Journal of Medicinal Chemistry

Article

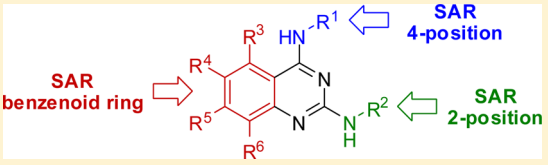
pubs.acs.org/jmc

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

Kurt S. Van Horn,[†] Xiaohua Zhu,[‡] Trupti Pandharkar,[‡] Sihyung Yang,[§] Brian Vesely,[∥] Manu Vanaerschot,[⊥] Jean-Claude Dujardin,[⊥,#] Suman Rijal,[∞] Dennis E. Kyle,[∥] Michael Zhuo Wang,[§] Karl A. Werbovetz,[‡] and Roman Manetsch*[,†]

[†]Department of Chemistry, University of South Florida, 4202 East Fowler Avenue, CHE 205, Tampa, Florida 33620, United States
[‡]Division of Medicinal Chemistry and Pharmacognosy, The Ohio State University, Columbus, Ohio 43210, United States
[§]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66047, United States
[∥]Department of Global Health, University of South Florida, Tampa, Florida 33620, United States
[⊥]Unit of Molecular Parasitology, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, 2000, Belgium
[#]Department of Biomedical Sciences, University of Antwerp, Antwerp, 2610, Belgium
[∞]B. P. Koirala Institute of Health Sciences, Ghopa, Dharan, 56700, Nepal

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 anthroponotic 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 Vianna 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 Brahmachari 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.[11] 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.[11,13] The high rate of resistance to pentavalent antimonials in India has led to the increasing use of amphotericin B and miltefosine

Received: January 8, 2014
Published: May 29, 2014

   dx.doi.org/10.1021/jm5000408 | *J. Med. Chem.* 2014, 57, 5141−5156

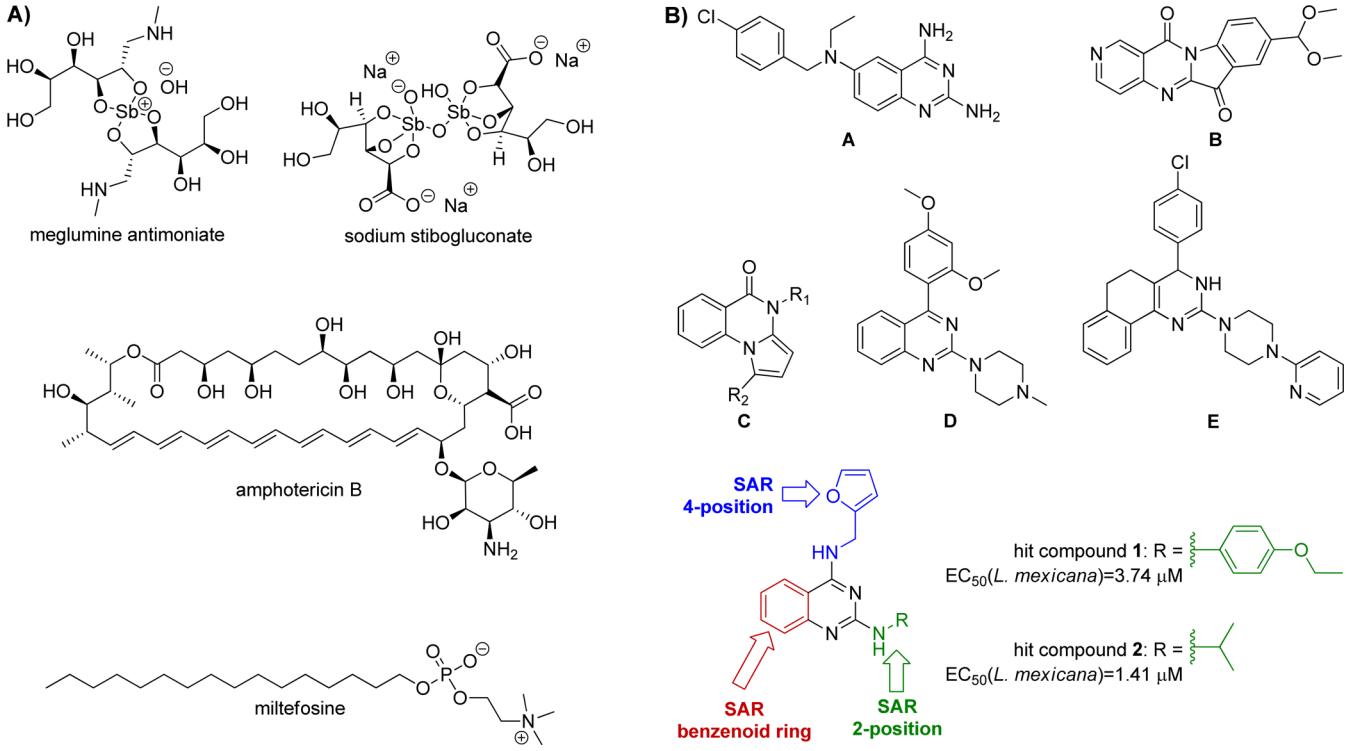


Figure 1. Antileishmanial compounds: (A) structures of currently used antileishmanial drugs;[12] (B) reported structures of quinazolines diplaying antileishmanial activity and including the hit compounds **1** and **2** and SAR studies targeting the major quinazoline sites.

against visceral leishmaniasis.[14] Since the 1960s, amphotericin B has been the second line treatment for visceral leishmaniasis.[11] 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.[7,11] Lipid formulations of amphotericin B have fewer side effects and are safer to use with the same cure rate.[7,11] Depending on the dose and formulation, the treatment regimen varies from 3 to 5 days to 8 weeks of administration on alternate days.[6,11] Miltefosine is the first oral drug to be released for leishmaniasis and is currently available in India, Germany, and Colombia.[11] Miltefosine is not recommended for women who are pregnant or may become pregnant because it is teratogenic.[1,11] 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.[1,11,15] 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.[16] 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.[16] 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.[17]

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_{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.[18] Bhattacharjee et al. (**B**), Ram et al. (**C**), and Shakya and Gupta et al. (**D** and **E**) have also tested quinazolines as antileishmanials.[19−22] This class of compounds has been reported as being dihydrofolate reductase (DHFR) inhibitors, although another mechanism of action may be involved with *Leishmania*.[18,23] 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^2,N^4-disubstituted quinazolines **1** and **2** (Figure 1B), which are structurally different from quinazoline series **A**−**E**. Antileishmanial testing of quinazolines **1** and **2** against *L. mexicana* axenic amastigotes revealed EC_{50} values in the single digit micromolar range, motivating us to investigate whether quinazolines structurally related to the hits **1** and **2** have potential to display potent antileishmanial activity.[24] 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).[25−27] Commercially

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

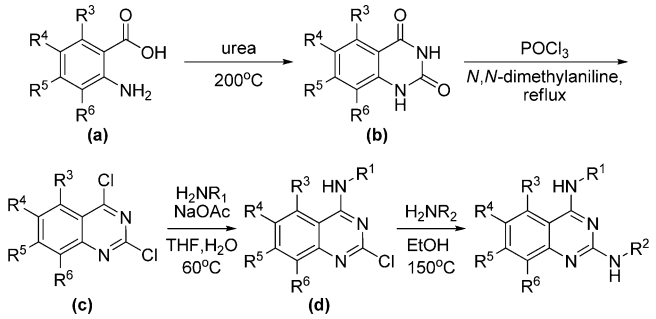
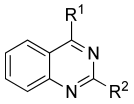


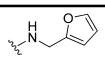
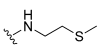
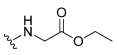
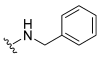
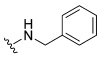
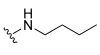
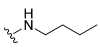
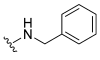
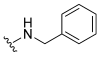
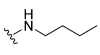
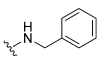
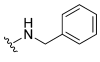
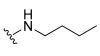
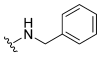
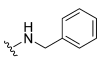
Figure 2. Synthesis of N^2,N^4-disubstituted quinazoline-2,4-diamines.

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 N^2,N^4-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[a]

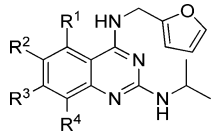


Compound	R^1	R^2	*L. donovani* EC_{50} μM	*L. amazonensis* EC_{50} μM	J774A.1 EC_{50} μM	SI
2			2.5 ± 0.4	3.7 ± 1.6	17 ± 6	6.8
3			3.6 ± 1.1	5.8 ± 2.3	23 ± 10	6.4
4			25 ± 18	> 50	> 33	> 1.3
5			3.7 ± 0.3	7.6 ± 3.2	> 33	> 8.9
6			4.3 ± 1.2	5.1 ± 0.9	20 ± 6	4.7
7			6.9 ± 1.8	20 ± 3	> 33	4.8
8			>50.0	> 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

[a]Amphotericin B is the internal control for the in vitro antileishmanial activity assay with EC_{50} = 40 ± 9 nM against *L. donovani* and EC_{50} = 89 ± 16 nM against *L. amazonensis*. Podophyllotoxin is the internal control for the in vitro cytotoxicity assay with EC_{50} = 250 ± 10 nM against J774A.1.

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

Table 2. SAR Study Focusing on the Benzenoid Ring of the Quinazoline Scaffold[a]



compd	R^1	R^2	R^3	R^4	EC_{50}, μM *L. donovani*	EC_{50}, μM *L. amazonensis*	EC_{50}, μM J774A.1	SI
2	-H	-H	-H	-H	2.5 ± 0.4	3.7 ± 1.6	17 ± 6	6.8
18	-H	-H	-H	-Cl	4.4 ± 1.3	18 ± 6	>33	>7.5
19	-H	-H	-Cl	-H	9.0 ± 3.7	23 ± 5	>100	>11
20	-H	-Cl	-H	-H	2.6 ± 1.9	25 ± 4	>33	>13
21	-Cl	-H	-H	-H	2.3 ± 0.6	12 ± 3	25 ± 3	11
22	-H	-H	-H	$-CH_3$	4.2 ± 2.1	4.4 ± 1.9	>33	>7.9
23	-H	-H	$-CH_3$	-H	0.83 ± 0.32	4.1 ± 1.2	>33	>40
24	-H	$-CH_3$	-H	-H	4.7 ± 2.5	15 ± 6	>33	>7.0
25	$-CH_3$	-H	-H	-H	0.95 ± 0.27	3.6 ± 1.5	20 ± 9	21
26	-H	-H	-H	$-OCH_3$	3.2 ± 1.2	7.3 ± 2.5	30 ± 5	9.4
27	-H	-H	$-OCH_3$	-H	1.2 ± 0.5	10 ± 2	16 ± 2	13
28	-H	$-OCH_3$	-H	-H	0.74 ± 0.37	9.6 ± 3.0	14 ± 1	19
29	$-OCH_3$	-H	-H	-H	0.97 ± 0.26	2.7 ± 1.4	18 ± 6	19

[a]Amphotericin B is the internal control for the in vitro antileishmanial activity assay with EC_{50} = 40 ± 9 nM against *L. donovani* and EC_{50} = 89 ± 16 nM against *L. amazonensis*. Podophyllotoxin is the internal control for the in vitro cytotoxicity assay with EC_{50} = 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 β-lactamase gene as outlined previously.[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_{50} (*L. donovani* or *L. amazonensis.*). Additionally, cytotoxicity against the macrophage cell line J774A.1 was determined as the effective concentration EC_{50} (J774A.1) and the selectivity index SI was calculated as the ratio of EC_{50} value for J774A.1 and the value for *L. donovani* (SI = EC_{50}(J774A.1)/EC_{50}(*L. donovani*)).

Structure–Activity Relationship Studies. To validate and optimize the antileishmanial activity of N^2,N^4-disubstituted quinazoline-2,4-diamines, two compound subseries were prepared and tested. The first subseries focused mainly on the optimization of the N^2- and N^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^4-furfuryl analogues **3** and **4** were prepared in which the N^2-isopropyl group was replaced by a short trifluoroalkyl or hydroxyalkyl 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^4-monosubstituted or N^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_{50} values of 670 nM against *L. donovani* and 1.4 μM 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^4-benzyl-N^2-methylquinazoline-2,4-diamine **12**, all of these compounds demonstrated submicromolar EC_{50} values against *L. donovani*. Among these six quinazolines tested, the N^2-benzylquinazoline-2,4-diamines **15**–**17** appeared to be at least 2-fold more potent against *L. donovani* than the N^4-benzyl counterparts **12**–**14**, suggesting that an N^2-benzyl is more favorable than an N^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_{50} of 150 nM against *L. donovani*. For *L. amazonensis*, the set of **12**–**17** displayed a similar activity trend, with N^2-benzylquinazolines **15** and **16** being the most potent compounds with submicromolar or single digit micromolar EC_{50} values.

N^4-Furfuryl-N^2-isopropyl-substituted quinazoline **2**, with EC_{50} values of 2.5 μM against *L. donovani* and 3.7 μM 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.[30] Overall, substitution on the benzenoid ring provided compounds with EC_{50} values in the single digit micromolar or submicromolar range against *L. donovani*. Among the chloro-substituted subseries, 5- and 6-substituted analogues **18** and **19** were less potent by a factor of 2 or more compared to reference **2**, while the 7- and 8-substituted quinazolines **20** and **21** displayed an activity on par with compound **2**. In contrast, the majority of the methyl- and methoxy-substituted quinazolines demonstrated a modest potency improvement over quinazoline **2**. For the methoxy-substituted quinazolines, potency increased according to substitutions in 8-position < 7-position < 5-position ~ 6-

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141–5156

position, whereas a potency dependence in the order of 8-position ~ 6-position < 7-position ~ 5-position was observed for the methyl-substituted compounds. 7-Methoxyquinazoline **28** with an EC_{50} of 740 nM against *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 *L. amazonensis* gave insight into a SAR, which differed from the one observed for *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_{50} of 20 μM 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 N^2-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 *L. donovani*. The potency of selected quinazolines against antimony-resistant *Leishmania* was assessed using murine peritoneal macrophages infected with two *L. donovani* clinical strains, BPK206/0 and BPK164/1 (Table 3).[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.[31] Compounds **15**, **16**, and **23** displayed similar activity against the antimony-susceptible and antimony-resistant parasites. The EC_{50} values against the clinical strains were 5- to 19-fold higher than their EC_{50} values against β-lactamase expressing *L. donovani* (Table 1). There are several possible reasons for the differences in susceptibility observed between the clinical *L. donovani* isolates and the β-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;[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 β-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.

Table 3. Activities of Compounds against *L. donovani* Clinical Isolates[a]

	EC_{50}, μM	
compd	BPK206/0	BPK164/1
sodium stibogluconate[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

[a]Mean ± range of two independent measurements. [b]Values for sodium stibogluconate are given in micrograms of pentavalent antimony per milliliter.

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.[18,23] *L. donovani* axenic amastigotes and J774A.1 (mouse macrophage cell line) were adapted to grow in media deficient in *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).[34,35] The presence of folinic acid dramatically increased the EC_{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_{50} of quinazoline **10** was 84.8 ± 2.2 and 84.2 ± 2.8 μM 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 *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 *D*, aqueous solubility, and permeability has been conducted with all compounds to assess potential physicochemical liabilities (Table 4). The log $D_{3.0}$ and log $D_{7.4}$, 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.[36] Solubility at pH 7.4 was 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 based on UV absorption.[37] Passive transcellular permeability was assessed in a standard parallel artificial membrane permeability assay (PAMPA) at pH 7.4 and pH 4.0. Generally, the aqueous solubility, the distribution coefficient log *D*, and the permeability of all quinazolines display a pH dependence (exemplified on log *D* or permeability in Table 4). The permeability is enhanced at neutral pH, while the aqueous solubility and log *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 μM, $P_e > 10 \times 10^{-6}$ cm·s^{-1}, $1 < \log D < 4$), 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 *L. donovani*, only compounds that displayed submicromolar in vitro activity against *L. donovani* with a SI value greater than 10 and a balanced combination of good physicochemical proper-

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141–5156

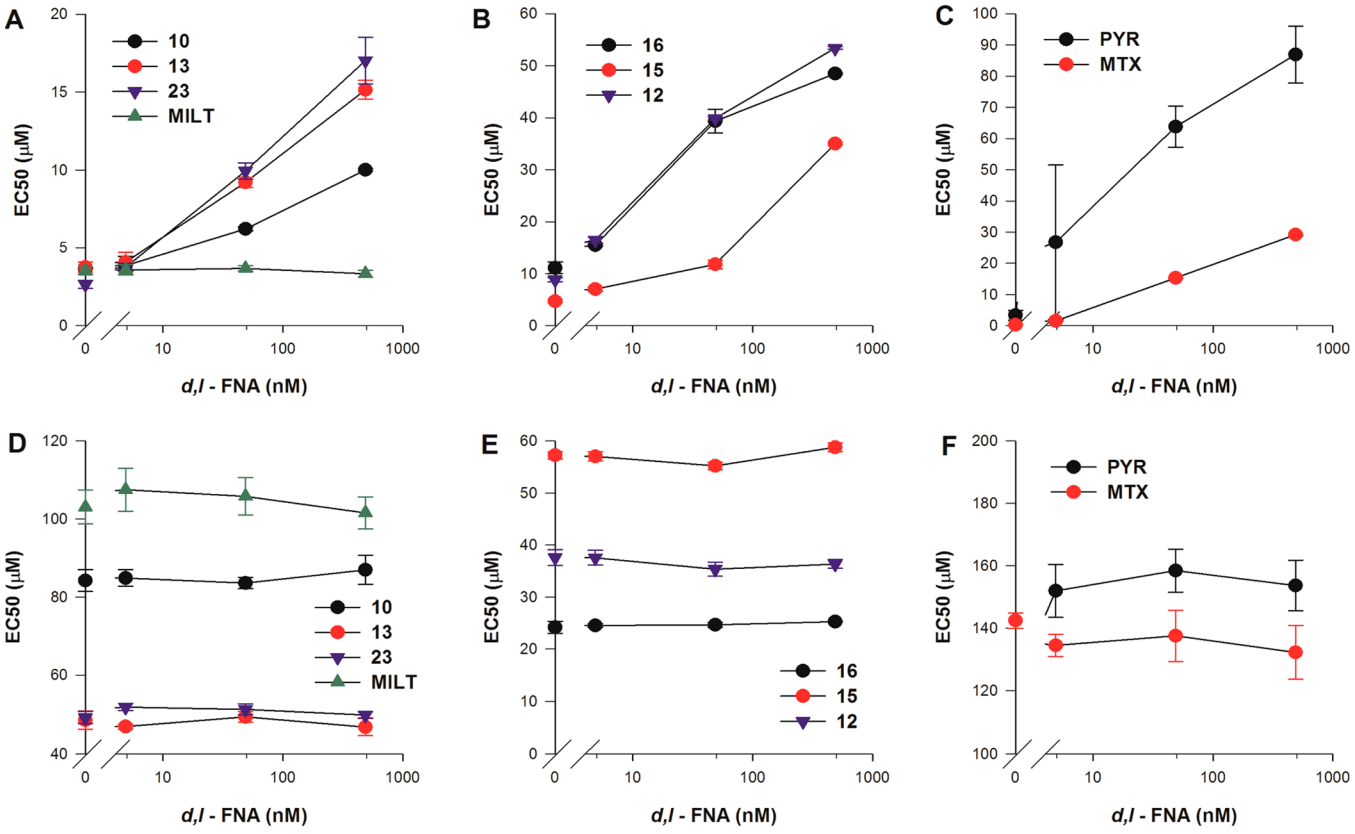


Figure 3. In vitro efficacy of quinazolines, methotrexate (MTX), pyrimethamine (PYR), and miltefosine (MILT) for axenic amastigotes of *Leishmania donovani* (A–C) and J774.A1 macrophages (D–F) in the absence or presence of D,L-folinic acid (FNA). Results are presented as the EC_{50} (μM) in the absence or presence of increasing concentrations of FNA.

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_{max} of 0.44 and 0.25 μM for **16** and **23**, respectively. The systemic and tissue exposure of **16** (AUC and C_{max}) were considerably greater than those of **23** (Table 4). After ip administration, plasma concentration reached a C_{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

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141–5156

Table 4. Physicochemical Properties of Quinazolines

Compound	Solubility[a]		Permeability Pe ·10^{-6} (cm/s)[b]		Log D	
	pH 7.4	pH 2.0	pH 7.4	pH 4.0	pH 7.4	pH 3.0
2	*****	*****	883	62.2	3.15	2.18
3	***	*****	979	67.7	4.20	2.02
4	****	*****	1130	26.4	2.19	1.48
5	*****	*****	780	19.7	3.19	2.40
6	****	*****	1480	28.8	3.02	2.06
7	****	*****	744	38.8	2.54	1.73
8	****	*****	783	112	4.69	3.56
9	***	*****	1120	72.3	3.13	1.64
10	**	****	383	312	3.82	2.82
11	***	*****	880	485	3.89	2.91
12	*****	*****	n/d	n/d	3.14	2.11
13	***	*****	1250	161	3.80	2.84
14	*	**	1480	271	4.17	2.78
15	***	*****	1720	57.1	2.96	2.00
16	***	*****	1020	341	3.84	2.86
17	*	*****	663	412	4.25	2.67
18	**	*****	1330	67.6	3.96	2.38
19	****	*****	1380	103	3.91	2.51
20	****	*****	1630	170	3.99	2.60
21	***	*****	1240	169	4.44	2.62
22	****	*****	666	45.9	3.55	1.57
23	****	*****	798	67.2	3.61	1.58
24	****	*****	789	67.2	3.63	1.55
25	***	*****	812	45.9	3.55	2.44
26	****	*****	1060	38.4	3.40	2.99
27	****	*****	1330	59.2	3.70	3.11
28	*****	*****	1010	80.2	3.58	2.95
29	****	*****	624	87.0	3.35	2.53

[a](*) For solubility ≤ 5 μM. (**) For 5 μM < solubility ≤10 μM. (***) For 10 μM < solubility ≤ 20 μM. (****) For 20 μM < solubility ≤ 30 μM. (*****) For solubility ≥ 30 μM. [b]For the determination of the P_e values, the following internal controls were utilized: carbazepine pH 4.0 permeability $P_e = 108 \times 10^{-6}$ cm/s and pH 7.4 permeability $P_e = 130 \times 10^{-6}$ cm/s; ranitidine·HCl pH 4.0 permeability $P_e = 5.2 \times 10^{-6}$ cm/s and pH 7.4 permeability $P_e = 2.2 \times 10^{-6}$ cm/s; verapamil·HCl pH 4.0 permeability $P_e = 20.6 \times 10^{-6}$ cm/s and pH 7.4 permeability $P_e = 1360 \times 10^{-6}$ cm/s. n/d: not determined.

possible to directly correlate drug exposure with antileishmanial activity in mice. In addition, it is worth pointing out that the terminal half-life of **23** after ip administration was considerably shorter than that after po administration (5 h vs 20 h; Table 5), and the liver concentration of **23** was detectable at 12 and 24 h after po administration, whereas it was below the detection limit after ip administration (Figure 6). These observations suggest flip-flop pharmacokinetics, where the rate of gastrointestinal absorption is slower than the rate of elimination due to dosage formulations (e.g., sustained release), excipients, physiological factors (e.g., intestinal mobility and pH), and/or drug characteristics.[38,39] The dramatic decrease (~12-fold) in the permeability of **23** from neutral to acidic pH (Table 4) could contribute to the slowed absorption of this compound in the upper gastrointestinal tract. In contrast, the permeability of **16** decreased only 3-fold from neutral to acidic pH (Table 4). Furthermore, **23** was metabolized quickly in the mouse liver microsomes ($t_{1/2}$ = 9.4 min; Table 5), suggesting that it is possible that the rate of elimination was greater than the rate of gastrointestinal absoption for **23**.

CONCLUSIONS

N^2,N^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 EC_{50} values in the submicromolar range against *L. donovani* were obtained with quinazoline-2,4-diamine scaffolds bearing either a N^2-benzyl-N^4-alkyl/phenyl or a N^2-ispropyl-N^4-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 EC_{50} 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 EC_{50} 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 N^2,N^4-disubstituted quinazoline-2,4-diamines a suitable platform for the future development of antileishmanial agents.

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

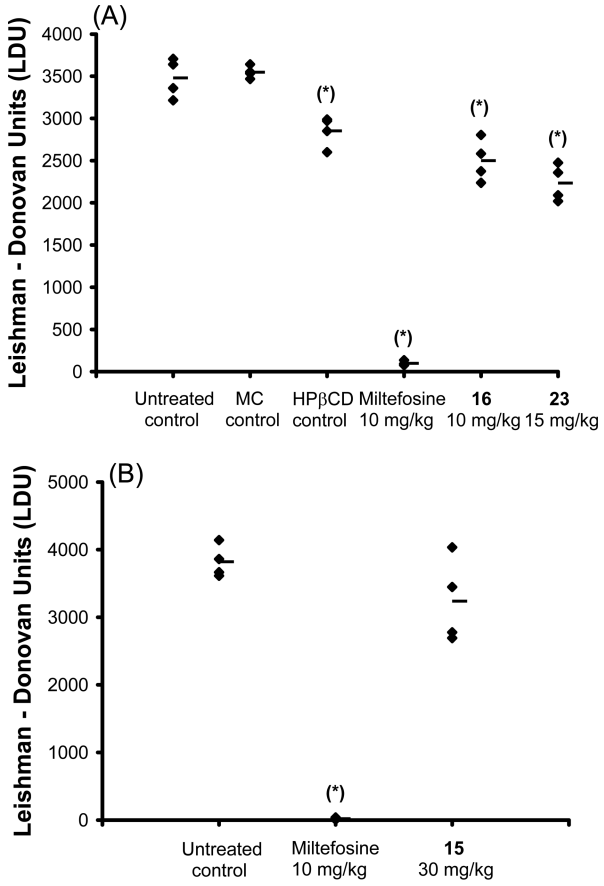


Figure 4. In vivo efficacy of quinazolines against LV82 in *L. donovani* infected BALB/c mice. Results are presented as the liver parasitemia (LDU) for each mouse (◆), and the average LDU in each group (−) was determined by microscopy (n = 4): (A) LDU for mice treated with **16** and **23**; (B) LDU 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 (HPβCD). (*) $p < 0.05$, compared with untreated control.

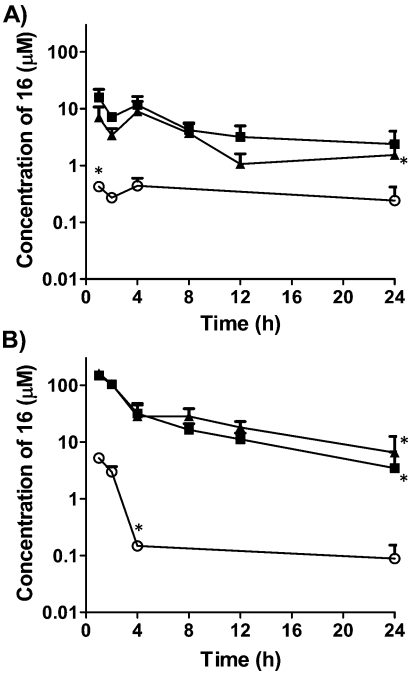


Figure 5. Plasma (open circles) and tissue (squares for liver and triangles for spleen) concentration−time profiles after po (A) and ip (B) administration of **16** in mice at a dose level of 100 μmol/kg (~30 mg/kg). Symbols and error bars represent the mean and standard error of triplicate determinations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

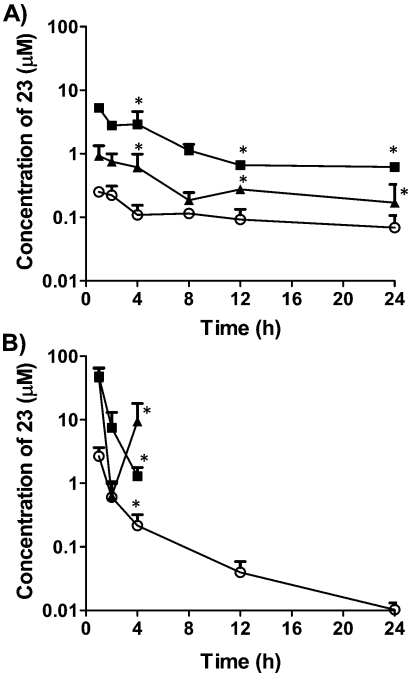


Figure 6. Plasma (open circles) and tissue (squares for liver and triangles for spleen) concentration−time profiles after po (A) and ip (B) administration of **23** in mice at a dose level of 100 μmol/kg (~30 mg/kg). Symbols and error bars represent the mean and standard error of triplicate determinations 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 μM) in the liver and spleen 12 and 24 h after ip administration.

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 δ 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 (δ 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

Table 5. Pharmacokinetic Outcomes of 16 and 23 after po and ip Administration to Mice

parameter[a]		po			ip		
		plasma	liver	spleen	plasma	liver	spleen
			Compound **16**				
C_{max}	(μM)	0.44	15.8	7.0	5.21	148	163
T_{max}	(h)	4	1	1	1	1	1
AUC_{last}	(μM·h)	7.8	110	68	11	500	620
$t_{1/2}$	(h)	NC[b]	ND[c]	ND[c]	20	ND[c]	ND[c]
Mic $t_{1/2}$ [d]	(min)			27			
			Compound 23				
C_{max}	(μM)	0.25	5.2	0.9	2.67	48	45.8
T_{max}	(h)	1	1	1	1	1	1
AUC_{last}	(μM·h)	2.5	29	7.4	4.6	42	71
$t_{1/2}$	(h)	24	ND[c]	ND[c]	5.4	ND[c]	ND[c]
Mic $t_{1/2}$ [d]	(min)			9.4			

[a]AUC (0−24 h) was calculated for plasma using noncompartmental analysis, whereas AUC (1−24 h) was calculated for tissues using trapezoid rule. AUC_{last}, AUC from the time of dose to the last measurable concentration. C_{max} and T_{max}, maximum concentration and time to reach C_{max}. $t_{1/2}$, terminal half-life. [b]NC, not calculable because of lack of a data point. [c]ND, not determined. [d]In vitro mouse liver microsomeal half-life.

version 5.3.2-4936. The purity of the final compounds was determined to be ≥95% by high pressure liquid chromatography (HPLC) using an Agilent 1100 LC/MSD-VL with electrospray ionization. Melting points were determined using a MEL-TEMP 3.0 instrument and are uncorrected. Low resolution mass spectrometry was performed on an Agilent 1100 LC/MSD-VL with electrospray ionization. High resolution mass spectrometry (HRMS) was performed on an Agilent LC/MSD TOF system G3250AA. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 precoated plates (0.25 mm) from EMD Chemical Inc., and components were visualized by ultraviolet light (254 nm). Reported R_f was determined for TLC. Silicycle silica gel 230−400 (particle size 40−63 μm) mesh was used for all flash column chromatography experiments.

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 mortar 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 mix 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_2SO_4. 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: Amine 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:

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

Purification 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 benzoylneurea (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). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29−8.24 (m, 1H), 8.02−7.99 (m, 2H), 7.74 (ddd, J = 6.3, 5.0, 3.2, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.25, 155.36, 152.60, 136.41, 129.61, 128.25, 126.42, 122.59. Mass (ESI): $[M + H]^+$ 199, 201; found 198.9 (100%), 200.9 (64%). R_f = 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 furfurylamine and purified according to method Ca to furnish 1.21 g (4.7 mmol) of the title compound in 93% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.75−7.55 (m, 3H), 7.34 (ddd, J = 8.1, 5.4, 2.9 Hz, 1H), 6.48 (s, 1H), 6.30−6.22 (m, 2H), 4.77 (d, J = 5.1 Hz, 2H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 160.57, 157.54, 150.82, 150.24, 142.56, 133.62, 127.66, 126.32, 121.09, 113.24, 110.65, 108.66, 38.43. Mass (ESI): $[M + H]^+$ 260; found 260.1. R_f = 0.14 (hexanes to ethyl acetate 4:1).

***N*^{4}-(Furan-2-ylmethyl)-*N*^{2}-isopropylquinazoline-2,4-diamine (2).** 1.05 g (4.04 mmol) of **2d** was reacted with isopropylamine and purified according to method Da to furnish 0.47 g (1.66 mmol) of the title compound as a light yellow solid in 41% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 7.94 (d, J = 8.1 Hz, 1H), 7.53−7.46 (m, 1H), 7.44−7.40 (m, 1H), 7.31 (s, 1H), 7.17−7.09 (m, 1H), 6.38−6.31 (m, 2H), 4.78 (s, 2H), 4.26 (sept, J = 6.5 Hz, 1H), 1.27 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 160.14, 154.22, 151.24, 142.01, 141.97, 134.05, 123.14, 123.04, 118.38, 110.17, 109.87, 107.44, 43.29, 37.65, 21.62. HRMS: m/z calcd for $C_{16}H_{19}N_4O$ $[M + H]^+$ 283.1553; found 283.1558. R_f = 0.44 (9:1 dichloromethane to methanol). Melting point 190−192 °C.

***N*^{4}-(Furan-2-ylmethyl)-*N*^{2}-(2,2,2-trifluoroethyl)quinazoline-2,4-diamine (3).** 0.045 g (0.17 mmol) of **2d** was reacted with 2,2,2-trifluoroethylamine and purified according to method Da to furnish 0.041 g (0.13 mmol) of the title compound as a white crystalline solid in 76% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (d, J = 8.2, 1H), 7.79 (dd, J = 8.4, 7.3, 1H), 7.51 (d, J = 7.1, 1H), 7.48−7.45 (m, 1H), 7.44 (dd, J = 7.3, 1.0, 1H), 6.39 (m, 2H), 4.89 (s, 2H), 4.38 (q, J = 9.0, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 160.64, 153.51, 150.23, 142.31,

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

138.72, 135.45, 125.29, 124.36 (q, J = 278.71), 123.64, 116.87, 110.16, 110.05, 107.89, 41.73 (q, J = 35.03), 38.11. HRMS: m/z calcd for $C_{15}H_{14}F_3N_4O$ $[M + H]^+$ 323.1114; found 323.1122. R_f = 0.14 (dichloromethane). Decomposed at 225 °C.

2-(4-(Furan-2-ylmethylamino)quinazolin-2-ylamino)ethanol (4). 0.12 g (0.46 mmol) of **2d** was reacted with 2-aminoethanol and purified according to method Da to furnish 0.048 g (0.17 mmol) of the title compound in 37% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 8.11 (d, J = 7.6 Hz, 1H), 7.76 (ddd, J = 8.4, 7.3, 1.3 Hz, 1H), 7.46 (d, J = 0.8 Hz, 1H), 7.40 (t, J = 7.5 Hz, 2H), 6.41 (d, J = 2.9 Hz, 1H), 6.40−6.36 (m, 1H), 3.77 (s, 2H), 3.70 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.87, 159.63, 151.51, 150.07, 142.05, 132.90, 124.21, 121.55, 121.39, 110.88, 110.45, 107.64, 63.77, 44.88, 37.89. HRMS: m/z calcd for $C_{15}H_{17}N_4O_2$ $[M + H]^+$ 285.1346; found 285.1350. R_f = 0.24 (dichloromethane to methanol 9:1). Melting point 219−221 °C.

***N*-2,2,2-Trifluoroethyl-2-chloroquinazolin-4-amine (5d).** 0.050 g (0.25 mmol) of **1c** was reacted with 2,2,2-trifluoroethylamine and purified according to method Cb to furnish 0.017 g (0.065 mmol) of the title compound in 26% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (d, J = 7.2 Hz, 1H), 7.82 (t, J = 7.4 Hz, 1H), 7.68 (d, J = 7.5 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 4.39 (q, J = 8.9 Hz, 2H). Mass (ESI): $[M + H]^+$ 235, 237; found 235.0 (100%), 237.1 (33%). R_f = 0.11 (hexanes to ethyl acetate 4:1).

***N*^{2}-Isopropyl-*N*^{4}-(2,2,2-trifluoroethyl)quinazoline-2,4-diamine (5).** 0.017 g (0.065 mmol) of **5d** was reacted with isopropylamine and purified according to method Db to furnish 0.005 g (0.018 mmol) of the title compound as a white crystalline solid in 28% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 7.91 (dd, J = 8.2, 1.1, 1H), 7.60 (ddd, J = 8.4, 7.0, 1.4, 1H), 7.38 (d, J = 8.4, 1H), 7.17 (ddd, J = 8.1, 7.0, 1.0, 1H), 4.38 (q, J = 9.3, 2H), 4.24 (sept, J = 6.5, 1H), 1.26 (d, J = 6.5, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 160.87, 157.71, 149.57, 133.28, 124.83 (q, J = 278.55), 122.38, 122.15, 121.53, 110.32, 42.57, 40.98 (q, J = 34.34), 21.71. HRMS: m/z calcd for $C_{13}H_{16}F_3N_4$ $[M + H]^+$ 285.1322; found 285.1325. Melting point 103−108 °C.

2-Chloro-*N*-(2-(methylthio)ethyl)quinazolin-4-amine (6d). 0.20 g (1.0 mmol) of **1c** was reacted with 2-(methylthio)ethylamine and purified according to method Cb to furnish 0.15 g (0.59 mmol) of the title compound in 59% yield. ^{1}H NMR (250 MHz, CD_3OD) δ 7.93 (dd, J = 8.3, 0.8 Hz, 1H), 7.65 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.50−7.44 (m, 1H), 7.38 (ddd, J = 8.3, 7.0, 1.3 Hz, 1H), 3.70 (dd, J = 7.5, 6.6 Hz, 2H), 2.75−2.65 (m, 2H), 2.08 (s, 3H). Mass (ESI): $[M + H]^+$ 254, 256; found 254.0 (100%), 256.0 (33%). R_f = 0.17 (hexanes to ethyl acetate 4:1).

***N*^{2}-Isopropyl-*N*^{4}-(2-(methylthio)ethyl)quinazoline-2,4-diamine (6).** 0.12 g (0.47 mmol) of **6d** was reacted with isopropylamine and purified according to method Db to furnish 0.056 g (0.20 mmol) of the title compound as a white solid in 43% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 7.81 (d, J = 8.1 Hz, 1H), 7.47 (t, J = 7.5 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 7.06 (t, J = 7.6 Hz, 1H), 4.20 (sept, J = 6.5 Hz, 1H), 3.76−3.71 (m, 2H), 2.79−2.74 (m, 2H), 2.11 (s, 3H), 1.22 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 160.39, 157.49, 148.19, 132.93, 122.28, 121.64, 121.48, 110.56, 42.62, 40.15, 32.36, 22.03, 14.11. HRMS: m/z calcd for $C_{14}H_{21}N_4S$ $[M + H]^+$ 277.1481; found 277.1488. R_f = 0.52 (dichloromethane to methanol 9:1). Melting point 87−90 °C.

Methyl 2-(2-Chloroquinazolin-4-ylamino)acetate (7d). 0.198 g (0.99 mmol) of **1c** was reacted with glycine methyl ester and purified according to method Ca to furnish 0.18 g (0.72 mmol) of the title compound in 73% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (d, J = 8.1 Hz, 1H), 7.62 (dd, J = 11.3, 4.1 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 4.26 (s, 2H), 3.71 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 170.77, 161.67, 157.12, 150.04, 133.76, 126.38, 125.93, 122.25, 113.24, 51.72, 42.35. R_f = 0.17 (hexanes to ethyl acetate 2:1).

Ethyl 2-(2-(Isopropylamino)quinazolin-4-ylamino)acetate (7). 0.12 g (0.47 mmol) of **7d** was reacted with isopropylamine and purified according to method Db to furnish 0.060 g (0.21 mmol) of the title compound as a white crystalline solid in 44% yield. A transesterification reaction occurred leading to the ethylester product. ^{1}H NMR (400 MHz, CD_3OD) δ 7.84 (d, J = 8.2 Hz, 1H), 7.57−7.51 (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). ^{13}C NMR (101 MHz, CD_3OD) δ 172.23, 162.13, 159.29, 150.86, 134.31, 123.71, 123.57, 122.61, 111.84, 62.22, 43.87, 43.66, 23.25, 14.52. HRMS: m/z calcd for $C_{15}H_{21}N_4O_2$ $[M + H]^+$ 289.1659; found 289.1668. R_f = 0.34 (dichloromethane to methanol 9:1).

2-Chloro-*N*-cyclohexylquinazolin-4-amine (8d). 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. ^{1}H NMR (250 MHz, CD_3OD) δ 8.05−7.99 (m, 1H), 7.60 (ddd, J = 8.4, 7.0, 1.4 Hz, 1H), 7.46−7.41 (m, 1H), 7.33 (ddd, 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). ^{13}C NMR (63 MHz, CD_3OD) δ 161.94, 159.05, 151.35, 134.67, 127.25, 126.90, 123.83, 114.81, 51.68, 33.32, 26.71, 26.48. Mass (ESI): $[M + H]^+$ 262, 264; found 262.1 (100%), 264.1 (33%). R_f = 0.43 (hexanes to ethyl acetate 2:1).

***N*^{4}-Cyclohexyl-*N*^{2}-isopropylquinazoline-2,4-diamine (8).** 0.10 g (0.40 mmol) of **8d** was reacted with isopropylamine and purified according to method Db to furnish 0.060 g (0.21 mmol) of the title compound as a white crystalline solid in 53% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (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 = 7.6 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), 1.41 (m, 5H), 1.25 (d, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ 159.82, 157.34, 147.57, 133.16, 122.65, 121.83, 121.25, 110.84, 50.46, 42.93, 32.26, 25.37, 21.93. HRMS: m/z calcd for $C_{17}H_{25}N_4$ $[M + H]^+$ 285.2074; found 285.2077. R_f = 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. ^{1}H NMR (400 MHz, CD_3OD) 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, 1H), 3.35 (s, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ 163.76, 156.03, 152.57, 133.10, 126.41, 125.89, 124.83, 114.07, 41.20. R_f = 0.35 (hexanes to ethyl acetate 4:1).

***N*^{2}-Isopropyl-*N*^{4},*N*^{4}-dimethylquinazoline-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. ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (101 MHz, CD_3OD) δ 162.97, 152.20, 143.16, 134.25, 127.59, 122.97, 117.82, 110.60, 43.69, 41.45, 21.46. HRMS: m/z calcd for $C_{13}H_{19}N_4$ $[M + H]^+$ 231.1604; found 231.1608. R_f = 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. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (ddd, 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). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.93, 157.93, 151.09, 137.48, 133.76, 129.11, 128.51, 128.21, 128.00, 126.46, 121.07, 113.38, 45.94. R_f = 0.21 (hexanes to ethyl acetate 4:1).

***N*^{2},*N*^{4}-Dibenzylquinazoline-2,4-diamine (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. ^{1}H NMR (500 MHz, DMSO-d_6) 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). ^{13}C NMR (126 MHz, DMSO-d_6) 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, 127.57, 124.93, 124.64, 117.19, 110.22, 110.12, 44.86, 44.35. HRMS: m/z calcd for $C_{22}H_{21}N_4$ $[M + H]^+$ 341.1761; found 341.1751. R_f = 0.45 (dichloromethane to methanol 9:1). Melting point 237−240 °C.

***N*-Butyl-2-chloroquinazolin-4-amine (11d).** 0.48 g (2.4 mmol) of **1c** was reacted with *n*-butylamine and purified according to method

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

Ca to furnish 0.49 g (2.1 mmol) of the title compound in 86% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.77−7.53 (m, 3H), 7.44−7.31 (m, 1H), 5.82 (s, NH), 3.61 (td, J = 7.1, 5.6, 2H), 1.64 (dt, J = 14.7, 7.2, 2H), 1.40 (td, J = 14.5, 7.2, 2H), 0.92 (t, J = 7.3, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.14, 150.99, 133.58, 128.10, 126.29, 121.19, 120.79, 113.42, 41.57, 31.47, 20.35, 14.02. Mass (ESI): $[M + H]^+$ 236, 238; found 236.0 (100%), 238.0 (34%). R_f = 0.21 (hexanes to ethyl acetate 4:1). Melting point 103−105 °C.

***N*^{2},*N*^{4}-Dibutylquinazoline-2,4-diamine (11).** 0.080 g (0.34 mmol) of **11d** was reacted with *n*-butylamine and purified according to method Da to furnish 0.031 g (0.114 mmol) of the title compound as a white solid in 34% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.78 (s, NH), 8.36 (d, J = 8.5 Hz, 1H), 8.14 (s, NH), 7.76 (t, J = 8.0 Hz, 1H), 7.42 (d, J = 6.6 Hz, 1H), 7.37 (t, J = 6.2 Hz, 1H), 3.58 (m, 2H), 3.45 (m, 2H), 1.65 (p, J = 7.5 Hz, 2H), 1.57 (p, J = 7.5, 2H), 1.36 (sept, J = 7.3 Hz, 4H), 0.92 (td, J = 7.3, 4.7 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.10, 153.50, 139.26, 135.43, 124.83, 124.36, 116.97, 110.02, 41.37, 40.72, 31.49, 30.69, 20.16, 19.93, 14.12, 14.07. HRMS: m/z calcd for $C_{16}H_{25}N_4$ $[M + H]^+$ 273.2074; found 273.2072. Melting point 151−154 °C.

***N*^{4}-Benzyl-*N*^{2}-methylquinazoline-2,4-diamine (12).** 0.088 g (0.326 mmol) of **10d** was reacted with 2.0 M methylamine in methanol and purified according to method Da to furnish 0.085 g (0.321 mmol) of the title compound as a cream colored solid in 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.37 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 9.0 Hz, 1H), 7.20−7.11 (m, 3H), 7.06 (t, J = 7.6 Hz, 1H), 4.80 (s, 2H), 2.93 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.11, 156.03, 142.69, 138.07, 133.96, 128.35, 127.94, 127.26, 123.74, 123.10, 119.20, 110.28, 45.06, 28.01. HRMS: m/z calcd for $C_{16}H_{17}N_4$ $[M + H]^+$ 265.1448; found 265.1454. R_f = 0.47 (dichloromethane to methanol 9:1).

***N*^{4}-Benzyl-*N*^{2}-butylquinazoline-2,4-diamine (13).** 0.10 g (0.37 mmol) of **10d** was reacted with *n*-butylamine and purified according to method Da to furnish 0.047 g (0.15 mmol) of the title compound as a white solid in 41% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (s, 1H), 8.43 (d, J = 8.1 Hz, 1H), 7.82 (s, 1H), 7.44−7.37 (m, 3H), 7.25−7.11 (m, 5H), 4.83 (d, J = 5.7 Hz, 2H), 3.39 (q, J = 6.8 Hz, 2H), 1.49 (p, J = 7.3 Hz, 2H), 1.31 (sext, J = 7.3 Hz, 2H), 0.85 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.08, 153.46, 137.43, 134.65, 128.41, 127.83, 127.40, 124.41, 124.12, 116.62, 109.99, 109.68, 45.31, 41.02, 31.30, 19.95, 13.66. HRMS: m/z calcd for $C_{19}H_{23}N_4$ $[M + H]^+$ 307.1917; found 307.1926. R_f = 0.33 (dichloromethane to methanol 9:1). Melting point 210−212 °C.

***N*^{4}-Benzyl-*N*^{2}-phenylquinazoline-2,4-diamine (14).** 0.10 g (0.37 mmol) of **10d** was reacted with aniline and purified according to method Da to furnish 0.117 g (0.358 mmol) of the title compound as a white crystalline solid in 97% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.46 (s, 2H), 8.46 (d, J = 8.3 Hz, 1H), 7.84 (ddd, J = 8.4, 7.3, 1.1 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.48 (td, J = 7.8, 1.0 Hz, 1H), 7.45−7.41 (m, 2H), 7.37−7.29 (m, 6H), 7.29−7.22 (m, 1H), 7.18 (t, J = 7.4 Hz, 1H), 4.76 (d, J = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.71, 151.94, 139.36, 138.02, 137.13, 135.93, 129.41, 128.89, 127.78, 127.67, 125.45, 125.31, 124.81, 122.71, 117.94, 110.70, 45.14. HRMS: m/z calcd for $C_{21}H_{19}N_4$ $[M + H]^+$ 327.1604; found 327.1613. R_f = 0.56 (dichloromethane to methanol 9:1). Decomposed at 310 °C.

***N*-Methyl-2-chloroquinazolin-4-amine (15d).** 1.0 g (5.0 mmol) of **1c** was reacted with methylamine and purified according to method Ca to furnish 0.89 g (4.6 mmol) of the title compound in 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74−7.66 (m, 3H), 7.41 (t, J = 6.9 Hz, 1H), 6.28 (s, NH), 3.20 (d, J = 4.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.82, 158.02, 150.80, 133.64, 127.90, 126.41, 121.02, 113.60, 28.74. Mass (ESI): $[M + H]^+$ 194; found 194. R_f = 0.12 (hexanes to ethyl acetate 4:1).

***N*^{2}-Benzyl-*N*^{4}-methylquinazoline-2,4-diamine (15).** 0.287 g (1.48 mmol) of **15d** was reacted with benzylamine and purified according to method Da to furnish 0.193 g (0.730 mmol) of the title compound as a white crystalline solid in 49% yield. ^{1}H NMR (250 MHz, CD_3OD) δ 8.06 (d, J = 8.1 Hz, 1H), 7.73 (t, J = 8.4 Hz, 1H), 7.50−7.20 (m, 7H), 4.75 (s, 2H), 3.14 (s, 3H). ^{13}C NMR (63 MHz, CD_3OD) δ 162.17, 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 $C_{16}H_{17}N_4$ $[M + H]^+$ 265.1453; found 265.1457. R_f = 0.31 (dichloromethane to methanol 9:1). Decomposed at 240 °C.

***N*^{2}-Benzyl-*N*^{4}-butylquinazoline-2,4-diamine (16).** 0.48 g (2.0 mmol) of **11d** was reacted with benzylamine and purified according to method Da to furnish 0.40 g (1.3 mmol) of the title compound as a white crystalline solid in 65% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.84 (s, 1H), 8.64 (s, 1H), 8.37 (d, J = 7.7 Hz, 1H), 7.76 (ddd, J = 8.4, 7.3, 1.1 Hz, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.4−7.3 (m, 5H), 7.24 (t, J = 7.1 Hz, 1H), 4.66 (d, J = 4.4 Hz, 2H), 3.49 (d, J = 6.1 Hz, 2H), 1.61−1.43 (m, 2H), 1.33−1.17 (m, 2H), 0.80 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.11, 153.48, 139.24, 139.11, 135.49, 128.83, 127.61, 127.51, 124.83, 124.54, 117.14, 110.15, 44.46, 41.46, 30.62, 20.11, 14.09. HRMS: m/z calcd for $C_{19}H_{23}N_4$ $[M + H]^+$ 307.1923; found 307.1921. R_f = 0.48 (dichloromethane to methanol 9:1). Melting point 194−196 °C.

2-Chloro-*N*-phenylquinazolin-4-amine (17d). 0.30 g (1.5 mmol) of **1c** was reacted with aniline and purified according to method Ca to furnish 0.36 g (1.4 mmol) of the title compound as a white solid in 93% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 8.33 (d, J = 8.3 Hz, 1H), 7.80 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H), 7.78−7.73 (m, 2H), 7.64 (dd, J = 8.4, 0.9 Hz, 1H), 7.56 (ddd, J = 8.2, 7.0, 1.2 Hz, 1H), 7.40−7.34 (m, 2H), 7.17 (t, J = 7.4 Hz, 1H). ^{13}C NMR (101 MHz, CD_3OD) δ 159.61, 156.96, 150.58, 137.99, 133.70, 128.28, 126.50, 126.00, 124.67, 122.55, 122.40, 113.65. R_f = 0.19 (hexanes to ethyl acetate 4:1).

***N*^{2}-Benzyl-*N*^{4}-phenylquinazoline-2,4-diamine (17).** 0.10 g (0.39 mmol) of **17d** was reacted with benzylamine and purified according to method Da to furnish 0.010 g (0.031 mmol) of the title compound as a white crystalline solid in 10% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.56 (s, 1H), 9.13 (s, 1H), 8.37 (d, J = 7.5 Hz, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.86 (d, J = 8.1 Hz, 2H), 7.66 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.39 (dd, J = 8.4, 7.4 Hz, 2H), 7.27 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H), 7.22 (dd, J = 8.4, 7.4 Hz, 2H), 7.13 (t, J = 7.4 Hz, 1H), 6.89 (t, J = 7.3 Hz, 1H), 1.89 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.01, 157.09, 152.33, 141.80, 140.10, 133.64, 129.12, 128.92, 126.26, 124.14, 123.77, 123.10, 122.52, 121.45, 119.58, 112.43, 21.76. HRMS: m/z calcd for $C_{21}H_{19}N_4$ $[M + H]^+$ 327.1604; found 327.1614. R_f = 0.53 (dichloromethane to methanol 9:1).

2,4,8-Trichloroquinazoline (18c). 0.5 g (2.9 mmol) of commercially available 2-amino-3-chlorobenzoic acid was reacted according to general procedure A to give crude **18b**. Without further purification, **18b** 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 232.9 (100%), 235.0 (86%).

2,8-Dichloro-*N*^{4}-(furan-2-ylmethyl)quinazolin-4-amine (18d). 0.10 g (0.43 mmol) of **18c** was reacted with furfurylamine according to general procedure Cb to give 0.11 g (0.37 mmol) of **18d** in 87% yield. ^{1}H NMR (250 MHz, CD_3OD) δ 7.89 (dd, J = 8.3, 1.2 Hz, 1H), 7.69 (dd, J = 7.7, 1.2 Hz, 1H), 7.33 (dd, J = 1.7, 1.0 Hz, 1H), 7.30−7.21 (m, 1H), 6.28−6.21 (m, 2H), 4.67 (s, 2H). ^{13}C NMR (63 MHz, CD_3OD) δ 162.61, 159.88, 152.48, 148.51, 143.44, 134.77, 132.04, 127.17, 122.63, 116.31, 111.47, 109.13, 39.00. Mass (ESI): $[M + H]^+$ 294, 296; found 294.0 (100%), 296.0 (64%).

8-Chloro-*N*^{4}-(furan-2-ylmethyl)-*N*^{2}-isopropylquinazoline-2,4-diamine (18). 0.047 g (0.16 mmol) of **18d** was reacted with isopropylamine according to general procedure Db to give 0.030 g (0.095 mmol) of the title compound as a yellow solid in 59% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (s, NH), 7.97 (d, J = 8.0 Hz, 1H), 7.63 (d, J = 7.5 Hz, 1H), 7.56 (s, 1H), 6.95 (t, J = 7.8 Hz, 1H), 6.65 (s, NH), 6.42−6.36 (m, 1H), 6.32 (s, 1H), 4.70 (d, J = 5.5 Hz, 2H), 4.28−4.14 (m, 1H), 1.17 (d, J = 5.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.87, 158.98, 151.43, 148.83, 142.16, 132.55, 128.98, 119.93, 119.78, 111.79, 110.46, 107.66, 42.93, 38.15, 22.99.HRMS: m/z calcd for $C_{16}H_{17}ClN_4O$ $[M + H]^+$ 317.1164; found 317.1173. 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

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

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 232.9 (100%), 235.0 (94%). R_f = 0.68 (hexanes to ethyl acetate 4:1).

2,7-Dichloro-*N*^{4}-(furan-2-ylmethyl)quinazolin-4-amine (19d). 0.30 g (1.3 mmol) of **19c** was reacted with furfurylamine according to general procedure Cb to give 0.35 g (1.2 mmol) of **19d** in 92% yield. ^{1}H NMR (250 MHz, CD_3OD) δ 8.13−8.06 (m, 1H), 7.62−7.58 (m, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.47−7.42 (m, 1H), 6.37−6.35 (m, 2H), 4.80 (s, 2H). ^{13}C NMR (63 MHz, CD_3OD) δ 163.24, 160.01, 156.93, 152.44, 143.49, 140.90, 128.01, 126.34, 125.69, 113.46, 111.47, 109.11, 38.87. Mass (ESI): $[M + H]^+$ 294, 296; found 294.0 (100%), 296.0 (63%). R_f = 0.27 (hexanes to ethyl acetate 4:1).

7-Chloro-*N*^{4}-(furan-2-ylmethyl)-*N*^{2}-isopropylquinazoline-2,4-diamine (19). 0.113 g (0.38 mmol) of **19d** was reacted with isopropylamine according to general procedure Db to give 0.085 g (0.27 mmol) of the title compound in 71% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.37 (s, NH), 8.03 (d, J = 8.7 Hz, 1H), 7.56−7.55 (m, 1H), 7.23 (s, 1H), 7.02 (dd, J = 8.7, 1.8 Hz, 1H), 6.57 (s, NH), 6.46−6.37 (m, 1H), 6.34 (s, 1H), 4.70 (d, J = 5.6 Hz, 2H), 4.16 (oct, J = 6.6 Hz, 1H), 1.15 (d, J = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.87, 153.82, 152.94, 142.36, 142.32, 137.40, 125.34, 123.78, 120.26, 110.95, 110.90, 107.56, 42.22, 37.32, 23.15. HRMS: m/z calcd for $C_{16}H_{18}ClN_4O$ $[M + H]^+$ 317.1164; found 317.1161. R_f = 0.63 (dichloromethane to methanol 9:1).

2,4,6-Trichloroquinazoline (20c). 1 g (5.8 mmol) of commercially available 2-amino-5-chlorobenzoic acid was reacted according to general procedure A to give crude **20b**. Without further purification, **20b** was reacted according to general procedure B to give 0.55 g (2.7 mmol) of the crude title compound (47% over two steps). ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.17 (d, J = 8.9 Hz, 1H), 7.94 (d, J = 2.0 Hz, 1H), 7.66 (dd, J = 8.9, 2.0 Hz, 1H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 163.85, 156.31, 152.68, 142.97, 130.45, 127.37, 127.07, 120.77. Mass (ESI): $[M + H]^+$ 233, 235; found 232.9 (100%), 235.0 (98%). R_f = 0.68 (hexanes to ethyl acetate 4:1).

2,6-Dichloro-*N*^{4}-(furan-2-ylmethyl)quinazolin-4-amine (20d). 0.30 g (1.3 mmol) of **20c** was reacted with furfurylamine according to general procedure Cb to give 0.072 g (0.24 mmol) of **20d** in 18% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (t, J = 5.1 Hz, NH), 8.44 (d, J = 2.1 Hz, 1H), 7.78 (dd, J = 8.9, 2.1 Hz, 1H), 7.65−7.54 (m, 2H), 6.42−6.34 (m, 2H), 4.69 (d, J = 5.4 Hz, 2H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 160.42, 158.82, 151.67, 139.79, 136.95, 128.95, 128.39, 128.14, 127.04, 126.91, 122.42, 111.58, 45.86. Mass (ESI): $[M + H]^+$ 294, 296; found 294.0 (100%), 296.0 (67%). R_f = 0.27 (hexanes to ethyl acetate 4:1).

6-Chloro-*N*^{4}-(furan-2-ylmethyl)-*N*^{2}-isopropylquinazoline-2,4-diamine (20). 0.041 g (0.14 mmol) of **20d** was reacted with isopropylamine according to general procedure Db to give 0.026 g (0.082 mmol) of the title compound in 59% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (s, NH), 8.15 (d, J = 2.3 Hz, 1H), 7.57 (d, J = 0.7 Hz, 1H), 7.46 (dd, J = 8.9, 2.3 Hz, 1H), 7.23 (d, J = 8.4 Hz, 1H), 6.56 (s, NH), 6.39 (dd, J = 3.0, 1.9 Hz, 1H), 6.34 (s, 1H), 4.68 (d, J = 5.4 Hz, 2H), 4.14 (oct, J = 6.6 Hz, 1H), 1.14 (d, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.22, 158.96, 151.64, 150.51, 142.01, 133.02, 126.36, 125.48, 120.96, 111.58, 110.43, 107.59, 42.72, 37.84, 23.16. HRMS: m/z calcd for $C_{16}H_{17}ClN_4O$ $[M + H]^+$ 317.1164; found 317.1165.

2,4,5-Trichloroquinazoline (21c). 0.36 g (2.1 mmol) of commercially available 2-amino-6-chlorobenzoic acid was reacted according to general procedure A to give crude **21b**. Without further purification, **21b** was reacted according to general procedure B to give 0.12 g (0.51 mmol) of the crude title compound (24% over two steps). Mass (ESI): $[M + H]^+$ 233, 235; found 235.0 (100%), 232.9 (84%). R_f = 0.68 (hexanes to ethyl acetate 4:1).

5-Chloro-*N*^{4}-(furan-2-ylmethyl)-*N*^{2}-isopropylquinazoline-2,4-diamine (21). 0.045 g (0.15 mmol) of **21c** was reacted with furfurylamine according to general procedure Cb. **21d** was reacted with isopropylamine according to general procedure Db to give 0.006 g (0.019 mmol) of the title compound in 13% yield over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.35 (dd, J = 1.8, 0.8 Hz, 1H), 7.28−7.24 (m, 2H), 6.98−6.88 (m, 1H), 6.31 (dd, J = 3.2, 1.9 Hz, 1H), 6.26 (dd, J = 3.2, 0.8 Hz, 1H), 4.93 (s, 1H), 4.73 (d, J = 5.0 Hz, 2H), 4.22 (oct, J = 6.5 Hz, 1H), 1.21 (d, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.17, 158.25, 155.04, 151.58, 142.07, 131.75, 128.92, 124.92, 122.93, 110.40, 108.71, 107.17, 42.66, 38.54, 23.17. HRMS: m/z calcd for $C_{16}H_{18}ClN_4O$ $[M + H]^+$ 317.1164; found 317.1169.

2,4,-Dichloro-8-methylquinazoline (22c). 1.0 g (6.6 mmol) of commercially available 2-amino-3-methylbenzoic acid was reacted according to general procedure A to give crude **22b**. Without further purification, **22b** was reacted according to general procedure B to give 1.1 g (5.2 mmol) of the crude title compound (78% over two steps). Mass (ESI): $[M + H]^+$ 213, 215; found 213.0 (100%), 215.0 (73%). R_f = 0.67 (hexanes to ethyl acetate 4:1).

2-Chloro-*N*^{4}-(furan-2-ylmethyl)-8-methylquinazolin-4-amine (22d). 0.50 g (2.3 mmol) of **22c** was reacted with furfurylamine according to general procedure Cb to give 0.43 g (1.6 mmol) of **22d** in 70% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (t, J = 5.5 Hz, NH), 8.07 (d, J = 8.2 Hz, 1H), 7.58−7.53 (m, 2H), 7.33 (t, J = 7.7 Hz, 1H), 6.39−6.35 (m, 1H), 6.32 (d, J = 3.0 Hz, 1H), 4.70 (d, J = 5.5 Hz, 2H), 2.45 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.92, 156.75, 152.08, 149.93, 142.85, 135.39, 134.26, 126.11, 121.26, 113.88, 111.21, 108.30, 38.10, 17.88. Mass (ESI): $[M + H]^+$ 274, 276; found 274.1 (100%), 276.1 (35%). R_f = 0.33 (hexanes to ethyl acetate 4:1).

***N*^{4}-(Furan-2-ylmethyl)-*N*^{2}-isopropyl-8-methylquinazoline-2,4-diamine (22).** 0.13 g (0.47 mmol) of **22d** was reacted with isopropylamine according to general procedure Db to give 0.10 g (0.34 mmol) of the title compound as a yellow solid in 74% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.53 (s, 1H), 7.94 (s, 1H), 7.35 (d, J = 7.3 Hz, 1H), 7.22−7.21 (m, 1H), 7.04 (t, J = 7.7 Hz, 1H), 6.27−6.19 (m, 2H), 4.80 (d, J = 5.2 Hz, 2H), 4.22 (oct, J = 6.7 Hz, 1H), 1.25 (d, J = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.37, 153.04, 150.27, 142.20, 138.12, 135.91, 126.12, 123.57, 121.16, 110.46, 109.19, 108.09, 43.89, 38.50, 22.34, 18.07. HRMS: m/z calcd for $C_{17}H_{21}N_4O$ $[M + H]^+$ 297.1710; found 297.1712. R_f = 0.50 (dichloromethane to methanol 9:1). Decomposed at 150 °C.

2,4-Dichloro-7-methylquinazoline (23c). 0.5 g (3.3 mmol) of commercially available 2-amino-4-methylbenzoic acid was reacted according to general procedure A to give crude **23b**. Without further purification, **23b** was reacted 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%). R_f = 0.55 (hexanes to ethyl acetate 4:1).

2-Chloro-*N*^{4}-(furan-2-ylmethyl)-7-methylquinazolin-4-amine (23d). 0.20 g (0.94 mmol) of **23c** was reacted with furfurylamine according to general procedure Cb to give 0.091 g (0.33 mmol) of **23d** in 35% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 (d, J = 8.4 Hz, 1H), 7.40 (s, 1H), 7.34 (s, 1H), 7.27 (d, J = 8.5 Hz, 1H), 6.32 (d, J = 1.2 Hz, 2H), 4.74 (s, 2H), 2.43 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.28, 157.55, 151.56, 150.46, 144.94, 142.11, 128.03, 125.11, 122.27, 111.37, 110.21, 107.66, 37.53, 20.59. Mass (ESI): $[M + H]^+$ 274, 276; found 274.1 (100%), 276.1 (36%). R_f = 0.32 (hexanes to ethyl acetate 4:1).

***N*^{4}-(Furan-2-ylmethyl)-*N*^{2}-isopropyl-7-methylquinazoline-2,4-diamine (23).** 0.068 g (0.25 mmol) of **23d** was reacted with isopropylamine according to general procedure Db to give 0.030 g (0.10 mmol) of the title compound in 40% yield. ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.00 (s, NH), 8.21 (d, J = 7.6 Hz, 1H), 8.04 (s, 1H), 7.60 (d, J = 1.3 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 6.43−6.32 (m, 2H), 4.77 (d, J = 4.9 Hz, 2H), 4.32−4.16 (m, 1H), 2.41 (s, 3H), 1.22 (d, J = 6.5 Hz, 6H). ^{13}C NMR (63 MHz, DMSO-d_6) δ 159.59, 151.02, 145.75, 142.30, 138.22, 135.88, 125.02, 124.02, 116.51, 112.23, 110.54, 107.67, 42.88, 37.55, 22.21, 21.34. HRMS: m/z calcd for $C_{17}H_{21}N_4O$ $[M + H]^+$ 297.1710; found 297.1716. R_f = 0.44 (dichloromethane to methanol 9:1).

2,4,-Dichloro-6-methylquinazoline (24c). 1 g (6.6 mmol) of commercially available 2-amino-5-methylbenzoic acid was reacted according to general procedure A to give crude **24b**. Without further purification, **24b** was reacted according to general procedure B to give

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

0.34 g (1.6 mmol) of the crude title compound (24% over two steps). Mass (ESI): $[M + H]^+$ 213; found 213.

2-Chloro-N^4-(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 0.19 g (0.69 mmol) of **24d** in 46% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.79 (s, 1H), 7.50 (dd, J = 8.5, 1.3 Hz, 1H), 7.41 (d, J = 8.3 Hz, 2H), 6.33 (d, J = 1.0 Hz, 2H), 4.74 (s, 2H), 2.41 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.06, 156.69, 151.55, 148.37, 142.11, 136.74, 135.25, 125.55, 121.59, 113.35, 110.21, 107.68, 37.56, 20.30. Mass (ESI): $[M + H]^+$ 274, 276; found 274.1 (100%), 276.1 (33%).

N^4-(Furan-2-ylmethyl)-N^2-isopropyl-6-methylquinazoline-2,4-diamine (24). 0.114 g (0.42 mmol) of **24d** was reacted with isopropylamine according to general procedure Db to give 0.105 g (0.35 mmol) of the title compound as a yellow solid in 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.40−7.29 (m, 3H), 6.33 (dd, J = 3.2, 1.9 Hz, 1H), 6.31 (d, J = 3.1 Hz, 1H), 4.82 (s, 2H), 4.27 (oct, J = 6.6 Hz, 1H), 2.36 (s, 3H), 1.27 (d, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.79, 154.12, 150.67, 142.21, 135.81, 133.04, 122.06, 119.30, 110.46, 109.44, 107.96, 43.45, 38.27, 22.63, 21.05.HRMS: m/z calcd for $C_{17}H_{21}N_4O$ $[M + H]^+$ 297.1710; found 297.1712. R_f = 0.43 (dichloromethane to methanol 9:1). Melting point 200−204 °C.

2,4,-Dichloro-5-methylquinazoline (25c). 1 g (6.6 mmol) of commercially available 2-amino-6-methylbenzoic acid was reacted according to general procedure A to give crude **25b**. Without further purification, **25b** was reacted according to general procedure B to give 0.60 g (2.8 mmol) of the crude title compound (42% over two steps). Mass (ESI): $[M + H]^+$ 213, 215; found 213.0 (100%), 215.0 (71%). R_f = 0.77 (hexanes to ethyl acetate 1:1).

2-Chloro-N^4-(furan-2-ylmethyl)-5-methylquinazolin-4-amine (25d). 0.30 g (1.4 mmol) of **25c** was reacted with furfurylamine according to general procedure Cb to give 0.055 g (0.20 mmol) of **25d** in 14% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.59−7.54 (m, 1H), 7.45−7.34 (m, 2H), 7.25 (d, J = 7.1 Hz, 1H), 6.36 (s, 2H), 4.79 (s, 2H), 2.82 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 162.25, 152.30, 142.08, 134.99, 134.03, 132.97, 129.84, 129.37, 126.90, 124.28, 110.25, 107.61, 38.32, 22.20. Mass (ESI): $[M + H]^+$ 274, 276; found 274.1 (100%), 276.1 (33%). R_f = 0.26 (hexanes to ethyl acetate 4:1).

N^4-(Furan-2-ylmethyl)-N^2-isopropyl-5-methylquinazoline-2,4-diamine (25). 0.10 g (0.37 mmol) of **25d** was reacted with isopropylamine according to general procedure Db to give 0.066 g (0.22 mmol) of the title compound as a yellow solid in 60% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.41 (d, J = 1.0 Hz, 1H), 7.28 (t, J = 7.8 Hz, 1H), 7.14 (d, J = 8.3 Hz, 1H), 6.78 (d, J = 7.1 Hz, 1H), 6.34 (dd, J = 3.0, 1.9 Hz, 1H), 6.27 (d, J = 2.8 Hz, 1H), 4.74 (s, 2H), 4.21(sept, J = 6.5 Hz, 1H), 2.69 (s, 3H), 1.21 (d, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, CD_3OD) δ 161.42, 158.32, 153.28, 152.67, 141.75, 134.10, 131.88, 124.40, 122.13, 111.02, 110.20, 106.52, 42.50, 38.16, 22.56, 22.30. HRMS: m/z calcd for $C_{17}H_{21}N_4O$ $[M + H]^+$ 297.1710; found 297.1710. R_f = 0.16 (ethyl acetate). Melting point 89−92 °C.

2,4,-Dichloro-8-methoxyquinazoline (26c). 1 g (6.0 mmol) of commercially available 2-amino-3-methoxybenzoic acid was reacted according to general procedure A to give crude **26b**. Without further purification, **26b** was reacted according to general procedure B to give 0.15 g (0.65 mmol) of the crude title compound (11% over two steps). Mass (ESI): $[M + H]^+$ 229, 231; found 229.0 (100%), 230.9 (68%). R_f = 0.26 (hexanes to ethyl acetate 4:1).

2-Chloro-N^4-(furan-2-ylmethyl)-8-methoxyquinazolin-4-amine (26d). 0.10 g (0.46 mmol) of **26c** was reacted with furfurylamine according to general procedure Cb to give 0.083 g (0.29 mmol) of **20d** in 63% yield. Mass (ESI): $[M + H]^+$ 290, 292; found 290.1 (100%), 292.0 (68%). R_f = 0.72 (dichloromethane to methanol 19:1).

N^4-(Furan-2-ylmethyl)-N^2-isopropyl-8-methoxyquinazoline-2,4-diamine (26). 0.069 g (0.24 mmol) of **26d** was reacted with isopropylamine according to general procedure Db to give 0.023 g (0.073 mmol) of the title compound as a yellow crystalline solid in 30% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 7.54 (dd, J = 7.2, 1.8 Hz, 1H), 7.44−7.42 (m, 1H), 7.17−7.09 (m, 2H), 6.37−6.33 (m, 2H), 4.81 (s, 2H), 4.30 (hept, J = 6.6 Hz, 1H), 3.92 (s, 3H), 1.29 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ 160.21, 153.10, 150.93, 147.67, 142.12, 131.49, 123.46, 114.22, 113.55, 110.15, 109.86, 107.53, 55.58, 43.46, 37.85, 21.51. HRMS: m/z calcd for $C_{17}H_{21}N_4O_2$ $[M + H]^+$ 313.1659; found 313.1654. R_f = 0.22 (dichloromethane to methanol 19:1). Melting point 157−162 °C.

2,4,-Dichloro-7-methoxyquinazoline (27c). 0.98 g (5.9 mmol) of commercially available 2-amino-4-methoxybenzoic acid was reacted according to general procedure A to give crude **27b**. Without further purification, **27b** was reacted according to general procedure B to give 0.09 g (0.39 mmol) of the crude title compound (7% over two steps). Mass (ESI): $[M + H]^+$ 229, 231; found 228.9 (100%), 231.0 (68%). R_f = 0.71 (hexanes to ethyl acetate 2:1).

2-Chloro-N^4-(furan-2-ylmethyl)-7-methoxyquinazolin-4-amine (27d). 0.075 g (0.33 mmol) of **27c** was reacted with furfurylamine according to general procedure Cb to give 0.083 g (0.29 mmol) of **27d** in 88% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 7.92 (d, J = 9.1 Hz, 1H), 7.43 (d, J = 1.0 Hz, 1H), 7.01 (dd, J = 9.1, 2.5 Hz, 1H), 6.91 (d, J = 2.3 Hz, 1H), 6.35 (s, 2H), 4.76 (s, 2H), 3.88 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 163.95, 160.75, 157.72, 152.39, 151.46, 141.90, 123.70, 117.16, 109.99, 107.40, 107.26, 105.06, 54.76, 37.32. Mass (ESI): $[M + H]^+$ 290, 292; found 290.1 (100%), 292.0 (33%). R_f = 0.44 (hexanes to ethyl acetate 2:1).

N^4-(Furan-2-ylmethyl)-N^2-isopropyl-7-methoxyquinazoline-2,4-diamine (27). 0.065 g (0.29 mmol) of **27d** was reacted with isopropylamine according to general procedure Db to give 0.007 g (0.022 mmol) of the title compound in 8% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, J = 21.0 Hz, 1H), 7.37−7.31 (m, 1H), 6.77 (s, 1H), 6.70 (dd, J = 9.0, 2.4 Hz, 1H), 6.32 (dd, J = 3.1, 1.9 Hz, 1H), 6.29 (d, J = 3.2 Hz, 1H), 4.80 (s, 2H), 4.27 (oct, J = 6.6 Hz, 1H), 3.81 (s, 3H), 1.27 (d, J = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.99, 159.57, 156.47, 151.19, 142.15, 127.72, 123.50, 113.25, 110.44, 107.73, 103.92, 101.78, 55.57, 43.17, 38.07, 22.83. HRMS: m/z calcd for $C_{17}H_{21}N_4O_2$ $[M + H]^+$ 313.1659; found 313.1667.

2,4,-Dichloro-6-methoxyquinazoline (28c). 0.20 g (1.2 mmol) of commercially available 2-amino-4-methoxybenzoic acid was reacted according to general procedure A to give crude **28b**. Without further purification, **28b** was reacted according to general procedure B to give 0.14 g (0.61 mmol) of the crude title compound (50% over two steps). Mass (ESI): $[M + H]^+$ 229, 231; found 229.0 (100%), 231.0 (67%). R_f = 0.53 (hexanes to ethyl acetate 4:1).

2-Chloro-N^4-(furan-2-ylmethyl)-6-methoxyquinazolin-4-amine (28d). 0.061 g (0.27 mmol) of **28c** was reacted with furfurylamine according to general procedure Cb to give 0.031 g (0.11 mmol) of **28d** in 41% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 7.45 (m, 2H), 7.40 (s, 1H), 7.28 (d, J = 9.0 Hz, 1H), 6.37 (d, J = 3.8 Hz, 2H), 4.77 (s, 2H), 3.86 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 160.52, 158.00, 155.06, 151.38, 144.99, 141.90, 127.01, 124.51, 113.88, 109.99, 107.59, 101.40, 54.99, 37.43. Mass (ESI): $[M + H]^+$ 290, 292; found 290.0 (100%), 292.0 (33%). R_f = 0.85 (dichloromethane to methanol 9:1).

N^4-(Furan-2-ylmethyl)-N^2-isopropyl-6-methoxyquinazoline-2,4-diamine (28). 0.14 g (0.48 mmol) of **28d** was reacted with isopropylamine according to general procedure Db to give 0.045 g (0.14 mmol) of the title compound as a yellow solid in 29% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.58 (dd, J = 1.8, 0.9 Hz, 1H), 7.54 (d, J = 2.4 Hz, 1H), 7.24 (d, J = 9.0 Hz, 1H), 7.18 (dd, J = 9.0, 2.5 Hz, 1H), 6.40 (dd, J = 3.1, 1.8 Hz, 1H), 6.35 (d, J = 2.8 Hz, 1H), 4.72 (d, J = 5.3 Hz, 2H), 4.13 (oct, J = 6.5 Hz, 1H), 3.79 (s, 3H), 1.15 (d, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.63, 157.27, 153.87, 152.97, 145.81, 142.36, 125.50, 123.66, 110.91, 107.59, 103.66, 56.05, 42.25, 37.36, 23.19. HRMS: m/z calcd for $C_{17}H_{21}N_4O_2$ $[M + H]^+$ 313.1659; found 313.1659. R_f = 0.28 (dichloromethane to methanol 9:1). Melting point 138−142 °C.

2,4,-Dichloro-5-methoxyquinazoline (29c). 1.0 g (6.2 mmol) of commercially available 2-amino-4-methoxybenzoic acid was reacted according to general procedure A to give crude **29b**. Without further purification, **29b** was reacted according to general procedure B to give 0.036 g (0.16 mmol) of the crude title compound (3% over two steps).

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

Mass (ESI): $[M + H]^+$ 229, 231; found 229.0 (100%), 231.0 (70%). R_f = 0.53 (hexanes to ethyl acetate 2:1).

2-Chloro-N^4-(furan-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 0.0045 g (0.11 mmol) of **29d** in 38% yield. ^{1}H NMR (250 MHz, CD_3OD) δ 7.58 (t, J = 8.3 Hz, 1H), 7.36 (d, J = 1.0 Hz, 1H), 7.08 (d, J = 8.2 Hz, 1H), 6.93 (d, J = 7.9 Hz, 1H), 6.33−6.20 (m, 2H), 4.70 (s, 2H), 3.95 (s, 3H). Mass (ESI): $[M + H]^+$ 290, 292; found 290.0 (100%), 292.0 (37%). R_f = 0.40 (hexanes to ethyl acetate 2:1).

N^4-(Furan-2-ylmethyl)-N^2-isopropyl-5-methoxyquinazoline-2,4-diamine (29). 0.0019 g (6.6 μmol) of **29d** was reacted with isopropylamine according to general procedure Db to give 0.0012 g (3.8 μmol) of the title compound in 58% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (t, J = 8.3 Hz, 1H), 7.41 (t, J = 6.7 Hz, 1H), 6.93 (d, J = 8.1 Hz, 1H), 6.78 (d, J = 8.2 Hz, 1H), 6.38−6.32 (m, 1H), 6.29 (s, 1H), 4.79 (s, 2H), 4.30−4.16 (m, 1H), 4.00 (s, 3H), 1.23 (d, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.20, 157.19, 150.24, 142.45, 142.31, 134.94, 127.73, 113.87, 110.56, 107.75, 104.07, 100.31, 56.50, 43.55, 38.44, 22.48. HRMS: m/z calcd for $C_{17}H_{21}N_4O_2$ $[M + H]^+$ 313.1659; found 313.1654. R_f = 0.31 (dichloromethane to methanol 9:1).

Physicochemical Assays. *Permeability Assay.* Permeability P_e has been determined by a standard parallel artificial membrane permeability assay (PAMPA by pION) as reported previously.[37]

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.[37] 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 10 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 an HPLC method adapted from the strategy reported by Donovan and Pescatore.[36] 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% acetonitrile, with buffer 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/L82[29] and *L. amazonensis* LV78[28] 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/BPK164/1[31] and antimony-susceptible isolate *L. donovani* MHOM/NP/03/BPK206/0[31] was also assessed. Briefly, second to third day stationary phase promastigotes grown in HOMEM (Invitrogen) supplemented with 20% fetal calf serum were used to infect murine primary peritoneal macrophages at a ratio of 10 parasites to 1 host cell and subsequently exposed to four concentrations ranging between 0.31 and 25 μM for compound **15** or 0.31−10 μM for compounds **16** and **23**. After 4 days of exposure to compounds, slides were fixed with methanol and stained with Giemsa to manually assess parasite survival (the percentage of infected macrophages) using conventional light microscopy. A standardized antimony-susceptibility test[31] was performed in parallel to confirm the validity of the test and the clinical isolates' antimony-susceptibility profiles.

Cytotoxicity Assays. Toxicity of compounds against the murine macrophage cell line J774A.1 was conducted using the 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described[40] except that cells were incubated with compounds for 72 h.

Murine Visceral Leishmaniasis Model. The in vivo antileishmanial efficacy of compounds was evaluated in a murine model of visceral leishmaniasis by the general method described earlier[29,41] with minor modifications. Briefly, BALB/c mice were inoculated with LV82 promastigotes on day 0 and then weighed and marked individually on day 6 in order to calculate the volume of solution to be administered to each mouse. In these studies, 45% (w/v) (2-hydroxypropyl)-β-cyclodextrin (HPβCD) vehicle was used to formulate **16**, and 0.5% methylcellulose/0.1% Tween 80 in water (MC) was used to dissolve **15** and **23**; all compounds and vehicles were administered intraperitoneally. After 5 consecutive days of treatment from day 7 to day 11, animals were sacrificed on day 14. Liver smear slides from each animal were made and stained, and the liver parasitemia expressed in Leishman−Donovan unit (LDU) was determined by microscopy.[41] The efficacy of each compound was calculated according to the method published previously.[41]

Pharmacokinetics. *Animals.* Protocols for pharmacokinetics studies in mice were approved by the Institutional Animal Care and Use Committee of the University of Kansas. Male Swiss Webster mice (weighing 20−25 g) were purchased from the Charles River Laboratories (Raleigh, NC). Mice were housed in a clean-room under filtered, pathogen-free air, in a 12 h light/dark cycle, and with food pellets and water available ad libitum.

*Pharmacokinetics and Metabolic Stability of **16** and **23** in Mice.* **16** (dissolved in 50% DMA) and **23** [dissolved in PEG400/DMA/water = 5:3:2 (v/v)] were administered at a dose of 100 μmol/kg body weight (or approximately 30 mg/kg; dose volume = 5 mL/kg) via oral gavage (po) or intraperitoneal (ip) administration to Swiss Webster mice. Blood sampling time schedules were 1, 2, 4, 8, 12, and 24 h (n = 3 at each blood sampling time point) after dosing. Blood and tissue samples were collected and processed for drug concentration determination as previously described.[42] Metabolic stability of **16** and **23** was evaluated using pooled mouse liver microsomes (XenoTech LLC, Lenexa, KS) 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 C_{18} 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 $t_{1/2}$) values were obtained by fitting the one-phase exponential decay equation to the percentage of substrate remaining versus time curves (GraphPad Prism, version

dx.doi.org/10.1021/jm5000408 | J. Med. Chem. 2014, 57, 5141−5156

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 with quantifiable drug levels without extrapolating back to t = 0 h or beyond the last quantifiable time point (GraphPad Prism, version 5.04).

AUTHOR INFORMATION

Corresponding Author

*Phone: 813-974-7306. E-mail: manetsch@usf.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded in part by the USF Center for Drug Discovery and Innovation and NIH Grant R21 AI101529. We also thank the University of South Florida for startup funding, Drs. R. Kiplin Guy and Fangyi Zhu for assisting us with PAMPA and solubility measurements, Dr. Andrii Monastyrskyi for conducting the PAMPA and solubility measurements, and Dr. Fred Buckner for providing the β-lactamase expressing *Leishmania* parasites. We also thank Dr. Arlene Bridges of the University of North Carolina at Chapel Hill for analytical support. Antimonial resistant clinical isolates of *L. donovani* were obtained through the Kaladrug-R project (EU-FP7 Grant 222895)

ABBREVIATIONS USED

DMA, dimethylacetamide; MTT, 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HPβCD, (2-hydroxypropyl)-β-cyclodextrin; C_{max}, maximum concentration; Mic $t_{1/2}$, microsomal half-life; PABA, *p*-aminobenzoic acid; P_e, permeability; SI, selectivity index; SRM, single reaction monitoring; SPR, structure−property relationship; w/v, weight per unit volume

REFERENCES

(1) Chappuis, F.; Sundar, S.; Hailu, A.; Ghalib, H.; Rijal, S.; Peeling, R. W.; Alvar, J.; Boelaert, M. Visceral leishmaniasis: What are the needs for diagnosis, treatment and control? *Nat. Rev. Microbiol.* **2007**, *5*, S7−S16.

(2) World Health Organization. Leishmaniasis. http://www.who.int/leishmaniasis/en/ (accessed January 7, 2014).

(3) Alvar, J.; Velez, I. D.; Bern, C.; Herrero, M.; Desjeux, P.; Cano, J.; Jannin, J.; den Boer, M.; Argaw, D.; Bhattacharya, S.; Ejov, M.; Elkhouri, A. N.; Ruiz-Postigo, J. A.; Serrano, J.; Denereaz, A.; Arana, B.; Robledo, S. M.; Mondragon, K.; Velez, A.; Lopez, L.; Acosta, L. A. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One* **2012**, *7*, e35671.

(4) World Health Organization. Leishmaniasis Fact Sheet No. 375. http://www.who.int/mediacentre/factsheets/fs375/en/ (accessed January 7, 2014).

(5) Desjeux, P. Leishmaniasis. Public health aspects and control. *Clin. Dermatol.* **1996**, *14*, 417−423.

(6) Pearson, R. D.; Sousa, A. Q. Clinical spectrum of Leishmaniasis. *Clin. Infect. Dis.* **1996**, *22*, 1−13.

(7) Guerin, P. J.; Olliaro, P.; Sundar, S.; Boelaert, M.; Croft, S. L.; Desjeux, P.; Wasunna, M. K.; Bryceson, A. D. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. *Lancet Infect. Dis.* **2002**, *2*, 494−501.

(8) McCall, L.-I.; Zhang, W.-W.; Matlashewski, G. Determinants for the development of visceral leishmaniasis disease. *PLoS Pathog.* **2013**, *9*, e1003053.

(9) Rey, L. Gaspar Vianna and the discovery of the treatment of leishmaniasis by antimonials. *Rev. Inst. Med. Trop. Sao Paulo* **1962**, *4*, 47−52.

(10) Peters, W. The treatment of kala-azar—new approaches to an old problem. *Indian J. Med. Res.* **1981**, *73* (Suppl.), 1−18.

(11) Maltezou, H. C. Drug resistance in visceral leishmaniasis. *J. Biomed. Biotechnol.* **2010**, *2010*, No. 617521.

(12) Frezard, F.; Martins, P. S.; Barbosa, M. C. M.; Pimenta, A. M. C.; Ferreira, W. A.; de Melo, J. E.; Mangrum, J. B.; Demicheli, C. New insights into the chemical structure and composition of the pentavalent antimonial drugs, meglumine antimonate and sodium stibogluconate. *J. Inorg. Biochem.* **2008**, *102*, 656−665.

(13) Sundar, S.; More, D. K.; Singh, M. K.; Singh, V. P.; Sharma, S.; Makharia, A.; Kumar, P. C.; Murray, H. W. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin. Infect. Dis.* **2000**, *31*, 1104−1107.

(14) Sundar, S.; Chakravarty, J. Leishmaniasis: an update of current pharmacotherapy. *Expert Opin. Pharmacother.* **2012**, *14*, 53−63.

(15) Perez-Victoria, F. J.; Sanchez-Canete, M. P.; Seifert, K.; Croft, S. L.; Sundar, S.; Castanys, S.; Gamarro, F. Mechanisms of experimental resistance of Leishmania to miltefosine: implications for clinical use. *Drug Resist. Updates* **2006**, *9*, 26−39.

(16) Sundar, S.; Singh, A.; Rai, M.; Prajapati, V. K.; Singh, A. K.; Ostyn, B.; Boelaert, M.; Dujardin, J.-C.; Chakravarty, J. Efficacy of miltefosine in the treatment of visceral leishmaniasis in india after a decade of use. *Clin. Infect. Dis.* **2012**, *55*, 543−550.

(17) Rijal, S.; Ostyn, B.; Uranw, S.; Rai, K.; Bhattarai, N. R.; Dorlo, T. P. C.; Beijnen, J. H.; Vanaerschot, M.; Decuypere, S.; Dhakal, S. S.; Das, M. L.; Karki, P.; Singh, R.; Boelaert, M.; Dujardin, J.-C. Increasing failure of miltefosine in the treatment of kala-azar in Nepal and the potential role of parasite drug resistance, reinfection, or non-compliance. *Clin. Infect. Dis.* **2013**, *56*, 1530−1538.

(18) Berman, J. D.; King, M.; Edwards, N. Antileishmanial activities of 2,4-diaminoquinazoline putative dihydrofolate reductase inhibitors. *Antimicrob. Agents Chemother.* **1989**, *33*, 1860−1863.

(19) Bhattacharjee, A. K.; Skanchy, D. J.; Jennings, B.; Hudson, T. H.; Brendle, J. J.; Werbovetz, K. A. Analysis of stereoelectronic properties, mechanism of action and pharmacophore of synthetic indolo[2,1-*b*]quinazoline-6,12-dione derivatives in relation to anti-leishmanial activity using quantum chemical, cyclic voltammetry and 3-D-QSAR CATALYST procedures. *Bioorg. Med. Chem.* **2002**, *10*, 1979−1989.

(20) Ram, V. J.; Goel, A.; Verma, M.; Kaul, I. B.; Kapil, A. Latent leishmanicidal activity of quinazolinones and 1,2,4-triazoloquinazolinones. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2087−2090.

(21) Agarwal, K. C.; Sharma, V.; Shakya, N.; Gupta, S. Design and synthesis of novel substituted quinazoline derivatives as antileishmanial agents. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5474−5477.

(22) Kumar, S.; Shakya, N.; Gupta, S.; Sarkar, J.; Sahu, D. P. Synthesis and biological evaluation of novel 4-(hetero)aryl-2-piperazinoquinazolines as anti-leishmanial and anti-proliferative agents. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2542−2545.

(23) Gilbert, I. H. Inhibitors of dihydrofolate reductase in leishmania and trypanosomes. *Biochem. Biophys. Acta* **2002**, *1587*, 249−257.

(24) Van Horn, K. S.; Vesely, B.; Srivastava, A.; Kyle, D. E.; Manetsch, R. Unpublished results.

(25) Feng, J.; Zhang, Z.; Wallace, M. B.; Stafford, J. A.; Kaldor, S. W.; Kassel, D. B.; Navre, M.; Shi, L.; Skene, R. J.; Asakawa, T.; Takeuchi, K.; Xu, R.; Webb, D. R.; Gwaltney, S. L., II. Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV. *J. Med. Chem.* **2007**, *50*, 2297−2300.

(26) Ife, R. J.; Brown, T. H.; Blurton, P.; Keeling, D. J.; Leach, C. A.; Meeson, M. L.; Parsons, M. E.; Theobald, C. J. Reversible inhibitors of the gastric (H+/K+)-ATPase. 5. Substituted 2,4-diaminoquinazolines and thienopyrimidines. *J. Med. Chem.* **1995**, *38*, 2763−2773.

(27) Kanuma, K.; Omodera, K.; Nishiguchi, M.; Funakoshi, T.; Chaki, S.; Semple, G.; Tran, T.-A.; Kramer, B.; Hsu, D.; Casper, M.; Thomsen, B.; Sekiguchi, Y. Lead optimization of 4-(dimethylamino)-quinazolines, potent and selective antagonists for the melanin-

concentrating hormone receptor 1. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3853−3856.
(28) Buckner, F. S.; Wilson, A. J. Colorimetric assay for screening compounds against Leishmania amastigotes grown in macrophages. *Am. J. Trop. Med. Hyg.* **2005**, *72*, 600−605.
(29) Zhu, X.; Pandharkar, T.; Werbovetz, K. Identification of new antileishmanial leads from hits obtained by high-throughput screening. *Antimicrob. Agents Chemother.* **2012**, *56*, 1182−1189.
(30) Topliss, J. G. Utilization of operational schemes for analog synthesis in drug design. *J. Med. Chem.* **1972**, *15*, 1006−1011.
(31) Rijal, S.; Yardley, V.; Chappuis, F.; Decuypere, S.; Khanal, B.; Singh, R.; Boelaert, M.; De Doncker, S.; Croft, S.; Dujardin, J.-C. Antimonial treatment of visceral leishmaniasis: Are current in vitro susceptibility assays adequate for prognosis of in vivo therapy outcome? *Microbes Infect.* **2007**, *9*, 529−535.
(32) Hailu, A.; Musa, A.; Wasunna, M.; Balasegaram, M.; Yifru, S.; Mengistu, G.; Hurissa, Z.; Hailu, W.; Weldegebreal, T.; Tesfaye, S.; Makonnen, E.; Khalil, E.; Ahmed, O.; Fadlalla, A.; El-Hassan, A.; Raheem, M.; Mueller, M.; Koummuki, Y.; Rashid, J.; Mbui, J.; Mucee, G.; Njoroge, S.; Manduku, V.; Musibi, A.; Mutuma, G.; Kirui, F.; Lodenyo, H.; Mutea, D.; Kirigi, G.; Edwards, T.; Smith, P.; Muthami, L.; Royce, C.; Ellis, S.; Alobo, M.; Omollo, R.; Kesusu, J.; Owiti, R.; Kinuthia, J. Geographical variation in the response of visceral leishmaniasis to paromomycin in east Africa: a multicentre, open-label, randomized trial. *PLoS Neglected Trop. Dis.* **2010**, *4*, e709.
(33) Berman, J. D.; Badaro, R.; Thakur, C. P.; Wasunna, K. M.; Behbehani, K.; Davidson, R.; Kuzoe, F.; Pang, L.; Weerasuriya, K.; Bryceson, A. D. Efficacy and safety of liposomal amphotericin B (AmBisome) for visceral leishmaniasis in endemic developing countries. *Bull. W. H. O.* **1998**, *76*, 25−32.
(34) El Fadili, A.; Richard, D.; Kündig, C.; Ouellette, M. Effect of polyglutamylation of methotrexate on its accumulation and the development of resistance in the protozoan parasite *Leishmania*. *Biochem. Pharmacol.* **2003**, *66*, 999−1008.
(35) Osorio, E.; Aguilera, C.; Naranjo, N.; Marin, M.; Muskus, C. Biochemical characterization of the bifunctional enzyme dihydrofolate reductase-thymidylate synthase from *Leishmania* (*Viannia*) and its evaluation as a drug target. *Biomedica* **2013**, *33*, 393−401.
(36) Donovan, S. F.; Pescatore, M. C. Method for measuring the logarithm of the octanol−water partition coefficient by using short octadecyl-poly(vinyl alcohol) high-performance liquid chromatography columns. *J. Chromatogr., A* **2002**, *952*, 47−61.
(37) Zhang, Y.; Clark, J. A.; Connelly, M. C.; Zhu, F.; Min, J.; Guiguemde, W. A.; Pradhan, A.; Iyer, L.; Furimsky, A.; Gow, J.; Parman, T.; El, M. F.; Phillips, M. A.; Kyle, D. E.; Mirsalis, J.; Guy, R. K. Lead optimization of 3-carboxyl-4(1*H*)-quinolones to deliver orally bioavailable antimalarials. *J. Med. Chem.* **2012**, *55*, 4205−4219.
(38) Zornoza, T.; Cano-Cebrian, M. J.; Hipolito, L.; Granero, L.; Polache, A. Evidence of a flip-flop phenomenon in acamprosate pharmacokinetics: an in vivo study in rats. *Biopharm. Drug Dispos.* **2006**, *27*, 305−311.
(39) Yanez, J. A.; Remsberg, C. M.; Sayre, C. L.; Forrest, M. L.; Davies, N. M. Flip-flop pharmacokinetics—delivering a reversal of disposition: challenges and opportunities during drug development. *Ther. Delivery* **2011**, *2*, 643−672.
(40) Yamada, T.; Goto, M.; Punj, V.; Zaborina, O.; Kimbara, K.; Das, G. T. K.; Chakrabarty, A. M. The bacterial redox protein azurin induces apoptosis in J774 macrophages through complex formation and stabilization of the tumor suppressor protein p53. *Infect. Immun.* **2002**, *70*, 7054−7062.
(41) Delfín, D.; Morgan, R.; Zhu, X.; Werbovetz, K. Redox-active dinitrodiphenylthioethers against Leishmania: Synthesis, structure−activity relationships and mechanism of action studies. *Bioorg. Med. Chem.* **2009**, *15*, 820−829.
(42) Wang, M. Z.; Zhu, X.; Srivastava, A.; Liu, Q.; Sweat, J. M.; Pandharkar, T.; Stephens, C. E.; Riccio, E.; Parman, T.; Munde, M.; Mandal, S.; Madhubala, R.; Tidwell, R. R.; Wilson, W. D.; Boykin, D. W.; Hall, J. E.; Kyle, D. E.; Werbovetz, K. A. Novel arylimidamides for treatment of visceral leishmaniasis. *Antimicrob. Agents Chemother.* **2010**, *54*, 2507−2516.
(43) Wang, M. Z.; Saulter, J. Y.; Usuki, E.; Cheung, Y. L.; Hall, M.; Bridges, A. S.; Loewen, G.; Parkinson, O. T.; Stephens, C. E.; Allen, J. L.; Zeldin, D. C.; Boykin, D. W.; Tidwell, R. R.; Parkinson, A.; Paine, M. F.; Hall, J. E. CYP4F enzymes are the major enzymes in human liver microsomes that catalyze the O-demethylation of the antiparasitic prodrug DB289 [2,5-bis(4-amidinophenyl)furan-bis-*O*-methylamidoxime]. *Drug Metab. Dispos.* **2006**, *34*, 1985−1994.