Novel Amino-pyrazole Ureas with Potent In Vitro and In Vivo Antileishmanial Activity


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Supporting Information

ABSTRACT: Visceral leishmaniasis is a severe parasitic disease that is one of the most neglected tropical diseases. Treatment options are limited, and there is an urgent need for new therapeutic agents. Following an HTS campaign and hit optimization, a novel series of amino-pyrazole ureas has been identified with potent in vitro antileishmanial activity. Furthermore, compound 26 shows high levels of in vivo efficacy (>90%) against Leishmania infantum, thus demonstrating proof of concept for this series.

INTRODUCTION

Visceral leishmaniasis (VL) is a systemic disease caused by the protozoan parasites Leishmania donovani and L. infantum.1,2 The parasites (promastigotes) are transmitted through the bite of female phlebotomine sand flies; in the human host, intracellular parasites (amastigotes) target the reticuloendothelial system, surviving and multiplying in different macrophage populations.1,4–7 VL occurs in Asia, East Africa, Latin America, the Mediterranean Basin, and the Middle East;1,4 90% of global cases occur in just six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan.1 It is estimated that there are 0.2–0.4 million new cases of VL per year and a case fatality of >20 000 per year.3 Most infections are asymptomatic, but in patients who develop symptoms, initial presentation is insidious, with development of splenomegaly, hepatomegaly, fever, anemia, pancytopenia, weight loss, and weakness occurring progressively over a period of weeks or even months; VL can be fatal if left untreated.1,4,5 Despite this, VL is one of the most neglected tropical diseases (NTD).1,3–5 Current treatment options for patients with VL include sodium stibogluconate (SSG; antimonial therapy), liposomal amphotericin B (LAB; polyene antibiotic), paromomycin (PM; aminoglycoside antibiotic), and miltefosine (phospholipid).1,4 All of these drugs have deficiencies in terms of safety, efficacy, ease-of-use, cost, or drug resistance.1–4 For example, SSG, LAB,
and PM are all delivered by long-duration, intravenous infusions, or intramuscular injections; only miltefosine is an oral drug, and all are associated with adverse events or toxicity. Research and development for the treatment of VL has evolved in recent years, including the discovery of two nitroimidazole drugs, fexinidazole (phase II) and (2R)-2-methyl-6-nitro-2-\{4-(trifluoromethoxy)phenoxy\}methyl\}-2H,3H-imidazo[2,1-b][1,3]oxazole (VL-2098, preclinical), from the Drugs for Neglected Diseases initiative (DNDi). The DNDi has also recently published the discovery of two new series of antileishmanial compounds: 2-substituted quinolines with potent in vitro activity and promising in vivo efficacy against VL, and aminothiazoles with excellent in vitro potency and good physicochemical properties. Current research toward VL is intensive, as judged by the volume and frequency of medicinal chemistry publications and the extent of a recent in-depth review. Many research groups have reported new chemical scaffolds with promising activity against VL and potential for the future development of antileishmanial agents. Furthermore, a significant number of recent publications have emerged from research groups in India and Brazil, highlighting the urgency with which new medicines are being sought in countries where VL is most prevalent. Overall, however, many of the newly published scaffolds contain undesirable structural alerts associated with adverse toxicity findings. Of note, several pyrazole-based series of antileishmanial compounds have been reported by research groups in Brazil, but these generally have modest weak activity against Leishmania species (e.g., L. amazonensis) that cause cutaneous leishmaniasis (CL), not VL. Consequently, there is an urgent need for additional research into more effective therapeutic options for the treatment of VL.

The overall goal of this project is to deliver a short-course oral treatment for VL that meets the published target product profile (TPP) to address the significant deficiencies of current standard-of-care medications. In this preliminary disclosure, we report the discovery of a novel series of pyrazole-based antileishmanial compounds with excellent in vitro potency against L. infantum. In addition, examples from this series show equivalent in vitro potency against L. donovani and high levels of in vivo efficacy (>90%) against L. infantum in a hamster model of VL. Compounds from the series also demonstrate promising in vivo pharmacokinetics and metabolic stability in human microsomes. Consequently, this series has the potential to deliver a breakthrough in the search for safe and effective oral agents for the treatment of VL.

**RESULTS AND DISCUSSION**

**Chemistry.** The synthesis of amino-pyrazole intermediates was straightforward (Scheme 1). Commercially available 3-(pyridin-2-yl)-1H-pyrazol-5-amine was chlorinated with NCS to provide chloro-substituted amino pyrazole 1. Methyl picolinate was treated with lithiated cyclopropylacetonitrile to give cyano ketone 2, which was cyclized with hydrazine to provide cyclopropyl-substituted amino pyrazole 3. Final compounds were synthesized from 3-(pyridin-2-yl)-1H-pyrazol-5-amine, chloro-derivative 1, or cyclopropyl-derivative 3, according to one of two general methods. Amides 4–10 were synthesized using the required amino-pyrazole intermediate and corresponding acid chloride, according to method A (Scheme 2). Ureas 14–26 were synthesized from the required amino-pyrazole intermediates, via their phenyl carbamates 11–13 (synthesized using method B), and the corresponding amine, according to method C (Scheme 3).

This pyrazole series was initially identified from an HTS campaign with approximately 95,000 compounds from a diverse subset, selected to represent the broad chemical space of Pfizer’s small-molecule compound collection. Compound 4 was a moderately potent hit (IC₅₀ = 10 μM) in vitro activity against L. donovani amastigotes in THP-1 cells (human monocytic leukemia cells). The primary HTS assay employed this intracellular assay, but some early characterization of the hit and initial analogues also showed activity (e.g., 4; IC₅₀ ≥ 10.9 μM) against axenic L. donovani amastigotes (Table 1). Initial simplification of the structure led to additional hit compounds 5 and 6 with improved potency (IC₅₀ = 3.71 and 0.738 μM, respectively) against axenic L. donovani amastigotes. However, 4–6 only showed weak activity (IC₅₀ > 40 μM) when tested in an in vitro macrophage assay using intracellular L. infantum amastigotes (Table 1), nor was there any evidence of cytotoxicity (CC₅₀ > 40 μM) toward the host cell, primary mouse macrophages (PMM). In contrast, the clinical agent miltefosine, used as a reference drug, demonstrated good activity (IC₅₀ = 7.26 μM) in this in vitro macrophage assay.

Although axenic amastigotes have been used for HTS (resulting in the identification of some starting points), occasional poor translation of activity from axenic to intracellular amastigotes has been reported. The axenic amastigote assay has advantages in terms of speed and cost, but the resource-intensive intracellular amastigote assay is thought to be the most relevant as the parasite survives and divides in macrophages to cause the actual disease. Consequently, additional compounds were designed, synthesized, and tested in the intracellular L. infantum amastigote assay to establish whether this scaffold represented a genuinely potent series (comparable to miltefosine) worthy of lead optimization.

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**Scheme 1. Synthesis of Amino-pyrazole Intermediates**

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\[ \begin{align*}
N & \rightarrow \text{Cl} \\
1 \rightarrow \text{Cl} & \rightarrow \text{NH}_2 \\
2 \rightarrow \text{CN} & \rightarrow \text{NH}_2
\end{align*} \]
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**Scheme 2. General Synthesis of Amides 4–10**

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\[ \begin{align*}
R^1 & = \text{H} \\
R^2 & = \text{O} \\
R^3 & = \text{NH}_2
\end{align*} \]
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**Scheme 3. Synthesis of Aminothiazoles 37–38**

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\[ \begin{align*}
\text{OMe} & \rightarrow \text{CN} \\
2 & \rightarrow \text{N} \rightarrow \text{NH}_2
\end{align*} \]
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exploration of the right-hand side of the molecule did not identify any alternative amide groups or substituents with the desired potency against intracellular amastigotes. However, subsequent investigation of the pyrazole core did result in compounds with much-improved potency. In particular, addition of a cyclopropyl group at the 4-position of the pyrazole ring provided 7 (IC$_{50}$ = 2.02 $\mu$M) (Table 2), which was significantly more potent than 6, its unsubstituted counterpart. Furthermore, 7 also demonstrated similar antileishmanial activity against L. donovani (IC$_{50}$ = 2.63 $\mu$M).

The encouraging potency of 7 prompted us to re-examine a variety of benzamide substituents with this new core, especially as we envisaged that the cyclopropyl group would sterically hinder the amide moiety and increase the likelihood of identifying metabolically stable compounds. Excellent potency was observed with 2-methoxy benzamide 8 (IC$_{50}$ = 0.064 $\mu$M). A variety of other benzamides showed levels of potency similar to 7, for example, the 2-fluoro (9; IC$_{50}$ = 0.965 $\mu$M) and 2,6-dichloro (10; IC$_{50}$ = 2.11 $\mu$M) analogues (Table 2).

Importantly, compounds 7–10 showed no evidence of cytotoxicity (CC$_{50}$ > 51 $\mu$M) in the counter-screen using the same PMM as those used in the intracellular amastigote assay (Table 2). In addition, encouraging stability (e.g., 7; Cl$_{int}$ = 12.75 $\mu$L/min/mg) was observed in human liver microsomes (HLM). Compounds 7–10 were significantly more potent than miltefosine, representing a key breakthrough for the series that prompted further hit optimization. However, one issue for the series concerned poor metabolic stability in hamster that precluded us from evaluating compounds in the in vivo hamster model of VL, recognized to be the most relevant model of VL as it closely mimics the pathology of the disease in human. Poor amide stability exclusive to hamster plasma (e.g., 7; 0.3% remaining after 6 h) could be overcome with the sterically hindered 2,6-dichloro analogue 10 (96.9% remaining after 6 h), but despite this, all analogues had poor stability (Cl$_{int}$ > 100 $\mu$L/min/mg) in hamster liver microsomes (HamLM). We concluded that even when sterically hindered with the bulky cyclopropyl group, the amide moiety was likely to be responsible for the high rate of metabolism in hamster and so decided to investigate ureas as a potential, more polar alternative. Initial benzyl urea analogues such as 14–16 (Table 2) had excellent, submicromolar potency (e.g., 16; IC$_{50}$ = 0.130 $\mu$M, AlogP = 3.15) with no evidence of cytotoxicity (CC$_{50}$ > 45 $\mu$M), although high levels of instability in HamLM (Cl$_{int}$ > 400 $\mu$L/min/mg) remained. Interestingly
though, and in contrast to the amide subseries, 14 did show that it was possible to obtain highly potent (IC$_{50}$ = 0.450 μM) ureas without a substituent on the pyrazole core. This provided an opportunity to synthesize additional urea analogues with reduced lipophilicity and potential for greater metabolic stability in HamLM (Cl$_{int}$ < 100 μL/min/mg) that would enable in vivo efficacy studies.

Phenyl piperidine urea 17 was quickly identified as a lead compound with excellent in vitro potency (IC$_{50}$ = 0.296 μM), good selectivity index (SI) over cytotoxicity (CC$_{50}$ > 64 μM; SI > 242), and good stability in HLM (Table 3). Furthermore, 17 showed equivalent antileishmanial activity against $L$. donovani (IC$_{50}$ = 0.078 μM). Compound 17 did not show any instability in hamster plasma (100% remaining after 6 h), but instability in HamLM was still high (Cl$_{int}$ = 216 μL/min/mg), prompting us to undertake in vitro metabolite identification studies in HLM and HamLM to understand this in more detail. While there was only one metabolite observed in HLM, seven metabolites were observed in HamLM. Overall, these data indicated that the majority of oxidative metabolism in both HLM and HamLM occurred on the right-hand side of the molecule, that is, the phenyl piperidine. This gave us the impetus to develop a medicinal chemistry design strategy that focused on retention of the pyridyl left-hand side and unsubstituted pyrazole urea, combined with substituted aryl piperidines and piperazines to block metabolism and/or lower lipophilicity.

In general, analogues 18–26 (which resulted from our design strategy) possessed excellent levels of antileishmanial activity and SI, combined with good metabolic stability in HLM, and, importantly, evidence of an improvement in HamLM stability. Substituted piperidines 18–22 possessed excellent—good potency (IC$_{50}$ = 0.224–2.862 μM) and good HLM stability (Cl$_{int}$ = 25.3–42.5 μL/min/mg) across an AlogP range of 3–4. Improved stability in HamLM was observed for chlorophenyl analogues 20 (Cl$_{int}$ = 43.8 μL/min/mg) and 21 (Cl$_{int}$ = 63.5 μL/min/mg), and cyanophenyl analogue 22 (Cl$_{int}$ = 80.8 μL/min/mg). As 20 and 21 had an AlogP (3.82) higher than that of 17 (3.15), it appeared that the improvement in metabolic stability was due to these substituents successfully blocking sites of metabolism. However, the choice of substituent seemed to be important as the fluorophenyl analogues 18 and 19 had poor stability in HamLM (Cl$_{int}$ = 272 and 207 μL/min/mg, respectively) that was similar to 17, despite having a lower AlogP (3.36) than 20 and 21. Aryl-oxy piperidines 23 and 24 retained excellent antileishmanial activity (IC$_{50}$ = 0.614 and 0.853 μM, respectively). Both compounds are more polar than 17 and had improved metabolic stability in HamLM, especially 24 (Cl$_{int}$ = 30.8 μL/min/mg), perhaps driven by a combination of lower AlogP (2.43) and addition of the 4-cyano substituent. The most polar compound 25 (AlogP = 1.94) had excellent stability in HLM and moderate stability in HamLM (Cl$_{int}$ = < 11.9 and 67.3 μL/min/mg, respectively), although potency was approximately 5-fold weaker than 23. It is interesting that, although 25 was the most stable compound in HLM, it was only the fifth most stable in HamLM, suggesting that different SAR exists for metabolic stability in these two species. Aryl piperazine 26 demonstrated good potency against both $L$. infantum and $L$. donovani (IC$_{50}$ = 2.37 and 1.31 μM, respectively), combined with good stability in HLM and HamLM (Cl$_{int}$ = 30.8 and 46.5 μL/min/mg, respectively). The pairwise comparison of 18 (AlogP = 3.36) and 26 (AlogP = 2.78) shows that piperazine is a suitable, more polar alternative to piperidine that is able to significantly improve HamLM stability. This may be due to the presence of the extra piperazine nitrogen atom, which could block a site of oxidative metabolism that is specific to hamster (HamLM).

Examples 20–26 combined good antileishmanial activity (and SI) with the desired improvement in HamLM stability and provided a number of options to evaluate this series in a hamster in vivo model of VL. Consequently, hamsters infected with $L$. infantum were treated with oral doses of a representative example (26) at 50 mg/kg b.i.d. for 5 days. Treatment with 26...
resulted in 92.7% and 95% reduction in parasite burden in liver and spleen, respectively, without any obvious signs of toxicity. For comparison, when dosed orally at 40 mg/kg q.d. for 5 days, miltefosine showed 97.8% (liver) and 99.6% (spleen) reduction in parasite burden. Pharmacokinetic studies in hamsters with a single oral dose of 50 mg/kg demonstrated that 26 rapidly achieved good levels of exposure ($T_{max} = 1.33$ h, $C_{max} = 5680$ ng/mL, $AUC_{\text{0-24h}} = 25376$ h*ng/mL, $t_{1/2} = 4.2$ h). Consequently, these data illustrate that compounds (e.g., 26) from this novel series are able to successfully treat VL infection in an in vivo setting.

**CONCLUSION**

We have identified two subsets of active compounds developed from a single HTS hit (4). Early hit-expansion activities identified potent amides (7–10) that seem to require a 4-substituent on the pyrazole ring for good activity against intracellular *L. infantum* amastigotes. Unfortunately, these compounds also have poor metabolic stability in hamster liver microsomes, which precluded their evaluation in our hamster model of VL. In contrast, the subsequent design of urea analogues (e.g., 14, 17–26) provided potent activity against intracellular *L. infantum* amastigotes without the need for a pyrazole-4-substituent, combined with evidence of much-improved metabolic stability in hamster liver microsomes. Furthermore, this novel series of pyrazole ureas has potent antileishmanial activity against both *L. infantum* and *L. donovani*, the two species of *Leishmania* parasites that cause VL in humans. Examples from the series (e.g., 26) demonstrate high levels of in vivo efficacy in a hamster model of VL, representing a positive in vivo proof of concept. Furthermore, compounds from the series already meet a number of the published criteria for the selection of a clinical drug candidate for the oral treatment of VL. Additional efforts are ongoing to optimize the series (e.g., physicochemical properties, pharmacokinetics, and solubility) and will be described in future publications.

**EXPERIMENTAL SECTION**

**Biology. Compounds and Reagents.** For in vitro assays, compound stock solutions were prepared in 100% DMSO at 20 mM. Compounds were serially prediluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to ensure a final in-test DMSO concentration of <1%. For in vivo efficacy studies, compound 26 was formulated in 4.5% (w/v) methylcellulose (viscosity = 15 cps) and 5% (v/v) Tween-80 in water at 25 mg/mL. Miltefosine was formulated in water at 20 mg/mL.

**Cell Cultures.** Primary peritoneal mouse macrophages (PMM) were collected 2 days after peritoneal stimulation with a 2% potato starch suspension. MRC5SV2 cells (diploid human embryonic lung fibroblasts) were cultured in minimal essential medium (MEM) containing Earle’s salts, supplemented with l-glutamine, NaHCO$_3$, and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO$_2$.

**Parasites.** *L. infantum* (MHOM/MA/67/ITMAP263) and *L. donovani* (MHOM/ET/67/L82) were maintained in the golden (Syrian) hamster (*Mesocricetus auratus*). Ex vivo amastigotes were collected from the spleen of an infected donor hamster using two centrifugation purification steps: 230g for 10 min, keeping the supernatant layer, and 4100g for 30 min, keeping the pellet. The spleen parasite burden was assessed using the Staubner technique. For the in vitro assays, the inoculum was prepared in RPMI-1640 medium, supplemented with 200 mM l-glutamine, 16.5 mM NaHCO$_3$, and 5% inactivated fetal calf serum. For the in vivo model, an infection inoculum containing $2 \times 10^7$ amastigotes/100 μL was prepared in phosphate buffered saline (PBS).

**Animals.** Female golden hamsters for the in vivo model of visceral leishmaniasis were purchased from Janvier, France (body weight 80–100 g). This study using laboratory rodents was carried out in strict accordance with all mandatory guidelines (EU directives, including the Revised Directive 2010/63/EU on the protection of Animals used for Scientific Purposes that came into force on 01/01/2013, and the declaration of Helsinki in its latest version) and was approved by the ethical committee of the University of Antwerp, Belgium (UA-ECD 2011-74). Female golden hamsters for the pharmacokinetic study were purchased from Vital River, Beijing, China. This study was conducted following institutional review and in accordance with institutional and national guidelines at WuXi AppTec (the Institutional Animal Care and Use Committee (IACUC)).

**In Vitro Axenic *L. donovani Amastigote Assay.**** The assay was performed as previously reported.

**In Vitro Intramacrophage *L. infantum Amastigote Assay.**** The assay was performed in sterile 96-well microtitre plates, each well containing 10 μL of the compound dilution and 190 μL of the PMM/amastigote inoculum (3 × 10$^5$ cells/4.5 × 10$^5$ parasites per well). Parasite multiplication was compared to untreated infected controls (100% growth) and uninfected controls (0% growth). After five-day incubation, total parasite burdens were microscopically assessed after staining the cells with a 10% Giemsa solution. The results were expressed as percentage reduction in parasite burden as compared to untreated control wells. IC$_{50}$ values were determined using an extended dose range (2-fold compound dilutions, 8-point concentration curve). Miltefosine was used as the reference drug. For selected compounds, this assay format was also run using the *L. donovani* inoculum.

**In Vitro MRC5 and PMM Cytotoxicity Assays.** Assays were performed in sterile 96-well microtitre plates, each well containing 10 μL of the compound dilution and 190 μL of MRC5SV2 or PMM inoculum (3 × 10$^5$ cells/mL). Cell growth was compared to untreated controls (100% growth) and assay-media controls (0% growth). After three-day incubation, cell viability was assessed fluorometrically by adding resazurin (50 μL/well of a stock solution in phosphate buffer (50 μg/mL)), incubating for 4 h, and measuring fluorescence ($\lambda_{ex} = 550$ nm, $\lambda_{em} = 590$ nm). The results were expressed as percentage reduction in cell growth as compared to untreated control wells. IC$_{50}$ values were determined using an extended dose range (2-fold compound dilutions, 8-point concentration curve) to a highest concentration of 64 μM. Tamoxifen was included as the reference drug.

**In Vivo Hamster Model of Visceral Leishmaniasis.** Female golden hamsters were randomly allocated to experimental groups of six animals each, based on body weight. At the start of the experiment (day 0), each animal was infected with *L. infantum* inoculum, delivered by intracardial injection. Six hamsters were assigned to the vehicle-treated, infected control group. Six hamsters were assigned per group (1 group/compound) for the evaluation of compound 26 and miltefosine. At day 21 postinfection (21 dpi), all animals in each group were dosed orally for five consecutive days (21–25 dpi): compound 26 was dosed at 50 mg/kg b.i.d.; miltefosine was dosed at 40 mg/kg q.d. At day 35 (10 days after the final oral dose), all animals were euthanized and autopsies were conducted. The study evaluated the following parameters:

1. Adverse effects: All animals were observed daily for the occurrence/presence of adverse effects.
2. Body weight: All animals were weighed twice per week to monitor general health.
3. Parasite burden: Amastigote burdens in each target organ (liver, spleen, and bone marrow) were determined at day 35. The organs of individual animals were weighed (except bone marrow). Impression smears were stained with Giemsa for microscopic examination of the total amastigote burden, defined as the mean number of amastigotes per cell multiplied by the number of cells counted (minimum 500 nuclei); results

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were expressed as a percentage reduction in amastigote burden as compared to vehicle-treated, infected control animals.

**Chemistry.** Unless otherwise indicated, all reactions were magnetically stirred under an inert atmosphere. All reagents, including solvents, were used as received. Anhydrous solvents were dried in-house by passing through activated alumina. Thin-layer chromatography was performed on glass-backed precoated silica gel 60 plates, and compounds were visualized using UV light or iodine. Silica gel column chromatography was performed using 200–300-mesh silica gel. Preparative HPLC was performed using Gilson-281 liquid handlers equipped with one of four columns, chosen from (1) Phenomenex Synergy C18 150 × 30 mm, 4 μm, (2) YMC-pack ODS-AQ 150 × 30 mm, 5 μm, (3) Agela Venusil ASB C18 150 × 21.2 mm, 5 μm, or (4) Boston Symmetry C18 ODS-R 150 × 30 mm, 5 μm; elution was performed with 0.225% (by volume) of formic acid in water (solvent A) and acetonitrile (solvent B); fractions containing product were lyophilized. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer equipped with electrospray ionization, quadruple MS. All compounds were isolated as amorphous solids without collection of melting point data.

3-Isopropoxy-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)-5-(pyrrolidine-1-carbonyl)benzamide (4). To a suspension of dimethyl 5-hydroxysophthalate (210.0 g, 0.1 mol) and anhydrous potassium carbonate solution (27.6 g, 0.2 mol) in DMF (100 mL) at room temperature was added 2-bromopropane (25.5 g, 0.15 mol), and the mixture was stirred at 80 °C for 16 h. After being cooled to room temperature, the mixture was diluted with water (250 mL) and extracted with ethyl acetate (150 mL × 2). The combined organic extracts were washed with brine (100 mL × 3), dried over anhydrous Na2SO4, and concentrated under reduced pressure to give dimethyl 5-isopropoxysophthalate as a white solid (25.0 g, crude). LC–MS (ESI) m/z = 253.1 [M + H]+, t = 1.250 min.

To a solution of dimethyl 5-isopropoxysophthalate (20.0 g, 79.4 mmol) in methanol (400 mL) was added aqueous NaOH solution (71 mL, 1 M, 71 mmol), and the mixture was stirred at 60 °C for 16 h. The methanol was removed under reduced pressure, and the residue was diluted with water (150 mL). The aqueous phase was adjusted to pH 6 and extracted twice with ethyl acetate (100 mL). The combined organic extracts were washed with brine (100 mL), dried over anhydrous Na2SO4, and concentrated under reduced pressure to give 3-isopropoxy-5-(methoxy carbonyl)benzoic acid as a white solid (14.0 g, 74%). LC–MS (ESI) m/z = 239.1 [M + H]+, t = 1.010 min. H NMR (400 MHz, DMSO-d6): δ 8.05 (t, J = 1.2 Hz, 1H), 7.63 (m, 2H), 4.74 (m, 1H), 3.87 (s, 1H), 1.29 (d, J = 6.0 Hz, 6H).

To a solution of 3-isopropoxy-5-(methoxy carbonyl)benzoic acid (14.0 g, 58.8 mmol) in DMF (140 mL) were added HATU (27.0 g, 71.0 mmol), triethylamine (16.4 mL, 117.6 mmol), and pyridylidine (5.0 g, 70 mmol). The mixture was stirred at room temperature for 16 h, then partitioned between ethyl acetate (200 mL) and water (200 mL). The aqueous phase was separated and extracted with further ethyl acetate (200 mL). The combined organic extracts were washed with saturated aqueous NH4Cl solution (200 mL) and brine (200 mL), dried over anhydrous Na2SO4, and concentrated under reduced pressure to give methyl 3-isopropoxy-5-(pyrrolidine-1-carbonyl)benzoate as a white solid (16.5 g, 96%). LC–MS (ESI) m/z = 292.2 [M + H]+, t = 1.020 min. H NMR (400 MHz, CDCl3): δ 8.73 (d, J = 7.6 Hz, 2H), 4.74 (m, 1H), 3.87 (s, 1H), 1.29 (d, J = 6.0 Hz, 6H).

A solution of 3-isopropoxy-5-(pyrrolidine-1-carbonyl)benzoic acid (800 mg, 2.9 mmol) in thionyl chloride (10 mL) was stirred at reflux for 2 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The residue was diluted with toluene (10 mL) and reconstituted under reduced pressure to give 3-isopropoxy-5-(pyrrolidine-1-carbonyl)benzoyl chloride as a white solid (860 mg, crude). The title compound was synthesized according to method A using 3-(pyridin-2-yl)-1H-pyrazol-5-amine (462 mg, 2.9 mmol) and 3-isopropoxy-5-(pyrrolidine-1-carbonyl)benzoyl chloride (860 mg, 2.9 mmol) in acetonitrile (40 mL), to yield 4 as a white solid (705 mg, 85%). HPLC: 99% pure. LC–MS (ESI) m/z = 420.2 [M + H]+, t = 0.850 min. H NMR (400 MHz, DMSO-d6): δ 10.02 (s, 1H), 9.25 (d, J = 8.0 Hz, 1H), 8.74 (s, 1H), 6.37 (m, 2H), 3.46 (t, J = 6.0 Hz, 4H), 3.87 (s, 1H), 1.84 (m, 4H), 1.28 (d, J = 6.0 Hz, 6H).

A solution of 3-isopropoxy-5-(pyrrolidine-1-carbonyl)benzoyl chloride (860 mg, 2.9 mmol) in thionyl chloride (10 mL) was stirred at reflux for 2 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The residue was diluted with toluene (10 mL) and reconstituted under reduced pressure to give 3-isopropoxy-5-(pyrrolidine-1-carbonyl)benzoyl chloride as a white solid (860 mg, crude). The title compound was synthesized according to method A using 3-(pyridin-2-yl)-1H-pyrazol-5-amine (100 mg, 0.63 mmol) and 4-chlorobenzoyl chloride (109 mg, 0.63 mmol) in acetonitrile (10...
The title compound was synthesized according to method A using 3-(pyridin-2-yl)-1H-pyrazol-5-amine (800 mg, 5.3 mmol) and 4-methoxybenzoyl chloride (895 mg, 5.3 mmol) in acetonitrile (100 mL), to yield 6 as a white solid (10.7 mmol). HPLC: 97% pure. MS (ESI) m/z = 299 [M + H]+. H NMR (400 MHz, DMSO-d6): δ 13.07 (s, 1H), 10.75 (s, 1H), 8.63 (d, J = 3.6 Hz, 1H), 8.04 (d, J = 8.4 Hz, 2H), 7.88 (m, 2H), 7.35 (m, 1H), 7.24 (s, 1H), 7.04 (d, J = 8.2 Hz, 2H), 7.35 (s, 1H), 7.28 (m, 2H).

N-(4-Cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-yl)-4-methoxybenzamide (7). The title compound was synthesized according to method A using 4-cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-amine (3) (300 mg, 1.5 mmol) and 4-methoxybenzoyl chloride (255 mg, 1.5 mmol) in acetonitrile (20 mL), to yield 7 as a white solid (80.7 mg, 16%). HPLC: 98% pure. MS (ESI) m/z = 335 [M + H]+. H NMR (400 MHz, DMSO-d6): δ 13.07 (m, 1H), 9.92 (s, 1H), 8.67 (d, J = 4.8 Hz, 1H), 8.00 (m, 4H), 7.39 (m, 1H), 7.07 (m, 2H), 3.84 (s, 3H), 1.82 (m, 1H), 0.75 (m, 2H), 0.40 (m, 2H).

N-(4-Cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-yl)-2-methoxybenzamide (8). The title compound was synthesized according to method A using 4-cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-amine (3) (200 mg, 1.0 mmol) and 2-methoxybenzoyl chloride (170 mg, 1.0 mmol) in acetonitrile (15 mL), to yield 8 as a white solid (121.5 mg, 36%). HPLC: 99% pure. MS (ESI) m/z = 335 [M + H]+. H NMR (400 MHz, DMSO-d6): δ 9.77 (s, 1H), 8.65 (d, J = 4.8 Hz, 1H), 7.95 (m, 2H), 7.83 (m, 1H), 7.54 (m, 1H), 7.37 (m, 1H), 7.22 (m, 1H), 7.09 (m, 1H), 3.96 (s, 3H), 1.88 (m, 1H), 0.82 (m, 2H), 0.46 (m, 2H).

N-(4-Cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-yl)-2-fluorobenzamide (9). The title compound was synthesized according to method A using 4-cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-amine (3) (200 mg, 1.0 mmol) and 2-fluorobenzoyl chloride (79 mg, 0.5 mmol) in acetonitrile (10 mL), to yield 9 as a white solid (40.4 mg, 25%). HPLC: 96% pure. MS (ESI) m/z = 323 [M + H]+. H NMR (400 MHz, DMSO-d6): δ 13.10 (s, 1H), 10.04 (s, 1H), 8.67 (d, J = 4.0 Hz, 1H), 1.97 (m, 2H), 7.71 (d, J = 8.0, 8.0 Hz, 1H), 7.56 (m, 1H), 7.37 (m, 3H), 1.85 (s, 1H), 0.82 (d, J = 7.2 Hz, 2H), 0.44 (d, J = 4.4 Hz, 2H).

2,6-Dichloro-N-(4-cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-yl)-benzamide (10). The title compound was synthesized according to method A using 4-cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-amine (3) (200 mg, 1.0 mmol) and 2,6-dichlorobenzoyl chloride (273 mg, 1.3 mmol) in acetonitrile (15 mL), to yield 10 as a white solid (23.1 mg, 6%). HPLC: 98% pure. MS (ESI) m/z = 373 [M + H]+. H NMR (400 MHz, DMSO-d6): δ 13.08 (m, 1H), 10.33 (s, 1H), 8.67 (d, J = 4.8 Hz, 1H), 7.96 (m, 2H), 7.57 (m, 2H), 7.50 (m, 1H), 7.48 (m, 1H), 1.86 (s, 1H), 0.86 (m, 2H), 0.44 (m, 2H).

Method B: Phenyl Carbamate Formation (11–13). To a solution of the corresponding amine (1.0 equiv) in pyridine at 0 °C was added phenyl chloroformate (1.0 equiv) dropwise over a period of 0.5 h. The reaction mixture was then stirred at room temperature for 12 h. The mixture was quenched with water, filtered, and the resulting solid washed with water to give the product, which was dried under vacuum.

Phenyl (3-(Pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (11). The title compound was synthesized according to method B using 3-(pyridin-2-yl)-1H-pyrazol-5-amine (25.0 g, 0.156 mol) and phenyl chloroformate (24.4 g, 0.156 mol) in pyridine (250 mL), to yield 11 as a gray solid (30.2 g, 69%). H NMR (400 MHz, DMSO-d6): δ 13.09 (br s, 1H), 10.61 (m, 1H), 8.61 (m, 1H), 7.86 (m, 2H), 7.44 (m, 2H), 7.35 (m, 1H), 7.27 (m, 1H), 7.22 (d, J = 7.8 Hz, 2H), 6.89 (m, 1H).

Phenyl (4-Chloro-3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (12). The title compound was synthesized according to method B using 4-chloro-3-(pyridin-2-yl)-1H-pyrazol-5-amine (1) (200 mg, 1.0 mmol) and phenyl chloroformate (160 mg, 1.0 mmol) in pyridine (5 mL), to yield 12 as a brown oil (300 mg, crude) that was used directly in the next step (to make 15).
The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)-carbamate (11) and 4-(chlorophenyl)piperidine, to yield 21 (3 mg). HPLC: 98.8% pure. MS (ESI) m/z = 382 [M + H]+, 395 [M + Na]+. 1H NMR (400 MHz, CDCl3): δ 12.54 (br s, 1H), 8.73 (m, 1H), 8.58 (d, J = 4.0 Hz, 1H), 7.82 (m, 2H), 7.30 (m, 1H), 6.87 (m, 1H), 4.27 (d, J = 13.2 Hz, 2H), 2.91 (t, J = 12.0 Hz, 2H), 2.81 (m, 1H), 1.82 (d, J = 12.0 Hz, 2H), 1.59 (m, 2H).

The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)-carbamate (11) and 4-(4-piperidinyl)benzonitrile, to yield 22 (3.6 mg). HPLC: 98.7% pure. MS (ESI) m/z = 373 [M + H]+, 396 [M + Na]+. 1H NMR (400 MHz, CDCl3): δ 11.11 (br s, 1H), 8.60 (d, J = 4.0 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 8.0 Hz, 2H), 7.45 (s, 1H), 7.31 (d, J = 8.4 Hz, 2H), 7.25 (m, 1H), 7.08 (s, 1H), 4.26 (d, J = 13.6 Hz, 2H), 3.00 (t, J = 12.4 Hz, 2H), 2.79 (m, 1H), 1.91 (d, J = 12.4 Hz, 2H), 1.71 (m, 2H).

The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)-carbamate (11) and 4-(4-piperidinyl)benzeneitrile, to yield 23 (3.2 mg). HPLC: 100% pure. MS (ESI) m/z = 364 [M + H]+, 386 [M + Na]+. 1H NMR (400 MHz, CDCl3): δ 11.19 (br s, 1H), 8.60 (d, J = 4.4 Hz, 1H), 7.75 (m, 1H), 7.67 (m, 1H), 7.42 (s, 1H), 7.28 (m, 1H), 7.08 (br s, 1H), 6.97 (t, J = 7.2 Hz, 1H), 6.93 (d, J = 8.0 Hz, 2H), 4.56 (m, 1H), 3.73 (m, 2H), 3.52 (m, 2H), 1.99 (m, 2H), 1.89 (m, 2H).

The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)-carbamate (11) and 4-(4-fluorophenyl)piperazine, to yield 24 (2.8 g, 10.0 mmol), 2-(piperidin-4-yloxy)pyridine (3.6 g, 24%). Mp = 223 °C. HPLC: 100% pure. MS (ESI) m/z = 389 [M + Na]+. 1H NMR (400 MHz, DMSO-d6): δ 12.77 (br s, 1H), 9.24 (br s, 1H), 8.60 (m, 1H), 7.78 (m, 2H), 7.30 (m, 1H), 7.05 (m, 4H), 6.82 (br s, 1H), 3.62 (m, 4H), 3.10 (m, 4H).

**ASSOCIATED CONTENT**

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01456.

**Compound details (CSV)**

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Paul A. Glossop prepared this manuscript on behalf of all of the authors. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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**ABBREVIATIONS USED**

CC50, half-maximum cytotoxic concentration; CL, cutaneous leishmaniasis; CLint, intrinsic clearance; DNDi, Drugs for Neglected Diseases initiative; HLM, human liver microsomes; HamLm, hamster liver microsomes; LAB, liposomal amphotericin B; L. donovani, Leishmania donovani; L. infantum, Leishmania infantum; MRC5, human fetal lung fibroblasts; NTD, neglected tropical disease; PM, paromomycin; PMM, primary mouse macrophages; SI, selectivity index; SSG, sodium stibogluconate; TPP, target product profile; VL, visceral leishmaniasis

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