Antileishmanial Activity of a Series of \(N^2,N^4\)-Disubstituted Quinazoline-2,4-diamines

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ABSTRACT: A series of \(N^2,N^4\)-disubstituted quinazoline-2,4-diamines has been synthesized and tested against \(Leishmania donovani\) and \(L. amazonensis\) intracellular amastigotes. A structure—activity and structure—property relationship study was conducted in part using the Topliss operational scheme to identify new lead compounds. This study led to the identification of quinazolines with EC\(_{50}\) values in the single digit micromolar or high nanomolar range in addition to favorable physicochemical properties. Quinazoline 23 also displayed efficacy in a murine model of visceral leishmaniasis, reducing liver parasitemia by 37% when given by the intraperitoneal route at 15 mg kg\(^{-1}\) day\(^{-1}\) for 5 consecutive days. Their antileishmanial efficacy, ease of synthesis, and favorable physicochemical properties make the \(N^2,N^4\)-disubstituted quinazoline-2,4-diamine compound series a suitable platform for future development of antileishmanial agents.

INTRODUCTION

Leishmaniasis is a debilitating disease that is prevalent across the globe with 350 million people in 88 countries at risk of acquiring leishmaniasis.\(^1,2\) A recent effort by the World Health Organization to provide a current estimate of the incidence of leishmaniasis concluded that between 0.2 and 0.4 million cases of visceral leishmaniasis occur each year, while from 0.7 to 1.2 million cases of cutaneous leishmaniasis occur each year.\(^3\) This study also estimated that from 20,000 to 40,000 deaths occur each year due to leishmaniasis. More than 20 \(Leishmania\) species are known to cause the disease in humans.\(^4\) Leishmaniasis manifests itself in numerous forms depending on which parasite species infects the host. The parasites are transferred from host to host by about 30 species of female sandfly vectors of the genera \(Phlebotomus\) and \(Lutzomyia\) that infect the host when taking a blood meal.\(^5\) Symptoms of leishmaniasis include unsightly spontaneously healing ulcers on the skin when cutaneous leishmaniasis is present, nonhealing lesions in the mucosa when mucocutaneous leishmaniasis is the affliction, and chronic, debilitating infection of the reticuloendothelial system that is fatal if left untreated due to visceral leishmaniasis.\(^1\) The majority of cases of visceral leishmaniasis are caused by \(L. donovani\) in East Africa and Asia, \(L. infantum\) in the Mediterranean region, and \(L. chagasi\) in Latin America.\(^6\) It should be noted that the last two are genetically identical.\(^7,8\) \(L. infantum\) and \(L. chagasi\) mainly affect children and immunocompromised individuals and are zoonotic parasites with canines being a major reservoir.\(^1\) \(L. donovani\), on the other hand, is an anthropoontic parasite that affects a broad range of ages.\(^1\)

For over 100 years, antimonials have been the drug of choice to combat leishmaniasis. In 1912 Gaspar Viana first reported the use of the trivalent antimonial tartar emetic for the treatment of cutaneous leishmaniasis caused by \(L. braziliensis\) in Brazil.\(^9\) Shortly thereafter McCombie Young and Upendranath Brahmacari used trivalent and pentavalent antimonials to treat visceral leishmaniasis in India with great success, decreasing the mortality rate of 95% to just 10% in 10 years (Figure 1A).\(^10\) Pentavalent antimonials such as meglumine antimoniate and sodium stibogluconate are currently the first line antileishmanial drugs in many areas.\(^1,11,12\) Treatment involves daily injections for up to a 30 day period.\(^13\) Problems with this treatment include a high rate of resistance that has been encountered in India, especially the state of Bihar, where up to 60% of infected individuals do not improve with treatment.\(^1,11,13\) The high rate of resistance to pentavalent antimonials in India has led to the increasing use of amphotericin B and miltefosine...
against visceral leishmaniasis. Since the 1960s, amphotericin B has been the second line treatment for visceral leishmaniasis. It has a cure rate of over 90% but is often accompanied by severe side effects such as nephrotoxicity that require administration in a hospital setting. Lipid formulations of amphotericin B have fewer side effects and are safer to use with the same cure rate. Depending on the dose and formulation, the treatment regimen varies from 3 to 5 days to 8 weeks of administration on alternate days. Miltefosine is the first oral drug to be released for leishmaniasis and is currently available in India, Germany, and Colombia. Miltefosine is not recommended for women who are pregnant or may become pregnant because it is teratogenic. Miltefosine resistance has been demonstrated in vitro, and its long half-life in the body, the 28-day treatment regimen, and it previously being available over the counter in India have led to concerns of clinical resistance. A recent study of 567 individuals in the Bihar state of India has been performed to determine the efficacy of miltefosine since its introduction in 2002. The 6-month cure rate was found to be roughly 90% and gastrointestinal intolerance was encountered in 64.5% of the cases with two deaths related to drug toxicity. Patients who did not improve with treatment were cured using amphotericin B. The authors of this study concluded that the failure rate of miltefosine has increased in the 10 years since its introduction for the treatment of visceral leishmaniasis in India. A recent study also showed that 20% of the visceral leishmaniasis patients in Nepal who were treated with miltefosine relapsed 12 months after treatment.

Because of increased parasite resistance, toxicity issues, increasing failure rates of current treatments, and the lack of effective clinical agents against cutaneous leishmaniasis, new drugs are needed to have an effective strategy for treating leishmaniasis. Quinazolines are a class of compounds that have shown potential as antileishmanials. Berman et al. reported a class of 2,4-diaminoquinazolines with EC\textsubscript{50} as low as 0.04 nM against L. major amastigotes in human monocyte-derived macrophages (A, Figure 1B); however the development of this compound series was abandoned because of toxicity issues. Bhattacharjee et al. (B), Ram et al. (C), and Shakya and Gupta et al. (D and E) have also tested quinazolines as antileishmanials. This class of compounds has been reported as being dihydrofolate reductase (DHFR) inhibitors, although another mechanism of action may be involved with Leishmania. Recently, we tested a small library of structurally diverse compounds, originally designed as potential anticancer probes, for antileishmanial activity in a L. mexicana axenic amastigote assay. Among this library were N\textsuperscript{2},N\textsuperscript{4}-disubstituted quinazolines and SAR studies targeting the major quinazoline sites. Herein, we report a detailed structure–activity relationship (SAR) study focusing on the 2-position, the 4-position, and the quinazoline’s benzenoid ring. All compounds were initially examined in L. donovani and L. amazonensis intracellular amastigote assays to preselect quinazoline candidates active against parasites responsible for causing visceral leishmaniasis and cutaneous leishmaniasis, respectively. Promising compounds have subsequently been tested for efficacy in a murine model of visceral leishmaniasis.

## RESULTS AND DISCUSSION

### Synthetic Chemistry.

The compounds were synthesized following known procedures (Figure 2). Commercially...
available anthranilic acids (a) were cyclized with urea, and the resulting quinazoline-2,4-dione (b) was reacted with phosphorus oxychloride to give the 2,4-dichloroquinazoline (c). Substitution with amines occurred selectively at position 4, yielding 4-amino-2-chloroquinazoline (d) followed by substitution at position 2 to give the 2,4-diamino-substituted quinazoline. In this synthetic sequence, only 4-amino-2-chloroquinazoline (d) and the final N²,N⁴-disubstituted quinazoline-2,4-diamine have been purified and characterized.

In Vitro Antileishmanial Efficacy and Cytotoxicity. All target compounds were tested against *L. donovani* and *L. amazonensis* intracellular amastigotes to identify molecules that are broadly active against these medically important *Leishmania*

Table 1. SAR Study Focusing on 2- and 4-Positions

| Compound | R¹ | R² | L. donovani EC₅₀ µM | L. donovani EC₅₀ µM | L. amazonensis EC₅₀ µM | J774A.1 EC₅₀ µM | SI |
|----------|----|----|---------------------|---------------------|-----------------------|-----------------|-----|
| 2        |    |    | 2.5 ± 0.4           | 3.7 ± 1.6           | 17 ± 6                | 6.8             |     |
| 3        |    |    | 3.6 ± 1.1           | 5.8 ± 2.3           | 23 ± 10               | 6.4             |     |
| 4        |    |    | 25 ± 18             | > 50                | > 33                  | > 1.3           |     |
| 5        |    |    | 3.7 ± 0.3           | 7.6 ± 3.2           | > 33                  | > 8.9           |     |
| 6        |    |    | 4.3 ± 1.2           | 5.1 ± 0.9           | 20 ± 6                | 4.7             |     |
| 7        |    |    | 6.9 ± 1.8           | 20 ± 3              | > 33                  | 4.8             |     |
| 8        |    |    | > 50.0              | > 50.0              | n.d.                  | n.d.            |     |
| 9        |    |    | 8.9 ± 1.0           | 19 ± 3              | > 33                  | > 3.7           |     |
| 10       |    |    | 0.67 ± 0.27         | 1.4 ± 0.5           | 5.5 ± 1.4             | 8.2             |     |
| 11       |    |    | 1.8 ± 0.4           | 2.1 ± 1.1           | 5.4 ± 1.4             | 3.0             |     |
| 12       |    |    | 1.5 ± 0.5           | 13 ± 3              | 18 ± 8                | 12              |     |
| 13       |    |    | 0.65 ± 0.10         | 1.8 ± 0.2           | 5.1 ± 1.5             | 7.8             |     |
| 14       |    |    | 0.64 ± 0.10         | 2.6 ± 0.7           | 6.8 ± 0.2             | 11              |     |
| 15       |    |    | 0.15 ± 0.02         | 0.90 ± 0.27         | 15 ± 1                | 100             |     |
| 16       |    |    | 0.34 ± 0.12         | 1.4 ± 0.2           | 4.9 ± 1.3             | 14              |     |
| 17       |    |    | 0.26 ± 0.15         | 2.2 ± 0.3           | 5.2 ± 1.3             | 20              |     |

"Amphotericin B is the internal control for the in vitro antileishmanial activity assay with EC₅₀ = 40 ± 9 nM against *L. donovani* and EC₅₀ = 89 ± 16 nM against *L. amazonensis*. Podophyllotoxin is the internal control for the in vitro cytotoxicity assay with EC₅₀ = 250 ± 10 nM against J774A.1."
species. The testing involved murine peritoneal macrophages as host cells, since compounds with potential for clinical use must be able to penetrate the infected macrophage in a human host. Antileishmanial activity was determined in an assay using transgenic parasites expressing a $\beta$-lactamase gene as outlined previously.\textsuperscript{28,29} Concentration response data for each compound was fitted by a nonlinear regression model, and the concentration that induces 50% inhibition was calculated as the effective concentration EC\textsubscript{50} (L. donovani or L. amazonensis). Additionally, cytotoxicity against the macrophage cell line J774A.1 was determined as the effective concentration EC\textsubscript{50} (J774A.1) and the selectivity index SI was calculated as the ratio of EC\textsubscript{50} value for J774A.1 and the value for L. donovani (SI = EC\textsubscript{50}(J774A.1)/EC\textsubscript{50}(L. donovani)).

Structure–Activity Relationship Studies. To validate and optimize the antileishmanial activity of N\textsuperscript{2},N\textsuperscript{4}-disubstituted quinazoline-2,4-diamines, two compound subseries were prepared and tested. The first subseries focused mainly on the optimization of the N\textsuperscript{2} and N\textsuperscript{4}-moieties (Table 1), whereas the second subseries was designed to investigate whether analogues being substituted at the quinazoline’s benzenoid ring display improved antileishmanial activity (Table 2).

Starting from hit compound 2, N\textsuperscript{2}-furfuryl analogues 3 and 4 were prepared in which the N\textsuperscript{2}-isopropyl group was replaced by a short trifluoroalkyl or hydroxalkyl group. While the 2,2,2-trifluoroethyl-substituted quinazoline 3 was slightly less potent than compound 2, alcohol 4 lost potency against L. donovani and L. amazonensis by a factor of 10 and >13, respectively. Testing of a small set of quinazoline-2,4-diamines 5–9 with N\textsuperscript{4}-monosubstituted or N\textsuperscript{4}-disubstituted with alkyl groups differing in size and polarity did not identify a particular structural motif improving the potency over 2. Replacement of the furfuryl and isopropyl groups in 2 by two benzyl or two n-butyl groups yielded quinazolines 10 and 11, of which both analogues were more potent than reference 2. Bis-benzyl-substituted quinazoline 10 displayed EC\textsubscript{50} values of 670 nM against L. donovani and 1.4 $\mu$M against L. amazonensis, whereas analogue 11 was approximately 2-fold less potent in comparison to compound 10. Consequently, a following set of six quinazolines 12–17 was designed in which one of the 2- and 4-positions was substituted by one benzylamine, while the remaining position was derivatized with an aniline, n-butylamine, or methylamine. Interestingly, with the exception of the N\textsuperscript{2}-benzyl-N\textsuperscript{4}-methylquinazoline-2,4-diamine 12, all of these compounds demonstrated submicromolar EC\textsubscript{50} values against L. donovani. Among these six quinazolines tested, the N\textsuperscript{2}-benzylquinazoline-2,4-diamines 15–17 appeared to be at least 2-fold more potent against L. donovani than the N\textsuperscript{2}-benzyl counterparts 12–14, suggesting that an N\textsuperscript{2}-benzyl is more favorable than an N\textsuperscript{4}-benzyl for antileishmanial activity. This observation is similar to previous results with quinazolines 2–11. Compound 15 was the most potent compound with an EC\textsubscript{50} of 150 nM against L. donovani. For L. amazonensis, the set of 12–17 displayed a similar activity trend, with N\textsuperscript{2}-benzylquinazolines 15 and 16 being the most potent compounds with submicromolar or single digit micromolar EC\textsubscript{50} values.

N\textsuperscript{2} Furfuryl-N\textsuperscript{4}-isopropyl-substituted quinazoline 2, with EC\textsubscript{50} values of 2.5 $\mu$M against L. donovani and 3.7 $\mu$M against L. amazonensis, was considered to be well suited as the key scaffold for a SAR study focusing on the benzenoid moiety of the quinazoline core. In a systematic approach following the Topliss operational scheme, compound 2 was monosubstituted with a chlorine atom, a methyl group, or a methoxy group in the 5-, 6-, 7-, and 8-positions to probe the benzenoid ring for steric and electronic effects.\textsuperscript{30} Overall, substitution on the benzenoid ring provided compounds with EC\textsubscript{50} values in the single digit micromolar or submicromolar range against L. donovani. For L. amazonensis, the set of 12–17 displayed a similar activity trend, with N\textsuperscript{2}-benzylquinazolines 15 and 16 being the most potent compounds with submicromolar or single digit micromolar EC\textsubscript{50} values.
position, whereas a potency dependence in the order of 8-position > 6-position > 7-position was observed for the methyl-substituted compounds. 7-Methoxyquinazoline 28 with an EC\text{50} of 740 nM against \textit{L. donovani} was the most potent analogue of the compound subseries substituted at the benzenoid ring. In contrast, the testing of the benzenoid ring substituted quinazolines against \textit{L. amazonensis} gave insight into a SAR, which differed from the one observed for \textit{L. donovani}. 8-Methoxy-substituted quinazoline 29 was the only analogue that was marginally more potent than reference compound 2, whereas all of the other analogues were equally or less potent than quinazoline 2.

Cytotoxicity. An important quality of the quinazolines is their selectivity to inhibit parasite growth over mammalian cells. Generally, the majority of the quinazolines 2–29 exhibited EC\text{50} of 20 \mu M or higher against J774A.1 (Tables 1 and 2). Quinazolines 18–20 and 22–24, whose benzenoid ring is substituted with one chloride or one methyl in 5-, 6-, or 7-position, were significantly less toxic than their reference 2. The quinazolines displaying potent antileishmanial activity, especially the \textit{N}-benzylquinazoline-2,4-diamines 15–17, were shown to have respectable SI values of 10 and larger, indicating that they are relatively selective, nontoxic chemotypes. The SI of 100 for compound 15 was particularly enticing and led to this compound being tested in vivo.

Activity against Antimony-Resistant \textit{L. donovani}. The potency of selected quinazolines against antimony-resistant \textit{Leishmania} was assessed using murine peritoneal macrophages infected with two \textit{L. donovani} clinical strains, BPK206/0 and BPK164/1 (Table 3).\textsuperscript{31} Strain BPK164/1 was isolated from a bone marrow aspirate taken from a Nepalese visceral leishmaniasis patient not responding to antimonial therapy; the BPK164/1 strain was originally found to be 6-fold resistant to sodium stibogluconate compared to the antimony-susceptible reference strain BPK206/0.\textsuperscript{31} Compounds 15, 16, and 23 displayed similar activity against the antimony-susceptible and antimony-resistant parasites. The EC\text{50} values against the clinical strains were 5- to 19-fold higher than their EC\text{50} values against \beta-lactamase expressing \textit{L. donovani} (Table 1). There are several possible reasons for the differences in susceptibility observed between the clinical \textit{L. donovani} isolates and the \beta-lactamase expressing genetically modified lab strain: (1) the former strains are from the Indian subcontinent, while the lab strain is of African origin. Antileishmanial drugs display differences in efficacy for treating Indian visceral leishmaniasis compared to African visceral leishmaniasis;\textsuperscript{32,33} strain differences could account for such differential susceptibility; (2) the clinical strains could have a greater fitness in an in vitro intracellular amastigote model than the lab adapted strain which has been in axenic culture for many passages, leading to lower compound susceptibility in the former; (3) the clinical strains were assayed using a different method under different conditions compared to the \beta-lactamase expressing lab strain, potentially contributing to distinctions in compound susceptibility. Nevertheless, these data confirm the in vitro activity of compounds 15, 16, and 23 against clinical strains currently circulating in endemic regions of the Indian subcontinent.

Mechanism of Action. We investigated the possibility that quinazolines inhibit folate metabolism in the parasite, since previous studies have shown DHFR inhibition with similar structures.\textsuperscript{18,23} \textit{L. donovani} axenic amastigotes and J774A.1 (mouse macrophage cell line) were adapted to grow in media deficient in \textit{p}-aminobenzoic acid (PABA) and folic acid prior to susceptibility testing. Susceptibility to quinazolines and miltefosine, as well as the known antifolate drugs methotrexate and pyrimethamine as positive controls, was assessed in the presence or absence of folinic acid (Figure 3).\textsuperscript{34,35} The presence of folinic acid dramatically increased the EC\text{50} values of each of the quinazolines as well as the methotrexate and pyrimethamine. For example, quinazoline 23 was 6.4-fold less potent in the presence of 488 nM folinic acid than in completely deficient media. Conversely, efficacy of miltefosine was not affected by addition of folinic acid to the media. Interestingly, we saw no antagonism of activity of quinazolines 10, 12, 13, 15, 16, or 23 for the mammalian cell line (J774A.1) in the presence of folinic acid. For example, the EC\text{50} of quinazoline 10 was 84.8 ± 2.2 and 84.2 ± 2.8 \mu M in the presence or absence of 488 nM folinic acid, respectively. In contrast, the efficacy of methotrexate and pyrimethamine was antagonized significantly by folinic acid in J774A.1. These results are consistent with the hypothesis that the quinazolines interfere with folate metabolism in \textit{L. donovani}.

Structure–Property Relationship Studies. In parallel to the testing of the compounds for antileishmanial activity in vitro, a structure–property relationship (SPR) study focusing on log \textit{D}_{\text{pION}} aqueous solubility, and permeability has been conducted with all compounds to assess potential physico-chemical liabilities (Table 4). The log \textit{D}_{\text{pION}} and log \textit{D}_{\text{aw}} permeability in Table 4). The log \textit{D}_{\text{pION}} and log \textit{D}_{\text{aw}} permeability of miltefosine were measured in a glassy carbon disk. The permeability of all quinazolines displayed a pH dependence (Table 4). Generally, the aqueous solubility, the distribution coefficient between octanol and water at pH 3.0 and pH 7.4, were experimentally determined via a previously described HPLC-based method.\textsuperscript{56} Solubility at pH 7.4 was determined using Biomek FX lab automation workstation with pION \mu SOL evolution software as reported previously and at pH 2.0 using an in-house HPLC assay based on UV absorption.\textsuperscript{57} Passive transcellular permeability was assessed in parallel artificial membrane permeability assay (PAMPA) at pH 7.4 and pH 4.0. Generally, the aqueous solubility, the distribution coefficient log \textit{D}_{\text{pION}} and permeability of all quinazolines display a pH dependence (exemplified on log \textit{D} or permeability in Table 4). The permeability is enhanced at neutral pH, while the aqueous solubility and log \textit{D} are better at lower pH ranges. However, since the aqueous solubility, the distribution coefficient, and the permeability are within the acceptable ranges (solubility of >20 \mu M, \textit{P}_{\text{aw}} > 10 \times 10^{-6} \text{ cm s}^{-1}, 1 < \log \text{D} < 4), the quinazoline compound series is considered to be suitable for the development of bioavailable antileishmanial compounds.

In Vivo Antileishmanial Efficacy Studies. For the selection of the candidates for in vivo efficacy against \textit{L. donovani}, only compounds that displayed submicromolar in vitro activity against \textit{L. donovani} with a SI value greater than 10 and a balanced combination of good physicochemical proper-

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Table 3. Activities of Compounds against \textit{L. donovani} Clinical Isolates\textsuperscript{a}

| compd | EC\text{50} \mu M |
|-------|------------------|
|       | BPK206/0 | BPK164/1 |
| sodium stibogluconate\textsuperscript{b} | 24 ± 6 | >60 |
| 15 | 2.8 ± 0.4 | 2.0 ± 0.4 |
| 16 | 2.0 ± 0.7 | 1.4 ± 0.3 |
| 23 | 4.2 ± 0.0 | 3.8 ± 0.6 |

\textsuperscript{a}Mean ± range of two independent measurements. \textsuperscript{b}Values for sodium stibogluconate are given in micrograms of pentavalent antimony per milliliter.
ties were chosen. Among the benzyl-substituted quinazolines 12−17, 15 and 16 appeared to be the best candidates for in vivo evaluation because of their combination of potency, selectivity, and favorable physicochemical properties. From the compound subseries substituted at the benzenoid ring, the physicochemical properties were not discriminatory, and hence, compound 23 was chosen as a viable candidate because of its submicromolar EC$_{50}$ against *L. donovani* as well as the outstanding SI value of this compound (>40).

The three compounds mentioned above were dissolved in an appropriate vehicle (15 and 23 dissolved in 0.5% methylcellulose and 0.1% Tween 80 in water; 16 dissolved in 45% (w/v) (2-hydroxypropyl)-β-cyclodextrin solution (HPβCD)) and administered to uninfected BALB/c mice intraperitoneally to determine a tolerated dose for in vivo efficacy studies. While 15 was well tolerated when given at 30 mg/kg ip for 5 consecutive days, 16 (slowed breathing) and 23 (hypoactivity) were toxic to animals when given at the same dosing regimen. Considering the toxicity of 16 and 23 when given at 30 mg/kg ip, lower doses of these compounds were administered in subsequent in vivo efficacy studies in a murine visceral leishmaniasis model (Figure 4). When tested at 5 × 15 mg/kg ip, 23 inhibited liver parasitemia by 37% compared to the vehicle control. However, 15 did not show significant antileishmanial efficacy when tested at 5 × 30 mg/kg/ip. There was also no significant difference in the parasite burden between mice in the group treated with compound 16 (5 × 10 mg/kg ip) and the vehicle control group ($P > 0.05$). As expected, the 45% HPβCD vehicle used to solubilize 16 itself resulted in 18% inhibition of liver parasitemia, consistent with our previous report.$^{29}$

**Pharmacokinetics of 16 and 23 after po and ip Administration in Mice.** Pharmacokinetic studies were conducted to determine the systemic and target tissue exposures of 16 and 23. The mean plasma and tissue concentration−time profiles of 16 and 23 after po and ip administration are shown in Figures 5 and 6, respectively. The relevant pharmacokinetic outcomes are listed in Table 5. After po administration at 100 μmol/kg (or approximately 30 mg/kg), both compounds were absorbed from the gastrointestinal tract of mice and plasma concentration reached a C$_{\text{max}}$ of 0.44 and 0.25 μM for 16 and 23, respectively. The systemic and tissue exposure of 16 (AUC and C$_{\text{max}}$) were considerably greater than those of 23 (Table 4). After ip administration, plasma concentration reached a C$_{\text{max}}$ of 5.2 and 2.7 μM for 16 and 23, respectively, before decreasing rapidly in the first 4 h, followed by a slower elimination process until 24 h. The rapid decline in plasma concentration was likely due to extensive tissue redistribution after absorption, as indicated by the high target tissue concentrations. The plasma and tissue exposures after ip administration were markedly greater than those after po administration (Table 4), suggesting significant first-pass metabolism and/or partial gastrointestinal absorption after oral administration of 16 and 23. The terminal elimination half-life ranged from 5 to 20 h. Minor reversible overt toxicity (hypoactivity) was observed after ip administration of 16; however, more severe toxicity was observed, unexpectedly, after a single ip administration of 23, which warranted euthanization of some mice within 15 min postdose. As efficacy was evaluated at lower doses than 30 mg/kg to avoid toxicity and dose linearity for pharmacokinetic outcomes were unknown, it is not
Furthermore, the permeability of 23 16 in the upper gastrointestinal tract. In contrast, the permeability of 23 16 could contribute to the slowed absorption of this compound in the permeability of 23 16 microsomes (2 and 16). The permeability of 23 16 has been tested with the aim to further optimize hit compounds and to conduct a detailed SAR study against L. amazonensis and to conduct a detailed SAR study against L. donovani and M. (4-Disubstituted quinazoline-2,4-diamines 1 and 2 were found to display antileishmanial activity in the single digit micromolar range. Subsequently a total of 28 molecules have been synthesized systematically by varying the substitutions in the 2-, 4-, 5-, 6-, 7-, and/or 8-positions. All quinazolines have been tested with the aim to further optimize hit compounds 1 and 2 and to conduct a detailed SAR study against L. donovani and L. amazonensis. The most potent activities with EC50 values in the submicromolar range against L. donovani were obtained with quinazoline-2,4-diamine scaffolds bearing either a N2-benzyl-N4-alkyl/phenyl or a N4-isopropyl-N4-furfuryl substituent combination. Furthermore, although the benzenoid ring of the quinazoline-2,4-diamine scaffold has been identified to play a secondary role for efficacy, quinazolines substituted at the 5- or 6-position with one methyl or one methoxy group have also been identified to possess submicromolar EC50 values against L. donovani. Although the quinazoline-2,4-diamines appear to display some cytotoxicity against the macrophage cell line J774A.1 yielding modest SI values, the SI values of the best antileishmanial quinazolines were larger than 10. In addition, assessment of key physicochemical properties confirmed that the quinazoline’s aqueous solubility, distribution coefficient, and passive transcellular permeability were in acceptable ranges. These promising results led to efficacy testing of the lead compounds 15, 16, and 23 in an in vivo murine visceral leishmaniasis model. While compounds 15 and 16 did not have activity translate from in vitro to in vivo, quinazoline 23 reduced parasitemia by 37% when 15 mg kg−1 day−1 was given via the intraperitoneal route for 5 consecutive days. Pharmacokinetic studies of compound 23 revealed a maximum plasma concentration that was 3-fold higher than the EC50 and a terminal half-life of 5 h after ip administration. Although a clear correlation between in vitro activity, in vitro physicochemical properties, and in vivo activity is not clearly observed, the potencies of front-runner compounds 15, 16, and 23 in conjunction with favorable physicochemical properties make N5,N5-disubstituted quinazoline-2,4-diamines a suitable platform for the future development of antileishmanial agents.

Table 4. Physicochemical Properties of Quinazolines

| Compound | Solubility* | Permeability Pe·10^6 (cm/s)^3 | Log D |
|----------|-------------|-------------------------------|-------|
|          | pH 7.4      | pH 4.0                        | pH 7.4 | pH 3.0 |
| 1        | 5          | 62.2                          | 3.15   | 2.18   |
| 2        | 5          | 67.7                          | 4.20   | 2.02   |
| 3        | 5          | 26.4                          | 2.19   | 1.48   |
| 4        | 5          | 19.7                          | 3.19   | 2.40   |
| 5        | 5          | 28.8                          | 3.02   | 2.06   |
| 6        | 5          | 38.8                          | 2.54   | 1.73   |
| 7        | 5          | 112                           | 4.69   | 3.56   |
| 8        | 5          | 72.3                          | 3.13   | 1.64   |
| 9        | 5          | 312                           | 3.82   | 2.82   |
| 10       | 5          | 485                           | 3.89   | 2.91   |
| 11       | 5          | n/d                           | 3.14   | 2.11   |
| 12       | 5          | 161                           | 3.80   | 2.84   |
| 13       | 5          | 271                           | 4.17   | 2.78   |
| 14       | 5          | 51                           | 2.96   | 2.00   |
| 15       | 5          | 341                           | 3.84   | 2.86   |
| 16       | 5          | 412                           | 4.25   | 2.67   |
| 17       | 5          | 67.6                          | 3.96   | 2.38   |
| 18       | 5          | 103                           | 3.91   | 2.51   |
| 19       | 5          | 170                           | 3.99   | 2.60   |
| 20       | 5          | 169                           | 4.44   | 2.62   |
| 21       | 5          | 45.9                           | 3.55   | 1.57   |
| 22       | 5          | 67.2                           | 3.61   | 1.58   |
| 23       | 5          | 67.2                           | 3.63   | 1.55   |
| 24       | 5          | 45.9                           | 3.55   | 2.44   |
| 25       | 5          | 38.4                           | 3.40   | 2.99   |
| 26       | 5          | 59.2                           | 3.70   | 3.11   |
| 27       | 5          | 80.2                           | 3.58   | 2.95   |
| 28       | 5          | 87.0                           | 3.35   | 2.53   |

* For solubility ≤ 5 μM. (***) For 5 μM < solubility ≤ 10 μM. (++++) For solubility ≥ 30 μM.

CONCLUSIONS

N5,N5-Disubstituted quinazoline-2,4-diamines 1 and 2 were found to display antileishmanial activity in the single digit micromolar range. Subsequently a total of 28 molecules have been synthesized systematically by varying the substitutions in the 2-, 4-, 5-, 6-, 7-, and/or 8-positions. All quinazolines have been tested with the aim to further optimize hit compounds 1 and 2 and to conduct a detailed SAR study against L. donovani and L. amazonensis. The most potent activities with EC50 values in the submicromolar range against L. donovani were obtained with quinazoline-2,4-diamine scaffolds bearing either a N2-benzyl-N4-alkyl/phenyl or a N4-isopropyl-N4-furfuryl substituent combination. Furthermore, although the benzenoid ring of the quinazoline-2,4-diamine scaffold has been identified to play a secondary role for efficacy, quinazolines substituted at the 5- or 6-position with one methyl or one methoxy group have also been identified to possess submicromolar EC50 values against L. donovani. Although the quinazoline-2,4-diamines appear to display some cytotoxicity against the macrophage cell line J774A.1 yielding modest SI values, the SI values of the best antileishmanial quinazolines were larger than 10. In addition, assessment of key physicochemical properties confirmed that the quinazoline’s aqueous solubility, distribution coefficient, and passive transcellular permeability were in acceptable ranges. These promising results led to efficacy testing of the lead compounds 15, 16, and 23 in an in vivo murine visceral leishmaniasis model. While compounds 15 and 16 did not have activity translate from in vitro to in vivo, quinazoline 23 reduced parasitemia by 37% when 15 mg kg−1 day−1 was given via the intraperitoneal route for 5 consecutive days. Pharmacokinetic studies of compound 23 revealed a maximum plasma concentration that was 3-fold higher than the EC50 and a terminal half-life of 5 h after ip administration. Although a clear correlation between in vitro activity, in vitro physicochemical properties, and in vivo activity is not clearly observed, the potencies of front-runner compounds 15, 16, and 23 in conjunction with favorable physicochemical properties make N5,N5-disubstituted quinazoline-2,4-diamines a suitable platform for the future development of antileishmanial agents.
For future compound design to be successful, optimization will focus not only on improving antileishmanial activity and key physicochemical properties but also on improving the pharmacokinetics and SI values for the entire quinazoline compound series.

**EXPERIMENTAL SECTION**

**Chemistry. General.** All reagents and solvents were obtained from Aldrich Chemical Co. and used without further purification. Anthranilic acids were purchased from Sigma-Aldrich, Oakwood Products, Inc., or TCI America. NMR spectra were recorded at ambient temperature on a 250 MHz Bruker, 400 MHz Varian, or 500 MHz Varian NMR spectrometer in the solvent indicated. All $^1$H NMR experiments are reported in $\delta$ units, parts per million (ppm) downfield of TMS, and were measured relative to the signals for chloroform ($7.26$ ppm), methanol ($3.31$ ppm), and dimethylsulfoxide ($2.50$ ppm). All $^{13}$C NMR spectra were reported in ppm relative to the signals for chloroform ($77$ ppm), methanol ($49$ ppm), and dimethylsulfoxide ($39.5$ ppm) with $^1$H decoupled observation. Data for $^1$H NMR are reported as follows: chemical shift ($\delta$ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, sext = sextet, sept = septet, oct = octet, m = multiplet), integration, and coupling constant (Hz), whereas $^{13}$C NMR analyses were reported in terms of chemical shift. NMR data were analyzed by using MestReNova Software, dx.doi.org/10.1021/jm5000408 [J. Med. Chem. 2014, 57, 5141−5156].

Figure 4. In vivo efficacy of quinazolines against LV82 in *L. donovani* infected BALB/c mice. Results are presented as the liver parasitemia (LDU) for each mouse (◆), and the average LDU in each group (−) was determined by microscopy (n = 4): (A) LDU for mice treated with 16 and 23; (B) LDU for mice treated with 15. All treatments were given by the ip route. Compounds 15 and 23 were dissolved in 0.5% methylcellulose and 0.1% Tween 80 (MC), while compound 16 was dissolved in 45% (w/v) (2-hydroxypropyl)-β-cyclodextrin solution (HP/βCD). (★) $p < 0.05$, compared with untreated control.

Figure 5. Plasma (open circles) and tissue (squares for liver and triangles for spleen) concentration–time profiles after po (A) and ip (B) administration of 16 in mice at a dose level of 100 $\mu$mol/kg (~30 mg/kg). Symbols and error bars represent the mean and standard error of triplicate determinations except for those labeled with asterisks where only one or two determinations were obtained because of sample loss.

Figure 6. Plasma (open circles) and tissue (squares for liver and triangles for spleen) concentration–time profiles after po (A) and ip (B) administration of 23 in mice at a dose level of 100 $\mu$mol/kg (~30 mg/kg). Symbols and error bars represent the mean and standard error of triplicate determinations except for those labeled with asterisks where only one or two determinations were obtained because of sample loss. 23 was below the detection limit (0.1 $\mu$M) in the liver and spleen 12 and 24 h after ip administration.
### Table 5. Pharmacokinetic Outcomes of 16 and 23 after po and ip Administration to Mice

| parameter<sup>a</sup> | po | ip |
|------------------------|----|----|
|                        | plasma | liver | spleen | plasma | liver | spleen |
| **Compound 16**        |       |       |       |       |       |       |
| C<sub>max</sub> (μM)   | 0.44  | 15.8  | 7.0   | 5.21  | 148  | 163   |
| T<sub>max</sub> (h)    | 4     | 1     | 1     | 1     | 1    | 1     |
| AUC<sub>last</sub> (μM·h) | 7.8  | 110   | 68    | 11    | 500  | 620   |
| t<sub>1/2</sub> (min)  | NC<sup>b</sup> | ND<sup>c</sup> | ND<sup>c</sup> | 20    | ND<sup>c</sup> | ND<sup>c</sup> |
| Mic t<sub>1/2</sub>     | 27    | 9.4   |       |       |       |       |

| **Compound 23**        |       |       |       |       |       |       |
| C<sub>max</sub> (μM)   | 0.25  | 5.2   | 0.9   | 2.67  | 48   | 45.8  |
| T<sub>max</sub> (h)    | 1     | 1     | 1     | 1     | 1    | 1     |
| AUC<sub>last</sub> (μM·h) | 2.5  | 29    | 7.4   | 4.6   | 42   | 71    |
| t<sub>1/2</sub> (min)  | 24    | ND<sup>c</sup> | ND<sup>c</sup> | 5.4   | ND<sup>c</sup> | ND<sup>c</sup> |
| Mic t<sub>1/2</sub>     |       | 9.4   |       |       |       |       |

<sup>a</sup>AUC (0–24 h) was calculated for plasma using noncompartmental analysis, whereas AUC (1–24 h) was calculated for tissues using trapezoid rule. AUC<sub>last</sub> AUC from the time of dose to the last measurable concentration. C<sub>max</sub> and T<sub>max</sub> maximum concentration and time to reach C<sub>max</sub>. t<sub>1/2</sub> terminal half-life. NC, not calculable because of lack of a data point. ND, not determined. <sup>b</sup>In vitro mouse liver microsomal half-life.

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**Particular Method Da.** The compound crystallized out of the cool solution and was filtered and rinsed with cold ethanol to yield pure product.

**Particular Method Db.** The solvent was evaporated, and the crude mixture was purified by flash chromatography using dichloromethane and methanol.

**2,4-Dichloroquinazoline (1c).** Commercially available benzoyleurea (0.12 mol) and N,N-dimethylaniline (0.12 mol) were mixed in 60 mL of phosphorus oxychloride and heated to reflux under an atmosphere of argon. After 5 h the solution was cooled and slowly added to 300 mL of ice. Once quenching was finished, the compound was extracted with chloroform (4 × 125 mL) and purified by flash chromatography using hexanes and ethyl acetate to yield the title compound in 61% yield (14.5 g, 73 mmol).<sup>1</sup> H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.29–8.24 (m, 1H), 8.02–7.99 (m, 2H), 7.74 (dd, J = 6.3, 5.0, 3.2, 1H).<sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 164.25, 155.36, 152.60, 136.41, 129.61, 128.25, 126.42, 122.59. Mass (ESI): [M + H]<sup>+</sup> 199, 201; found 198.9 (100%), 200.9 (64%). R<sub>f</sub> = 0.56 (hexanes to ethyl acetate 4:1).

**2-Chloro-N-(furan-2-ylmethyl)quinazolin-4-amine (2d).** 1 g (5.0 mmol) of 1c was reacted with fururylurea and purified according to method Ca to furnish 1.21 g (4.7 mmol) of the title compound. The mixture in chloroform was washed three times with an equal amount of water and dried over Na<sub>2</sub>SO<sub>4</sub>. The crude was then purified by either method Ca or Cb:

**Purification Method Ca.** The compound was recrystallized with ethanol and water, filtered, and rinsed with cold ethanol to yield pure product.

**Purification Method Cb.** The crude was purified by flash chromatography using hexanes and ethyl acetate.

**General Procedure A: Cyclization of Anthranilic Acids to the Corresponding Quinazoline-2,4-diones.** 1 equiv of anthranilic acid and 3.5 equiv of urea were mortared and pestled to a powder and heated to 200 °C in a round-bottom flask open to the atmosphere. After 2 h, the mixture was cooled, triturated with water, and filtered to give the product as crude. No further purification was performed.

**General Procedure B: Chlorination of Quinazoline-2,4-diones to the Corresponding 2,4-Dichloroquinazolines.** 1 equiv of quinazoline-2,4-dione and 1 equiv of N,N-dimethylaniline were mixed in 12 equiv of phosphorus oxychloride, and the mixture was refluxed under an argon atmosphere until starting material was no longer present by TLC (3–16 h). The mixture was then cooled and added to ice in the amount of 10 times the reaction volume. The solution was filtered to give crude product.

**General Procedure C: Amine Substitution of 2,4-Dichloroquinazolines to Yield 4-Amino-Substituted 2-Chloroquinazolines.** 1.1 equiv of amine and sodium acetate were mixed with 1 equiv of 2,4-dichloroquinazoline at 0.1 M concentration in a 3 to 1 mixture of tetrahydrofuran and water and heated to 65 °C. When the reaction was observed to be finished by TLC, the solution was diluted with ethyl acetate, the layers were separated, and the organic phase was washed three times with an equal amount of water and dried over Na<sub>2</sub>SO<sub>4</sub>. The crude was then purified by either method Ca or Cb:

**Purification Method Ca.** The compound was recrystallized with ethanol and water, filtered, and rinsed with cold ethanol to yield pure product.

**Purification Method Cb.** The crude was purified by flash chromatography using hexanes and ethyl acetate.

**General Procedure D: Amin substitution of 4-Amino-substituted-2-chloroquinazolines to Yield 2,4-Diamino-Substituted Quinazolines.** 1.5 equiv of amine was mixed with 1 equiv of 4-amino-substituted 2-chloroquinazoline at 0.2 M concentration in ethanol in a sealed tube and heated to 150 °C. When the reaction was finished as observed by TLC, the compound was purified by either method Da or Db:
(t, J = 7.8 Hz, 1H), 7.32 (d, J = 8.2 Hz, 1H), 7.10 (t, J = 7.8 Hz, 1H), 4.26–4.14 (m, 5H), 1.25 (t, J = 7.1 Hz, 3H), 1.21 (d, J = 6.5 Hz, 6H).

13C NMR (101 MHz, CD3OD) δ 172.23, 162.13, 159.29, 150.86, 134.31, 123.71, 123.72, 112.61, 111.84, 62.22, 43.87, 43.66, 23.25, 14.52. HRMS: m/z calcd for C19H13F2N2O (M + H+) 289.1659; found 289.1668. Rf = 0.34 (dichloromethane to methanol 9:1).

2-Chloro-N-cyclohexylquinazolin-4-amine (6d). 0.16 g (0.80 mmol) of 1c was reacted with cyclohexylamine and purified according to method Cb to furnish 0.15 g (0.55 mmol) of the title compound in 69% yield. 1H NMR (250 MHz, CD3OD) δ 8.05–7.99 (m, 1H), 7.60 (dd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.46–7.41 (m, 1H), 7.33 (dd, J = 8.3, 7.0, 1.3 Hz, 1H), 4.17–4.01 (m, 1H), 1.97–1.84 (m, 2H), 1.70 (d, J = 2.6 Hz, 2H), 1.65–1.52 (m, 1H), 1.38–1.25 (m, 4H), 1.20–1.00 (m, 1H).

13C NMR (63 MHz, CD3OD) δ 161.94, 159.05, 151.35, 134.67, 127.25, 126.90, 123.83, 114.81, 51.68, 33.22, 26.71, 26.48. Mass (ESI): [M + H]+ 262, found 262.1 (100%), 264 (3%). Rf = 0.43 (hexanes to ethyl acetate 2:1).

N4-Cyclohexyl-N4-isopropylquinazolin-2-4-diamine (8). 0.10 g (0.40 mmol) of 8d was reacted with isopropylamine and purified according to method Db to furnish 0.06 g (0.21 mmol) of the title compound as a white crystalline solid in 69% yield. 1H NMR (400 MHz, CD3OD) δ 7.89 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 7.5 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.14 (t, J = 6.8 Hz, 1H), 4.20 (sept, J = 6.5 Hz, 2H), 2.04 (d, J = 2.2 Hz, 2H), 1.83 (d, J = 4.5 Hz, 2H), 1.70 (d, J = 12.4 Hz, 1H). 13C NMR (101 MHz, CD3OD) δ 159.82, 157.34, 147.57, 131.16, 122.65, 121.63, 121.25, 110.84, 54.66, 42.93, 32.26, 25.73, 21.93. HRMS: m/z calcd for C19H13F2N2 (M + H+) 285.2074; found 285.2077. Rf = 0.38 (dichloromethane to methanol 9:1). Decomposed at 345 °C.

N,N-Dimethyl-2-chloroquinazolin-4-amine (9d). 0.054 g (0.27 mmol) of 1c was reacted with dimethylamine and purified according to method Ca to furnish 0.037 g (0.18 mmol) of the title compound in 69% yield. 1H NMR (400 MHz, CD3OD) δ 8.06 (d, J = 8.5 Hz, 1H), 7.68–7.62 (m, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.39–7.33 (m, 3H), 3.35 (s, 6H).

13C NMR (101 MHz, CD3OD) δ 163.76, 156.03, 152.57, 133.10, 126.41, 125.89, 124.83, 114.07, 41.20. Rf = 0.35 (hexanes to ethyl acetate 1:4).

N4-Isopropyl-N4,N4-dimethyl-2-chloroquinazolin-2-4-diamine (9). 0.030 g (0.14 mmol) of 9d was reacted with isopropylamine and purified according to method Db to furnish 0.015 g (0.065 mmol) of the title compound as a white solid in 46% yield. 1H NMR (400 MHz, CD3OD) δ 8.11 (d, J = 8.3 Hz, 1H), 7.69 (t, J = 8.2 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.31 (t, J = 8.2 Hz, 1H), 4.31–4.19 (m, 1H), 3.48 (s, 6H), 1.29 (d, J = 6.5 Hz, 6H).

13C NMR (101 MHz, CD3OD) δ 162.97, 152.20, 143.16, 134.25, 125.79, 125.97, 122.47, 110.60, 43.69, 41.45, 21.46. HRMS: m/z calcd for C19H14F2N2 (M + H+) 311.1604; found 311.1608. Rf = 0.33 (dichloromethane to methanol 9:1).

Melting point 110–115 °C.

N-Benzyl-2-chloroquinazolin-4-amine (10d). 0.30g (1.5 mmol) of 1c was reacted with benzylamine and purified according to method Ca to furnish 0.345 g (1.28 mmol) of the title compound in 85% yield. 1H NMR (400 MHz, CD3OD) δ 7.70 (dd, J = 11.3, 8.4, 4.9 Hz, 3H), 7.48–7.26 (m, 5H), 6.27 (s, 1H), 4.83 (d, J = 5.3 Hz, 2H).

13C NMR (101 MHz, CD3OD) δ 160.93, 157.93, 151.09, 137.48, 133.76, 129.11, 128.51, 128.21, 128.00, 124.66, 121.07, 113.38, 45.94. Rf = 0.21 (hexanes to ethyl acetate 4:1).

N4-Dibenzylnitrazil-2-chloroquinazolin-4-amine (10). 0.10 g (0.37 mmol) of 10d was reacted with benzylamine and purified according to method Da to furnish 0.101 g (0.297 mmol) of the title compound as a white crystalline solid in 80% yield. 1H NMR (500 MHz, DMSO-d6) δ 10.46 (s, NH), 8.64 (s, NH), 8.45 (d, J = 8.0 Hz, 1H), 7.74 (dt, J = 7.8, 1.5 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.36 (dt, J = 7.8, 1.3 Hz, 1H), 7.36–7.12 (m, 10H), 4.75 (d, J = 5.5 Hz, 2H), 4.63 (d, J = 4.8 Hz, 2H).

13C NMR (126 MHz, DMSO-d6) δ 160.26, 153.49, 139.44, 138.85, 138.29, 135.67, 129.93, 128.85, 128.80, 128.24, 128.12, 127.72, 127.64, 124.93, 124.64, 117.32, 110.22, 110.12, 44.86, 44.35. HRMS: m/z calcd for C28H22N2 (M + H+) 341.1761; found 341.1751. Rf = 0.45 (dichloromethane to methanol 9:1).

Melting point 237–240 °C.

N-Benzyl-2-chloroquinazolin-4-amine (11d). 0.48 g (2.4 mmol) of 1c was reacted with n-butyllamine and purified according to method to furnish 0.23 g (0.78 mmol) of the title compound as a white crystalline solid in 44% yield. A transesterification reaction occurred leading to the etherester product.

1H NMR (400 MHz, CD3OD) δ 7.84 (d, J = 8.2 Hz, 1H), 7.57–7.51
Ca to furnish 0.49 g (2.1 mmol) of the title compound in 86% yield. 1H NMR (250 MHz, CDCl3) 1.67 (s, 3H), 4.02 (s, 2H), 4.10 (t, J = 7.7 Hz, 2H), 7.25 (s, 5H). 13C NMR (100 MHz, CDCl3) δ 141.29, 139.65, 138.70, 135.50, 128.96, 128.72, 128.04, 127.87, 127.54, 126.51, 125.31, 124.96, 123.15, 122.68, 121.50, 121.16, 113.26, 108.70, 108.36, 105.04. HRMS: m/z calcd for C25H23N4 [M + H]+ 375.1728; found 375.1722.  [C33H28N6]18d: 0.10 g (0.043 mmol) of 18c was reacted with furfurylamine according to general procedure D to give 0.011 g (0.038 mmol) of the title compound as white amorphous solid in 30% yield. 1H NMR (250 MHz, CDCl3) δ 1.60 (s, 3H), 3.69 (s, 2H), 4.16 (s, 2H), 4.71 (d, J = 7.7 Hz, 2H), 7.12 (s, 5H). 13C NMR (63 MHz, CDCl3) δ 161.27, 154.54, 140.08, 139.51, 136.27, 129.70, 128.69, 128.59, 125.92, 124.85, 117.86, 111.30, 45.91, 29.04. HRMS: m/z calcd for C7H7N3 [M + H]+ 165.1435; found 165.1457. Rf 0.21 (hexanes to ethyl acetate 4:1). Decomposed at 150 °C. 2-Chloro-N-furfural-2-yminol)-N-isopropylquinazoline-4,4-diamine (18e). 0.047 g (0.16 mmol) of 18d was reacted with isopropylamine according to general procedure D to give 0.030 g (0.095 mmol) of the title compound as a yellow solid in 59% yield. 1H NMR (400 MHz, DMSO-d6) δ 8.08 (s, 1H), 7.73 (t, J = 8.4 Hz, 1H), 7.50–7.20 (m, 7H). 13C NMR (63 MHz, CDCl3) δ 162.17, 154.54, 140.08, 139.51, 136.27, 129.70, 128.59, 125.92, 124.85, 117.86, 111.30, 45.91, 29.04. HRMS: m/z calcd for C6H7N2 [M + H]+ 125.1076; found 125.0836. Rf 0.33 (hexanes to methyl cellosolve 9:1). 2-Chloro-N-furan-2-ylmethyl)-N-isopropylquinazoline-4,4-diamine (18f). 0.047 g (0.16 mmol) of 18d was reacted with isopropylamine according to general procedure D to give 0.030 g (0.095 mmol) of the title compound as a yellow solid in 59% yield. 1H NMR (400 MHz, DMSO-d6) δ 8.08 (s, 1H), 7.73 (t, J = 8.4 Hz, 1H), 7.50–7.20 (m, 7H). 13C NMR (63 MHz, CDCl3) δ 162.17, 154.54, 140.08, 139.51, 136.27, 129.70, 128.59, 125.92, 124.85, 117.86, 111.30, 45.91, 29.04. HRMS: m/z calcd for C5H6N2 [M + H]+ 125.1076; found 125.1076. Rf 0.31 (dichloromethane to methanol 9:1). Decomposed at 240 °C. 2,4,7-Trichloroquinazoline (19c). 0.75 g (4.4 mmol) of commercially available 2-amino-4-chlorobenzoic acid was reacted according to general procedure A to give crude 19b. Without further purification, 19b was reacted according to general procedure B to give 0.15 g (0.6 mmol) of the crude title compound (20% over two steps). Mass (ESI): [M + H]+ 233, 235; found 232.99 (100%), 235.00 (56%). 2-Chloro-N-(furan-2-ylmethyl)-N-isopropylquinazoline-4,4-diamine (18e). 0.047 g (0.16 mmol) of 18d was reacted with furfurylamine according to general procedure D to give 0.011 g (0.038 mmol) of the title compound as white amorphous solid in 30% yield. 1H NMR (250 MHz, CDCl3) δ 1.60 (s, 3H), 3.69 (s, 2H), 4.16 (s, 2H), 4.71 (d, J = 7.7 Hz, 2H), 7.12 (s, 5H). 13C NMR (63 MHz, CDCl3) δ 161.27, 154.54, 140.08, 139.51, 136.27, 129.70, 128.59, 125.92, 124.85, 117.86, 111.30, 45.91, 29.04. HRMS: m/z calcd for C7H7N3 [M + H]+ 165.1435; found 165.1457. Rf 0.21 (hexanes to ethyl acetate 4:1). Decomposed at 150 °C. 2,4,7-Trichloroquinazoline (19c). 0.75 g (4.4 mmol) of commercially available 2-amino-4-chlorobenzoic acid was reacted according to general procedure A to give crude 19b. Without further...
purification, 19b was reacted according to general procedure B to give 0.64 g (2.7 mmol) of the crude title compound (61% over two steps). Mass (ESI): [M + H]+ 233, 235; found 233.0 (100%), 235.0 (84%). Rf = 0.68 (hexanes to ethyl acetate 4:1).

2-Chloro-9-N2-(furan-2-ylmethyl)quinazolin-4-amine (21c). 0.36 g (2.1 mmol) of 20c was reacted with furfurylamine according to general procedure Cb to give 0.21 g (0.94 mmol) of the title compound in 40% yield over two steps. Mass (ESI): [M + H]+ 274, 276; found 274.1 (100%), 276.1 (35%). Rf = 0.33 (hexanes to ethyl acetate 4:1).

2-Chloro-N2-(furan-2-ylmethyl)-8-methylquinazolin-4-amine (22d). 0.50 g (2.3 mmol) of 22c was reacted with furfurylamine according to general procedure B to give 0.34 g (1.6 mmol) of the crude title compound (48% over two steps). Mass (ESI): [M + H]+ 213, 215; found 213.0 (100%), 215.0 (63%). Rf = 0.55 (hexanes to ethyl acetate 4:1).

N2-(Furan-2-ylmethyl)-N2-isopropyl-8-methylquinazolin-4-amine (23c). 0.5 g (3.3 mmol) of commercially available 2-amino-4-methylbenzoic acid was reacted according to general procedure B to give 0.9 g (4.6 mmol) of the crude title compound (72% over two steps). Mass (ESI): [M + H]+ 274, 276; found 274.1 (100%), 276.1 (35%). Rf = 0.32 (hexanes to ethyl acetate 4:1).

N2-(Furan-2-ylmethyl)-N2-isopropyl-7-methylquinazolin-4-amine (23d). 0.20 g (0.94 mmol) of 23c was reacted with furfurylamine according to general procedure B to give 0.09 g (0.33 mmol) of 23d in 35% yield. Mass (ESI): [M + H]+ 215, 217; found 215.0 (100%), 217.0 (63%). Rf = 0.32 (hexanes to ethyl acetate 4:1).
2-Chloro-N^2-(furan-2-ylmethyl)-6-methylquinazolin-4-amine (24d). 0.32 g (1.5 mmol) of 24c was reacted with furfurylamine according to general procedure Cb to give 0.105 g (0.53 mmol) of the crude title compound as a yellow solid in 33% yield. ^1H NMR (400 MHz, CDCl₃) δ 7.54 (dd, J = 2.8 Hz, 1H), 7.47 (s, 1H), 2.12 (s, 3H). ^13C NMR (101 MHz, CDCl₃) δ 159.02, 136.73, 128.04, 122.22, 118.49, 117.64, 107.31, 107.00, 38.97, 22.03. HRMS: m/z calcd for C₁₇H₁₇N₄O₂ [M+H]^+ 313.1659; found 313.1654.

2-Chloro-N^2-(furan-2-ylmethyl)-6-methylquinazolin-4-amine (24d). 0.29 g (2.3 mmol) of 24c was reacted with furfurylamine according to general procedure Cb to give 0.098 g (0.29 mmol) of the crude title compound as a yellow solid in 32% yield. ^1H NMR (400 MHz, DMSO-d₆) δ 7.48 (s, 1H), 7.34 (s, 1H), 7.14 (s, 1H), 6.92 (s, 1H), 2.13 (s, 3H). ^13C NMR (101 MHz, DMSO-d₆) δ 158.36, 142.62, 123.22, 122.14, 117.62, 107.61, 107.06, 104.55, 27.86. HRMS: m/z calcd for C₁₇H₁₇N₄O₂ [M+H]^+ 313.1659; found 313.1654.

2-Chloro-N^2-(furan-2-ylmethyl)-6-methylquinazolin-4-amine (24d). 0.20 g (1.2 mmol) of 24c was reacted with furfurylamine according to general procedure Cb to give 0.105 g (0.53 mmol) of the title compound as a yellow solid in 33% yield. ^1H NMR (400 MHz, CDCl₃) δ 7.54 (dd, J = 2.8 Hz, 1H), 7.47 (s, 1H), 2.12 (s, 3H). ^13C NMR (101 MHz, CDCl₃) δ 159.02, 136.73, 128.04, 122.22, 118.49, 117.64, 107.31, 107.00, 38.97, 22.03. HRMS: m/z calcd for C₁₇H₁₇N₄O₂ [M+H]^+ 313.1659; found 313.1654.

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2-Chloro-N^2-(furan-2-ylmethyl)-6-methylquinazolin-4-amine (24d). 0.20 g (1.2 mmol) of 24c was reacted with furfurylamine according to general procedure Cb to give 0.105 g (0.53 mmol) of the title compound as a yellow solid in 33% yield. ^1H NMR (400 MHz, CDCl₃) δ 7.54 (dd, J = 2.8 Hz, 1H), 7.47 (s, 1H), 2.12 (s, 3H). ^13C NMR (101 MHz, CDCl₃) δ 159.02, 136.73, 128.04, 122.22, 118.49, 117.64, 107.31, 107.00, 38.97, 22.03. HRMS: m/z calcd for C₁₇H₁₇N₄O₂ [M+H]^+ 313.1659; found 313.1654.
Mass (ESI): [M + H]+ 229, 231; found 229.0 (100%), 231.0 (70%). Rf = 0.53 (hexanes to ethyl acetate 2:1).

2-Chloro-N'-[2-(furanyl)-2-ylmethyl]-5-methoxyquinazolin-4-amine (29d). 0.0094 g (41 μmol) of 29c was reacted with furfurylamine according to general procedure Cb to give 0.0012 g (3.8 μmol) of the title compound in 38% yield. 1H NMR (400 MHz, CD3OD) δ 7.92 (s, 3H), 4.79 (s, 2H), 4.30 (s, 2H), 3.98 (d, J = 8.2 Hz, 1H), 7.41 (t, J = 6.7 Hz, 1H), 7.39 (d, J = 8.7 Hz, 1H), 6.78 (d, J = 8.2 Hz, 1H), 6.38–6.32 (m, 1H), 4.79 (s, 2H), 4.30–4.16 (m, 1H), 4.00 (s, 3H), 1.23 (d, J = 6.5 Hz, 6H). 13C NMR (101 MHz, CDCl3) δ 160.20, 157.19, 150.24, 142.45, 142.31, 134.97, 123.77, 113.87, 110.75, 107.45, 104.03, 101.31, 56.50, 43.55, 38.44, 22.48. HRMS: [M + H]+ 290, 292; found 290.0 (100%), 292.0 (70%).

Physicochemical Assays. Permeability Assay. Permeability P was determined by a standard parallel artificial membrane permeability assay (PAMPA by pION) as reported previously.29 Aqueous Solubility Assay. Solubility at pH 7.4 has been determined using Biomek FX lab automation workstation with pION μSOL evolution software as reported previously and at pH 2.0 using an in-house HPLC assay.32 For pH 2.0, a calibration curve was made by plotting the area under the curve at 254 nm (UV by HPLC) against the concentration of each compound injected after performing a serial dilution (25–0.781 μM) using a solvent in which the compound is soluble (DMSO). A 100 μM solution was then made for each compound in a buffer at 2.0 by performing a 1:100 serial dilution using a 0.1 mM DMSO stock solution of each compound. This solution was incubated at 21 °C for 18 h, filtered using a filter plate, and injected into the HPLC to compare the area found at wavelength 254 nm with the previously made calibration curve.

Partition Coefficient Assay. The log D was also determined in-house via a HPLC method adapted from the strategy reported by Donovan and Pescatore.30 Two buffers were made at a concentration of 50 μM each: ammonium acetate at pH 7.4 and ammonium formate at pH 3.0. A set of compounds with known log D values between −0.36 and 5.68 were used to make a calibration curve at each pH by using a linear gradient between 0% and 100% acetone-water at the specific pH used as the second solvent. The curve was made by plotting the log D value against the retention time. Quinazolines were then injected into the HPLC instrument, and the retention time was compared to the calibration curve previously determined.

Efficacy Studies. Intracellular Amastigote Assays. In vitro antileishmanial potency of compounds against intracellular L. donovani MHOM/ET/67/L8229 and L. amazonensis LV7833 parasites was measured using the colorimetric assay as described previously. The potency of compounds 15, 16, and 23 against intracellular antimony-resistant clinical isolate L. donovani MHOM/NP/02/BPK164.13 and antimony-susceptible isolate L. donovani MHOM/NP/03/BPK206.04 was also assessed. Briefly, second to third day stationary phase promastigotes grown in HOMEM (Invitrogen) supplemented with NADPH. Microsomal incubations were carried out according to a protocol described previously.43 Substrate and microsomal protein concentrations during incubations were 3 μM and 0.5 mg/mL, respectively. Substrate depletion over a 60 min incubation time was quantified using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) as described below.

Analytical Assays. The processed samples (plasma, tissue homogenates, and microsomal incubation mixtures) were analyzed for drug concentration using an API3000 triple quadrupole mass spectrometer equipped with a Turbo IonSpray interface (AB Sciex, Foster City, CA). Samples (5 μL) were introduced to the mass spectrometer using a thermostatic (4 °C) autosampler (CTC PAL, LEAP Technologies, Carrboro, NC) and a Shimadzu HPLC system (Shimadzu, Kyoto, Japan). All equipment was controlled using Analyst software (version 1.3). Compounds were separated on a ZORBAX Bonus-RP C8 HPLC column (2.1 mm × 50 mm, 3.5 μm particle size; Agilent, Milford, MA). HPLC mobile phases consisted of water containing 0.1% formic acid (A) and methanol containing 0.1% formic acid (B) with a flow rate of 0.35 mL/min. After a 0.4 min initial hold at 15% B, mobile phase composition started with 15% B and was increased to 95% B over 1.1 min. Then the column was washed with 95% B for 1.5 min and was re-equilibrated with 15% B for 1.0 min before injecting the next sample. The characteristic single reaction monitoring (SRM) transitions for 16 and 23 were m/z 297.1 → 81.0 and 307.1 → 91.0 under positive ion mode. They were used as internal standard for each other during quantification. The calibration curves for 16 and 23 ranged from 0.01 to 10 μM for plasma and from 0.10 to 50 μM for tissue homogenates. For microsomal stability samples, substrate depletion was calculated based on the relative MS signal of the analyte normalized by the internal standard.

Data Analysis. Microsomal half-life (Mic t1/2) values were obtained by fitting the one-phase exponential decay equation to the percentage of substrate remaining versus time curves (GraphPad Prism, version 5.154.
5.04, San Diego, CA). Noncompartmental pharmacokinetic analysis of plasma concentration versus time curves was performed to obtain the area under the concentration–time curve (AUC), C_{max}, t_{max} and terminal half-life (t_1/2) (WinNonlin 6.3, Pharsight, Mountain View, CA). Tissue AUC was calculated using the trapezoid rule from 1 h (first sampling time) to the last time point (GraphPad Prism, version 5.04).

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Notes

The authors declare no competing financial interest.

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**ABBREVIATIONS USED**

DNA, dimethylacetamide; MTT, 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HP/CD, (2-hydroxypropyl)-β-cycloextrin; C_{max} maximum concentration; Mic t_{1/2}, microsomal half-life; PABA, p-aminobenzoic acid; P_{o/w} permeability; SI, selectivity index; SRM, single reaction monitoring; SPR, structure–property relationship; w/v, weight per unit volume

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