aniline (8f).

Brown oil, 0.29 g, yield 99% starting from 0.33 g **7f** (1.19 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40–1.52 (m, 2H), 1.70–1.89 (m, 4H), 3.08–3.41 (brs, $ArNH_2$), 3.86 (t, J = 6.2 Hz, 2H), 3.94 (t, J = 7.1 Hz, 2H), 6.62 (d, J = 8.9 Hz, 2H), 6.71 (d, J = 8.9 Hz, 2H), 6.90 (s, 1H), 7.05 (s, 1H), 7.47 (s, 1H).

4-((6-(1*H*-imidazole-1-yl)hexyl)oxy)aniline (8g).

Brown oil, 0.30 g, yield 92% starting from 0.35 g **7g** (1.20 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29–1.41 (m, 2H), 1.42–1.54 (m, 2H), 1.67–1.86 (m, 4H), 2.80–3.35 (brs, $ArNH_2$), 3.86 (t, J = 6.3 Hz, 2H), 3.93 (t, J = 7.1 Hz, 2H), 6.63 (d, J = 8.9 Hz, 2H), 6.72 (d, J = 8.9 Hz, 2H), 6.89 (s, 1H), 7.05 (s, 1H), 7.46 (s, 1H).

4-((8-(1*H*-imidazole-1-yl)octyl)oxy)aniline (8h).

Orange powder, 0.23 g, yield 85%, starting from 0.30 g **7h** (0.95 mmol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23–1.36 (m, 6H), 1.37–1.46 (m, 2H), 1.67–1.80 (m, 4H), 3.18–3.58 (brs,

ArNH$_2$), 3.86 (t, *J* = 6.5 Hz, 2H), 3.90 (t, *J* = 7.1 Hz, 2H), 6.62 (brd, *J* = 8.7 Hz m, 2H), 6.72 (brd, *J* = 8.7 Hz, 2H), 6.89 (s, 1H), 7.04 (s, 1H), 7.44 (s, 1H).

4-((8-(1*H*-imidazole-1-yl)octyl)oxy)-3-chloroaniline (8i).

Yellow oil, 0.17 g, yield 93%, starting from 0.20 g **7i** (0.57 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24–1.39 (m, 6H), 1.40–1.51 (m, 2H), 1.70–1.82 (m, 4H), 2.95–3.70 (brs, *ArNH*$_2$), 3.91 (t, *J* = 6.7 Hz, 4H), 6.52 (dd, *J* = 8.6, 2.8 Hz, 1H), 6.73 (d, *J* = 2.8 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 6.89 (s, 1H), 7.04 (s, 1H), 7.45 (s, 1H).

4-((8-(1*H*-imidazole-1-yl)octyl)oxy)-2-chloroaniline (8j).

Yellow oil, 0.23 g, yield 97%, starting from 0.26 g **7j** (0.74 mmol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23–1.45 (m, 8H), 1.66–1.81 (m, 4H), 3.61–3.80 (brs, *ArNH*$_2$, partially under other peaks), 3.84 (t, *J* = 6.5 Hz, 2H), 3.91 (t, *J* = 7.1 Hz, 2H), 6.62–6.71 (m, 2H), 6.83 (d, *J* = 2.4 Hz, 1H), 6.89 (s, 1H), 7.04 (s, 1H), 7.46 (s, 1H).

4-((8-(1*H*-1,2,4-triazol-1-yl)octyl)oxy)aniline (8k).

Buff powder, 0.21 g, yield 93%, starting from 0.25 g **7k** (0.78 mmol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23–1.46 (m, 8H), 1.66–1.75 (m, 2H), 1.83–1.91 (m, 2H), 3.26–3.53 (brs, *ArNH*$_2$), 3.85 (t, *J* = 6.5 Hz, 2H), 4.14 (t, *J* = 7.1 Hz, 2H), 6.62 (brd, *J* = 8.8 Hz, 2H), 6.72 (brd, *J* = 8.8 Hz, 2H), 7.92 (s, 1H), 8.03 (s, 1H).

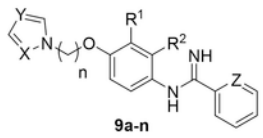
4-((10-(1*H*-imidazole-1-yl)decyl)oxy)aniline (8l).

Buff powder, 0.20 g, yield 88%, starting from 0.25 g **7l** (0.72 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.47 (m, 12H), 1.67–1.81 (m, 4H), 3.07–3.64 (brs, *ArNH*$_2$), 3.83–3.95 (m, 4H), 6.63 (d, *J* = 9.0 Hz, 2H), 6.73 (d, *J* = 8.9 Hz, 2H), 6.89 (t, *J* = 1.2 Hz, 1H), 7.05 (s, 1H), 7.45 (s, 1H).

4-((8-(1*H*-pyrrol-1-yl)octyl)oxy)aniline (8m).

The general procedure listed above was used to prepare **8m**, but this material was also prepared via a Zn-mediated reduction which is described here. To a solution of **7m** (170 mg, 0.54 mmol) in MeOH (10 mL) was added ammonium chloride (285 mg, 5.30 mmol) and Zn (350 mg, 5.40 mmol) and the mixture was stirred at room temperature for 3 h. After the reaction was complete, the reaction mixture was filtered through celite. The celite was washed with MeOH (10 mL), then the combined MeOH filtrate was evaporated under reduced pressure to obtain a crude solid. To the crude solid was added water (20 mL), followed by extraction with DCM (2×20 mL). The organic layers were collected, dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain the title product as yellow oil (130 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.47 (m, 8H), 1.68–1.80 (m, 4H), 3.83–3.89 (m, 4H), 6.13 (t, *J* = 2.1 Hz, 2H), 6.64 (t, *J* = 2.1 Hz, 2H), 6.67 (d, *J* = 8.8 Hz, 2H), 6.74 (d, *J* = 8.8 Hz, 2H).

Synthesis of hybrid target compounds 9a-n.



9a-n

9a: n=2, X=CH, Y=Z=N, R^1=R^2=H
9b: n=2, X=Y=Z=N, R^1=R^2=H
9c: n=3, X=CH, Y=Z=N, R^1=R^2=H
9d: n=4, X=CH, Y=Z=N, R^1=R^2=H
9e: n=4, X=Y=Z=N, R^1=R^2=H
9f: n=5, X=CH, Y=Z=N, R^1=R^2=H
9g: n=6, X=CH, Y=Z=N, R^1=R^2=H
9h: n=8, X=CH, Y=Z=N, R^1=R^2=H
9i: n=8, X=CH, Y=Z=N, R^1=Cl, R^2=H
9j: n=8, X=CH, Y=Z=N, R^1=H, R^2=Cl
9k: n=8, X=Y=Z=N, R^1=R^2=H
9l: n=10, X=CH, Y=Z=N, R^1=R^2=H
9m: n=8, X=Y=CH, Z=N, R^1=R^2=H
9n: n=8, X=Z=CH, Y=N, R^1=R^2=H

Synthesis of AIA azole hybrid target compounds followed previously published procedures for the synthesis of arylimidamides.[6, 7, 56] Two equivalents of *S*-(2-naphthylmethyl)-2-pyridyl thioimidate hydrobromide (0.76–1.38 mmol) or *S*-(2-naphthylmethyl) benzimidothioate hydrobromide (1.04 mmol) were added to one equivalent of a cooled solution of the arylamines **8a-m** (0.38–0.69 mmol) in dry acetonitrile:ethanol (3:10 mL) in an ice bath for 15 minutes, then this solution was warmed to room temperature. The reaction mixture was stirred at room temperature for 24–48 h. After the disappearance of the starting material, the organic solvent was evaporated under reduced pressure to yield a crude oil product. Dry ether (100 mL) was added to the crude material and the mixture was stirred at room temperature overnight. The precipitate was filtered and washed with dry ether. The solid was dissolved in ethanol (2 mL), then the solution was cooled to 0 °C in an ice bath and 10% NaOH was added until the pH reached approximately 11. The free base was extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with distilled water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure yielding a waxy or solid product which was further purified by column chromatography on triethylamine neutralized silica using DCM:MeOH as an eluent (100:0.5 to 100:3). The product was further crystalized using hexanes/ethyl acetate to yield the final product in 33–70% yield.

N-(4-(2-(1*H*-imidazol-1-yl)ethoxy)phenyl) picolinimidamide (9a).

Yellow powder; 63 mg; yield 42% (starting from 0.10 g of **8a**, 0.49 mmol); mp 141–144 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.21 (t, J = 5.2 Hz, 2H), 4.35 (t, J = 5.1 Hz, 2H), 6.26–6.59 (brs, *imidamide NHs*), 6.82–6.93 (m, 5H), 7.25 (t, J = 1.2 Hz, 1H), 7.53 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 7.68 (s, 1H), 7.93 (td, J = 7.7, 1.8 Hz, 1H), 8.29 (d, J = 7.9 Hz, 1H), 8.61 (ddd, J = 4.8, 1.7, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 45.7, 67.4, 115.3, 119.7, 121.1, 122.4, 125.3, 128.3, 137.0, 137.6, 143.6, 148.0, 151.6, 151.8, 153.4; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{17}H_{18}N_5O$, 308.15059; found, 308.15057; Anal. Calcd for $C_{17}H_{17}N_5O$. C, 66.43; H, 5.58; N, 22.79. Found: C, 66.15; H, 5.71; N, 22.49.

N-(4-(2-(1*H*-1,2,4-triazol-1-yl)ethoxy)phenyl) picolinimidamide (9b).

Buff powder; 107 mg; yield 59% (starting from 0.12 g of **8b**, 0.58 mmol); mp 115–118 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.33 (t, J = 5.0 Hz, 2H), 4.56 (t, J = 5.0 Hz, 2H), 5.40–6.21 (brs, *imidamide NHs*), 6.86 (brd, J = 8.8 Hz, 2H), 6.93 (brd, J = 8.6 Hz, 2H), 7.38 (ddd, J =

7.4, 4.9, 1.1 Hz, 1H), 7.80 (td, *J* = 7.8, 1.7 Hz, 1H), 7.96 (s, 1H), 8.23 (s, 1H), 8.38 (d, *J* = 8.0 Hz, 1H), 8.56 (br d, *J* = 4.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 49.6, 66.2, 115.9, 121.6, 122.8, 125.3, 136.9, 144.2, 148.0, 151.7, 152.2, 153.1, 154.0; HRMS (ESI) *m/z* (M +H)$^+$ calcd for $C_{16}H_{17}N_6O$, 309.14584; found, 309.14569; Anal. Calcd for $C_{16}H_{16}N_6O$: C, 62.32; H, 5.23; N, 27.26. Found: C, 62.39; H, 5.28; N, 27.18.

N-(4-(3-(1*H*-imidazol-1-yl)propoxy)phenyl) picolinimidamide (9c).

Yellow powder; 85 mg; yield 38% (starting from 0.15 g of **8c**, 0.69 mmol); mp 115–118 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 2.20–2.25 (m, 2H), 3.91 (t, *J* = 5.7 Hz, 2H), 4.21 (t, *J* = 6.8 Hz, 2H), 5.35–6.49 (brs, *imidamide NHs*), 6.90 (d, *J* = 8.8 Hz, 2H), 6.94 (t, *J* = 1.2 Hz, 1H), 6.97 (brd, *J* = 8.6 Hz, 2H), 7.07 (s, 1H), 7.39 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H), 7.49 (s, 1H), 7.81 (td, *J* = 7.7, 1.7 Hz, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.57 (ddd, *J* = 4.8, 1.7, 0.9 Hz, 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 31.0, 43.6, 64.1, 115.7, 119.1, 121.6, 122.8, 125.3, 129.8, 136.9, 137.6, 143.5, 148.0, 151.7, 153.2, 154.7; HRMS (ESI) *m/z* (M +H)$^+$ calcd for $C_{18}H_{20}N_5O$, 322.16624; found, 322.16592; Anal. Calcd for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79. Found: C, 67.42; H, 5.98; N, 21.78.

N-(4-(4-(1*H*-imidazol-1-yl)butoxy)phenyl) picolinimidamide (9d).

Buff powder; 132 mg; yield 65% (starting from 0.14 g of **8d**, 0.60 mmol); mp 103–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74–1.82 (m, 2H), 1.96–2.05 (m, 2H), 3.97 (t, *J* = 6.0 Hz, 2H), 4.03 (t, *J* = 7.0 Hz, 2H), 5.43–6.30 (brs, *imidamide NHs*), 6.89 (d, *J* = 8.9 Hz, 2H), 6.92–6.98 (m, 3H), 7.07 (t, *J* = 0.9 Hz, 1H), 7.38 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 1H), 7.49 (s, 1H), 7.80 (td, *J* = 7.7, 1.7 Hz, 1H), 8.40 (d, *J* = 7.9 Hz, 1H), 8.56 (ddd, *J* = 4.8, 1.7, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.5, 28.2, 46.9, 67.5, 115.7, 118.9, 121.6, 122.7, 125.2, 129.7, 136.9, 137.2, 143.3, 148.0, 151.8, 153.1, 154.9; HRMS (ESI) *m/z* (M +H)$^+$ calcd for $C_{19}H_{22}N_5O$, 336.18189; found, 336.18155; Anal. Calcd for $C_{19}H_{21}N_5O$: C, 68.04; H, 6.31; N, 20.88. Found: C, 68.20; H, 6.35; N, 20.77.

N-(4-(4-(1*H*-1,2,4-triazol-1-yl)butoxy)phenyl) picolinimidamide (9e).

Buff powder; 141 mg; yield 70% (starting from 0.14 g of **8e**, 0.60 mmol); mp 109–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75–1.83 (m, 2H), 2.08–2.16 (m, 2H), 3.99 (t, *J* = 6.0 Hz, 2H), 4.27 (t, *J* = 7.0 Hz, 2H), 5.32–6.31 (brs, *imidamide NHs*), 6.89 (d, *J* = 8.9 Hz, 2H), 6.96 (brd, *J* = 8.7 Hz, 2H), 7.38 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 1H), 7.80 (td, *J* = 7.8, 1.8 Hz, 1H), 7.95 (s, 1H), 8.08 (s, 1H), 8.40 (d, *J* = 7.9 Hz, 1H), 8.56 (ddd, *J* = 4.8, 1.7, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.3, 27.1, 49.5, 67.5, 115.6, 121.62, 122.7, 125.2, 136.9, 143.1, 143.3, 148.0, 151.8, 152.1, 153.1, 154.9; HRMS (ESI) *m/z* (M +H)$^+$ calcd for $C_{18}H_{21}N_6O$, 337.17714; found, 337.17712; Anal. Calcd for $C_{18}H_{20}N_6O$: C, 64.27; H, 5.99; N, 24.98. Found: C, 64.43; H, 6.00; N, 24.93.

N-(4-((5-(1*H*-imidazol-1-yl)pentyl)oxy)phenyl) picolinimidamide (9f).

Light brown powder; 70 mg; yield 33% (starting from 0.15 g of **8f**, 0.61 mmol); mp 128–131 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.47–1.53 (m, 2H), 1.78–1.83 (m, 2H), 1.84–1.89 (m, 2H), 3.94 (t, *J* = 6.2 Hz, 2H), 3.97 (t, *J* = 7.2 Hz, 2H), 5.37–6.48 (brs, *imidamide NHs*), 6.89 (d, *J* = 8.8 Hz, 2H), 6.91 (t, *J* = 1.1 Hz, 1H) 6.95 (brd, *J* = 7.5 Hz, 2H), 7.06 (s, 1H),

7.38 (ddd, J = 7.5, 4.8, 1.1 Hz, 1H), 7.47 (s, 1H), 7.80 (td, J = 7.7, 1.7 Hz, 1H), 8.40 (d, J = 8.0 Hz, 1H), 8.57 (ddd, J = 4.8, 1.6, 0.8 Hz, 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 23.4, 28.9, 31.0, 47.0, 67.8, 115.6, 118.9, 121.6, 122.6, 125.2, 129.6, 136.9, 137.2, 143.2, 148.0, 151.8, 153.1, 155.1; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{20}H_{24}N_5O$, 350.19754; found, 350.19757; Anal. Calcd for $C_{20}H_{23}N_5O$: C, 68.74; H, 6.63; N, 20.04. Found: C, 68.45; H, 6.60; N, 19.90.

N-(4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)phenyl) picolinimidamide (9g).

Light brown powder; 70 mg; yield 33% (starting from 0.15 g of **8g**, 0.57 mmol); mp 127–129 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33–1.43 (m, 2H), 1.48–1.56 (m, 2H), 1.73–1.87 (m, 4H), 3.92–3.97 (m, 4H), 5.61–6.12 (brs, *imidamide NHs*), 6.88–6.98 (m, 5H), 7.06 (s, 1H), 7.39 (ddd, J = 7.4, 4.9, 1.0 Hz, 1H), 7.46 (s, 1H), 7.81 (td, J = 7.8, 1.7 Hz, 1H), 8.41 (d, J = 8.0 Hz, 1H), 8.57 (d, J = 4.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 26.5, 29.3, 31.2, 47.1, 68.0, 115.7, 118.9, 121.6, 122.6, 125.2, 129.6, 136.9, 137.2, 143.1, 148.0, 151.9, 153.1, 155.2; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{21}H_{26}N_5O$, 364.21319; found, 364.21298; Anal. Calcd for $C_{21}H_{25}N_5O$: C, 69.40; H, 6.93; N, 19.27. Found: C, 69.43; H, 6.81; N, 19.13.

N-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)phenyl) picolinimidamide (9h).

Light brown powder; 85 mg; yield 44% (starting from 0.14 g of **8h**, 0.49 mmol); mp 89–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28–1.40 (m, 6H), 1.41–1.50 (m, 2H), 1.73–1.82 (m, 4H), 3.90–3.97 (m, 4H), 5.47–6.30 (brs, *imidamide NHs*), 6.89–6.93 (m, 3H), 6.96 (d, J = 8.9 Hz, 2H), 7.05 (t, J = 1.0 Hz, 1H), 7.39 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 7.45 (s, 1H), 7.81 (td, J = 7.8, 1.8 Hz, 1H), 8.42 (d, J = 8.0 Hz, 1H), 8.57 (ddd, J = 4.9, 1.7, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1, 26.6, 29.1, 29.3, 29.4, 31.2, 47.2, 68.3, 115.7, 118.9, 121.7, 122.7, 125.2, 129.6, 136.9, 137.2, 142.7, 148.0, 151.8, 153.2, 155.4; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{23}H_{30}N_5O$, 392.24449; found, 392.24451; Anal. Calcd for $C_{23}H_{29}N_5O$: C, 70.56; H, 7.47; N, 17.89. Found: C, 70.27; H, 7.29; N, 17.70.

N-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-3-chlorophenyl) picolinimidamide (9i).

Yellow powder; 99 mg; yield 54% (starting from 0.14 g of **8i**, 0.43 mmol); mp 102–104 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.41 (m, 6H), 1.45–1.55 (m, 2H), 1.74–1.86 (m, 4H), 3.92 (t, J = 7.1 Hz, 2H), 4.01 (t, J = 6.4 Hz, 2H), 5.42–6.39 (brs, *imidamide NHs*), 6.86 (dd, J = 8.6, 2.4 Hz, 1H), 6.90 (t, J = 1.2 Hz, 1H), 6.93 (d, J = 8.6 Hz, 1H), 7.05 (s, 1H), 7.08 (d, J = 2.4 Hz, 1H), 7.39 (ddd, J = 7.5, 4.8, 1.2 Hz, 1H), 7.45 (s, 1H), 7.81 (td, J = 7.7, 1.7 Hz, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.57 (ddd, J = 4.8, 1.7, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.0, 26.6, 29.1, 29.2, 29.3, 31.2, 47.2, 69.7, 115.0, 118.9, 120.8, 121.7, 123.6, 123.9, 125.4, 129.6, 137.0, 137.2, 143.6, 148.0, 150.7, 151.5, 153.3; HRMS (ESI) *m/z* $(M+H)^+$ and $((M+2)+H)^+$ calcd for $C_{23}H_{29}N_5ClO$, 426.20551 and 428.20261; found, 426.20490 and 428.20236; Anal. Calcd for $C_{23}H_{28}N_5ClO$: C, 64.85; H, 6.63; N, 16.44. Found: C, 64.81; H, 6.67; N, 16.32.

N-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-2-chlorophenyl) picolinimidamide (9j).

White powder; 71 mg; yield 39% (starting from 0.14 g of **8j**, 0.43 mmol); mp 74–76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.40 (m, 6H), 1.40–1.50 (m, 2H), 1.72–1.83 (m, 4H), 3.89–3.95 (m, 4H), 5.40–6.38 (brs, *imidamide NHs*), 6.82 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.90 (s, 1H), 6.95 (d, *J* = 8.7 Hz, 1H), 7.00 (d, *J* = 2.7 Hz, 1H), 7.05 (s, 1H), 7.40 (ddd, *J* = 7.4, 4.8, 1.0 Hz, 1H), 7.46 (s, 1H), 7.82 (td, *J* = 7.7, 1.6 Hz, 1H), 8.46 (d, *J* = 8.0 Hz, 1H), 8.58 (d, *J* = 4.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.0, 26.6, 29.1, 29.27, 29.30, 31.2, 47.2, 68.6, 114.7, 116.2, 118.9, 122.0, 123.4, 125.4, 126.7, 129.6, 137.0, 137.2, 139.7, 148.0, 151.3, 153.4, 155.6; HRMS (ESI) *m/z* $(M+H)^+$ and $((M+2)+H)^+$ calcd for $C_{23}H_{29}N_5ClO$, 426.20551 and 428.20261; found, 426.20663 and 428.20416; Anal. Calcd for $C_{23}H_{28}N_5ClO$: C, 64.85; H, 6.63; N, 16.44. Found: C, 64.68; H, 6.58; N, 16.38.

N-(4-((8-(1*H*-1,2,4-triazol-1-yl)octyl)oxy)phenyl) picolinimidamide (9k).

Light brown powder; 85 mg; yield 39% (starting from 0.16 g of **8k**, 0.55 mmol); mp 98–100 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.28–1.39 (m, 6H), 1.42–1.48 (m, 2H), 1.74–1.79 (m, 2H), 1.87–1.92 (m, 2H), 3.94 (t, *J* = 6.4 Hz, 2H), 4.16 (t, *J* = 7.1 Hz, 2H), 5.29–6.44 (brs, *imidamide NHs*), 6.91 (d, *J* = 8.8 Hz, 2H), 6.96 (brd, *J* = 8.6 Hz, 2H), 7.38 (ddd, *J* = 7.4, 4.9, 1.0 Hz, 1H), 7.80 (td, *J* = 7.7, 1.7 Hz, 1H), 7.93 (s, 1H), 8.04 (s, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.56 (d, *J* = 4.7 Hz, 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 26.1, 26.5, 29.1, 29.3, 29.4, 29.9, 49.8, 68.3, 115.7, 121.7, 122.7, 125.2, 136.9, 142.9, 148.0, 151.7, 152.0, 153.2, 155.4; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{22}H_{29}N_6O$, 393.23974; found, 393.23932; Anal. Calcd for $C_{22}H_{28}N_6O$: C, 67.32; H, 7.19; N, 21.41. Found: C, 67.18; H, 7.16; N, 21.47.

N-(4-((10-(1*H*-imidazol-1-yl)decyl)oxy)phenyl) picolinimidamide (9l).

Light brown powder; 61 mg; yield 38% (starting from 0.12 g of **8l**, 0.38 mmol); mp 104–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.39 (m, 10H), 1.41–1.50 (m, 2H), 1.73–1.81 (m, 4H), 3.89–3.97 (m, 4H total, overlapped), 3.91 (t, *J* = 7.1 Hz, overlapped), 3.94 (t, *J* = 6.5 Hz, overlapped), 5.38–6.29 (brs, *imidamide NHs*), 6.89–6.98 (m, 5H), 7.05 (s, 1H), 7.38 (ddd, *J* = 7.4, 4.8, 1.1 Hz, 1H), 7.45 (s, 1H), 7.80 (td, *J* = 7.7, 1.7 Hz, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.56 (ddd, *J* = 4.8, 1.7, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2, 26.7, 29.2, 29.46, 29.47, 29.50, 29.55, 31.2, 47.2, 68.4, 115.7, 118.9, 121.7, 122.7, 125.2, 129.5, 136.9, 137.2, 142.5, 148.0, 151.8, 153.2, 155.4; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{25}H_{34}N_5O$, 420.27579; found, 420.27637; Anal. Calcd for $C_{25}H_{33}N_5O$: C, 71.57; H, 7.93; N, 16.69. Found: C, 71.27; H, 7.66; N, 16.47.

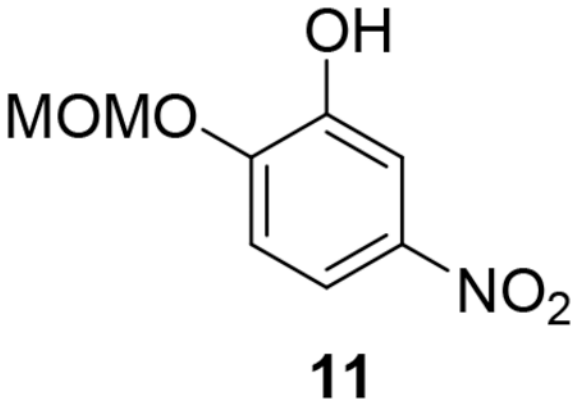
N-(4-((8-(1*H*-pyrrol-1-yl)octyl)oxy)phenyl) picolinimidamide (9m).

The synthesis of **9m** follows the general synthesis of **9a-l** with the modification of using 1.5 equivalents of *S*-(2-naphthylmethyl)-2-pyridyl thioimidate hydrobromide and, after completion of the reaction, no diethyl ether treatment was performed. The compound was purified by column chromatography using hexanes/DCM (1:1.5) on triethylamine neutralized silica. After column chromatography purification step, the powder was triturated with diethyl ether/hexanes (3 × 10 mL) followed by filtration. The filtrate was further crystalized using hexanes/ethyl acetate to yield the final product Light brown powder, 58

mg, yield 17% (starting from 0.25 g of **8m**, 0.87 mmol); mp 93–95 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26–1.40 (m, 6H), 1.42–1.51 (m, 2H), 1.73–1.82 (m, 4H), 3.87 (t, *J* = 7.2 Hz, 2H), 3.94 (t, *J* = 6.5 Hz, 2H), 5.52–6.09 (brs, *imidamide NHs*), 6.14 (t, *J* = 2.1 Hz, 2H), 6.65 (t, *J* = 2.1 Hz, 2H), 6.89–6.99 (m, 4H), 7.39 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H), 7.81 (td, *J* = 7.8, 1.7 Hz, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.57 (d, *J* = 4.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2, 26.9, 29.3, 29.4, 29.5, 31.7, 49.8, 68.4, 107.9, 115.7, 120.6, 121.6, 122.6, 125.2, 136.9, 143.0, 148.0, 151.9, 153.0, 155.4; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{24}H_{31}N_4O$, 391.24924; found, 391.24988; Anal. Calcd for $C_{24}H_{30}N_4O$: C, 73.81; H, 7.74; N, 14.35. Found: C, 74.03; H, 7.89; N, 14.25.

N-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)phenyl)benzimidamide (9n).

Buff powder; 45 mg; yield 22% (starting from 0.15 g of **8h**, 0.52 mmol); mp 107–109 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.28–1.38 (m, 6H), 1.42–1.48 (m, 2H), 1.74–1.80 (m, 4H), 3.90–3.95 (m, 4H), 4.68–5.18 (brs, *imidamide NHs*), 6.87–6.94 (m, 5H), 7.04 (s, 1H), 7.41–7.48 (m, 4H), 7.85 (brs, 2H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 26.1, 26.6, 29.1, 29.3, 29.4, 31.2, 47.1, 68.3, 115.6, 118.9, 122.7, 126.9, 128.7, 129.5, 130.6, 136.1, 137.2, 142.6, 155.3; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{24}H_{31}N_4O$, 391.24924; found, 391.24905; Anal. Calcd for $C_{24}H_{30}N_4O$: C, 73.81; H, 7.74; N, 14.35. Found: C, 73.53; H, 7.80; N, 14.26.

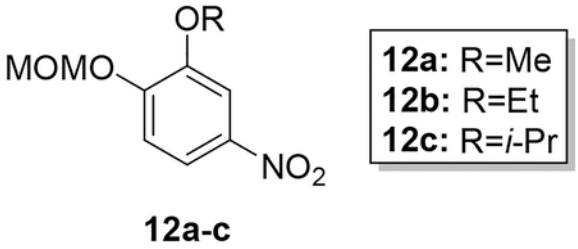


11

2-(Methoxymethoxy)-5-nitrophenol (11).

Compound **11** was synthesized following a previously published procedure.[29] To a solution of 4-nitrocatechol (**10**, 1.0 g, 6.44 mmol) in dry DMF (10 mL) was added 1.2 equivalent of potassium carbonate (1.06 g, 7.67 mmol) and 1.1 equivalent of MOMCl (0.57 g, 7.08 mmol). The reaction mixture was heated to 40 °C for 2 hours. After completion of the reaction, the solvent was removed under reduced pressure, water was added and the product was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated under reduced pressure. The crude product was purified by column chromatography using hexanes/ethyl acetate (4:1) as eluent yielding the product as a buff powder, 0.61 g, 48%.

Synthesis of 2-alkoxy-1-(methoxymethoxy)-4-nitrobenzenes (12a-c).



12a-c

To a solution of **11** (1.30–1.50 mmol) in dry acetonitrile (5 mL) was added 2 equivalents of potassium carbonate (2.60–3.00 mmol) and 3 equivalents of alkyl iodide (3.90–4.50 mmol). The mixture was heated in a sealed tube at 80 °C for 4–5 hours. After the reaction was complete, the solution was cooled and the solvent was removed under reduced pressure. Ice was added to precipitate the pure product, which was filtered as a white powder in 91–97% yield.

2-Methoxy-1-(methoxymethoxy)-4-nitrobenzene (12a).

0.29 g, yield 97%, starting from 0.28 g **11** (1.40 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51 (s, 3H), 3.96 (s, 3H), 5.32 (s, 2H), 7.21 (d, J = 8.9 Hz, 1H), 7.77 (d, J = 2.6 Hz, 1H), 7.86 (dd, J = 8.9, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.5, 56.8, 95.3, 107.1, 114.4, 117.6, 142.5, 149.6, 152.2.

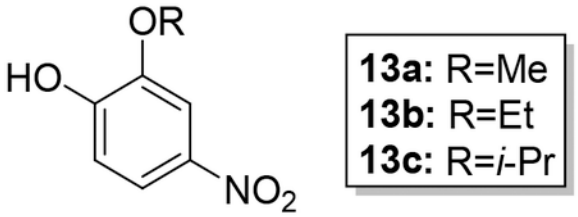
2-Ethoxy-1-(methoxymethoxy)-4-nitrobenzene (12b).

0.28 g, yield 95%, starting from 0.26 g **11** (1.30 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (t, J = 7.0 Hz, 3H), 3.51 (s, 3H), 4.18 (q, J = 7.0 Hz, 2H), 5.31 (s, 2H), 7.20 (d, J = 9.0 Hz, 1H), 7.75 (d, J = 2.6 Hz, 1H), 7.84 (dd, J = 9.0, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7, 56.7, 65.1, 95.4, 108.2, 115.0, 117.4, 142.6, 149.0, 152.4.

2-Isopropoxy-1-(methoxymethoxy)-4-nitrobenzene (12c).

0.33 g, yield 91%, starting from 0.30 g **11** (1.50 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 (d, J = 6.1 Hz, 6H), 3.51 (s, 3H), 4.62 (sep, J = 6.1 Hz, 1H), 5.28 (s, 2H), 7.18 (d, J = 9.0 Hz, 1H), 7.77 (d, J = 2.6 Hz, 1H), 7.83 (dd, J = 9.0, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.0, 56.7, 72.4, 95.3, 110.7, 115.5, 117.5, 142.6, 148.1, 153.4.

Synthesis of 2-alkoxy-4-nitrobenzenes (13a-c).



13a-c

To a cooled solution of **12a-c** (1.11–1.31 mmol) in methanol/DCM (6:3) at 0 °C was added 4N HCl (1 mL) and the mixture was stirred for 30 min. The mixture was then warmed to rt and allowed to stir for another 2 hours. After the reaction was complete, ethyl acetate (30 mL) was added and the product was extracted (2 × 30 mL) with ethyl acetate. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated under reduced pressure. The crude product was purified by column chromatography affording the product in 82–89% yield.

2-Methoxy-4-nitrophenol (13a).[57]

Chromatography solvent: hexanes/DCM 1:1, yellow powder, 0.18 g, yield 81% starting from 0.28 g **12a** (1.31 mmol).

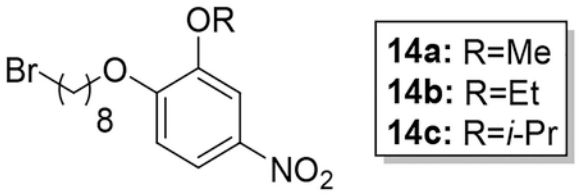
2-Ethoxy-4-nitrophenol (13b).[58]

Chromatography solvent: hexanes/DCM 2:1 to 2:3, yellow powder, 0.18 g, yield 89% starting from 0.25 g **12b** (1.11 mmol).

2-Isopropoxy-4-nitrophenol (13c).

Chromatography solvent: hexanes/DCM 2:1, yellow waxy oil, 0.23 g, yield 90% starting from 0.31 g **12c** (1.29 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (d, J = 6.1 Hz, 6H), 4.71 (sep, J = 6.1 Hz, 1H), 6.31 (brs, 1H), 6.97 (d, J = 8.8 Hz, 1H), 7.75 (d, J = 2.5 Hz, 1H), 7.84 (dd, J = 8.8, 2.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.0, 72.7, 108.4, 114.1, 118.4, 141.2, 144.3, 152.6.

Synthesis of 1-((8-bromooctyl)oxy)-2-alkoxy-4-nitrobenzenes (14a-c).



14a-c

Compounds **14a-c** were prepared from **13a-c** (0.92–1.11 mmol) following the general synthesis of compounds **6a-i**.

1-((8-Bromooctyl)oxy)-2-methoxy-4-nitrobenzene (14a).

Yellowish white powder, 0.33 g, yield 91% starting from 0.17 g **13a** (1.00 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30–1.54 (m, 8H), 1.80–1.93 (m, 4H), 3.40 (t, J = 6.8 Hz, 2H), 3.94 (s, 3H), 4.10 (t, J = 6.7 Hz, 2H), 6.88 (d, J = 9.0 Hz, 1H), 7.73 (d, J = 2.6 Hz, 1H), 7.88 (dd, J = 8.9, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 28.2, 28.7, 28.9, 29.2, 32.8, 34.0, 56.4, 69.6, 106.8, 110.9, 117.9, 141.4, 149.2, 154.3.

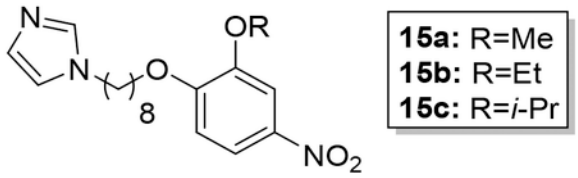
1-((8-Bromooctyl)oxy)-2-ethoxy-4-nitrobenzene (14b).

Yellowish white powder, 0.32 g, yield 92% starting from 0.17 g **13b** (0.92 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31–1.52 (m, 11H total, overlapped), 1.48 (t, *J* = 7.0 Hz, overlapped) 1.80–1.92 (m, 4H), 3.40 (t, *J* = 6.8 Hz, 2H), 4.09 (t, *J* = 6.7 Hz, 2H, overlapped), 4.15 (q, *J* = 7.0 Hz, 2H, overlapped), 6.88 (d, *J* = 8.9 Hz, 1H), 7.73 (d, *J* = 2.6 Hz, 1H), 7.87 (dd, *J* = 8.9, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7, 25.9, 28.2, 28.8, 29.0, 29.2, 32.9, 34.0, 65.1, 69.6, 108.2, 111.2, 117.8, 141.4, 148.6, 154.7.

1-((8-Bromooctyl)oxy)-2-isopropoxy-4-nitrobenzene (14c).

White powder, 0.36 g, yield 84% starting from 0.22 g **13c** (1.11 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31–1.54 (m, 14H total, overlapped), 1.38 (d, *J* = 6.1 Hz, overlapped), 1.80–1.92 (m, 4H), 3.41 (t, *J* = 6.8 Hz, 2H), 4.07 (t, *J* = 6.5 Hz, 2H), 4.57 (sep, *J* = 6.0 Hz, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 7.76 (d, *J* = 2.6 Hz, 1H), 7.87 (dd, *J* = 9.0, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.1, 25.9, 28.2, 28.8, 29.0, 29.2, 32.9, 34.0, 69.5, 72.8, 111.7, 111.7, 118.3, 141.3, 147.5, 156.0.

Synthesis of 1-(8(2-alkoxy-4-nitrophenoxy)octyl)-1*H*-imidazoles (15a-c).



15a-c

Compounds **15a-c** were synthesized from **14a-c** (0.83–0.98 mmol) following the general synthesis of compounds **7a-l**.

1-(8(2-Methoxy-4-nitrophenoxy)octyl)-1*H*-imidazole (15a).

Yellowish white powder, 0.23 g, yield 74% starting from 0.32 g **14a** (0.89 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.50 (m, 8H), 1.71–1.90 (m, 4H), 3.88–3.94 (m, 5H total, overlapped), 3.94 (s, overlapped), 4.08 (t, *J* = 6.7 Hz, 2H), 6.85–6.89 (m, 2H), 7.04 (s, 1H), 7.44 (s, 1H), 7.72 (d, *J* = 2.6 Hz, 1H), 7.87 (dd, *J* = 8.9, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.9, 26.6, 28.9, 29.1, 29.2, 31.1, 47.1, 56.4, 69.5, 106.8, 110.9, 117.9, 118.8, 129.5, 137.2, 141.4, 149.2, 154.3.

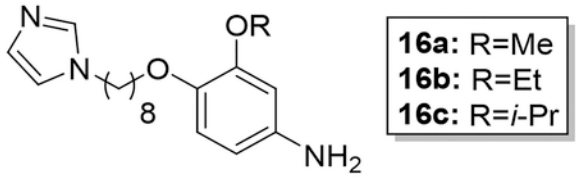
1-(8(2-Ethoxy-4-nitrophenoxy)octyl)-1*H*-imidazole (15b).

Yellowish white powder, 0.22 g, yield 73% starting from 0.31 g **14b** (0.83 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.51 (m, 11H total, overlapped), 1.47 (t, *J* = 7.0 Hz, overlapped), 1.72–1.90 (m, 4H), 3.92 (t, *J* = 7.1 Hz, 2H), 4.07 (t, *J* = 6.7 Hz, 2H), 4.14 (q, *J* = 7.0 Hz, 2H), 6.84–6.90 (m, 2H), 7.04 (s, 1H), 7.45 (s, 1H), 7.72 (d, *J* = 2.6 Hz, 1H), 7.86 (dd, *J* = 8.9, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.7, 25.9, 26.6, 28.9, 29.1, 29.2, 31.2, 47.1, 65.1, 69.5, 108.2, 111.2, 117.8, 118.9, 129.5, 137.2, 141.4, 148.6, 154.6.

1-(8(2-Isopropoxy-4-nitrophenoxy)octyl)-1*H*-imidazole (15c).

Yellow oil, 0.33 g, yield 90% starting from 0.38 g **14c** (0.98 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24–1.50 (m, 14H total, overlapped), 1.36 (d, *J* = 6.1 Hz, overlapped) 1.71–1.88 (m, 4H), 3.91 (t, *J* = 7.1 Hz, 2H), 4.05 (t, *J* = 6.6 Hz, 2H), 4.55 (sep, *J* = 6.1 Hz, 1H), 6.84–6.89 (m, 2H), 7.03 (s, 1H), 7.43 (s, 1H), 7.74 (d, *J* = 2.7 Hz, 1H), 7.85 (dd, *J* = 9.0, 2.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.1, 25.9, 26.6, 28.9, 29.1, 29.2, 31.1, 47.1, 69.4, 72.7, 111.6, 111.7, 118.2, 118.8, 129.5, 137.1, 141.3, 147.5, 155.9.

Synthesis of 4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-3-alkoxyanilines (16a-c).



16a-c

Compounds **16a-c** were synthesized from **15a-c** (0.49–0.86 mmol) following the general synthesis of compounds **8a-m**.

4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-3-methoxyaniline (16a).

Orange oil, 0.27 g, yield 99% starting from 0.30 g **15a** (0.86 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.46 (m, 8H), 1.70–1.81 (m, 4H), 3.00–3.39 (brs, *ArNH*$_2$), 3.79 (s, 3H), 3.86–3.93 (m, 4H), 6.19 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.29 (d, *J* = 2.6 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 1H), 6.88 (t, *J* = 1.1 Hz, 1H), 7.04 (s, 1H), 7.44 (s, 1H).

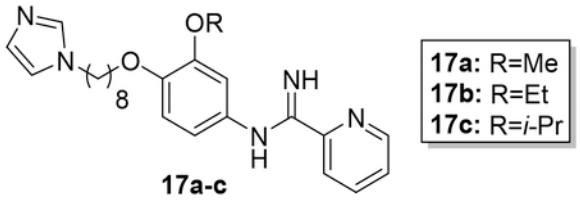
4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-3-ethoxyaniline (16b).

Brown oil, 0.16 g, yield 98% starting from 0.18 g **15b** (0.49 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24–1.48 (m, 11H total, overlapped), 1.40 (t, *J* = 7.0 Hz, overlapped), 1.68–1.82 (m, 4H), 1.92–2.43 (brs, *ArNH*$_2$), 3.86–3.95 (m, 4H), 4.01 (q, *J* = 7.0 Hz, 2H), 6.20 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.30 (d, *J* = 2.7 Hz, 1H), 6.72 (d, *J* = 8.4 Hz, 1H), 6.89 (brs, 1H), 7.04 (s, 1H), 7.45 (s, 1H).

1-((8-(1*H*-imidazol-1-yl)octyl)oxy)-3-isopropoxyaniline (16c).

Brown oil, 0.27 g, yield 98% starting from 0.30 g **15c** (0.79 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22–1.47 (m, 14H total, overlapped), 1.30 (d, *J* = 6.1 Hz, overlapped), 1.66–1.80 (m, 4H), 3.08–3.43 (brs, *ArNH*$_2$), 3.84–3.93 (m, 4H), 4.42 (sep, *J* = 6.1 Hz, 1H), 6.22 (dd, *J* = 8.4, 2.7 Hz, 1H), 6.31 (d, *J* = 2.7 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 6.88 (t, *J* = 1.1 Hz, 1H), 7.03 (s, 1H), 7.44 (s, 1H).

Synthesis of *N*-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)3-alkoxyphenyl) picolinimidamides (17a-c).



17a-c

Compounds **17a-c** were synthesized from **16a-c** (0.45–0.69 mmol) following the general synthesis of compounds **9a-m**.

N-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)3-methoxyphenyl) picolinimidamide (17a).

Yellowish white powder, 130 mg, yield 45% starting from 220 mg **16a** (0.69 mmol); mp 117–119 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28–1.39 (m, 6H), 1.41–1.51 (m, 2H), 1.74–1.87 (m, 4H), 3.84 (s, 3H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.99 (t, *J* = 6.8 Hz, 2H), 5.50–6.34 (brs, *imidamide NHs*), 6.55 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.62 (d, *J* = 2.3 Hz, 1H), 6.87–6.91 (m, 2H), 7.05 (s, 1H), 7.39 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 1H), 7.45 (s, 1H), 7.81 (td, *J* = 7.7, 1.7 Hz, 1H), 8.41 (d, *J* = 8.0 Hz, 1H), 8.57 (ddd, *J* = 4.8, 1.7, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.0, 26.6, 29.1, 29.3, 29.4, 31.2, 47.2, 56.0, 69.6, 106.5, 112.8, 114.6, 118.9, 121.6, 125.3, 129.6, 136.9, 137.2, 143.5, 144.6, 148.0, 150.6, 151.7, 153.2; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{24}H_{32}N_5O_2$, 422.25505; found, 422.25616; Anal. Calcd for $C_{24}H_{31}N_5O_2$: C, 68.38; H, 7.41; N, 16.61. Found: C, 68.18; H, 7.46; N, 16.38.

N-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)3-ethoxyphenyl) picolinimidamide (17b).

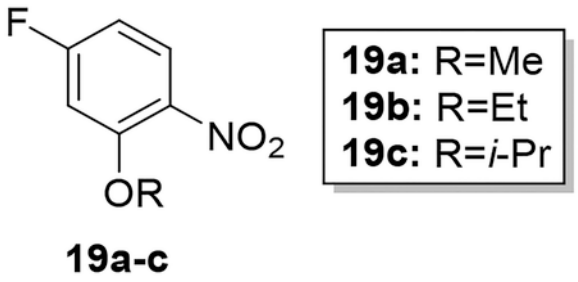
Yellowish white powder, 65 mg, yield 33% starting from 150 mg **16b** (0.45 mmol); mp 100–102 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.51 (m, 11H total, overlapped), 1.42 (t, *J* = 7.0 Hz, overlapped), 1.74–1.84 (m, 4H), 3.92 (t, *J* = 7.1 Hz, 2H), 3.98 (t, *J* = 6.7 Hz, 2H), 4.06 (q, *J* = 7.0 Hz, 2H), 5.49–6.23 (brs, *imidamide NHs*), 6.54 (d, *J* = 8.3 Hz, 1H), 6.61 (s, 1H), 6.87–6.91 (m, 2H), 7.05 (s, 1H), 7.38 (ddd, *J* = 7.5, 4.8, 1.1 Hz, 1H), 7.45 (s, 1H), 7.80 (td, *J* = 7.8, 1.7 Hz, 1H), 8.40 (d, *J* = 7.9 Hz, 1H), 8.56 (d, *J* = 4.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.0, 26.1, 26.6, 29.1, 29.3, 29.5, 31.2, 47.2, 64.5, 70.0, 108.0, 113.0, 115.7, 118.9, 121.6, 125.2, 129.6, 136.9, 137.2, 143.9, 145.0, 148.0, 150.1, 151.8, 153.1; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{25}H_{34}N_5O_2$, 436.27070; found, 436.27029; Anal. Calcd for $C_{25}H_{33}N_5O_2$: C, 68.94; H, 7.64; N, 16.08. Found: C, 69.09; H, 7.54; N, 16.03.

N-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)3-isopropoxyphenyl) picolinimidamide (17c).

Yellowish white powder, 90 mg, yield 31% starting from 220 mg **16c** (0.63 mmol); mp 88–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28–1.39 (m, 12H), 1.42–1.52 (m, 2H), 1.74–1.82 (m, 4H), 3.92 (t, *J* = 7.2 Hz, 2H), 3.97 (t, *J* = 6.6 Hz, 2H), 4.48 (sep, *J* = 6.1 Hz, 1H), 5.50–6.30 (brs, *imidamide NHs*), 6.57 (d, *J* = 8.4 Hz, 1H), 6.64 (s, 1H), 6.88–6.91 (m, 2H), 7.05 (s, 1H), 7.38 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 7.45 (s, 1H), 7.80 (td, *J* = 7.8, 1.7 Hz, 1H), 8.40 (d, *J* = 8.0 Hz, 1H), 8.57 (ddd, *J* = 4.8, 1.6, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 26.1, 26.7, 29.2, 29.3, 29.6, 31.2, 47.2, 70.0, 72.1, 111.7, 114.1, 116.3, 118.9, 121.6, 125.2, 129.6, 136.9, 137.2, 143.9, 146.4, 148.0, 149.1, 151.8, 153.1; HRMS (ESI) *m/z* (M

+H)$^+$ calcd for $C_{26}H_{36}N_5O_2$, 450.28635; found, 450.28671; Anal. Calcd for $C_{26}H_{35}N_5O_2$: C, 69.46; H, 7.85; N, 15.58. Found: C, 69.19; H, 7.77; N, 15.28.

Synthesis of 4-fluoro-2-alkoxy-1-nitrobenzenes (19a-c).



19a-c

To a solution of **18** (0.50–0.55 g, 3.18–3.50 mmol) in dry DMF (5 mL) was added 1.5 equivalent of potassium carbonate (4.77–5.25 mmol) and 3 equivalent of alkyl iodide (9.54–10.50 mmol) and the mixture was heated in a sealed tube at 80 °C for 4–5 hours. After the reaction was complete, the solution was cooled down and the solvent was removed under reduced pressure. The crude product was purified by column chromatography to afford the products **24a-c** in 64–82% yield.

4-Fluoro-2-methoxy-1-nitrobenzene (19a).[59]

Chromatography solvent: hexanes/DCM 3:1 yellow powder, 0.43 g, yield 79% starting from 0.50 g **18** (3.18 mmol).

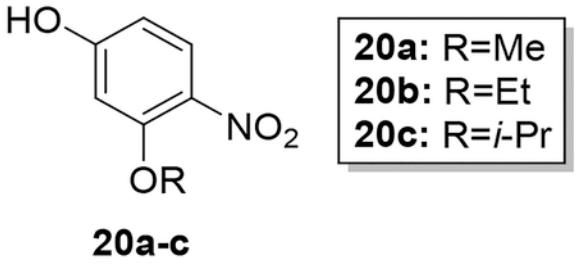
4-Fluoro-2-ethoxy-1-nitrobenzene (19b).[59]

Chromatography solvent: hexanes/DCM 4:1 yellow powder, 0.53 g, yield 82% starting from 0.55 g **18** (3.50 mmol).

4-Fluoro-2-isopropoxy-1-nitrobenzene (19c).[59]

Chromatography solvent: hexanes/DCM 4:1 yellow oil, 0.42 g, yield 64% starting from 0.52 g **18** (3.30 mmol).

Synthesis of 3-alkoxy-4-nitrophenols (20a-c).



20a-c

Compounds **20a-c** were prepared according to a previously published procedure.[30] To a solution of **19a-c** (1.30–1.80 mmol) in DMSO (5 mL) was added NaOH (0.5 g) dissolved in distilled water (5 mL) and the mixture was stirred vigorously at 80 °C for 20 hours. After completion of the reaction, the solution was cooled and the pH was rendered acidic with 6N

HCl. The product was extracted with ethyl acetate (3 × 30 mL), washed with water and brine and dried over sodium sulfate. The combined organic layer was evaporated under reduced pressure then purified by column chromatography using hexanes/ethyl acetate 2:1 as eluent to afford the pure product in 74–85% yield.

3-Methoxy-4-nitrophenol (20a).[60]

Yellow powder, 0.32 g, yield 83%, starting from 0.39 g **19a** (2.27 mmol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.86 (s, 3H), 6.47 (dd, J = 9.1, 2.4 Hz, 1H), 6.60 (d, J = 2.4 Hz, 1H), 7.88 (dd, J = 9.1 Hz, 1H), 10.87 (brs); ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.4, 100.4, 107.5, 128.3, 130.9, 155.7, 164.0.

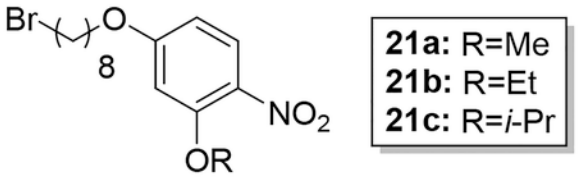
3-Ethoxy-4-nitrophenol (20b).

Yellow powder, 0.36 g, yield 74%, starting from 0.49 g **19b** (2.64 mmol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.35 (t, J = 6.9 Hz, 3H), 4.13 (q, J = 6.9 Hz, 2H), 6.46 (dd, J = 9.0, 2.3 Hz, 1H), 6.58 (d, J = 2.3 Hz, 1H), 7.86 (d, J = 9.0 Hz, 1H), 10.83 (brs); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.3, 64.8, 101.0, 107.5, 128.1, 131.1, 154.8, 163.8.

3-Isopropoxy-4-nitrophenol (20c).[61]

Yellow powder, 0.30 g, yield 85%, starting from 0.36 g **19c** (1.80 mmol).

Synthesis of 4-((8-bromooctyl)oxy)-2-alkoxy-1-nitrobenzenes (21a-c).



21a-c

Compounds **21a-c** were synthesized from **20a-c** (1.32–2.06 mmol) following the general synthesis of compounds **6a-i**.

4-((8-Bromooctyl)oxy)-2-methoxy-1-nitrobenzene (21a).

Yellow powder, 0.67 g, yield 90% starting from 0.35 g **20a** (2.06 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33–1.53 (m, 8H), 1.75–1.91 (m, 4H), 3.41 (t, J = 6.8 Hz, 2H), 3.94 (s, 3H), 4.02 (t, J = 6.5 Hz, 2H), 6.48 (dd, J = 9.0, 2.5 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 7.98 (d, J = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 28.2, 28.8, 29.1, 29.2, 32.9, 34.0, 56.6, 68.9, 100.2, 105.3, 128.6, 132.9, 155.9, 164.5.

4-((8-Bromooctyl)oxy)-2-ethoxy-1-nitrobenzene (21b).

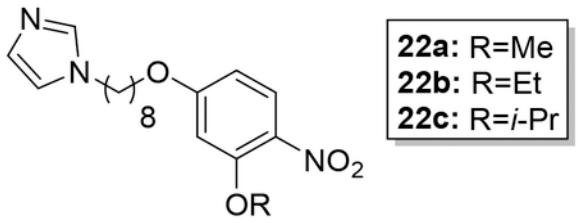
Yellow powder, 0.51 g, yield 76% starting from 0.33 g **20b** (1.80 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.32–1.52 (m, 11H total, overlapped), 1.48 (t, J = 7.0 Hz, overlapped) 1.75–1.91 (m, 4H), 3.41 (t, J = 6.8 Hz, 2H), 4.00 (t, J = 6.5 Hz, 2H), 4.15 (q, J = 7.0 Hz, 2H), 6.46 (dd, J = 9.0, 2.4 Hz, 1H), 6.50 (d, J = 2.3 Hz, 1H), 7.95 (d, J = 9.0 Hz, 1H); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 14.7, 26.0, 28.2, 28.8, 29.1, 29.2, 32.9, 34.1, 65.5, 68.8, 101.0, 105.3, 128.4, 133.2, 155.2, 164.3.

4-((8-Bromooctyl)oxy)-2-isopropoxy-1-nitrobenzene (21c).

White powder, 0.48 g, yield 94% starting from 0.26 g **20c** (1.32 mmol); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30–1.50 (m, 14 H total, overlapped), 1.39 (d, *J* = 6.1 Hz, overlapped) 1.74–1.89 (m, 4H), 3.40 (t, *J* = 6.8 Hz, 2H), 3.99 (t, *J* = 6.5 Hz, 2H), 4.62 (sep, *J* = 6.1 Hz, 1H), 6.45 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.50 (d, *J* = 2.5 Hz, 1H), 7.89 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.0, 25.9, 28.1, 28.7, 29.1, 29.2, 32.8, 34.0, 68.7, 72.8, 102.5, 105.4, 128.2, 134.2, 154.1, 164.0.

Synthesis of 1-(8(3-alkoxy-4-nitrophenoxy)octyl)-1*H*-imidazoles (22a-c).



22a-c

Compounds **22a-c** were synthesized from **21a-c** (0.85–1.38 mmol) following the general synthesis of compounds **7a-l**.

1-(8-(3-Methoxy-4-nitrophenoxy)octyl)-1*H*-imidazole (22a).

Yellow powder, 0.28 g, yield 81% starting from 0.36 g **21a** (1.00 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24–1.50 (m, 8H), 1.72–1.84 (m, 4H), 3.89–3.95 (m, 5H total, overlapped), 3.93 (s, overlapped), 4.00 (t, *J* = 6.5 Hz, 2H), 6.47 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.89 (s, 1H), 7.05 (s, 1H), 7.45 (s, 1H), 7.98 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0, 26.6, 29.09, 29.11, 29.3, 31.2, 47.1, 56.6, 68.8, 100.2, 105.3, 118.9, 128.6, 129.6, 132.9, 137.2, 155.9, 164.5.

1-(8-(3-Ethoxy-4-nitrophenoxy)octyl)-1*H*-imidazole (22b).

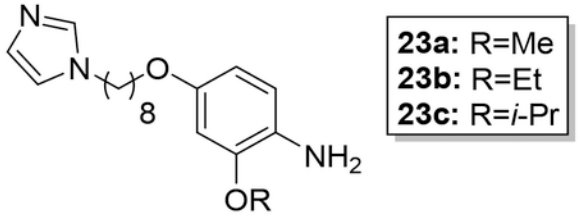
Yellow powder, 0.25 g, yield 81% starting from 0.32 g **21b** (0.85 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25–1.50 (m, 11H total, overlapped), 1.47 (t, *J* = 7.0 Hz, overlapped), 1.72–1.83 (m, 4H), 3.92 (t, *J* = 7.1 Hz, 2H), 3.99 (t, *J* = 6.5 Hz, 2H), 4.14 (q, *J* = 7.0 Hz, 2H), 6.45 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 6.89 (s, 1H), 7.04 (s, 1H), 7.45 (s, 1H), 7.94 (d, *J* = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 25.9, 26.6, 29.08, 29.10, 29.2, 31.1, 47.1, 65.5, 68.7, 101.0, 105.3, 118.9, 128.4, 129.6, 133.3, 137.2, 155.1, 164.3.

1-(8-(3-Isopropoxy-4-nitrophenoxy)octyl)-1*H*-imidazole (22c).

Yellow oil, 0.35 g, yield 79% starting from 0.46 g **21c** (1.18 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25–1.48 (m, 14H total, overlapped), 1.40 (d, *J* = 6.1 Hz, overlapped), 1.73–1.81 (m, 4H), 3.92 (t, *J* = 7.1 Hz, 2H), 3.98 (t, *J* = 6.5 Hz, 2H), 4.61 (sep, *J* = 6.1 Hz, 1H), 6.45

(dd, *J* = 9.1, 2.5 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 6.89 (s, 1H), 7.04 (s, 1H), 7.45 (s, 1H), 7.90 (d, *J* = 9.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.0, 26.0, 26.6, 29.10, 29.11, 29.3, 31.2, 47.1, 68.7, 72.8, 102.5, 105.4, 118.9, 128.2, 129.6, 134.3, 137.2, 154.1, 164.0.

Synthesis of 4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-3-alkoxyanilines (23a-c).



23a-c

Compounds **23a-c** were synthesized from **22a-c** (0.66–0.86 mmol) following the general synthesis of compounds **8a-m**.

4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-2-methoxyaniline (23a).

Yellow oil, 0.26 g, yield 95% starting from 0.30 g **22a** (0.86 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23–1.48 (m, 8H), 1.67–1.82 (m, 4H), 2.88–3.30 (brs, *ArNH*$_2$), 3.83 (s, 3H), 3.87 (t, *J* = 6.4 Hz, 2H), 3.91 (t, *J* = 7.1 Hz, 2H), 6.33 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.44 (d, *J* = 2.6 Hz, 1H), 6.62 (d, *J* = 8.4 Hz, 1H), 6.89 (t, *J* = 1.1 Hz, 1H), 7.05 (s, 1H), 7.45 (s, 1H).

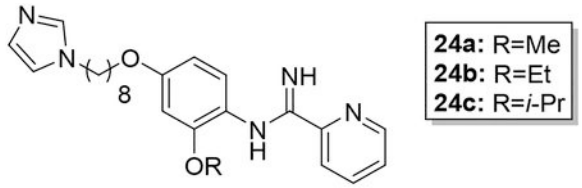
4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-2-ethoxyaniline (23b).

Brown oil, 0.21 g, yield 95% starting from 0.24 g **22b** (0.66 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26–1.47 (m, overlapped, 11H total), 1.42 (t, *J* = 7.0 Hz),1.67–1.83 (m, 4H), 2.34–3.02 (brs, *ArNH*$_2$), 3.86 (t, *J* = 6.6 Hz, 2H), 3.92 (t, *J* = 7.1 Hz, 2H), 4.03 (q, *J* = 7.0 Hz, 2H), 6.32 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.43 (d, *J* = 2.6 Hz, 1H), 6.63 (d, *J* = 8.4 Hz, 1H), 6.89 (s, 1H), 7.05 (s, 1H), 7.46 (s, 1H).

4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-2-isopropoxyaniline (23c).

Brown oil, 0.24 g, yield 100% starting from 0.26 g **22c** (0.69 mmol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25–1.47 (m, overlapped, 14H total), 1.34 (d, *J* = 6.0 Hz), 1.67–1.82 (m, 4H), 3.02–3.36 (brs, *ArNH*$_2$), 3.85 (t, *J* = 6.5 Hz, 2H), 3.91 (t, *J* = 7.1 Hz, 2H), 4.48 (sep, *J* = 6.1 Hz, 1H), 6.32 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.44 (d, *J* = 2.6 Hz, 1H), 6.62 (d, *J* = 8.5 Hz, 1H), 6.89 (s, 1H), 7.04 (s, 1H), 7.45 (s, 1H).

Synthesis of *N*-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)2-alkoxyphenyl) picolinimidamides (24a-c).



24a-c

Compounds **24a-c** were synthesized from **23a-c** (0.54–0.63 mmol) following the general synthesis of compounds **9a-m**.

***N*-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-2-methoxyphenyl) picolinimidamide (24a).**

Yellowish white powder, 120 mg, yield 45% starting from 200 mg **23a** (0.63 mmol); mp 109–112°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26–1.40 (m, 6H), 1.41–1.51 (m, 2H), 1.73–1.83 (m, 4H), 3.80 (s, 3H), 3.90–3.97 (m, 4H), 5.53–6.18 (brs, *imidamide NHs*), 6.50 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.56 (d, *J* = 2.6 Hz, 1H), 6.88–6.99 (brs, 2H total, overlapped), 6.90 (t, *J* = 1.2 Hz), 7.05 (t, *J* = 1.0 Hz, 1H), 7.37 (ddd, *J* = 1.2, 4.8, 7.5 Hz, 1H), 7.46 (s, 1H), 7.80 (td, J = 7.7, 1.7 Hz, 1H), 8.47 (d, *J* = 8.0 Hz, 1H). 8.56 (ddd, *J* = 0.9, 1.7, 4.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1, 26.6, 29.2, 29.4, 29.5, 31.2, 47.2, 56.0, 68.3, 100.7, 105.6, 118.9, 122.0, 123.0, 125.2, 129.6, 136.9, 137.2, 147.9, 151.7, 152.0, 153.6, 156.5; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{24}H_{32}N_5O_2$, 422.25505; found, 422.25452; Anal. Calcd for $C_{24}H_{31}N_5O_2$: C, 68.38; H, 7.41; N, 16.61. Found: C, 68.47; H, 7.54; N, 16.61.

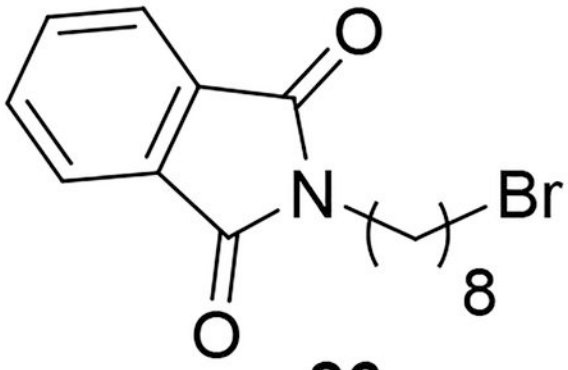
***N*-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-2-ethoxyphenyl) picolinimidamide (24b).**

Buff powder, 100 mg, yield 42% starting from 180 mg **23b** (0.54 mmol); mp 86–89°C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.28–1.38 (m, 9H total, overlapped), 1.34 (t, *J* = 7.0 Hz), 1.43–1.48 (m, 2H), 1.74–1.81 (m, 4H), 3.91–3.95 (m, 4H), 4.03 (q, *J* = 7.0 Hz, 2H), 5.51–6.41 (brs, *imidamide NHs*), 6.51 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.56 (d, *J* = 2.5 Hz, 1H), 6.90 (s, 1H), 6.98 (brs, 1H), 7.05 (s, 1H), 7.39 (ddd, *J* = 0.9, 4.8, 7.4 Hz, 1H), 7.45 (s, 1H), 7.81 (td, *J* = 7.8, 1.6 Hz, 1H), 8.47 (d, *J* = 7.8 Hz, 1H), 8.57 (d, *J* = 4.6 Hz, 1H);^{13}C NMR (176 MHz, $CDCl_3$) δ 15.0, 26.1, 26.6, 29.1, 29.3, 29.5, 31.2, 47.1, 64.5, 68.3, 102.2, 106.1, 118.9, 122.0, 123.5, 125.2, 129.6, 137.0, 137.2, 148.0, 151.2, 151.5, 153.5, 156.5; HRMS (ESI) *m/z* $(M+H)^+$ calcd for $C_{25}H_{34}N_5O_2$, 436.27070; found, 436.27139; Anal. Calcd for $C_{25}H_{33}N_5O_2$: C, 68.94; H, 7.64; N, 16.08. Found: C, 68.66; H, 7.65; N, 15.86.

***N*-(4-((8-(1*H*-imidazol-1-yl)octyl)oxy)-2-isopropoxyphenyl) picolinimidamide (24c).**

Yellow powder, 92 mg, yield 34% starting from 210 mg **23c** (0.60 mmol); mp 82–85 °C; ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.25 (brd, *J* = 5.9 Hz, 6H), 1.28–1.38 (m, 6H), 1.42–1.48 (m, 2H), 1.74–1.80 (m, 4H), 3.92 (t (apparent), *J* = 6.9 Hz, 4H), 4.44 (sep, *J* = 5.9 Hz, 1H), 5.38–6.16 (brs, *imidamide NHs*), 6.55 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.57 (d, *J* = 2.5 Hz, 1H), 6.80–6.98 (brs, 2H total, overlapped), 6.90 (s), 7.05 (s, 1H), 7.36–7.39 (m, 1H), 7.46 (s, 1H), 7.78–7.82 (m, 1H), 8.43 (brs, 1H), 8.57 (d, *J* = 3.9 Hz, 1H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 22.4, 26.1, 26.6, 29.2, 29.3, 29.5, 31.2, 47.2, 68.3, 72.1, 105.9, 107.7, 118.9, 121.8, 123.7,

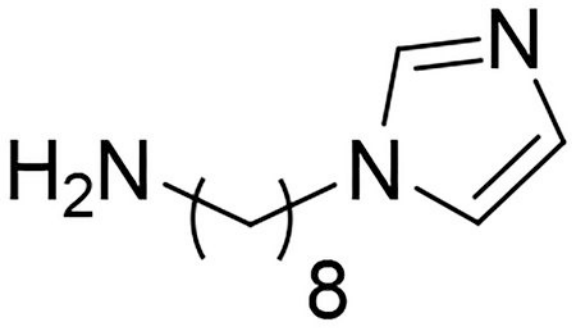
125.0, 129.6, 133.8, 136.8, 137.2, 147.9, 149.8, 152.0, 152.6, 156.1; HRMS (ESI) *m/z* (M +H)$^+$ calcd for $C_{26}H_{36}N_5O_2$, 450.28635; found, 450.28778; Anal. Calcd for $C_{26}H_{35}N_5O_2$: C, 69.46; H, 7.85; N, 15.58. Found: C, 69.40; H, 7.90; N, 15.37.



26

2-(8-bromooctyl) isoindoline-1, 3-dione (26).

To a solution of 1,8-dibromooctane (8.8 g, 32.4 mmol) in dry DMF (30 mL) was added phthalimide potassium salt (2.0 g, 10.8 mmol) and the mixture was stirred at 90 °C for 24h. The reaction mixture was extracted with CH_2Cl_2 (2 × 30 mL) and washed with 0.1N NaOH (50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography using hexanes/ethyl acetate 15:1 as eluent to afford the pure product as a white powder, 2.2 g, yield 60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.46 (m, 8H), 1.62–1.72 (m, 2H), 1.79–1.87 (m, 2H), 3.38 (t, *J* = 6.9 Hz, 2H), 3.67 (t, *J* = 7.3 Hz, 2H), 7.67–7.73 (m, 2H), 7.81–7.86 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8, 28.2, 28.6, 28.7, 29.1, 32.9, 34.1, 38.1, 123.3, 132.3, 134.0, 168.6.

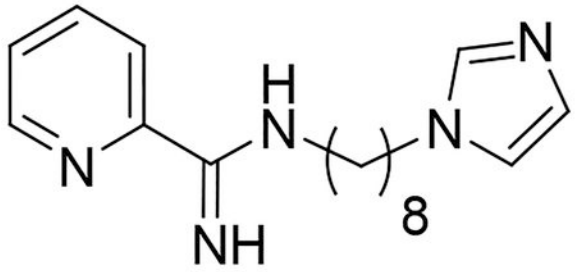


27

8-(1*H*-imidazol-1-yl)octan-1-amine (27).

Compound **27** was prepared over two steps with slight modification to a previously published procedure.[31] To a solution of **26** (2.0 g, 5.91 mmol) in dry DMF (20 mL) was

added 1.5 equivalents K_2CO_3 (1.22 g, 8.86 mmol) and 2 equivalents of imidazole (0.80g, 11.82 mmol) and the mixture was stirred at 80 °C for 12h. After the reaction was completed, the suspension was filtered and the filtrate was concentrated under reduced pressure. The crude product was subjected to silica gel chromatography using DCM/MeOH 10:0.3 as the eluent to afford the pure product as a white powder, 1.20 g, yield 62%. The 2-(8-(1H-imidazol-1-yl)octyl)isoindoline-1,3-dione product (1.1. g, 3.38 mmol) was heated to reflux with 6 equivalents of 80% $NH_2NH_2{\cdot}H_2O$ (1.2 mL, 20.28 mmol) in absolute ethanol (40 mL) under a N_2 atmosphere for 8h. The mixture was cooled and treated with 4N HCl (12 mL) and heated to reflux for a further 6 h. The suspension was filtered and the filtrate was concentrated under reduced pressure. The solution was rendered alkaline with 2N NaOH then the product was extracted with DCM (100 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford the product as yellow oil which was taken to the next step without further purification, 0.44 g, yield 67%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19–1.34 (m, 8H), 1.35–1.48 (m, 2H), 1.67–1.87 (m, 4H), 2.66 (t, J = 7.0 Hz, 2H), 3.89 (t, J = 7.1 Hz, 2H), 6.87 (s, 1H), 7.02 (s, 1H), 7.44 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6, 26.8, 29.1, 29.3, 31.1, 33.6, 42.2, 47.1, 118.8, 129.4, 137.2



28

N-(8-(1*H*-imidazol-1-yl)octyl)picolinimidamide (28).

The synthesis of **28** follows the general synthesis and workup procedure for **9a-l** with the modification of using 2.2 equivalents of *S*-(2-naphthylmethyl)-2-pyridyl thioimidate hydrobromide. The compound was further purified by column chromatography using DCM/methanol/triethylamine (10:1.5:0.5) followed by trituration from hexanes/ether (3 × 10 mL). White powder, 65 mg, yield 28% (starting from 0.15 g of **26**, 0.77 mmol); mp 65–67 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.16–1.40 (m, 8H), 1.53–1.62 (m, 2H), 1.64–1.73 (m, 2H), 3.15 (t, J = 7.0 Hz, 2H), 3.93 (t, J = 7.1 Hz, 2H), 6.86 (s, 1H), 7.14 (t, J = 1.1 Hz, 1H), 7.45 (ddd, J = 7.4, 4.8, 1.1 Hz, 1H), 7.59 (s, 1H), 7.86 (td, J = 7.7, 1.7 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.55 (ddd, J = 4.8, 1.6, 0.9 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.9, 27.0, 28.5, 28.8, 30.0, 30.6, 45.4, 45.9, 119.2, 120.5, 124.7, 128.3, 136.8, 137.2, 147.8, 151.9, 154.1; HRMS (ESI) m/z $(M+H)^+$ calcd for $C_{17}H_{26}N_5$, 300.21827; found, 300.21811; Anal. Calcd for $C_{17}H_{25}N_5$: C, 68.19; H, 8.42; N, 23.39. Found: C, 68.30; H, 8.33; N, 23.35.

Biological Assays

IC_{50} determinations (general).

For in vitro cell-based susceptibility assays, validity criteria concerning Z' scores and R^2 values for dose-response curves were as defined in Abdelhameed et al.[52] For colorimetric assays employing J774 macrophages and HepG2 cells, an additional validity criterion was that the mean absorbance of the positive control wells was ≥ 1.0. Absolute IC_{50} values were determined for all assays as described earlier.[11]

L. donovani-infected macrophage assay.

The species identity of the LV82 strain *L. donovani* parasites used in this work was verified by *Hae*III-mediated restriction fragment length polymorphism (RFLP) analysis of the ribosomal internal transcribed spacer region obtained by PCR from promastigote genomic DNA.[62] Evaluation of the activity of hybrid compounds against intracellular *L. donovani* was performed as outlined by Abdelhameed et al.[52]

J774 macrophage toxicity assay.

J774 murine macrophages were confirmed to be of mouse origin by species-specific PCR evaluation and to be free of *Mycoplasma* contamination by IDEXX BioResearch (Columbia, MO). The assay to determine the toxicity of hybrid compounds on J774 murine macrophages was conducted as described by Zhu et al.[63]

HepG2 toxicity assay.

HepG2 cells were authenticated as an exact match to ATCC HB-8065 (HepG2) by the American Type Culture Collection (ATCC, Manassas, VA) using Short Tandem Repeat analysis.[64] The HepG2 assay was performed as described previously[65] with minor modifications. HepG2 cells were maintained in a complete MEM Alpha medium (MEM Alpha (Life Technologies) containing 10% heat-inactivated FBS [Sigma-Aldrich], 50 U/mL penicillin, and 50 μg/mL streptomycin (Life Technologies)). Cells were trypsinized with TrypLE Express (Life Technologies) and were plated at a density of 5×10^3 cells/well in 100 μL volume in 96-well plates. Cells were allowed to adhere overnight at 37 °C in a 5% CO_2 atmosphere. The medium was then replaced with medium containing either compound or control drug and test plates were allowed to incubate for approximately 72 hours at 37 °C in a 5% CO_2 atmosphere. Following this incubation, medium was removed and replaced with 100 μL/well of fresh medium and 25 μL/well of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL in sterile PBS). After an additional 2–3 hour incubation, 100 μL of SDS lysis buffer (100 mg/mL SDS in 50% aqueous DMF) was added to lyse the cells. After an additional 3–5 hour incubation, the absorbance in each well was read at 570 nm with the aid of a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA).

L. donovani CYP51 binding spectra and dissociation constant determination.

To examine the binding of hybrid compounds to *L. donovani* CYP51 (XP_003859085.1), a plasmid containing an N-terminal truncated construct (CYP51-32c; removal of 31 N-

terminal amino acids to increase solubility) and a C-terminal histidine tag (6xHis; to facilitate affinity purification) was selected for heterologous expression in *Escherichia coli* and protein purification as described previously for *L. infantum* CYP51[48] with modifications. Emulgen 911, instead of Triton X-100, was used as detergent to solubilize CYP51 from *E. coli* cell homogenate. Purified CYP51 was analyzed by SDS-PAGE and Western blot (anti-His tag) analysis for purity and by carbon monoxide (CO)-difference spectra upon reduction by sodium dithionite as previously described.[32] To determine dissociation constants (K_d) of CYP-ligand complexes, titration of *L. donovani* CYP51 with selected AA hybrid compounds was performed as described previously[66] with modifications. Titration of CYP51 (0.5 μM) was carried out in 30 mM potassium phosphate buffer, pH7.4, containing 0.1 mM EDTA and 20% glycerol. The difference spectra were obtained by recording the absorbance in the sample cuvette versus the absorbance in the reference cuvette. For compounds without absorbance interference in the 350 to 500 nm range, both reference and sample cuvettes contained the same amount of the protein. Compounds were added to the sample cuvette from stock solutions in DMSO and the corresponding volume of DMSO was added to the reference cuvette. For compounds with absorbance interference in 350 to 500 nm range, only sample cuvette contained protein solution. Compounds were then added to both sample and reference cuvettes from a stock solution in DMSO. The dissociation constants were calculated by fitting the equation $y = \frac{B_{max} \times [S]}{K_d + [S]}$ to the $\Delta(A_{max} - A_{min})$ versus substrate concentration curves.

L. donovani CYP51 inhibition assay.

A fluorescence-based inhibition assay was developed for the *L. donovani* CYP51 using 7-benzyloxy-4-trifluoromethylcoumarin (BFC; Corning Inc.) as substrate and its fluorescent metabolite 7-hydroxy-4-trifluoromethylcoumarin (HFC, excitation 410 nm, emission 538 nm). Cumene hydroperoxide (CuOOH) was used as a cofactor to enable the CYP-catalyzed reactions in place of CYP natural cofactors (NADPH:P450 oxidoreductase and NADPH).[34] Inhibition assays were performed in 96-well plates in a 200 μL volume. A stock solution of fluorogenic substrate BFC (5 mM) was prepared in 1:1 [v/v] DMSO:DMA (dimethylacetamide). The hybrid compounds (1–10 mM) were dissolved in DMSO. The reaction mixture contained CYP51 (60 nM P450), BFC (50 μM), and various concentrations (0.01–100 μM) of hybrid compounds in a phosphate buffer (100 mM, pH 7.4) containing $MgCl_2$ (3.3 mM). DMSO was used as negative control. The reaction was initiated with the addition of 100 μM CuOOH (Alfa Aesar) and monitored at 37°C for emission at 538 nm under excitation at 410 nm on a Tecan Infinite® M200 Pro microplate reader. For each compound, the percent inhibition at different concentrations was calculated as $(1 - RFU_{compound}/RFU_{negative\ control}) \times 100$, where RFU is the relative fluorescence unit. The following two-parameter logistic equation was fitted to the percent inhibition versus the logarithm of the compound concentration curves to obtain the IC_{50} values.

$$y = \frac{100}{1 + \left(\frac{x}{IC_{50}}\right)^{Hill\ Slope}}$$

Microsomal stability determinations.

Metabolic stability of selected hybrid compounds (3 μM) was evaluated using human and mouse liver microsomes (0.5 mg/mL) supplemented with NADPH (1 mM). Microsomal incubations were carried out as described previously[10, 11] with modifications. Reactions were allowed to proceed for up to 60 min at 37°C, then aliquots (10 μL each) were removed and quenched with ice-cold acetonitrile (200 uL) containing an internal standard (2 nM). After centrifugation (2,000 × *g*), the supernatants (100 μL) were dried down with nitrogen gas, then reconstituted with 15% methanol (150 μL) and analyzed by UPLC-MS/MS to quantify the amount of hybrid compound remaining. For LC-UV detection, reaction aliquots (200 μL) were removed and quenched with ice-cold acetonitrile (100 ul) containing an internal standard (5 μM). After centrifugation (2,000 × *g*), the supernatants (250 μL) were dried down with nitrogen gas, then reconstituted with 15% methanol (100 μL) and quantified. Control incubations were run as described above in the absence of NADPH. Microsomal half-life ($t_{1/2}$) values were obtained by fitting the one-phase exponential decay equation ($C = C_0 \times e^{-kt}$; $t_{1/2} = 0.693/k$) to percent substrate remaining versus time curves.

In vivo experiments.

Experiments assessing the in vivo toxicity and antileishmanial efficacy of compound **24c** were conducted according to procedures approved by the Institutional Animal Care and Use Committee at The Ohio State University. In vivo toxicity. The toxicity of compound **24c** to 6–8 week old female BALB/c mice was assessed as outlined by Joice et al.[11] Compound **24c** was dissolved in 50% PEG400/50% water and was administered by oral gavage. No particles or cloudiness were noted when the required solution of **24c** (10 mg/mL) was prepared. In vivo efficacy. The efficacy of compound **24c** and miltefosine in a murine visceral leishmaniasis model were determined according to the methods described by Joice et al.[11] In the present study, the uninfected vehicle control group received PEG-400, compound **24c** was dissolved in 50% PEG400/50% water, and miltefosine was dissolved in water. Administration of the test agents and vehicle was by oral gavage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

AIA	arylimidamide
BFC	7-benzyloxy-4-trifluoromethylcoumarin

CuOOH	cumene hydroperoxide
HFC	7-hydroxy-4-(trifluoromethyl)coumarin
LDU	Leishman-Donovan units
S.E.M.	standard error of the mean

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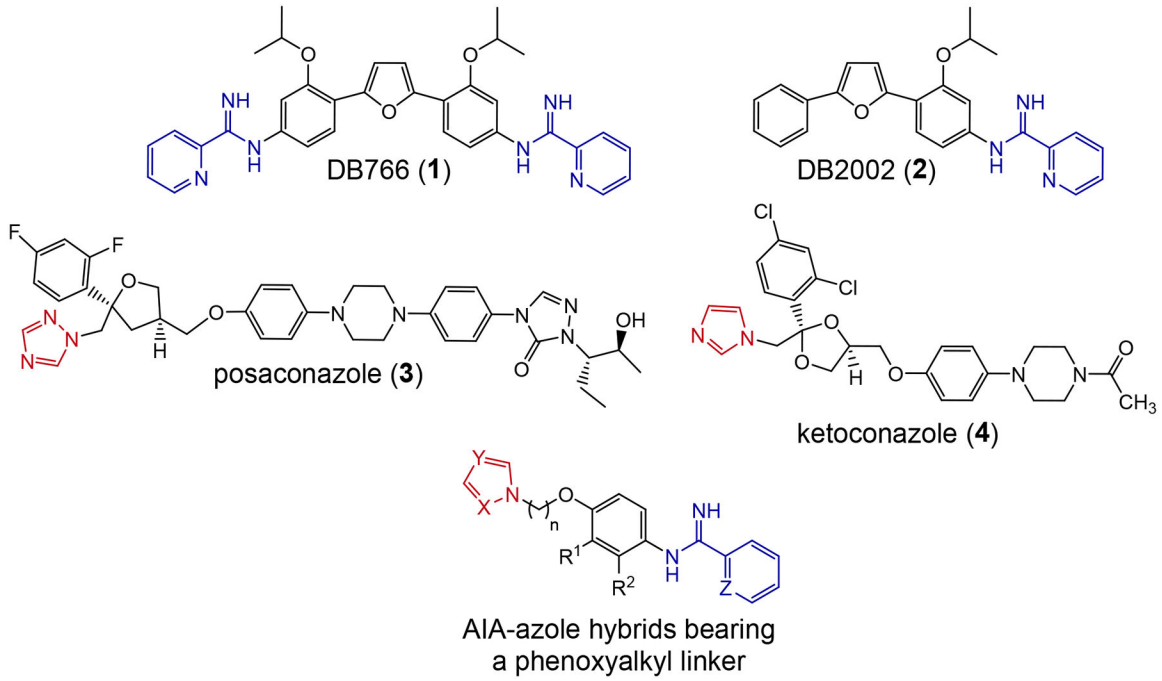


Figure 1.
Structures of **1**-**4** and the AIA-azole scaffold explored here.

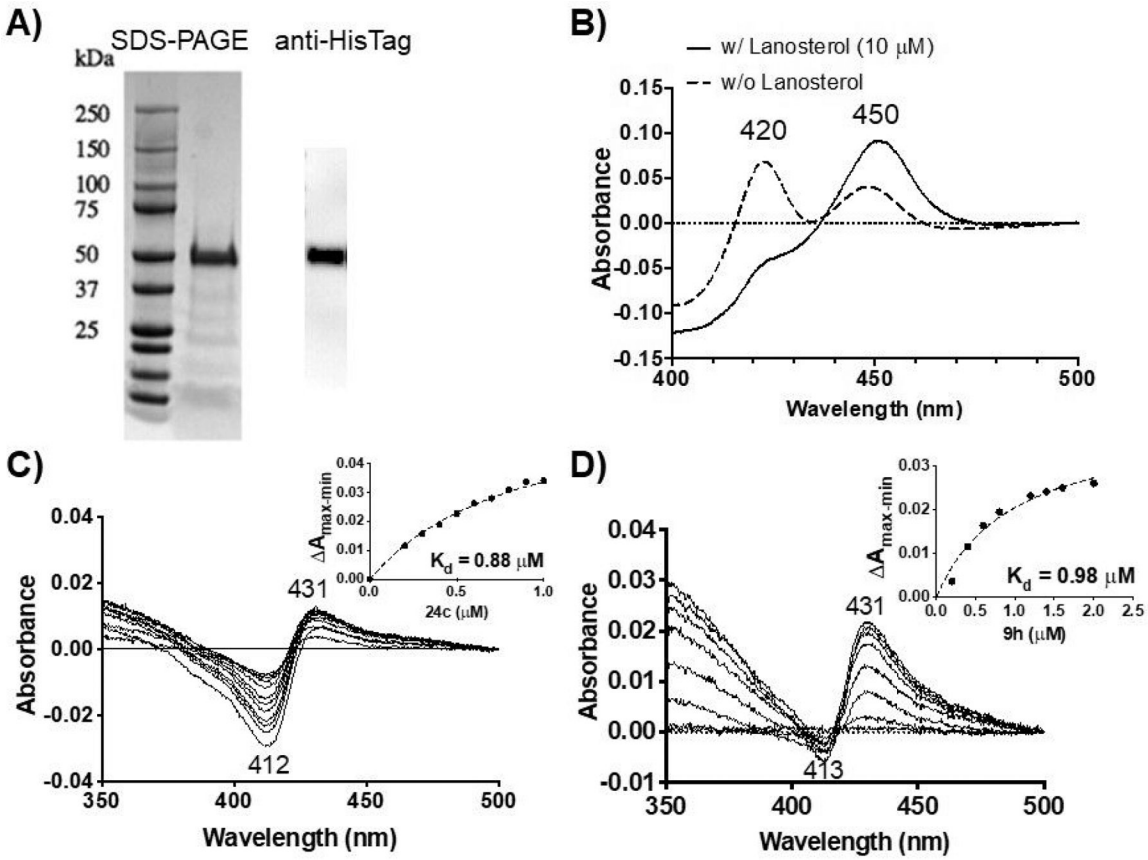


Figure 2.

Gel electrophoresis (A), reduced CO-difference spectra (B), and ligand binding spectra (C and D) of the purified *L. donovani* CYP51. The expected molecular weight of the CYP51-32c construct is 53 kDa. The P450 content was determined to be 7.1 nmol/mg for the batch of purified CYP51 used in this experiment. Various concentrations of **24c** (C) and **9h** (D) were titrated into CYP51 before recording binding spectra and determining dissociation constants (K_d).

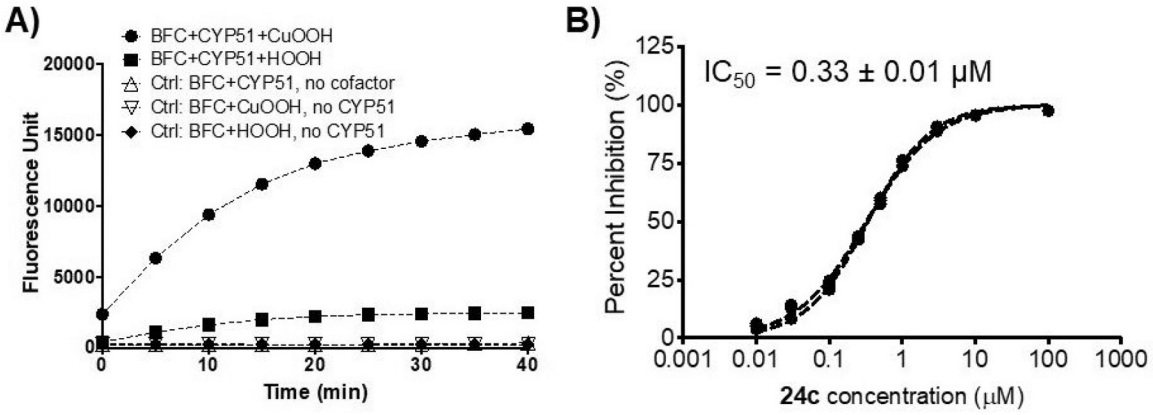


Figure 3.

Fluorescence-based inhibition assay for *L. donovani* CYP51 (A) and inhibition by **24c** (B). The fluorogenic substrate BFC (50 μM) was incubated with purified CYP51-32c (60 nM) in the presence of cofactor CuOOH (0.1 mM) or hydrogen peroxide (HOOH; 25 mM) at 37°C. Control incubations did not contain either the cofactor (but with CYP) or the CYP protein (but with CuOOH or HOOH). IC_{50} value (mean ± SE of triplicate determinations) is shown for **24c**.

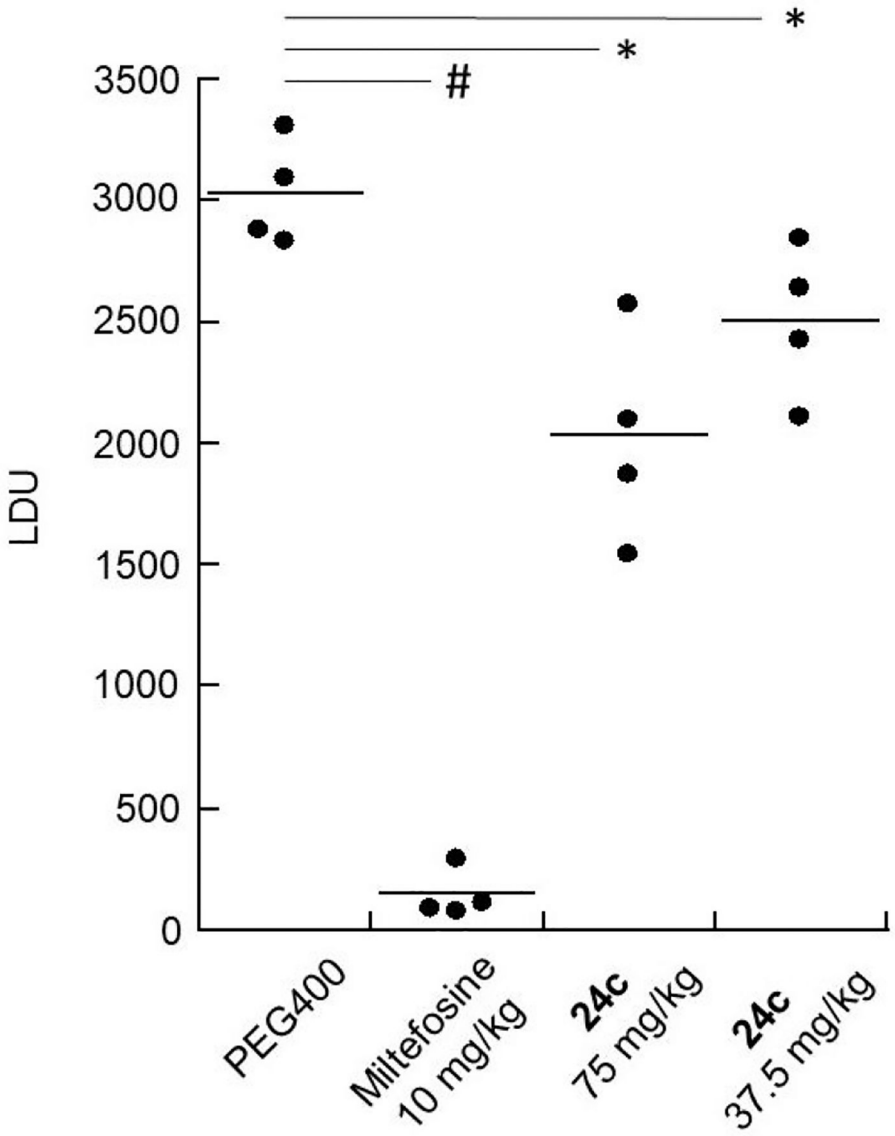
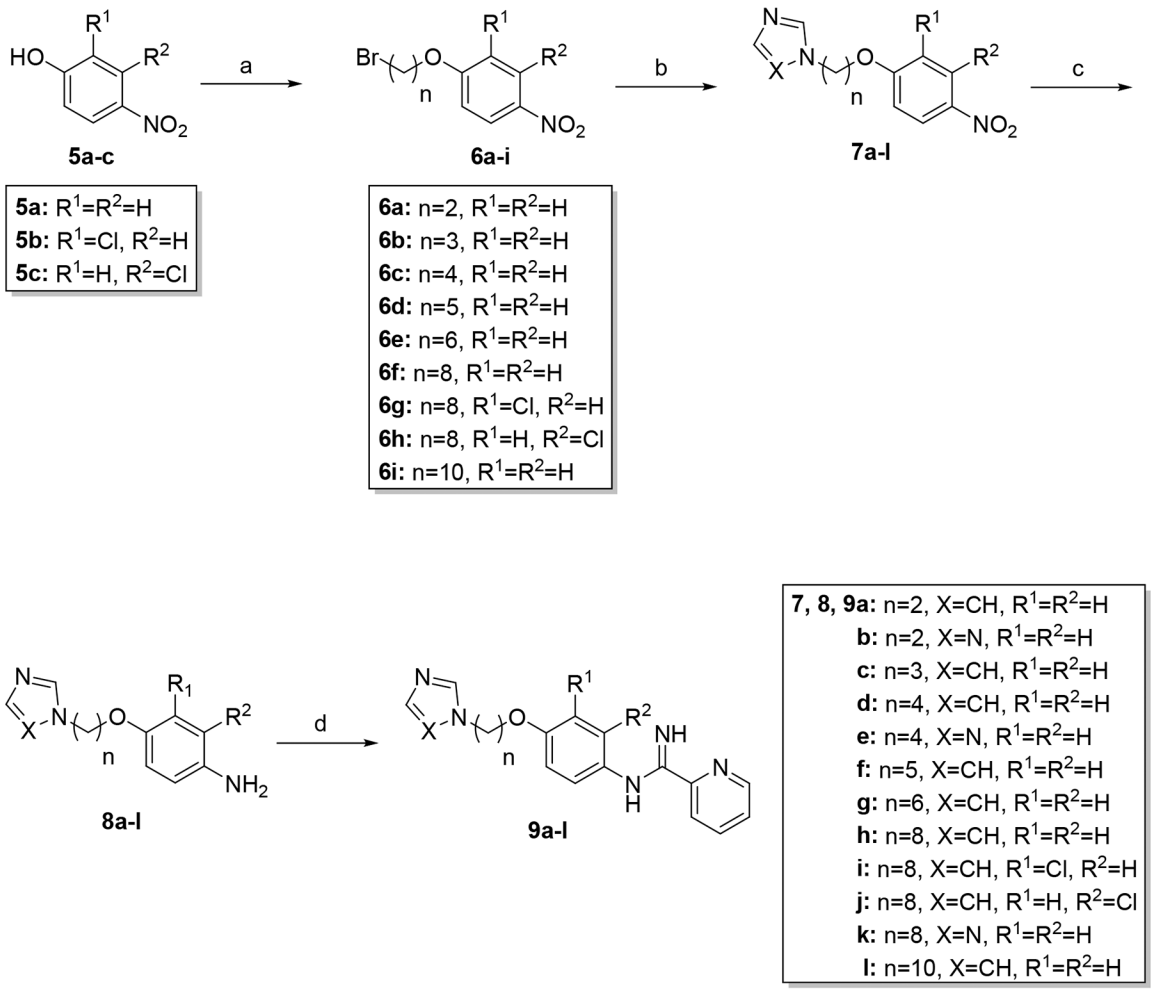


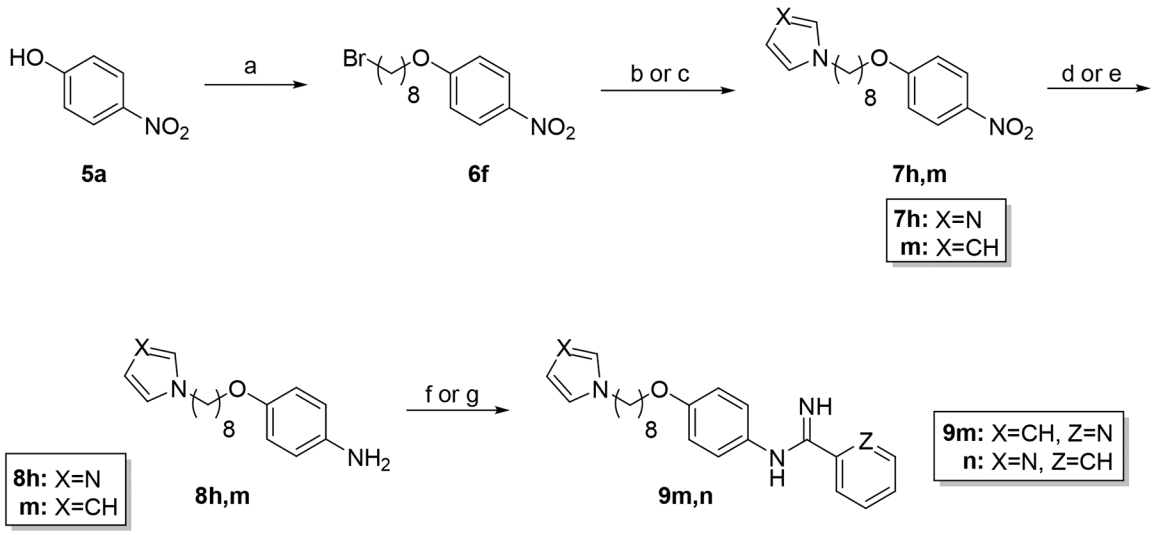
Figure 4.

Efficacy of compound **24c** in the murine model of visceral leishmaniasis. Compound **24c**, miltefosine, and vehicle were administered by oral gavage for five consecutive days to groups of four *L. donovani*-infected female BALB/c mice. Liver parasite burdens are expressed as Leishman-Donovan units (LDU), with horizontal bars indicating the mean for each group. #, $p < 0.001$ compared to PEG400 control group; *, $p < 0.05$ compared to PEG400 control group as assessed by two-tailed Student's t test.



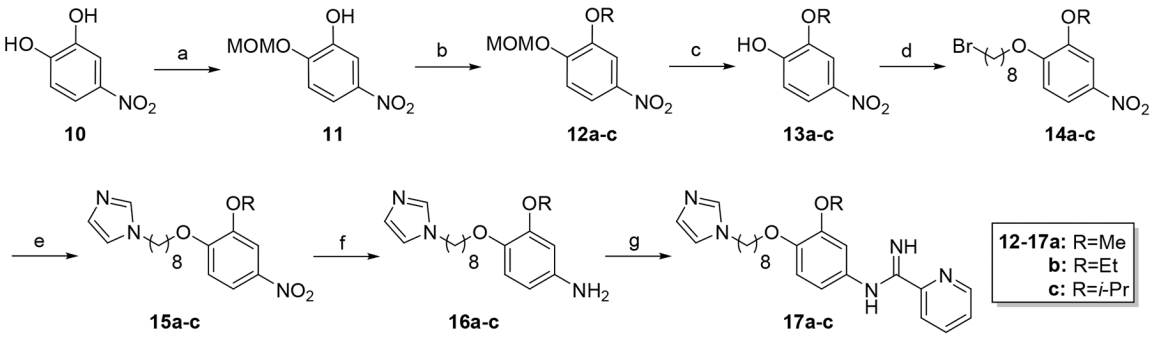
Scheme 1.

Synthesis of **9a-l**. Reagents and conditions: a) dibromoalkane, K_2CO_3, CH_3CN, reflux (62–93%); b) imidazole or 1,2,4-triazole, K_2CO_3, CH_3CN (49–86%); c) $SnCl_2{\cdot}2H_2O$, EtOAc (81–99%); d) naphthalen-2-ylmethyl pyridine-2-carbimidothioate·HBr, CH_3CN/EtOH (1:3), rt (33–70%).



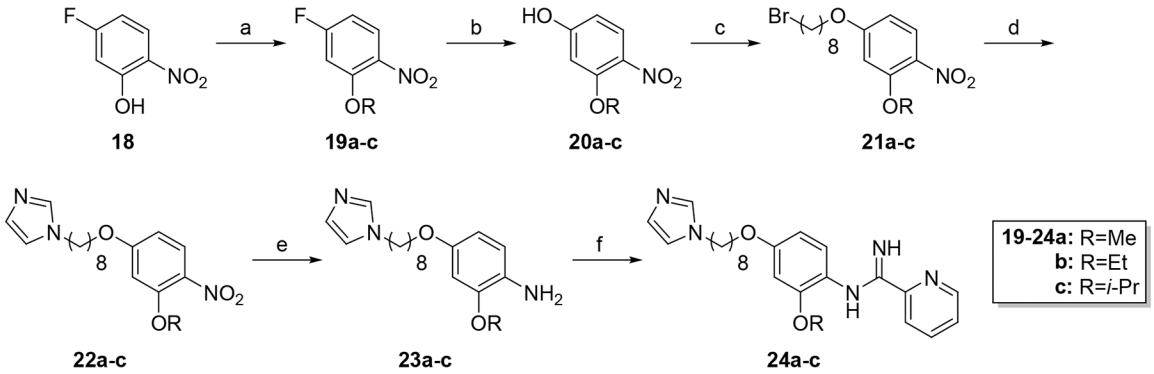
Scheme 2.

Synthesis of **9m,n**. Reagents and conditions: a) 1,8-dibromooctane, K_2CO_3, CH_3CN, reflux (92%); b) imidazole, K_2CO_3, CH_3CN, reflux (86% **7h**); c) pyrrole, KOH, DMF, reflux (35% **7m**); d) $SnCl_2{\cdot}2H_2O$, EtOAc (85% **8h**); e) Zn, NH_4Cl, MeOH (85% **8m**); f) naphthalen-2-ylmethyl benzimidothioate·HBr, CH_3CN/EtOH (1:3), rt (22% **9n**); g) naphthalen-2-ylmethyl pyridine-2-carbimidothioate·HBr, CH_3CN/EtOH (1:3), rt (17% **9m**).



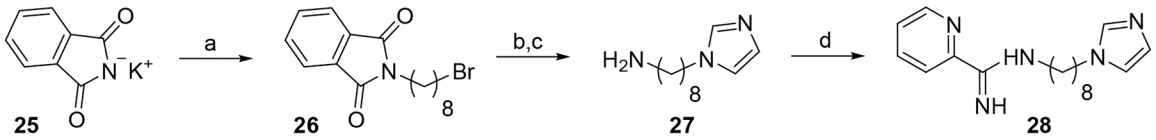
Scheme 3.

Synthesis of **17a-c**. Reagents and conditions: a) MOMCl, K_2CO_3, DMF, 30°C (48%); b) RI, K_2CO_3, CH_3CN, sealed tube, 80°C (91–97%); c) HCl, MeOH/CH_2Cl_2, rt (81–90%); d) 1,8-dibromooctane, K_2CO_3, CH_3CN, reflux (84–92%); e) imidazole, K_2CO_3, CH_3CN, reflux (73–90%); f) $SnCl_2{\cdot}2H_2O$, EtOAc, reflux (98–99%); g) naphthalen-2-ylmethyl pyridine-2-carbimidothioate·HBr, CH_3CN/EtOH (1:3), rt (31–45%).



Scheme 4.

Synthesis of **24a-c**. Reagents and conditions: a) RI, K_2CO_3, sealed tube, DMF, 80°C (64–82%); b) NaOH, DMSO/H_2O, reflux (74–85%); c) 1,8-dibromooctane, K_2CO_3, CH_3CN, reflux (76–94%); d) imidazole, K_2CO_3, CH_3CN, reflux (79–81%); e) $SnCl_2 \cdot 2H_2O$, EtOAc, reflux (95–100%); f) naphthalen-2-ylmethyl pyridine-2-carbimidothioate·HBr, CH_3CN/EtOH (1:3), rt (34–45%).



Scheme 5.

Synthesis of **28**. Reagents and conditions: a) 1,8-dibromooctane, DMF, 90°C (60%); b) imidazole, K_2CO_3, DMF, 80°C (62%); c) $NH_2NH_2.H_2O$, EtOH, reflux (67%); d) naphthalen-2-ylmethyl pyridine-2-carbimidothioate·HBr, CH_3CN/EtOH (1:3), rt (28%).

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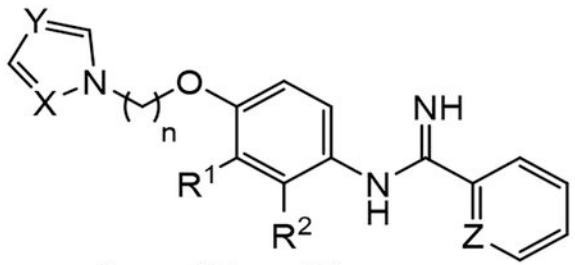
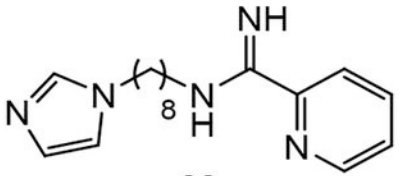
Table 1.

In Vitro Antileishmanial Activity, Mammalian Cell Toxicity, and Inhibition of *L. donovani* CYP51 Activity for AIA-Azole Hybrids



9a-n, 17a-c, 24a-c



28

Compound	X	Y	Z	R^1	R^2	n	IC_{50} against *L. donovani* (μM)[a]	IC_{50} against J774 macrophages (μM)[a]	IC_{50} against HepG2 (μM)[a]	IC_{50} against recombinant *L. donovani* CYP51 (μM)[a]
9a	CH	N	N	H	H	2	>50	>50	>50	21 ± 1
9b	N	N	N	H	H	2	>100	>50	>100	35 ± 2
9c	CH	N	N	H	H	3	>25	>50	>50	22 ± 0
9d	CH	N	N	H	H	4	18 ± 3	>50	>20	6.4 ± 0.1
9e	N	N	N	H	H	4	>100	>50	>100	89 ± 4
9f	CH	N	N	H	H	5	13 ± 5	>50	42 ± 4	4.8 ± 0.1
9g	CH	N	N	H	H	6	8.8 ± 0.6	>40	20 ± 3	1.3 ± 0.1
9h	CH	N	N	H	H	8	1.0 ± 0.0	11 ± 1	12 ± 3	0.36 ± 0.03
9i	CH	N	N	Cl	H	8	1.3 ± 0.2	6.8 ± 0.7	7.7 ± 1.5	0.18 ± 0.01
9j	CH	N	N	H	Cl	8	2.1 ± 0.2	8.6 ± 1.2	8.6 ± 1.2	0.15 ± 0.01
9k	N	N	N	H	H	8	51 ± 5%[b]	36 ± 1	32 ± 5	1.9 ± 0.1
9l	CH	N	N	H	H	10	1.4 ± 0.2	4.9 ± 0.9	6.9 ± 1.0	0.22 ± 0.01
9m	CH	CH	N	H	H	8	1.7 ± 0.3	11 ± 1	13 ± 2	14 ± 1
9n	CH	N	CH	H	H	8	1.2 ± 0.1[c]	0.48 ± 0.02	4.9 ± 1.0	0.34 ± 0.01
17a	CH	N	N	OMe	H	8	2.2 ± 0.3	16 ± 1	23 ± 2	0.57 ± 0.01

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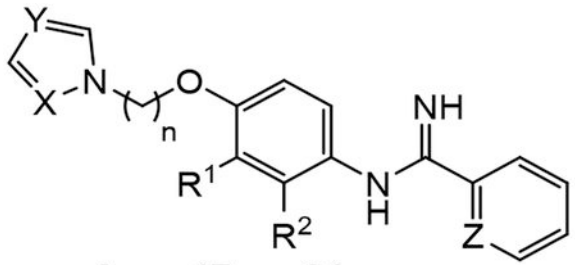
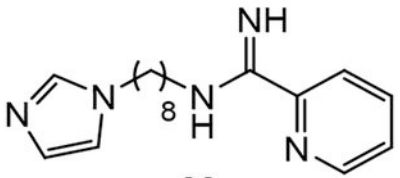
				 **9a-n, 17a-c, 24a-c**			 **28**			
Compound	**X**	**Y**	**Z**	**R^1**	**R^2**	**n**	**IC_{50} against *L. donovani* (μM)[a]**	**IC_{50} against J774 macrophages (μM)[a]**	**IC_{50} against HepG2 (μM)[a]**	**IC_{50} against recombinant *L. donovani* CYP51 (μM)[a]**
17b	CH	N	N	OEt	H	8	1.9 ± 0.3	9.0 ± 1.3	13 ± 0	0.53 ± 0.02
17c	CH	N	N	O*i*Pr	H	8	0.97 ± 0.07	7.8 ± 0.3	18 ± 1	0.29 ± 0.01
24a	CH	N	N	H	OMe	8	1.5 ± 0.4	9.3 ± 0.5	16 ± 2	0.36 ± 0.01
24b	CH	N	N	H	OEt	8	0.73 ± 0.15	6.8 ± 0.1	12 ± 2	0.34 ± 0.01
24c	CH	N	N	H	O*i*Pr	8	0.53 ± 0.05	6.8 ± 0.2	12 ± 2	0.33 ± 0.01
28	-	-	-	-	-	-	>100	Not tested	>100[c]	Not tested
Amphotericin B							0.045 ± 0.003	Not tested	Not tested	Not tested
Podophyllotoxin							Not tested	0.025 ± 0.001	Not tested	Not tested
Doxorubicin							Not tested	Not tested	0.20 ± 0.02	Not tested
Quinacrine							Not tested	Not tested	5.5 ± 0.7	Not tested
Ketoconazole							50 ± 7%[d]	Not tested	Not tested	0.048 ± 0.004
Posaconazole							47 ± 2%[e]	Not tested	Not tested	0.059 ± 0.001
Fluconazole							16 ± 3%[d]	Not tested	Not tested	0.96 ± 0.03

[a] Mean ± standard error of the mean (S.E.M., n=3);

[b] Inhibition of parasitemia at 12.5 μM (mean ± S.E.M. (n=3));

[c] Mean ± range (n=2);

[d] Inhibition of parasitemia at 50 μM (mean ± S.E.M. (n=3));

[e] Inhibition of parasitemia at 7.5 μM (mean ± S.E.M. (n=3)).

Table 2.

In vitro metabolic stability of selected hybrid compounds.

Compound	Human liver microsome $t_{1/2}$ or percent remaining[a]	Mouse liver microsome $t_{1/2}$ or percent remaining[a]
9h	100% after 60 min	70 min
9l	57 min	68 min
17a	100% after 60 min	100% after 60 min
17c	>80% after 60 min	100% after 60 min
24a	>80% after 60 min	>80% after 60 min
24b	>80% after 60 min	100% after 60 min
24c	52 min[b]	67 min[b]
1	>50% after 120 min[c]	>50% after 120 min[c]
2	100%, 111% after 60 min[d]	44%, 61% after 60 min[d]

[a]Substrate concentration 3 μM, measured by LC-MS detection unless otherwise noted

[b]Measured by UV detection

[c]Measured at substrate concentrations of 1 μM and 10 μM, from Wang et al.[10]

[d]Measured by UV detection at substrate concentrations of 1 μM and 10 μM, respectively.