

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

Charles E. Mowbray,^{*,†} Stéphanie Braillard,[†] William Speed,[†] Paul A. Glossop,^{‡,▲} Gavin A. Whitlock,[‡] Karl R. Gibson,[‡] James E. J. Mills,[‡] Alan D. Brown,[§] J. Mark F. Gardner,^{||} Yafeng Cao,[⊥] Wen Hua,[⊥] Garreth L. Morgans,^{#,Δ} Pim-Bart Feijens,[○] An Matheussen,[○] and Louis J. Maes[○]

[†]Drugs for Neglected Diseases initiative (DNDi), 15 Chemin Louis-Dunant, 1202 Geneva, Switzerland

[‡]Sandexis Medicinal Chemistry Ltd., Innovation House, Discovery Park, Ramsgate Road, Sandwich, Kent CT13 9ND, United Kingdom

[§]Pfizer Worldwide Medicinal Chemistry, The Portway Building, Granta Park, Great Abington, Cambridge CB21 6GS, United Kingdom

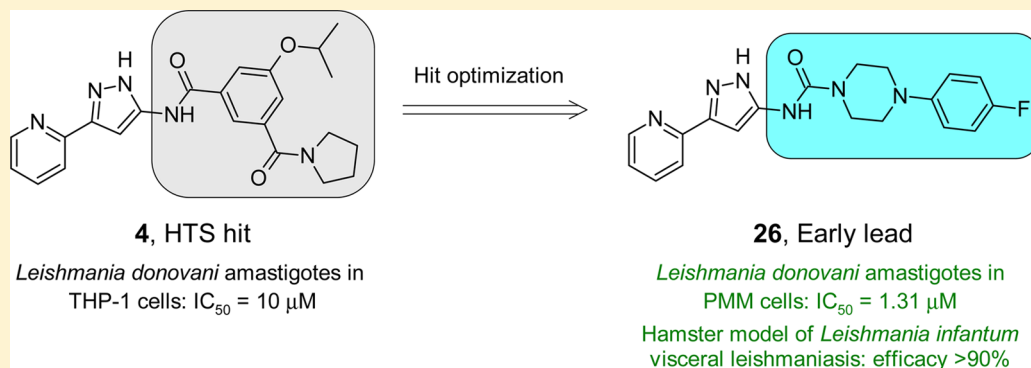
^{||}AMG Consultants Ltd., Discovery Park House, Discovery Park, Ramsgate Road, Sandwich, Kent CT13 9ND, United Kingdom

[⊥]WuXi AppTec (Wuhan) Company Ltd., 666 Gaoxin Road, East Lake High-Tech Development Zone, Wuhan 430075, People's Republic of China

[#]iThemba Pharmaceuticals, Building T5, Pinelands Site, High Street, Modderfontein 1609, Gauteng, South Africa

[○]Laboratory for Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, S7, Universiteitsplein 1, 2610 Wilrijk, Antwerp, Belgium

S 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} VL occurs in Asia, East Africa, Latin America, the Mediterranean Basin, and the Middle East;¹ 90% of global cases occur in just six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan.⁵ 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.⁵ 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}

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,

Received: August 5, 2015

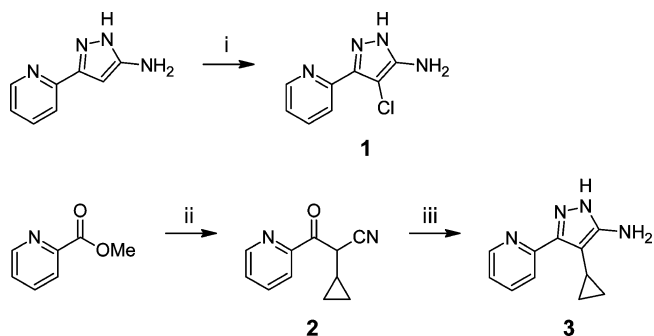
and PM are all delivered by long-duration, intravenous infusions, or intramuscular injections; only miltefosine is an oral drug, and all are associated with adverse events or toxicity.^{1,3,4} Research and development for the treatment of VL has evolved in recent years, including the discovery of two nitroimidazole drugs, fexinidazole (phase II) and (2*R*)-2-methyl-6-nitro-2-{{4-(trifluoromethoxy)phenoxy}methyl}-2*H*,3*H*-imidazo[2,1-*b*][1,3]oxazole (VL-2098, preclinical), from the Drugs for Neglected Diseases initiative (DNDi).^{6–11} 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,^{12,13} and aminothiazoles with excellent *in vitro* potency and good physicochemical properties.¹⁴ 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.² Many research groups have reported new chemical scaffolds with promising activity against VL and potential for the future development of antileishmanial agents.^{15–21} 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.^{5,12–14,22–28} Overall, however, many of the newly published scaffolds contain undesirable structural alerts associated with adverse toxicity findings.²⁹ 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.^{1,28,30–35} Consequently, there is an urgent need for additional research into more effective therapeutic options for the treatment of VL.^{1–4}

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.^{3,36} 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

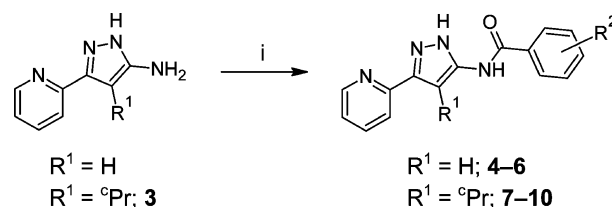
Chemistry. The synthesis of amino-pyrazole intermediates was straightforward (Scheme 1). Commercially available 3-(pyridin-2-yl)-1*H*-pyrazol-5-amine was chlorinated with NCS to provide chloro-substituted amino pyrazole **1**. Methyl picolinate was treated with lithiated cyclopropylacetonitrile to give cyano ketone **2**, which was cyclized with hydrazine to provide cyclopropyl-substituted amino-pyrazole **3**. Final compounds were synthesized from 3-(pyridin-2-yl)-1*H*-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–**

Scheme 1. Synthesis of Amino-pyrazole Intermediates^a



^aReagents and conditions: (i) NCS, DMF, 0 °C, 0.5 h; (ii) 2-cyclopropylacetonitrile, LDA, dry THF, –78 °C to rt, 18 h; (iii) hydrazine hydrate, EtOH, reflux, 16 h.

Scheme 2. General Synthesis of Amides **4–10**^a

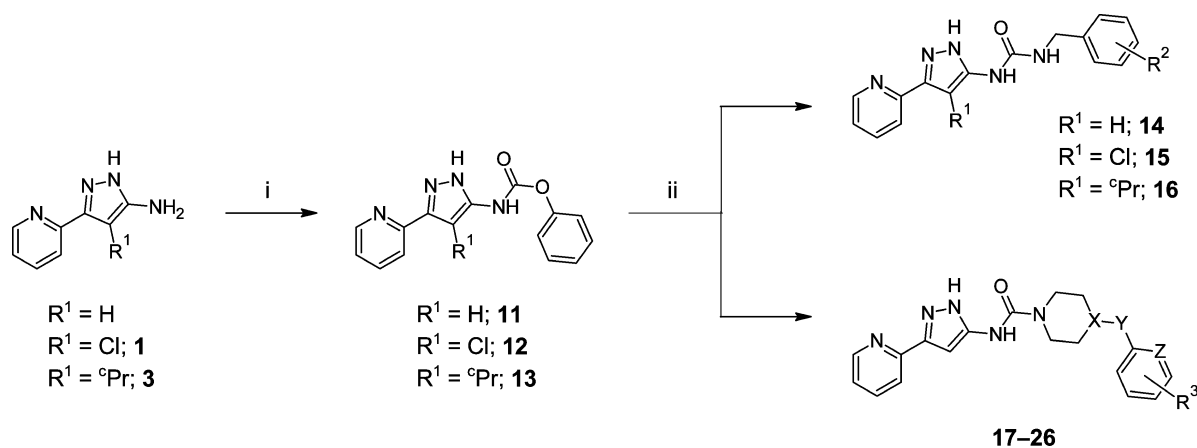


^aReagents and conditions: (i) Method A, corresponding acyl chloride, MeCN, rt to reflux, 16 h.

13 (synthesized using method B), and the corresponding amine, according to method C (Scheme 3).

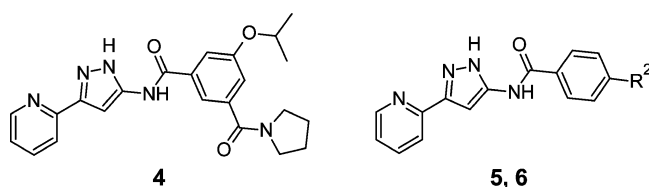
This pyrazole series was initially identified from an HTS campaign with approximately 95 000 compounds from a diverse subset, selected to represent the broad chemical space of Pfizer's small-molecule compound collection.³⁷ Compound **4** was a moderately potent hit (IC₅₀ = 10 μM) with *in vitro* activity 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.³ 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.² 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. Initial

Scheme 3. General Synthesis of Ureas 14–26^a

^aReagents and conditions: (i) Method B, phenyl chloroformate, pyridine, 0 °C to rt, 12.5 h; (ii) Method C, corresponding amine, triethylamine, chloroform, reflux, 16 h.

Table 1. Structure and Antileishmanial Activity of Hits 4–6



compound	R ²	axenic <i>L. donovani</i> amastigote IC ₅₀ (μM) ^a	intramacrophage <i>L. infantum</i> amastigote IC ₅₀ (μM) ^b	PMM ^c CC ₅₀ (μM)
4		≥10.9	>42.4	>40.3
5	Cl	3.71	>64 ^c	>64 ^c
6	OMe	0.738	>40.3	>40.3
miltefosine		n.d.	7.26	33.1

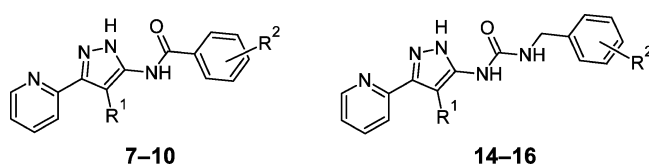
^aGeometric-mean value of at least three independent tests.

^bGeometric-mean value of at least three independent tests in primary mouse macrophages (PMM). ^cSingle test value ($n = 1$). n.d., not determined.

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** (IC₅₀ = 2.02 μM) (Table 2), which was significantly more potent than **6**, its unsubstituted counterpart. Furthermore, **7** also demonstrated similar antileishmanial activity against *L. donovani* (IC₅₀ = 2.63 μM). 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** (IC₅₀ = 0.064 μM). A variety of other benzamides showed levels of potency similar to **7**, for example, the 2-fluoro (**9**; IC₅₀ = 0.965 μM) and 2,6-dichloro (**10**; IC₅₀ = 2.11 μM) analogues (Table 2). Importantly, compounds **7–10** showed no evidence of cytotoxicity (CC₅₀ > 51 μM) 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} =

Table 2. Structure and Antileishmanial Activity of Amides 7–10 and Ureas 14–16



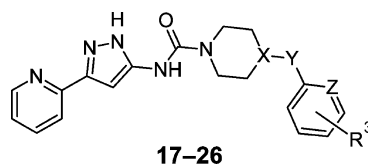
compound	R ¹	R ²	intramacrophage <i>L. infantum</i> amastigote IC ₅₀ (μM) ^a	PMM ^a CC ₅₀ (μM)
7	^o Pr	4-OMe	2.02	>64
8	^o Pr	2-OMe	0.064	>51
9	^o Pr	2-F	0.965	>64
10	^o Pr	2,6-diCl	2.11	>64
14	H	3-Cl	0.450	>64
15	Cl	H	0.066	>64
16	^o Pr	H	0.130	>45
miltefosine			7.26	33.1

^aGeometric-mean value of at least two independent tests in PMM.

12.75 μL/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.^{3,38} 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 μL/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**; IC₅₀ = 0.130 μM, AlogP = 3.15) with no evidence of cytotoxicity (CC₅₀ > 45 μM), although high levels of instability in HamLM (Cl_{int} > 400 μL/min/mg) remained. Interestingly

Table 3. Structure, Antileishmanial Activity, Cytotoxicity, SI, HLM/HamLM Stability, and AlogP for Ureas 17–26



compound	X	Y	Z	R ³	intramacrophage <i>L. infantum</i> amastigote IC ₅₀ (μM) ^a	PMM ^a CC ₅₀ (μM)	MRC5 ^b		HLM	HamLM	AlogP ³⁹
							CC ₅₀ (μM)	SI ^c	Cl _{int} (μL/min/mg)		
17	CH		CH	H	0.296	>64	>64	>242	15.3	216	3.15
18	CH		CH	4-F	0.530	>64	>64	>120	42.5	272	3.36
19	CH		CH	3-F	0.224	>45	>64	>286	25.3	207	3.36
20	CH		CH	4-Cl	1.105	>55	>62	>74	30.0	43.8	3.82
21	CH		CH	3-Cl	0.396	>22	>64	>161	30.8	63.5	3.82
22	CH		CH	4-CN	2.862	>64	>64	>22	25.3	80.8	3.03
23	CH	O	CH	H	0.614	>40	>64	>109	27.3	98.5	2.55
24	CH	O	CH	4-CN	0.853	>64	>63	>74	14.7	30.8	2.43
25	CH	O	N	H	2.884	>64	>63	>21	<11.9	67.3	1.94
26	N		CH	4-F	2.370	>57	>64	>44	30.8	46.5	2.78
14					0.450	>64	>64	>142	184	434	2.99
miltefosine					7.26	33.1	24.7	3.26	n.d.	n.d.	3.73

^aGeometric-mean value of at least two independent tests in PMM. ^bGeometric-mean value of at least two independent tests in human fetal lung fibroblasts. ^cSelectivity index representing the ratio of CC₅₀/IC₅₀; data are presented as the geometric-mean value of ratios calculated using separate data from at least two independent tests. n.d., not determined.

though, and in contrast to the amide subseries, **14** did show that it was possible to obtain highly potent (IC₅₀ = 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 (IC₅₀ = 0.296 μM), good selectivity index (SI) over cytotoxicity (CC₅₀ > 64 μM; SI > 242), and good stability in HLM (Table 3). Furthermore, **17** showed equivalent antileishmanial activity against *L. donovani* (IC₅₀ = 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 (IC₅₀ = 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 (IC₅₀ = 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* (IC₅₀ = 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, $AUC_{0-24h} = 25\,376$ 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.^{3,36} 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. MRC5_{SV2} cells (diploid human embryonic lung fibroblasts) were cultured in minimal essential medium (MEM) containing Earle's salts, supplemented with L-glutamine, NaHCO₃, and 5% inactivated fetal calf serum. All cultures and assays were conducted at 37 °C under an atmosphere of 5% CO₂.

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 Stauber technique.⁴⁰ For the in vitro assays, the inoculum was prepared in RPMI-1640 medium, supplemented with 200 mM L-glutamine, 16.5 mM NaHCO₃, and 5% inactivated fetal calf serum. For the in vivo model, an infection

inoculum containing 2×10^7 amastigotes/100 μ L 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.⁴¹

In Vitro Intramacrophage *L. infantum* Amastigote Assay. The assay was performed in sterile 96-well microtiter plates, each well containing 10 μ L of the compound dilution and 190 μ L of the PMM/amastigote inoculum (3×10^4 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. IC₅₀ values were determined using an extended dose range (2-fold compound dilutions, 8-point concentration curve). Miltefosine was used as the reference drug.⁴² 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 microtiter plates, each well containing 10 μ L of the compound dilution and 190 μ L of MRC5_{SV2} or PMM inoculum (3×10^4 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 μ L/well of a stock solution in phosphate buffer (50 μ g/mL)), incubating for 4 h, and measuring fluorescence (λ_{ex} 550 nm, λ_{em} 590 nm). The results were expressed as percentage reduction in cell growth as compared to untreated control wells. IC₅₀ values were determined using an extended dose range (2-fold compound dilutions, 8-point concentration curve) to a highest concentration of 64 μ M. 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

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 Synergi C18 150 × 30 mm, 4 μm, (2) YMC-pack ODS-AQ 150 × 30 mm, 5 μm, (3) Agella Venusil ASB C18 150 × 21.2 mm, 5 μm, or (4) Boston Symmetrix 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 in the solvents specified. LCMS spectra were recorded on an Agilent 1200 or Shimadzu 2020 spectrometer equipped with electrospray ionization, quadrupole MS detector and Chromolith Flash RP-18e 25 × 2.0 mm column, eluting with 0.0375% (by volume) of TFA in water (solvent A), and 0.01875% (by volume) of TFA in acetonitrile (solvent B). Analytical HPLC was performed using a Shimadzu LC20AB machine and one of two columns, chosen from (1) Xtimate C18 2.1 × 30 mm, 3 μm, or (2) CHROM-MATRIX Innovation C18 2.1 × 30 mm, 2.6 μm; elution was performed with 0.0375% (by volume) of TFA in water (solvent A), and 0.01875% (by volume) of TFA in acetonitrile (solvent B). Purity of final compounds was ≥96%, as determined by HPLC. Unless otherwise stated, final compounds were isolated as amorphous solids without collection of melting point data.

4-Chloro-3-(pyridin-2-yl)-1H-pyrazol-5-amine (1). To a solution of 3-(pyridin-2-yl)-1H-pyrazol-5-amine (1.6 g, 10.0 mmol) in DMF (16 mL) at 0 °C was added NCS (1.47 g, 11.0 mmol) in portions. The mixture was then stirred at 0 °C for 0.5 h. Saturated aqueous NH₄Cl solution (150 mL) was then added, and the resulting precipitate was collected by filtration and dried under vacuum to yield **1** as a gray solid (1.3 g, 67%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.45 (m, 1H), 8.63 (d, *J* = 4.8 Hz, 1H), 7.90 (s, 2H), 7.36 (s, 1H), 4.94 (m, 2H).

2-Cyclopropyl-3-oxo-3-(pyridin-2-yl)propanenitrile (2). To a solution of 2-cyclopropylacetonitrile (5.6 g, 76.7 mmol) in dry THF (70 mL) at –78 °C was added LDA (80.6 mmol, 40.3 mL) dropwise, and the mixture was stirred for 1 h. A solution of methyl picolinate (10 g, 73.0 mmol) in dry THF (30 mL) was then added dropwise, maintaining the temperature below –60 °C. After addition, the mixture was warmed slowly to room temperature and stirred for 18 h. The mixture was quenched with 10% citric acid (150 mL), extracted with ethyl acetate (100 mL × 2), dried over anhydrous Na₂SO₄, and concentrated under reduced pressure to give **2** as a yellow oil (14.8 g, crude). ¹H NMR (400 MHz, CDCl₃): δ 8.73 (m, 1H), 8.17 (td, *J* = 7.9, 1.0 Hz, 1H), 7.93 (dt, *J* = 7.7, 1.8 Hz, 1H), 7.58 (ddd, *J* = 7.6, 4.8, 1.2 Hz, 1H), 4.92 (d, *J* = 7.9 Hz, 1H), 1.48 (m, 1H), 0.69 (m, 4H).

4-Cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-amine (3). To a solution of 2-cyclopropyl-3-oxo-3-(pyridin-2-yl)propanenitrile (**2**) (14.8 g, 73.0 mmol) in ethanol (200 mL) was added hydrazine hydrate (4.3 mL, 79.6 mmol), and the mixture was stirred at reflux for 16 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The residue was washed with petroleum ether/ethyl acetate (10/1, 50 mL) to give **3** as a yellow solid (11.5 g, crude). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.86 (s, 1H), 8.59 (td, *J* = 4.6, 1.3 Hz, 1H), 7.88 (m, 2H), 7.30 (m, 1H), 4.38 (s, 2H), 1.69 (m, 1H), 0.86 (m, 2H), 0.36 (m, 2H).

Method A: Amide Formation (4–10). To a solution of the corresponding amine (1.0 equiv) in acetonitrile at room temperature was added dropwise a solution of the corresponding acyl chloride (1.0 equiv) in acetonitrile. The reaction mixture was then stirred at reflux for 16 h. After being cooled to room temperature, the mixture was concentrated under reduced pressure to give the crude product, which was purified by HPLC.

3-Isopropoxy-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)-5-(pyrrolidine-1-carbonyl)benzamide (4). To a suspension of dimethyl 5-hydroxyisophthalate (21.0 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-isopropoxyisophthalate 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-isopropoxyisophthalate (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-(methoxycarbonyl)benzoic acid as a white solid (14.0 g, 74%). LC–MS (ESI) *m/z* = 239.1 [M + H]⁺, *t* = 1.010 min. ¹H NMR (400 MHz, DMSO-*d*₆): 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-(methoxycarbonyl)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 pyrrolidine (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.

To a solution of methyl 3-isopropoxy-5-(pyrrolidine-1-carbonyl)benzoate (16.5 g, 56.7 mmol) in methanol (200 mL) was added aqueous NaOH solution (56.7 mL, 2 M, 113.4 mmol), and the mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure, and the residue was diluted with water (150 mL). The aqueous phase was adjusted to pH 5 with aqueous 2 M HCl solution and extracted twice with ethyl acetate (100 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give 3-isopropoxy-5-(pyrrolidine-1-carbonyl)benzoic acid as a white solid (13.5 g, 86%). LC–MS (ESI) *m/z* = 278.1 [M + H]⁺, *t* = 0.932 min. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.55 (s, 1H), 7.44 (d, *J* = 0.8 Hz, 1H), 7.24 (s, 1H), 4.71 (m, 1H), 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)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 reconcentrated 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, 47%). HPLC: 99% pure. LC–MS (ESI) *m/z* = 420.2 [M + H]⁺, *t* = 0.850 min. ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.20 (m, 1H), 11.00 (m, 1H), 8.63 (d, *J* = 3.6 Hz, 1H), 7.88 (m, 2H), 7.66 (m, 2H), 7.36 (s, 1H), 7.24 (s, 1H), 7.17 (s, 1H), 4.78 (m, 1H), 3.48 (t, *J* = 6.4 Hz, 2H), 3.40 (t, *J* = 6.4 Hz, 2H), 1.86 (m, 4H), 1.29 (m, 6H).

4-Chloro-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)benzamide (5). 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

mL), to yield **5** as a white solid (76 mg, 41%). HPLC: 99% pure. MS (ESI) $m/z = 299$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6): δ 11.06 (s, 1H), 8.64 (d, $J = 4.4$ Hz, 1H), 8.04 (d, $J = 8.8$ Hz, 2H), 7.94 (m, 2H), 7.59 (d, $J = 8.4$ Hz, 2H), 7.40 (m, 1H), 7.17 (s, 1H).

4-Methoxy-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)benzamide (6). The title compound was synthesized according to method A using 3-(pyridin-2-yl)-1H-pyrazol-5-amine (850 mg, 5.3 mmol) and 4-methoxybenzoyl chloride (895 mg, 5.3 mmol) in acetonitrile (100 mL), to yield **6** as a white solid (1.04 g, 67%). HPLC: 99% pure. MS (ESI) $m/z = 295$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6): δ 13.15 (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 (s, 1H), 7.24 (s, 1H), 7.04 (d, $J = 8.2$ Hz, 2H), 3.84 (s, 3H).

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) $m/z = 335$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6): 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) $m/z = 335$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6): 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**) (100 mg, 0.5 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) $m/z = 323$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6): δ 13.10 (s, 1H), 10.04 (s, 1H), 8.67 (d, $J = 4.0$ Hz, 1H), 7.97 (m, 2H), 7.71 (dd, $J = 8.0, 8.0$ 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) $m/z = 373$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6): 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 g, 69%). 1H NMR (400 MHz, DMSO- d_6): 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**).

Phenyl (4-Cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (13). The title compound was synthesized according to method B using 4-cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-amine (**3**) (200 mg, 1.0 mmol) and phenyl chloroformate (160 mg, 1.0 mmol) in pyridine (5 mL), to yield **13** as a brown oil (300 mg, crude) that was used directly in the next step (to make **14**).

Method C: Urea Formation (14–26). A mixture of the corresponding phenyl carbamate (1.0 equiv), the corresponding amine (1.0 equiv), and triethylamine (2.0 equiv) in chloroform was stirred at reflux for 16 h. After being cooled to room temperature, the mixture was concentrated under reduced pressure to give the crude product, which was purified by HPLC.

1-(3-Chlorophenyl)methyl-3-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)urea (14). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**11**) and 3-chlorobenzylamine, to yield **14** (4 mg). HPLC: 100% pure. MS (ESI) $m/z = 328$ $[M + H]^+$, 350 $[M + Na]^+$. 1H NMR (400 MHz, DMSO- d_6): δ 12.83 (br s, 1H), 8.97 (br s, 1H), 8.59 (m, 1H), 7.82 (m, 2H), 7.32 (m, 6H), 6.71 (br s, 1H), 4.36 (d, 2H).

1-(3-Chloro-3-(pyridin-2-yl)-1H-pyrazol-5-yl)urea (15). The title compound was synthesized according to method C using phenyl (4-chloro-3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**12**) (300 mg, 0.95 mmol) and benzylamine (153 mg, 1.43 mmol) in chloroform (10 mL), to yield **15** as a white solid (129 mg, 41%). HPLC: 100% pure. MS (ESI) $m/z = 328$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6): δ 13.38 (m, 1H), 8.67 (m, 2H), 7.95 (s, 2H), 7.41 (m, 1H), 7.34 (m, 4H), 7.26 (m, 2H), 4.34 (d, $J = 6.0$ Hz, 2H).

1-Benzyl-3-(4-cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-yl)urea (16). The title compound was synthesized according to method C using phenyl (4-cyclopropyl-3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**13**) (150 mg, 0.47 mmol) and benzylamine (76 mg, 0.70 mmol) in chloroform (5 mL), to yield **16** as a light yellow solid (45.8 mg, 29%). HPLC: 97% pure. MS (ESI) $m/z = 334$ $[M + H]^+$. 1H NMR (400 MHz, DMSO- d_6): δ 12.80 (m, 1H), 8.63 (d, $J = 4.8$ Hz, 1H), 8.01 (m, 3H), 7.35 (m, 7H), 4.35 (d, $J = 5.6$ Hz, 2H), 1.77 (m, 1H), 0.84 (m, 2H), 0.35 (m, 2H).

4-Phenyl-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperidine-1-carboxamide (17). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**11**) (3.0 g, 10.7 mmol), 4-phenylpiperidine (1.9 g, 11.8 mmol), and triethylamine (3.7 mL, 26.8 mmol) in chloroform (80 mL) to yield the crude product, which was precipitated from ethyl acetate (100 mL), filtered, and recrystallized from DMSO (20 mL) to yield **17** as a white solid (1.09 g, 29%). Mp = 237 °C. HPLC: 99% pure. MS (ESI) $m/z = 348$ $[M + H]^+$. 1H NMR (400 MHz, CDCl₃): δ 12.66 (m, 1H), 8.61 (d, $J = 4.8$ Hz, 1H), 7.74 (m, 1H), 7.68 (m, 2H), 7.50 (s, 1H), 7.32 (m, 2H), 7.27 (m, 4H), 7.09 (m, 1H), 4.24 (d, $J = 13.6$ Hz, 2H), 3.00 (td, $J = 12.8, 2.4$ Hz, 2H), 2.72 (m, 1H), 1.91 (d, $J = 11.2$ Hz, 2H), 1.72 (m, 2H).

4-(4-Fluorophenyl)-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperidine-1-carboxamide (18). 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)piperidine, to yield **18** (4 mg). HPLC: 100% pure. MS (ESI) $m/z = 366$ $[M + H]^+$, 388 $[M + Na]^+$. 1H NMR (400 MHz, CDCl₃): δ 10.77 (br s, 1H), 8.60 (m, 1H), 7.77 (t, 1H), 7.67 (d, 1H), 7.26 (m, 4H), 7.10 (m, 3H), 4.25 (d, 2H), 3.03 (t, 2H), 2.75 (m, 1H), 1.93 (d, 2H), 1.71 (m, 2H).

4-(3-Fluorophenyl)-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperidine-1-carboxamide (19). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**11**) and 4-(3-fluorophenyl)piperidine, to yield **19** (4.5 mg). HPLC: 100% pure. MS (ESI) $m/z = 366$ $[M + H]^+$, 388 $[M + Na]^+$. 1H NMR (400 MHz, CDCl₃): δ 11.16 (br s, 1H), 8.62 (d, $J = 4.4$ Hz, 1H), 7.77 (t, $J = 7.6$ Hz, 1H), 7.68 (d, $J = 8.0$ Hz, 1H), 7.45 (s, 1H), 7.26 (m, 2H), 7.09 (s, 1H), 7.00 (d, $J = 7.6$ Hz, 1H), 6.93 (m, 2H), 4.26 (d, $J = 12.8$ Hz, 2H), 3.02 (t, $J = 12.4$ Hz, 2H), 2.75 (m, 1H), 1.94 (d, $J = 12.8$ Hz, 2H), 1.70 (m, 2H).

4-(4-Chlorophenyl)-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperidine-1-carboxamide (20). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-

yl)carbamate (**11**) (2.6 g, 9.28 mmol), 4-(4-chlorophenyl)piperidine (2.2 g, 9.52 mmol), and triethylamine (3.2 mL, 23.2 mmol) in chloroform (70 mL), to yield the crude product, which was precipitated from ethyl acetate (100 mL), filtered, and recrystallized from DMSO (25 mL) to yield **20** as a white solid (0.998 g, 28%). Mp = 231 °C. HPLC: 98% pure. MS (ESI) m/z = 382 [M + H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆, *t* = 80 °C): δ 12.54 (m, 1H), 8.73 (m, 1H), 8.58 (d, *J* = 4.0 Hz, 1H), 7.82 (m, 2H), 7.30 (m, 5H), 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).

4-(3-Chlorophenyl)-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperidine-1-carboxamide (**21**). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**11**) and 4-(3-chlorophenyl)piperidine, to yield **21** (3 mg). HPLC: 98.8% pure. MS (ESI) m/z = 382 [M + H]⁺, 404 [M + Na]⁺. ¹H NMR (400 MHz, CDCl₃): δ 10.78 (br s, 1H), 8.60 (m, 1H), 7.77 (m, 1H), 7.67 (m, 1H), 7.24 (m, 5H), 7.10 (m, 2H), 4.27 (m, 2H), 3.03 (m, 2H), 2.75 (m, 1H), 1.95 (m, 2H), 1.72 (m, 2H).

4-(4-Cyanophenyl)-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperidine-1-carboxamide (**22**). 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)benzotrile, to yield **22** (3.6 mg). HPLC: 98.7% pure. MS (ESI) m/z = 373 [M + H]⁺, 395 [M + Na]⁺. ¹H NMR (400 MHz, CDCl₃): δ 11.13 (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).

4-Phenoxy-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperidine-1-carboxamide (**23**). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**11**) and 4-(phenoxy)-piperidine, to yield **23** (393 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, 3H), 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).

4-(4-Cyanophenoxy)-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperidine-1-carboxamide (**24**). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**11**) (3.2 g, 11.4 mmol), 4-(piperidin-4-yloxy)-benzotrile (2.6 g, 12.9 mmol), and triethylamine (3.96 mL, 28.6 mmol) in chloroform (80 mL), to yield the crude product, which was purified by silica gel chromatography (dichloromethane/methanol, 50:1 to 10:1) and recrystallized from acetonitrile/methanol (4:1, 25 mL) to yield **24** as a white solid (1.05 g, 24%). Mp = 223 °C. HPLC: 98% pure. MS (ESI) m/z = 389 [M + H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 11.37 (m, 1H), 8.62 (d, *J* = 5.2 Hz, 1H), 7.76 (ddd, *J* = 8.0, 8.0, 1.6, 1H), 7.60 (m, 4H), 7.25 (d, *J* = 6.0 Hz, 1H), 7.08 (m, 1H), 6.94 (d, *J* = 8.8 Hz, 2H), 4.61 (td, *J* = 6.40, 2.8 Hz, 1H), 3.70 (m, 2H), 3.51 (m, 2H), 2.01 (m, 2H), 1.85 (td, *J* = 6.4, 4.0 Hz, 2H).

N-(3-(Pyridin-2-yl)-1H-pyrazol-5-yl)-4-(pyridin-2-yloxy)piperidine-1-carboxamide (**25**). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**11**) (2.8 g, 10.0 mmol), 2-(piperidin-4-yloxy)pyridine hydrochloride (2.5 g, 11.7 mmol), and triethylamine (3.47 mL, 25.0 mmol) in chloroform (80 mL) to yield the crude product, which was purified by silica gel chromatography (dichloromethane/methanol, 50:1 to 10:1) and recrystallized from acetonitrile/methanol (4:1, 25 mL) to yield **25** as a white solid (1.46 g, 40%). Mp = 216 °C. HPLC: 98% pure. MS (ESI) m/z = 365 [M + H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 11.07 (m, 1H), 8.60 (d, *J* = 4.8 Hz, 1H), 8.14 (d, *J* = 4.8 Hz, 1H), 7.76 (m, 1H), 7.67 (m, 1H), 7.58 (t, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 7.21–7.26 (m, 1H), 7.09 (m, 1H), 6.87 (t, *J* = 6.0 Hz, 1H), 6.73 (d, *J* = 8.4 Hz, 1H), 5.31 (m, 1H), 3.79 (m, 2H), 3.47 (m, 2H), 2.08 (m, 2H), 1.86 (m, 2H).

4-(4-Fluorophenyl)-N-(3-(pyridin-2-yl)-1H-pyrazol-5-yl)piperazine-1-carboxamide (**26**). The title compound was synthesized according to method C using phenyl (3-(pyridin-2-yl)-1H-pyrazol-5-yl)carbamate (**11**) and 1-(4-fluorophenyl)-piperazine, to yield **26** (842

mg). HPLC: 100% pure. MS (ESI) m/z = 389 [M + Na]⁺. ¹H NMR (400 MHz, DMSO-*d*₆): 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)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +41 (0)22 906 9220. E-mail: cmowbray@dndi.org.

Present Address

▲Mojozi Consulting (Pty) Ltd., 88 Tenth Avenue, Sydenham, Johannesburg 2092, Gauteng, South Africa.

Author Contributions

▲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.

■ ACKNOWLEDGMENTS

The DNDi thanks Pfizer Ltd. for generously providing access to the original library of compounds used for the HTS, and also for ongoing advice and support from staff in Pfizer Worldwide Medicinal Chemistry. The DNDi also thank the staff of SCYNEXIS Inc., WuXi AppTech, iThemba Pharmaceuticals, and LMPH for their individual practical contributions to this work. In addition, we thank Robert T. Jacobs and Jason Speake of SCYNEXIS Inc. for their contributions to the initial hit confirmation and early expansion of the amide series. We gratefully acknowledge financial support for this work from United Kingdom Department for International Development (DFID), Dutch Ministry of Foreign Affairs (DGIS), Swiss Agency for Development and Cooperation (SDC), Bill & Melinda Gates Foundation, German Federal Ministry of Education and Research (BMBF) through KfW, and Médecins Sans Frontières (Doctors without Borders).

■ ABBREVIATIONS USED

CC₅₀, half-maximum cytotoxic concentration; CL, cutaneous leishmaniasis; Cl_{int}, 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

■ REFERENCES

- (1) WHO. Control of the Leishmaniases. *World Health Organ. Technol. Rep. Ser.* **2010**, *949*, 1–186.
- (2) Hussain, H.; Al-Harrasi, A.; Al-Rawahi, A.; Green, I. R.; Gibbons, S. Fruitful Decade for Antileishmanial Compounds from 2002 to Late 2011. *Chem. Rev.* **2014**, *114*, 10369–10428.

- (3) Freitas-Junior, L. H.; Chatelain, E.; Kim, H. A.; Siqueira-Neto, J. L. Visceral Leishmaniasis Treatment: What Do We Have, What Do We Need and How to Deliver It? *Int. J. Parasitol.: Drugs Drug Resist.* **2012**, *2*, 11–19.
- (4) WHO. Research Priorities for Chagas Disease, Human African Trypanosomiasis and Leishmaniasis. *World Health Organ. Technol. Rep. Ser.* **2012**, *975*, 1–100.
- (5) WHO. Leishmaniasis. *World Health Organ. Fact Sheet*, 2014; Vol. 375.
- (6) Wyllie, S.; Patterson, S.; Stojanovski, L.; Simeons, F. R. C.; Norval, S.; Kime, R.; Read, K. D.; Fairlamb, A. H. The Anti-Trypanosome Drug Fexinidazole Shows Potential for Treating Visceral Leishmaniasis. *Sci. Transl. Med.* **2012**, *4*, 119re1.
- (7) DNDi. Phase II Proof of Concept Trial to Determine Efficacy of Fexinidazole in Visceral Leishmaniasis Patients in Sudan; <http://clinicaltrials.gov/show/NCT01980199> (accessed Oct 3, 2014).
- (8) Gupta, S.; Yardley, V.; Vishwakarma, P.; Shivahare, R.; Sharma, B.; Launay, D.; Martin, D.; Puri, S. K. Nitroimidazo-Oxazole Compound DNDI-VL-2098: An Orally Effective Preclinical Drug Candidate for the Treatment of Visceral Leishmaniasis. *J. Antimicrob. Chemother.* **2015**, *70*, 518–527.
- (9) Mukkavilli, R.; Pinjari, J.; Patel, B.; Sengottuvelan, S.; Mondal, S.; Gadekar, A.; Verma, M.; Patel, J.; Pothuri, L.; Chandrashekar, G.; Koiram, P.; Harisudhan, T.; Moinuddin, A.; Launay, D.; Vachharajani, N.; Ramanathan, V.; Martin, D. In Vitro Metabolism, Disposition, Preclinical Pharmacokinetics and Prediction of Human Pharmacokinetics of DNDI-VL-2098, a Potential Oral Treatment for Visceral Leishmaniasis. *Eur. J. Pharm. Sci.* **2014**, *65*, 147–155.
- (10) Patterson, S.; Wyllie, S. Nitro Drugs for the Treatment of Trypanosomatid Diseases: Past, Present, and Future Prospects. *Trends Parasitol.* **2014**, *30*, 289–298.
- (11) DNDi. R&D Portfolio: Patient Needs-Driven Collaborative R&D Model for Neglected Diseases; <http://www.dndi.org/pdf-portfolio/> (accessed Oct 3, 2014).
- (12) Gopinath, V. S.; Pinjari, J.; Dere, R. T.; Verma, A.; Vishwakarma, P.; Shivahare, R.; Moger, M.; Kumar Goud, P. S.; Ramanathan, V.; Bose, P.; Rao, M. V. S.; Gupta, S.; Puri, S. K.; Launay, D.; Martin, D. Design, Synthesis and Biological Evaluation of 2-Substituted Quinolines as Potential Antileishmanial Agents. *Eur. J. Med. Chem.* **2013**, *69*, 527–536.
- (13) Gopinath, V. S.; Rao, M.; Shivahare, R.; Vishwakarma, P.; Ghose, S.; Pradhan, A.; Hindupur, R.; Sarma, K. Das; Gupta, S.; Puri, S. K.; Launay, D.; Martin, D. Design, Synthesis, ADME Characterization and Antileishmanial Evaluation of Novel Substituted Quinoline Analogs. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2046–2052.
- (14) Bhuniya, D.; Mukkavilli, R.; Shivahare, R.; Launay, D.; Dere, R. T.; Deshpande, A.; Verma, A.; Vishwakarma, P.; Moger, M.; Pradhan, A.; Pati, H.; Gopinath, V. S.; Gupta, S.; Puri, S. K.; Martin, D. Aminothiazoles: Hit to Lead Development to Identify Antileishmanial Agents. *Eur. J. Med. Chem.* **2015**, *102*, 582–593.
- (15) Van Horn, K. S.; Zhu, X.; Pandharkar, T.; Yang, S.; Vesely, B.; Vanaerschot, M.; Dujardin, J.-C.; Rijal, S.; Kyle, D. E.; Wang, M. Z.; Werbovetz, K. A.; Manetsch, R. Antileishmanial Activity of a Series of N2,N4-Disubstituted Quinazoline-2,4-Diamines. *J. Med. Chem.* **2014**, *57*, 5141–5156.
- (16) Zhu, X.; Van Horn, K. S.; Barber, M. M.; Yang, S.; Wang, M. Z.; Manetsch, R.; Werbovetz, K. A. SAR Refinement of Antileishmanial N(2),N(4)-Disubstituted Quinazoline-2,4-Diamines. *Bioorg. Med. Chem.* **2015**, *23*, 5182–5189.
- (17) Hutton, J. A.; Goncalves, V.; Brannigan, J. A.; Paape, D.; Wright, M. H.; Waugh, T. M.; Roberts, S. M.; Bell, A. S.; Wilkinson, A. J.; Smith, D. F.; Leatherbarrow, R. J.; Tate, E. W. Structure-Based Design of Potent and Selective Leishmania N-Myristoyltransferase Inhibitors. *J. Med. Chem.* **2014**, *57*, 8664–8670.
- (18) Castera-Ducros, C.; Paloque, L.; Verhaeghe, P.; Casanova, M.; Cantelli, C.; Hutter, S.; Tanguy, F.; Laget, M.; Remusat, V.; Cohen, A.; Crozet, M. D.; Rathelot, P.; Azas, N.; Vanelle, P. Targeting the Human Parasite Leishmania Donovanii: Discovery of a New Promising Anti-Infectious Pharmacophore in 3-nitroimidazo[1,2-a]pyridine Series. *Bioorg. Med. Chem.* **2013**, *21*, 7155–7164.
- (19) Galiana-Roselló, C.; Bilbao-Ramos, P.; Dea-Ayuela, M. A.; Rolón, M.; Vega, C.; Bolás-Fernández, F.; García-España, E.; Alfonso, J.; Coronel, C.; González-Rosende, M. E. In Vitro and in Vivo Antileishmanial and Trypanocidal Studies of New N-Benzene- and N-Naphthalenesulfonamide Derivatives. *J. Med. Chem.* **2013**, *56*, 8984–8998.
- (20) Sobarzo-Sánchez, E.; Bilbao-Ramos, P.; Dea-Ayuela, M.; González-Díaz, H.; Yañez, M.; Uriarte, E.; Santana, L.; Martínez-Sernández, V.; Bolás-Fernández, F.; Ubeira, F. M. Synthetic Oxoisoalloxazine Alkaloids: In Vitro, in Vivo and in Silico Assessment of Antileishmanial Activities. *PLoS One* **2013**, *8*, e77560.
- (21) Reid, C. S.; Farahat, A. A.; Zhu, X.; Pandharkar, T.; Boykin, D. W.; Werbovetz, K. A. Antileishmanial Bis-Arylimidamides: DB766 Analogs Modified in the Linker Region and Bis-Arylimidamide Structure-Activity Relationships. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6806–6810.
- (22) Pinto, E. G.; Santos, I. O.; Schmidt, T. J.; Borborema, S. E. T.; Ferreira, V. F.; Rocha, D. R.; Tempone, A. G. Potential of 2-Hydroxy-3-Phenylsulfanyl-methyl-[1,4]-Naphthoquinones against Leishmania (L.) Infantum: Biological Activity and Structure-Activity Relationships. *PLoS One* **2014**, *9*, e105127.
- (23) Shivahare, R.; Korthikunta, V.; Chandasana, H.; Suthar, M. K.; Agnihotri, P.; Vishwakarma, P.; Chaitanya, T. K.; Kancharla, P.; Khaliq, T.; Gupta, S.; Bhatta, R. S.; Pratap, J. V.; Saxena, J. K.; Gupta, S.; Tadigoppula, N. Synthesis, Structure-Activity Relationships, and Biological Studies of Chromenochalcones as Potential Antileishmanial Agents. *J. Med. Chem.* **2014**, *57*, 3342–3357.
- (24) Sharma, M.; Chauhan, K.; Shivahare, R.; Vishwakarma, P.; Suthar, M. K.; Sharma, A.; Gupta, S.; Saxena, J. K.; Lal, J.; Chandra, P.; Kumar, B.; Chauhan, P. M. S. Discovery of a New Class of Natural Product-Inspired Quinazolinone Hybrid as Potent Antileishmanial Agents. *J. Med. Chem.* **2013**, *56*, 4374–4392.
- (25) Suryawanshi, S. N.; Kumar, S.; Shivahare, R.; Pandey, S.; Tiwari, A.; Gupta, S. Design, Synthesis and Biological Evaluation of Aryl Pyrimidine Derivatives as Potential Leishmanicidal Agents. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5235–5238.
- (26) Bonano, V. I.; Yokoyama-Yasunaka, J. K. U.; Miguel, D. C.; Jones, S. A.; Dodge, J. A.; Uliana, S. R. B. Discovery of Synthetic Leishmania Inhibitors by Screening of a 2-Arylbenzothiofene Library. *Chem. Biol. Drug Des.* **2014**, *83*, 289–296.
- (27) Morais, S. M. de; Vila-Nova, N. S.; Bevilacqua, C. M. L.; Rondon, F. C.; Lobo, C. H.; Moura, A. de A. A.; Sales, A. D.; Rodrigues, A. P. R.; Figueiredo, J. R. de; Campello, C. C.; Wilson, M. E.; de Andrade, H. F. Thymol and Eugenol Derivatives as Potential Antileishmanial Agents. *Bioorg. Med. Chem.* **2014**, *22*, 6250–6255.
- (28) Faria, J. V.; dos Santos, M. S.; Bernardino, A. M. R.; Becker, K. M.; Machado, G. M. C.; Rodrigues, R. F.; Canto-Cavalheiro, M. M.; Leon, L. L. Synthesis and Activity of Novel Tetrazole Compounds and Their Pyrazole-4-Carbonitrile Precursors against Leishmania Spp. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6310–6312.
- (29) Blagg, J. Structure–Activity Relationships for In Vitro and In Vivo Toxicity. *Annu. Rep. Med. Chem.* **2006**, *41*, 353–368.
- (30) Dos Santos, M. S.; Oliveira, M. L. V.; Bernardino, A. M. R.; de Léo, R. M.; Amaral, V. F.; de Carvalho, F. T.; Leon, L. L.; Canto-Cavalheiro, M. M. Synthesis and Antileishmanial Evaluation of 1-Aryl-4-(4,5-Dihydro-1H-Imidazol-2-yl)-1H-Pyrazole Derivatives. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7451–7454.
- (31) Dos Santos Faiões, V.; Leon, L. L.; Canto-Cavalheiro, M. M.; Torres-Santos, E. C.; Bernardino, A. M. R.; Vegi, P. F.; dos Santos, M. S. Effectiveness of Novel 5-(5-Amino-1-Aryl-1H-Pyrazol-4-yl)-1H-Tetrazole Derivatives against Promastigotes and Amastigotes of Leishmania Amazonensis. *Chem. Biol. Drug Des.* **2014**, *83*, 272–277.
- (32) Bernardino, A. M. R.; Gomes, A. O.; Charret, K. S.; Freitas, A. C. C.; Machado, G. M. C.; Canto-Cavalheiro, M. M.; Leon, L. L.; Amaral, V. F. Synthesis and Leishmanicidal Activities of 1-(4-X-Phenyl)-N'-[(4-Y-Phenyl)methylene]-1H-Pyrazole-4-Carbohydrazides. *Eur. J. Med. Chem.* **2006**, *41*, 80–87.

(33) Santos, M. S. dos; Gomes, A. O.; Bernardino, A. M. R.; Souza, M. C. de; Khan, M. A.; Brito, M. A. de; Castro, H. C.; Abreu, P. A.; Rodrigues, C. R.; Léo, R. M. M. de; Leon, L. L.; Canto-Cavalheiro, M. M. Synthesis and Antileishmanial Activity of New 1-Aryl-1H-Pyrazole-4-Carboximidamides Derivatives. *J. Braz. Chem. Soc.* **2011**, *22*, 352–358.

(34) Borges, J. C.; Carvalho, A. V.; Bernardino, A. M. R.; Oliveira, C. D.; Pinheiro, L. C. S.; Marra, R. K. F.; Castro, H. C.; Wardell, S. M. S. V.; Wardell, J. L.; Amaral, V. F.; Canto-Cavalheiro, M. M.; Leon, L. L.; Genestra, M. Synthesis and in Vitro Evaluation of New Benzenesulfonamides as Antileishmanial Agents. *J. Braz. Chem. Soc.* **2014**, *25*, 980–986.

(35) Marra, R. K. F.; Bernardino, A. M. R.; Proux, T. A.; Charret, K. S.; Lira, M.-L. F.; Castro, H. C.; Souza, A. M. T.; Oliveira, C. D.; Borges, J. C.; Rodrigues, C. R.; Canto-Cavalheiro, M. M.; Leon, L. L.; Amaral, V. F. 4-(1H-Pyrazol-1-yl) Benzenesulfonamide Derivatives: Identifying New Active Antileishmanial Structures for Use against a Neglected Disease. *Molecules* **2012**, *17*, 12961–12973.

(36) DNDi. Leishmaniasis; Target Product Profile for VL; <http://www.dndi.org/diseases-projects/diseases/vl/tpp/tpp-vl.html> (accessed Jan 22, 2015).

(37) Siqueira-Neto, J. L.; Moon, S.; Jang, J.; Yang, G.; Lee, C.; Moon, H. K.; Chatelain, E.; Genovesio, A.; Cechetto, J.; Freitas-Junior, L. H. An Image-Based High-Content Screening Assay for Compounds Targeting Intracellular Leishmania Donovanii Amastigotes in Human Macrophages. *PLoS Neglected Trop. Dis.* **2012**, *6*, e1671.

(38) Gupta, S. Visceral Leishmaniasis: Experimental Models for Drug Discovery. *Indian J. Med. Res.* **2011**, *133*, 27–39.

(39) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. Prediction of Hydrophobic (Lipophilic) Properties of Small Organic Molecules Using Fragmental Methods: An Analysis of ALOGP and CLOGP Methods. *J. Phys. Chem. A* **1998**, *102*, 3762–3772.

(40) Stauber, L. A. Characterization of Strains of Leishmania Donovanii. *Exp. Parasitol.* **1966**, *18*, 1–11.

(41) Mercer, L.; Bowling, T.; Perales, J.; Freeman, J.; Nguyen, T.; Bacchi, C.; Yarett, N.; Don, R.; Jacobs, R.; Nare, B. 2,4-Diaminopyrimidines as Potent Inhibitors of Trypanosoma Brucei and Identification of Molecular Targets by a Chemical Proteomics Approach. *PLoS Neglected Trop. Dis.* **2011**, *5*, e956.

(42) Maes, L.; Vanden Berghe, D.; Germonprez, N.; Quirijnen, L.; Cos, P.; De Kimpe, N.; Van Puyvelde, L. In Vitro and in Vivo Activities of a Triterpenoid Saponin Extract (PX-6518) from the Plant Maesa Balansae against Visceral Leishmania Species. *Antimicrob. Agents Chemother.* **2004**, *48*, 130–136.