Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

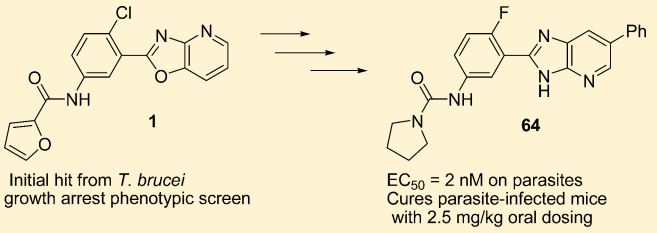
Substituted 2-Phenylimidazopyridines: A New Class of Drug Leads for Human African Trypanosomiasis

Hari Babu Tatipaka,[†] J. Robert Gillespie,[‡] Arnab K. Chatterjee,[∥,∞] Neil R. Norcross,[†] Matthew A. Hulverson,[‡] Ranae M. Ranade,[‡] Pendem Nagendar,[†] Sharon A. Creason,[‡] Joshua McQueen,[†] Nicole A. Duster,[‡] Advait Nagle,[∥] Frantisek Supek,[∥] Valentina Molteni,[∥] Tanja Wenzler,[⊥,#] Reto Brun,[⊥,#] Richard Glynne,[∥] Frederick S. Buckner,*[,‡] and Michael H. Gelb*[,†,§]

Departments of [†]Chemistry, [‡]Medicine, and [§]Biochemistry, University of Washington, Seattle, Washington 98195, United States
[∥]Genomics Institute of the Novartis Foundation, 10675 John Jay Hopkins Drive, San Diego, California 92121, United States
[⊥]Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4002 Basel, Switzerland
[#]University of Basel, 4003 Basel, Switzerland

Supporting Information

ABSTRACT: A phenotypic screen of a compound library for antiparasitic activity on *Trypanosoma brucei*, the causative agent of human African trypanosomiasis, led to the identification of substituted 2-(3-aminophenyl)oxazolopyridines as a starting point for hit-to-lead medicinal chemistry. A total of 110 analogues were prepared, which led to the identification of **64**, a substituted 2-(3-aminophenyl)imidazopyridine. This compound showed antiparasitic activity in vitro with an EC_{50} of 2 nM and displayed reasonable druglike properties when tested in a number of in vitro assays. The compound was orally bioavailable and displayed good plasma and brain exposure in mice. Compound **64** cured mice infected with *Trypanosoma brucei* when dosed orally down to 2.5 mg/kg. Given its potent antiparasitic properties and its ease of synthesis, compound **64** represents a new lead for the development of drugs to treat human African trypanosomiasis.



INTRODUCTION

Two protozoan agents cause human African trypanosomiasis (HAT): *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. Natural transmission occurs in 36 countries of sub-Saharan Africa by the bite of infected tsetse flies (WHO Fact Sheet No. 259). After the initial cutaneous inoculation, early stage (hemolymphatic) infection occurs. In the form occurring in West and Central Africa, (Gambian HAT), the first stage can last for months to years before progressing to second stage infection in the central nervous system. In the form occurring in East and Southern Africa (Rhodesian HAT), the first stage lasts only a few weeks to months before causing late-stage disease. Once the parasites enter the central nervous system (second stage), the patients suffer neurological effects that culminate in coma and death if untreated.

Better HAT drugs are desperately needed especially for second stage HAT.[1] The recent introduction of nifurtimox−eflornithine combination therapy for Gambian HAT allows for the use of fewer doses of eflornithine, but these still need to be given by iv injections, and the treatment remains very expensive. Melarsoprol, a trivalent arsenical, is the only drug effective for second stage Rhodesian HAT. Unfortunately, toxic encephalopathy occurs in 5−18% of patients with associated mortality of 3−12% from treatment itself. Because second stage HAT has 100% mortality, melarsoprol remains as first line treatment despite this toxicity. An additional concern is the increasing incidence of melarsoprol resistant strains.[2]

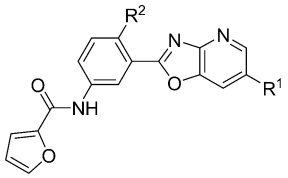
Better drugs are also desirable for treating first stage disease. Current treatment options pentamidine and suramin must be given by injection, although most patients with early stage HAT are able to take oral medicines. Both drugs have considerable toxicity and are unsafe in pregnancy.[3−5]

Two promising drug candidates have recently entered early clinical development for treating HAT: a nitroheterocyle fexinidazole and a benzoxaborole SCYX-7158.[6,7] These candidates follow on the heels of the diamidine drug, pafuramidine (DB-289), where efficacy was established in phase III studies of HAT; however, nephrotoxicity observed in an expanded phase I safety trial led to abandonment.[8] As evidenced by pafuramidine, the attrition rate of clinical drug candidates is high (nearly 90% upon entering phase I),[9] underscoring the importance of maintaining an adequate pipeline of drug candidates.

Received: October 13, 2013

   dx.doi.org/10.1021/jm401178t | *J. Med. Chem.* XXXX, XXX, XXX−XXX

Table 1. 2-(3-Aminophenyl)oxazolopyridines Containing a N-Linked 2-Furanoyl Group



compd	R^1	R^2	EC_{50} [a] (μM)	CC_{50} [b] (μM)
1	H	Cl	0.22	>50[c,d,e]
2	H	F	0.12	>50[c]
3	H	Me	0.43	>50[c]
4	H	CN	0.4	>50[c]
5	H	OMe	>10	>50[c]
6	H	4-fluorophenyl	>50	>50[c]
7	H	3,4-difluorophenyl	>50	>50[c]
8	H	4-pyridyl	>20	
9	Br	F	0.23	>50[c]
10	CN	F	0.38	>50[c]
11	phenyl	F	0.04 (0.08)	7[c]
12	4-fluorophenyl	F	0.05 (0.1)	>50,[d] 32[e]
13	3-chlorophenyl	F	0.05	>50[c]
14	4-MeO-phenyl	F	0.17	
15	4-phenylphenyl	F	0.42	>50[c]
pentamidine[f]			1.7	
quinacrine[g]				3.9,[d] 9.8[e]
podophyllotoxin[g]				0.014[c]

[a]Concentration of compound required to inhibit growth by 50% (EC_{50}) of *T. brucei* strain BF427 or *T. brucei rhodesiense* (numbers in parentheses). [b]Concentration of compound required to inhibit growth by 50% (CC_{50}) of mammalian cell lines. [c]Rat myoblasts (L6). [d]Human lymphoblasts (CRL-8155). [e]Human hepatocytes (HepG2). [f]Control compound for *T. brucei* assays: average EC_{50} was 1.74 ± 0.15 SEM (n = 57 assays). [g]Control compounds for cytotoxicity assays, average CC_{50} and SEM: for CRL-8155 cells, 3.92 ± 0.91 μM (n = 8 assays); for HepG2 cells, 9.78 μM ± 1.27 μM (n = 10 assays); for L6 myoblasts, 0.014.3 ± 0.00065 μM (n = 15 assays).

RESULTS AND DISCUSSION

A high throughput screen was designed to identify new small molecules with antiparasitic activity toward *T. brucei* within a library of 700 000 compounds. This library was assembled with a particular focus on druglike properties and structural diversity. The library had been previously profiled in more than 60 other high throughput screens, which included both biochemical and cell-based assays against human and pathogen targets and has been an excellent source of attractive starting points for medicinal chemistry in these other programs. Further, the screen history of this library also allowed rapid identification and elimination of compounds with a "frequent hitter" profile.

Compounds from this library were tested for inhibitory activity on *T. brucei* at 3.6 μM. The screen yielded 3889 primary hits (0.6% hit rate) that inhibited growth by >50%. Data from more than 95% of the assay plates had $Z' > 0.6$, using DMSO as the negative control and 1 μM pentamidine as the positive control. Primary hits from the screen were further characterized using a dose−response assay format to determine the EC_{50}. In parallel, cytotoxicities of these compounds were determined against a proliferating human hepatoma cell line (Huh7). The final set of confirmed hits consisted of compounds that had $EC_{50} < 3.6$ μM against *T. brucei*, as well as a limited or nonobservable Huh7 cytotoxicity ($CC_{50} > 10$ μM or SI > 10; SI = CC_{50}/EC_{50}). The final set of confirmed *T. brucei* hits consisted of 1035 inhibitors. Of this set, over 95% of the identified hits did not carry a "frequent hitter" liability (identified as a hit in >5 screens out of a total of 60−65 screens). The 1035 confirmed and selective hits could be grouped into about 115 distinct scaffolds, with 144 compounds having a EC_{50} on *T. brucei* of less than 100 nM and a further 446 compounds having a EC_{50} of less than 500 nM.

Properties of Lead Compound 1. Lead compound **1** was selected from the available hits based on ease of synthesis, including a lack of stereogenic carbons, as well as druglike features including low MW and Lipinski rule-of-five compliance. Compound **1** blocks the growth of *T. brucei* in vitro with an EC_{50} of 220 nM (Table 1). All compounds in this study were tested for anti-parasite activity on the cattle virulent *T. brucei brucei* strain BF427 in vitro, and selected examples were also tested on the human virulent *T. brucei rhodesiense* strain STIB900. In all cases, EC_{50} values for both parasite strains were within a factor of 2−3 of each other. Compound **1** was not cytotoxic to mammalian cells, as the EC_{50} was >50 μM for rat myoblasts (L6 cells), human lymphoblasts (CRL-8155 cells), and human hepatocytes (HepG2 cells). The compound was reasonably soluble in aqueous buffer (20 μM at pH 7.4 and 22 μM at pH 2.0) (Supporting Information Table 1).

Supporting Information Table 1 summarizes in vitro ADME studies and pharmacokinetics (PK) studies in mice. When injected ip into mice, analysis of brain and plasma compound levels 15 and 60 min postinjection showed a brain-to-plasma concentration ratio of 0.54 and 0.38, respectively, indicating exposure into the central nervous system. We also measured permeability of **1** across a tight monolayer of Madin−Darby canine kidney cells that have been transfected with the human MDR1 (P-glycoprotein) drug efflux pump gene (MDCK-MDR1 cells), and permeability was measured in the presence and absence of the MDR1 inhibitor GF-120918 to gauge whether **1** is a substrate for the efflux pump.[10−12] Values of P_{app} in the absence

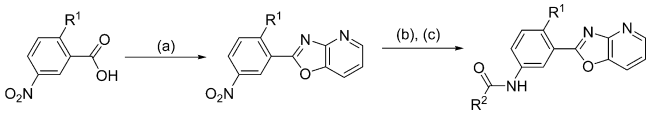
dx.doi.org/10.1021/jm401178t | J. Med. Chem. XXXX, XXX, XXX−XXX

and presence of GF-120918 were 417 and 362 nm/s, respectively. The corresponding value for propanolol, a readily permeable, non-MDR1 substrate comparator compound, was 444−469 nm/s in the presence and absence of GF-120918. The additional comparator compound amprenavir is highly permeable by passive diffusion and is also a potent MDR1 substrate and shows P_{app} values of 252 and 27 nm/s in this assay. The high permeability of **1** and its poor tendency to be pumped by MDR1 in this in vitro assay are consistent with central nervous system exposure in mice.

A liability of **1** is its poor metabolic stability in liver microsomes. The half-life for loss of **1** in mouse and human liver microsomes was 0.8 and 6.4 min, respectively. A PK study was carried out by dosing **1** by oral gavage in mice at 50 mg/kg. The C_{max} value in plasma was low, 0.06 μM (T_{max} was 30 min). The area under the plasma concentration−time curve (AUC) was 187 min·μM. The half-life for elimination from the plasma was 53 min, and the plasma clearance was 98 mL min^{-1} kg^{-1}. The low exposure of **1** in mice is consistent with the high rate of metabolism seen in liver microsomes. In vitro metabolite identification studies in liver microsomes showed a major metabolite due to amide hydrolysis of the 4-chloroanilide group (not shown). Given this result, we tested the hydrolysis product (the aniline derived from **1**) and found no inhibition of *T. brucei* growth in vitro when tested up to 20 μM. Thus, the anilide and not its hydrolysis product was causing the antiparasitic activity. It could be possible that **1** kills parasites by acylating its biological target (given that the 4-chloroaniline is a relatively good leaving group); however, studies with analogues where the anilide is replaced with a urea (described below) suggest that this mechanism is unlikely. In summary, **1** was selected for hit-to-lead optimization because of good whole-cell activity on *T. brucei*, no cytotoxicity on mammalian cell lines, reasonable exposure into the central nervous system, and acceptable solubility. Moving forward, the goal was to design and synthesize chemical analogues to further optimize the antitrypanosomal potency while improving metabolic stability.

Synthesis of 1 and Its Analogues. Scheme 1 shows the synthetic route to make **1** and its analogues (substituted 2-(3-aminophenyl)oxazolopyridines). The substituted 3-nitrobenzoic acid is condensed with 2-amino-3-hydroxypyridine to generate the oxazole ring. When R^1 is chloro, it can be replaced with phenyl or substituted phenyl by Suzuki cross coupling (not shown). Reduction of the nitro group is followed by acylation.

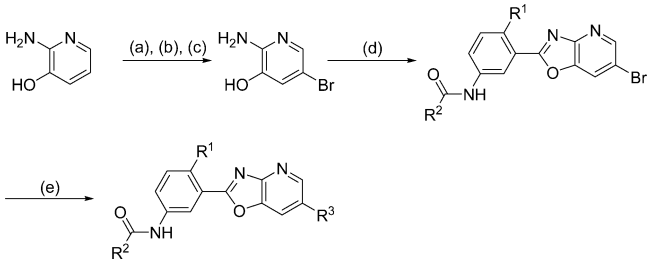
Scheme 1. Synthesis of Substituted 2-(3-Aminophenyl)oxazolopyridines[a]



[a]Reagents and conditions: (a) 2-amino-3-hydroxypyridine, polyphosphoric acid, 160 °C, 5 h; (b) $SnCl_2·2H_2O$, EtOAc, reflux; (c) R^2COCl, pyridine, cat. DMAP, rt or R^2CO_2H, EDCI, HOBt, CH_2Cl_2, rt.

Scheme 2 shows the preparation of compounds containing a phenyl substituent at the 6-position of the oxazolopyridine ring. 2-Amino-3-hydroxy-5-bromopyridine is made in three steps and then converted into the oxazolopyridine as in Scheme 1. Bromine at the 6-position is replaced by phenyl or substituted phenyl by Suzuki cross coupling.

Scheme 2. Synthesis of Substituted 2-(3-Aminophenyl)oxazolopyridines Containing 6-Substituents[a]



[a]Reagents and conditions: (a) *N*,*N*′-disuccinimidyl carbonate, THF, rt; (b) DMF, Br_2, rt; (c) NaOH, H_2O, reflux 5 h; (d) as in Scheme 1; (e) K_2CO_3, R^3-boronic acid, tetrakis(triphenylphosphine)Pd(0) (catalytic), toluene, ethanol, reflux.

Structure−Activity Studies of Analogues of 1. Table 1 shows a number of analogues of **1** and their antiparasitic activity on *T. brucei* in vitro. Replacement of the aryl chlorine (R^2) with fluorine (**2**) leads to a 2-fold improvement in potency. In contrast, replacement with methyl (**3**) or cyano (**4**) reduces potency about 2-fold, and replacement by methoxy (**5**) abrogates activity. Replacement of chloro with various aryl groups (**6**−**8**) abrogates anti-parasite activity.

When the 6-position (R^1) of the oxazolopyridine ring in compound **2** is substituted with Br or CN, there is a 2- to 3-fold decrease in potency (**9**, **10**) with respect to **2**, but in contrast a phenyl group improves potency, with or without halogen substitution at the 3 or 4 position of the phenyl ring (**11**, **12**, **13**). When the phenyl is substituted with a 4-methoxy, there is no potency gain (**14**) and larger substitutions lead to a decrease in potency (**15**).

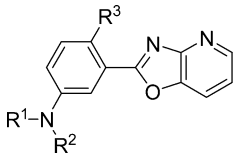
Table 2 shows the effect of substituting or replacing the 2-furanoyl group with other moieties. While methylation of the 2-furan ring at the 3- and 5-position is tolerated (**16**, **17**), larger substitutions such as benzofuran (**18**) are not tolerated. The 3-furoyl group is equally tolerated (**19**), but other amides including aliphatics, aromatics, and heteroaromatics lead to a mild to profund decline in activity (**20**−**29**) including closely related tetrahydrofuran (**22**) and oxazole (**24**). Also inactives are sulfonamides (**30**, **31**), while thioamide retains some activity (**32**). Interestingly, while methylation at the amide nitrogen is not tolerated (**33**), acylation is (**34**, **35**). Complete removal of the amide (**36**) and either monobenzylation or dibenzylation lead to loss and decrease of activity, respectively (**37**, **38**). In an effort to identify additional R^1 moieties with increased metabolic stability, the replacement of the furanoyl amide with ureas was explored. Terminally monosubstituted and disubstituted ureas (**39**−**44**) were investigated, and we discovered that some of the disubstutued ureas are tolerated with the pyrrolidine urea (**43**) showing slightly better potency than the similar 2-furanoyl derivatives.

Additional structure−activity relationships came from a study of the analogues shown in Scheme 3 where changes at the core were explored. The results show that the oxazolopyridine bicycle cannot be easily replaced with a monocycle (**45**−**49**). Moreover, the N at the 4-position of the oxazolopyridines is essential for activity, as it cannot be replaced with C or moved to the 5-position (**50**−**57**).

An alternative to the oxazolopyridine core came by replacing the oxygen with a nitrogen in the bicycle, leading to the imidazopyridine core (Table 3). These analogues were made as

dx.doi.org/10.1021/jm401178t | J. Med. Chem. XXXX, XXX, XXX−XXX

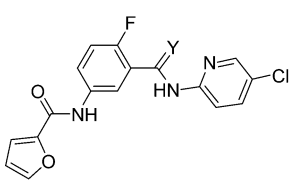
Table 2. 2-(3-Aminophenyl)oxazolopyridines Containing Variations of the N-Linked Group[a]



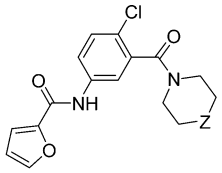
compd	R^1	R^2	R^3	EC_{50} [b] (μM)	CC_{50} [c] (μM)
16	5-methylfuran-2-carbonyl	H	F	0.20	>50[d]
17	3-methylfuran-2-carbonyl	H	F	0.1	>50[d]
18	benzofuran-2-carbonyl	H	F	>10	>50[d]
19	3-furanoyl	H	F	0.15	>50[d]
20	acetyl	H	F	>20	>50[d]
21	trifluoroacetyl	H	Cl	(>50)	
22	tetrahydrofuran-2-carbonyl-	H	F	>10	
23	benzoyl	H	Cl	7.1	>50[d]
24	oxazole-5-carbonyl-	H	F	1.9	>50[d]
25	2-thiophenoyl	H	Cl	1.5	>50[d]
26	3-pyridinecarbonyl-	H	F	7.0	>50[d]
27	1-methyl-1*H*-imidazole-5-carbonyl	H	F	>20	>50[d]
28	pyrazine-2-carbonyl-	H	F	0.9	>50[d]
29	*N*-methylpyrrole-2-carbonyl-	H	F	1.1	>50[d]
30	methylsulfonyl	H	Cl	6.1	>50[d]
31	phenylsulfonyl	H	Cl	>20	>50[d]
32	2-furancarbothioyl-	H	F	0.41	>50[d]
33	2-furanoyl	methyl	F	>10	>50[d]
34	2-furanoyl	2-acetyl	F	0.5	
35	2-furanoy	2-furanoyl	F	0.12	>50[d,e,f]
36	H	H	Cl	>20	
37	benzyl	H	F	>20	
38	benzyl	benzyl	F	1.1	>50[d]
39	methylcarbamoyl	H	F	3.8	>50[d]
40	isopropylcarbamoyl-	H	Cl	1.0	>50[d]
41	phenylcarbamoyl-	H	Cl	12.0	>50[d]
42	dimethylcarbamoyl-	H	Cl	0.4	>50[d]
43	1-pyrrolidinoyl-	H	Cl	0.09	>50[e,f]
44	1-piperidinoyl-	H	Cl	1.9	

[a]Controls are shown at bottom of Table 1. [b]Concentration of compound required to inhibit growth by 50% (EC_{50}) of *T. brucei* strain BF427 or *T. brucei rhodesiense* (numbers in parentheses). [c]Concentration of compound required to inhibit growth by 50% (CC_{50}) of mammalian cell lines. [d]Rat myoblasts (L6). [e]Human lymphoblasts (CRL-8155). [f]Human hepatocytes (HepG2).

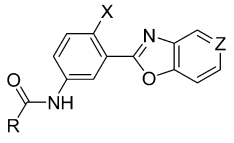
Scheme 3



45 Y = O; EC_{50} = 7 μM
46 Y = H; EC_{50} > 10 μM



47 Z = O; EC_{50} > 20 μM
48 Z = NH; EC_{50} > 20 μM
49 Z = N-CH_3; EC_{50} > 20 μM



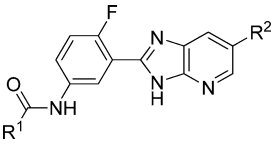
50-56 Z= CH; X=Cl; R = 2-furan, Ph, 2-F-Ph, 2-Me-Ph, 3-F-Ph, 3-Me-Ph, cylohexyl; EC_{50} > 20 μM
57 Z= N; X=F; R = 2-furan; EC_{50} > 20 μM

in Scheme 1 starting from the appropriately substituted 2,3-diaminopyridines (not shown). Compound **58** showed comparable potency to **2**, and chloro and fluoro substitution at C6 (R^2) of the core was also beneficial (**59**, **60**). As expected from the previous SAR, chloro substitution at C5 (**61**) led to an inactive compound and it was shown that chloro substitution was also tolerated at C7 (**62**). In general the SAR for the imidazopyridines was found to be similar to that for the oxazolopyridines (only selected compounds are shown). The pyrrolidine urea was a good replacement for the furanoyl amide, as was the case for the oxazolopyridines (**63**). An expected improvement in potency was further achieved by adding a phenyl group at the 6 position of the core which led to compound **64** with an EC_{50} against *T. brucei* of 2 nM. A wide variety of substituents can be added to this 6-phenyl group, and the phenyl can also be replaced with several heterocyclic groups with maintenance of anti-parasite potency (**65**–**86**).

Compound **64** was studied in detail. It produced a very steep dose response curve for antiparasitic activity on *T. brucei* in vitro with an EC_{90} of 2 nM, and complete growth inhibition was

dx.doi.org/10.1021/jm401178t | *J. Med. Chem.* XXXX, XXX, XXX–XXX

Table 3. 2-(3-Aminophenyl)imidazopyridines Containing a N-Linked 2-Furanoyl Group[a]



compd	R^1	R^2	EC_{50} [b] (μM)	CC_{50} [c] (μM)
58	2-furanyl	H	0.2	
59	2-furanyl	Cl	0.07	>50,[d,f] 1.7[e]
60	2-furanyl	F	0.2	
61	2-furanyl	5-Cl	10	
62	2-furanyl	7-Cl	0.12	
63	*N*-pyrrolidinyl	Cl	0.05	15,[d] >50[e,f]
64	*N*-pyrrolidinyl	phenyl	0.002	24,[e] 30[f]
65	*N*-pyrrolidinyl	3-methoxyphenyl	0.01	3.6,[e] 35[f]
66	*N*-pyrrolidinyl	2-methoxyphenyl	0.005	13,[e] >50[f]
67	*N*-pyrrolidinyl	3-Cl-phenyl	0.002	2.8,[e] >50[f]
68	*N*-pyrrolidinyl	2-chlorophenyl	0.005	
69	*N*-pyrrolidinyl	3-acetylphenyl	0.003	
70	*N*-pyrrolidinyl	3-Me-phenyl	0.002	5,[e] 10[f]
71	*N*-pyrrolidinyl	3-trifluoromethoxyphenyl	0.03	11,[e] >50[f]
72	*N*-pyrrolidinyl	3-methyl-4-fluorophenyl	0.02	>50[e]
73	*N*-pyrrolidinyl	3-NH_2-phenyl	0.01	5,[e] 40[f]
74	*N*-pyrrolidinyl	3-furanyl	0.004	25,[e] >50[f]
75	*N*-pyrrolidinyl	3-thiophenyl	0.002	6,[e] >50[f]
76	*N*-pyrrolidinyl	2-thiophenyl	0.004	9[e]
77	*N*-pyrrolidinyl	3-pyridyl	0.005	14,[e] >50[f]
78	*N*-pyrrolidinyl	5-(2-chloropyridyl)	0.01	3.8,[e] >50[f]
79	*N*-pyrrolidinyl	4-(2-chloropyridyl)	0.004	>50[e,f]
80	*N*-pyrrolidinyl	5-(3-methylpyridyl)	0.01	>50[e,f]
81	*N*-pyrrolidinyl	5-(2-methoxypyridyl)	0.005	>50[e,f]
82	*N*-pyrrolidinyl	5-(3-pyrrolidino)	0.02	8,[e] >50[f]
83	*N*-pyrrolidinyl	5-(3-chloropyrimidinyl)	0.005	38,[e] >50[f]
84	*N*-pyrrolidinyl	5-pyrimidinyl	0.03	12,[e] >50[f]
85	*N*-pyrrolidinyl	5-(2-methoxypyrimidinyl)	0.06	3.9,[e] 9.8[f]
86	*N*-pyrrolidinyl	5-(2-chloropyrimidinyl)	0.04	14.3[d]

[a]Controls are shown at bottom of Table 1. [b]Concentration of compound required to inhibit growth by 50% (EC_{50}) of *T. brucei* strain BF427 or *T. brucei rhodesiense* (numbers in parentheses). [c]Concentration of compound required to inhibit growth by 50% (CC_{50}) of mammalian cell lines. [d]Rat myoblasts (L6). [e]Human lymphoblasts (CRL-8155). [f]Human hepatocytes (HepG2).

observed. Cytotoxicity toward mammalian cells was minimal (24−30 μM, Table 3). Along with other analogues, it was characterized in in vitro ADME assays (Supporting Information Table 1) and showed aqueous solubility at pH 7.4 of 8.1 μM which increased to 32 μM at pH 2.0, presumably because of imidazole ring protonation. Compound **64** was metabolized upon incubation with mouse and human liver microsomes in vitro with half-lives of 10 and 31 min, respectively. This represented a significant improvement in metabolic stability over the initial lead **1**, presumably because the latter is more sensitive to hydrolysis. Compound **64** was a modest inhibitor of hepatic cytochrome P450 3A4-isoform in vitro with an IC_{50} of 1.5 μM. Oral gavage dosing of **64** in mice at 50 mg/kg gave a maximal concentration in the plasma (C_{max} of 4.3 μM) with a time to C_{max} (T_{max}) of 30 min. The plasma concentration versus time curve is provided as Figure 1 of Supporting Information. The area under the plasma concentration versus time curve (AUC) after oral dosing was 1125 min·μM. The half-life for elimination from the plasma was 204 min, and the plasma clearance (Cl_p) was 14.8 mL min^{-1} kg^{-1}. The in vivo clearance was substantially lower than what was expected from the in vitro metabolism in liver microsmes likely because of the high plasma protein binding (95% in mouse plasma). Compound **64** showed excellent permeability across MDCK-MDR1 cells and was not a significant substrate for the MDR1 efflux pump (P_{app} = 401 and 503 (+MDR1 inhibitor) nm/s). Consistent with this is the significant distribution of **64** into mouse brain, with a brain-to-plasma concentration ratio 60 min after ip dosing of 0.5 and 1.1 (two independent mouse experiments). PK studies of **64** in rats are summarized in Supporting Information Figure 2 and Table 2. Briefly, rats were dosed by iv injection and by oral gavage and the bioavailability of **64** was 35% upon delivery of a 21.3 mg/kg oral dose. The apparent volume of distribution during steady state (V_{SS}) was 2.4 L/kg. PK data in mice for additional compounds are provided in Supporting Information Table 2.

Analysis of Efficacy of 64 in Parasite-Infected Mice. Given the good physiochemical and PK properties of **64**, we decided to test it for efficacy in a mouse model of acute HAT infection. Mice were injected with *T. brucei rhodesiense* on day 0, and **64** was given by oral gavage 48 h after parasite injection. Mice were dosed for a total of 5 days using the dosing regimens shown in Table 4. Parasitemia in the blood and survival were followed out to 60 days. Cures in all mice were obtained with once and twice dosing at 20 and 50 mg/kg (Table 4). With 5 mg/kg

dx.doi.org/10.1021/jm401178t | J. Med. Chem. XXXX, XXX, XXX−XXX

Table 4. Efficacy of 64 in *T. brucei* Infected Mice[a]

expt	dose (mg/kg)	doses per day	cured	day of euthanasia[b]
1	50	2	5/5	60
1	20	2	5/5	60
1	5	2	5/5	60
1	50	1	5/5	60
1	Veh	2	0/5	4
2	20	1	5/5	60
2	5	2	5/5	60
2	5	1	2/5	13, 18, 27, 60, 60
2	2.5	2	2/5	16, 16, 20, 60, 60
2	1	2	0/5	4, 4, 9, 11, 13
2	Veh	2	0/5	4

[a]Mice were infected on day 0 and treated for 5 consecutive days beginning 48 h after infection when patent parasitemia was detectable. [b]Mice that cleared parasites and remained parasite free through day 60 after infection were declared cured.

dosing, all mice were cured with twice daily dosing, and 2 of 5 mice were cured with once daily dosing. Twice daily dosing at 2.5 mg/kg cured 2 of 5 mice, and twice daily dosing with 1 mg/kg cured 0 of 5 mice. These results show that **64** is highly efficacious in a mouse model of acute trypanosomiasis.

Mice dosed with **64** orally at 50 mg/kg twice per day for 14 days had no difference in weight change compared to vehicle treated mice and no observed clinical toxicity, indicating good tolerability of the compound above fully efficacious doses.

CONCLUDING REMARKS

A high throughput phenotypic screen successfully identified the oxazolopyridines as antiparasitic agents against *T. brucei*. Medicinal chemistry optimization to improve potency and PK properties led to the discovery of the imidazopyridines as a novel class of potent antiparasitic *T. brucei* compounds. The substituted 2-phenylimidazopyridine **64** is a highly potent antiparasitic agent against *T. brucei* and cures parasite-infected mice when given orally when dosed down to 2.5 mg/kg. Thus, **64** represents a significant lead compound for the development of a new drug class to treat HAT. Studies are in progress to further optimize the druglike properties of **64** and to explore its potential in the treatment of second stage (central nervous system involvement) HAT. Additional toxicology studies are also in progress as part of a preclinical drug development program.

EXPERIMENTAL SECTION

The synthesis of all compounds is given in Supporting Information. All target compounds were purified by preparative HPLC and are judged to be >95% pure.

Phenotypic Screen for *T. brucei* Growth Arrest Compounds. To screen compound libraries for antiparasitic activity on *T. brucei*, we employed the bloodstream form of the well-characterized *T. brucei* isolate, Lister 427. During the screen, the parasite was grown in 1536-well plates in 5.5 μL of HMI-9 medium in the presence of library compounds. All wells including negative controls contained a final of 0.4% DMSO. After incubation of the plates at 37 °C for 48 h, the parasite density was determined using the CellTiter-Glo reagent (Promega), a firefly luciferase assay system that measures the amount of cellular ATP present in plate wells.

Solubility Measurement. A glass tube containing 8 mL of buffer (20 mM ammonium acetate, pH 7.4) was placed in a 37 °C water bath. The test compound was added from a DMSO stock solution to give the desired concentration of compound with 1% DMSO final concentration. The tube was incubated at 37 °C for 5−10 min. Sufficient compound was used to give a visibly cloudy solution. After incubation overnight at 37 °C, the tube was centrifuged at 1000*g* for 20 min at 37 °C. An amount of 7 mL of supernatant was transferred to a new tube, and buffer was removed in a Speed-Vac concentrator. Then 1 mL of distilled water was added to the residue, and the sample was concentrated to dryness as above. To the residue was added 0.3 mL of water and 0.3 mL of methanol. The amount of compound was determined by HPLC using a C18 reverse-phase column. HPLC of a standard amount of test compound was used to convert the UV peak area to moles of compound. In some cases, solubility was carried out in 0.01 M HCl.

Distribution of Compounds between Mouse Plasma and Brain. Mice (in groups of 3) were injected with test compounds (5 mg/kg ip) and sacrificed at the indicated times for collection of plasma and brain. Compound was dissolved in 0.4 mL of dosing solution (7% Tween 80, 3% ethanol, 5% DMSO, 0.9% saline) for ip injections. The brains were weighed and immediately frozen, then later homogenized in acetonitrile using a Dounce homogenizer. Prior to animal studies, recovery of test compound was carried out by adding a known amount to a mouse brain in the test extraction solvent and performing the homogenization. Compound recovery was determined by liquid chromatography/tandem mass spectrometry analysis relative to a standard compound amount. Blood was taken from the same mice in heparinized capillary tubes (Fisherbrand) for determination of compound concentration in plasma. The concentration of compound in the brain was obtained by dividing the moles of compound in the brain by the brain volume (obtained from the brain weight assuming 1 g is 1 mL) and correcting for the brain vasculature volume of 2.5% by weight.

In Vitro Parasite Growth Arrest Assay. Compounds were tested for antiparasitic activity on *T. brucei brucei* (strain BF427) as described.[13] Cells were tested in triplicate in the presence of serial dilutions of compound, and growth is quantified with AlamarBlue.[14] Pentamidine isothionate (Aventis, Dagenham, U.K.) was included in each assay as a positive control (EC_{50} = 1.2 ± 0.3 nM).

Mammalian Cell Cytotoxicity Assay. Selected compounds were tested for cytotoxicity at University of Washington against CRL-8155 cells (human lymphoblasts) and HepG2 cells (human hepatocellular carcinoma) as previously described (PMID, 22720744). Briefly, cells were exposed to serial dilutions of compounds for 48 h and cytotoxicity was assayed using the AlamarBlue (Life Technologies). Each dilution was assayed in quadruplicate with standard error of the mean values averaging <15%. Concentrations causing 50% growth inhibition (CC_{50}) were calculated by nonlinear regression using GraphPad Prism software (San Diego, CA). L6 rat myoblasts were tested by similar methods at the Swiss TPH as previously described (PMID, 12512078).

In Vitro Liver Microsome Studies. Liver microsome metabolism studies were carried out as described.[15,16] Compounds were incubated with liver microsomes (mouse or human), and samples were extracted at time points for liquid chromatography/tandem mass spectrometry analysis. Reference drugs dextromethorphan and testosterone were used to standardize each assay.

Inhibition of Liver Cytochrome P450s. Inhibition of human cytochrome P450 (3YP3A4 isoform) by test compounds was measured with a commercial kit (BD Biosciences) according to the manufacturer's instructions.

Permeability across Monolayers of MDCKII-MDR1 Cells. This assay uses Madin−Darby canine kidney cells that were transfected with the human MDR1 (P-gp) gene.[10−12] Permeability across these monolayers was measured in triplicate as described.[17] The assay was performed with and without the addition of GF-120918, an inhibitor of the MDR1 efflux pump to determine if the compound is a pump substrate. Propranolol was used as a permeable, non-MDR1 substrate control, and amprenavir was used as a permeable, MDR1 substrate control.

PK Studies in Mice. Test compound was administered to mice by oral gavage followed by blood sampling at intervals of 30, 60, 120, 180, 240, and 360 min. Compound was dosed orally at 50 mg/kg in 0.2 mL of dosing solution (7% Tween 80, 3% ethanol, 5% DMSO, 0.9% saline).

Experiments were performed with groups of three mice per compound as published.[17,18] Plasma was separated and extracted with

dx.doi.org/10.1021/jm401178t | J. Med. Chem. XXXX, XXX, XXX−XXX

acetonitrile for measurements of compound concentrations by liquid chromatography/tandem mass spectrometry.

PK Studies in Rats. Test compound was administered to Sprague–Dawley jugular canulated rats (Charles River) by either oral gavage or iv injection followed by blood sampling from the jugular vein at 5, 15, 30, 60, 90, 120, 240, 360, 480, 720, and 1440 min. The oral dose was administered to each rat at 20 mg/kg at time = 0 in a 1 mL volume of dosing solution (7% Tween 80, 3% EtOH, 5% DMSO, 09.% saline). The iv injections were administered at 5 mg/kg from time = 0 to time = 3 min in a 1 mL volume of dosing solution, and blood was sampled at the same time points via the jugular vein. Experiments were performed with groups of two rats each for the oral and iv dosing. Plasma was separated and extracted with acetonitrile for measurements of compound concentrations by liquid chromatography/tandem mass spectrometry. PK calculations were performed using Phoenix WinNonlin software (Pharsight).

Plasma Protein Binding. Dialysis membrane sheet (MW cutoff 3.5 kDa, HTDialysis catalog no. 1135) was soaked for 1 h in distilled water and then in 20% ethanol for 30 min. The membrane was clamped between two Teflon plates containing a row of opposing wells. Test compound in DMSO was added to 0.12 mL of serum to give 9 μM. A small aliquot was taken as a 100% recovery standard, and the solution was placed on one side of the membrane. The well on the other side of the membrane was charged with an equal volume of dialysis buffer (14.2 g/L Na_2HPO_4, 8,77 g/L NaCl, pH 7.4), and the block was placed on an orbital shaker for 18 h at 37 °C. An aliquot was taken from each side of the membrane. One-fourth volume of acetonitrile was added to each aliquot, and the samples were centrifuged to precipitate protein. Test compound in the supernatants was quantified by liquid chromatography/tandem mass spectrometry to determine the concentration on each of the membrane and the total recovery of test compound from the device. A control dialysis was carried out with dialysis buffer on both sides of the membrane to ensure that equilibration of test compound across the membrane was achieved. The fraction of compound bound to protein was calculated as bound/(unbound + bound), and the value was taken only if compound recovery was >50% (recovery was 84% for **64**).

Anti-Parasite Efficacy Studies in Mice. Studies were carried out using the standard operating procedure used by WHO screening centers.[19] The studies were done in compliance with University of Washington IACUC approved protocol. Female Swiss-Webster mice age 6–8 weeks in groups of 5 were infected with 2×10^4 *T. brucei rhodesiense* STIB 900 strain on day 0, then administered compound (in three dose levels) or vehicle for 5 days. Treatments were administered orally at a dose and schedule anticipated to maintain plasma concentrations well above the EC_{50} (see Table 4). The first dose was 48 h after parasite injection, and dosing was 12 or 24 h apart. Dosing was carried out by oral gavage as for PK studies (see above). Parasitemia was monitored for 60 days by microscopic analysis of blood collected from tail bleeds. Cures were defined by sustained clearance of microscopic parasitemia through the end of the 60-day observation period. Mice were euthanized when high levels of parasitemia were evident on peripheral blood slides.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of compounds and pharmacokinetics data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*F.S.B.: phone, 206-616-9214; e-mail, fbuckner@u.washington.edu.

*M.H.G.: phone, 206-543-7142; e-mail, gelb@chem.washington.edu.

Present Address

[∞]A.K.C.: California Institute for Biomedical Research, 11119 N. Torrey Pines Road, No. 100, La Jolla, CA 92037.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (Grants AI054384, AI070218, and AI106850) and The Consortium for Parasitic Drug Development (CPDD).

■ ABBREVIATIONS USED

AUC, area under the plasma concentration–time curve; Cl_p, plasma clearance; HAT, human African trypanosomiasis; MDCK, Madin–Darby canine kidney cells; MDCK-MDR1, MDCK cells transfected with human P-glycoprotein; PK, pharmacokinetics

■ REFERENCES

(1) Lutje, V.; Seixas, J.; Kennedy, A. Chemotherapy for second-stage human African trypanosomiasis. *Cochrane Database Syst. Rev.* **2010**, CD006201.

(2) Baker, N.; de Konng, H. P.; Maser, P.; Horn, D. Drug resistance in African trypanosomiasis: the melarsoprol and pentamidine story. *Trends Parasitol.* **2013**, *29*, 110–118.

(3) Brun, R.; Don, R.; Jacobs, R. T.; Wang, M. Z.; Barrett, M. P. Development of novel drugs for African trypanosomiasis. *Future Microbiol.* **2011**, *6*, 677–791.

(4) Harstad, T. W.; Little, B. B.; Bawdon, R. E.; Knoll, K.; Roe, D.; Gilstrap, L. C. Pharmacokinetics of pentamidine in Sprague-Dawley rats in late pregnancy. *Am. J. Obstet. Gynecol.* **1990**, *163*, 912–916.

(5) Freeman, S. J.; Lloyd, J. B. Evidence that suramin and aurothiomalate are teratogenic in rat by disturbing yolk sac-mediated embryonic protein nutrition. *Chem.-Biol. Interact.* **1986**, *58*, 149–160.

(6) Torreele, E.; Bourdin, T. B.; Tweats, D.; Kaiser, M.; Brun, R.; Mazue, G.; Bray, M. A.; Pecoul, B. Fexinidazole—a new oral nitroimidazole drug candidate entering clinical development for the treatment of sleeping sickness. *PLoS Neglected Trop. Dis.* **2010**, *4*, e293.

(7) Jacobs, R. T.; Nare, B.; Wring, S. A.; Orr, M. D.; Chen, D.; Sligar, J. M.; Jenks, M. X.; Noe, R. A.; Bowling, T. S.; Mercer, L. T.; Rewerts, C.; Gaukel, E.; Owens, J.; Parham, R.; Randolph, R.; Beaudet, B.; Bacchi, C. J.; Yarlett, N.; Plattner, J. J.; Freund, Y.; Ding, C.; Akama, T.; Zhang, Y. K.; Brun, R.; Kaiser, N.; Scandale, I.; Don, R. SCYX-7158, an orally-active benzoxaborole for the treatment of stage 2 human African trypanosomiasis. *PLoS Neglected Trop. Dis.* **2011**, *5*, e1151.

(8) Harrill, A. h.; Desmet, K. D.; Wolf, K. K.; Bridges, A. S.; Eaddy, J. S.; Kurtz, C. LO.; Hall, J. E.; Paine, M. F.; Tidwell, R. R.; Watkins, P. B. A mouse diversity panel approach reveals the potential for clinical kidney injury due to DB289 not predicted by classical rodent models. *Toxicol. Sci.* **2012**, *130*, 416–426.

(9) Pammolli, F.; Magazzini, L.; Riccaboni, M. The productivity crisis in pharmaceutical R & D. *Nat. Rev. Drug Discovery* **2011**, *10*, 428–438.

(10) Evers, R.; Cnubben, N. H.; Wijnholds, J.; van Deemter, L.; van Bladeren, P. J.; Borst, P. Transport of glutathione prostaglandin A conjugates by the multidrug resistance protein 1. *FEBS Lett.* **1997**, *419*, 112–116.

(11) Shibata, S.; Gillespie, J. R.; Ranade, R. M.; Koh, C. Y.; Kim, J. E.; Laydbak, J. U.; Zucker, F. H.; Hol, W. G.; Verlinde, C. L.; Buckner, F. S.; Fan, E. Urea-based inhibitors of *Trypanosoma brucei* methionyl-tRNA synthetase: selectivity and in vivo characterization. *J. Med. Chem.* **2012**, *55*, 6342–6351.

(12) Hyafil, F.; Vergely, C.; Du, V. P.; Grand-Perret, T. In vitro and in vivo reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. *Cancer Res.* **1993**, *53*, 4595–4602.

(13) Aronov, A. M.; Suresh, S.; Buckner, F. S.; Van Voorhis, W. C.; Verlinde, C. L. M.; Opperdoes, F. R.; Hol, W. G. J.; Gelb, M. H. Structure-based design of submicromolar, biologically active inhibitors of trypanosomatid glyceraldehyde-3-phosphate dehydrogenase. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4273–4278.

(14) Raz, B.; Iten, M.; Buhler-Grether, Y.; Kaminsky, R.; Brun, R. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T.b. rhodesiense* and *T.b. gambiense*) in vitro. *Acta Tropica* **1997**, *68*, 139−147.
(15) Bendale, P.; Olepu, S.; Suryadevara, P. K.; Bulbule, V.; Rivas, K. L.; Nallan, L.; Smart, B. P.; Yokoyama, K.; Ankala, S.; Pendyala, P. R.; Floyd, D.; Lombardo, L. J.; Floyd, D.; Williams, D. K.; Buckner, F. S.; Chakrabarti, D.; Verlinde, C. L. M. J.; Van Voorhis, W. C.; Gelb, M. H. Second generation tetrahydroquinoline-based protein farnesyltransferase inhibitors as antimalarials. *J. Med. Chem.* **2007**, *50*, 4585−4605.
(16) Van Voorhis, W. C.; Rivas, K.; Bendale, P.; Nallan, L.; Hornéy, C.; Barrett, L. K.; Ankala, S.; Hucke, O.; Verlinde, C. L. M. J.; Chakrabarti, D.; Strickland, C.; Yokoyama, K.; Buckner, F. S.; Hamilton, A.; Williams, D. K.; Lombardo, L. J.; Floyd, D.; Gelb, M. H. Efficacy, pharmacokinetics, and metabolism of tetrahydroquinoline inhibitors of *Plasmodium falciparum* protein farnesyltransferase. *Antimicrob. Agents Chemother.* **2007**, *51*, 3659−3671.
(17) Suryadevara, P. K.; Olepu, S.; Lockman, J. W.; Ohkanda, J.; Karimi, M.; Verlinde, C. L.; Kraus, J. M.; Schoepe, J.; Van Voorhis, W. C.; Hamilton, A. D.; Buckner, F. S.; Gelb, M. H. Structurally simple inhibitors of lanosterol 14alpha-demethylase are efficacious in a rodent model of acute Chagas disease. *J. Med. Chem.* **2009**, *52*, 3703−3715.
(18) Kraus, J. M.; Verlinde, C. L. M. J.; Karimi, M.; Lepesheva, G. I.; Gelb, M. H.; Buckner, F. S. Rational modification of a candidate cancer drug for use against Chagas disease. *J. Med. Chem.* **2009**, *52*, 1639−1647.
(19) Dardonville, C.; Barrett, M. P.; Brun, R.; Kaiser, M.; Tanious, F.; Wilson, W. D. DNA binding affinity of bisguanidine and bis(2-aminoimidazoline) derivatives with in vivo antitrypanosomal activity. *J. Med. Chem.* **2006**, *49*, 3748−3752.
(20) Ferrins, L.; Rahmani, R.; Sykes, M. L.; Jones, A. J.; Avery, V. M.; Teston, E.; Almohaywi, B.; Yin, J.; Smith, J.; Hyland, C.; White, K. L.; Ryan, E.; Campbell, M.; Charman, S. A.; Kaiser, M.; Baell, J. B. 3-(Oxazolo[4,5-b]pyridin-2-yl)anilides as a novel class of potent inhibitors for the kinetoplastid Trypanosoma brucei, the causative agent for human African trypanosomiasis. *Eur. J. Med. Chem.* **2013**, *66*, 450−465.

NOTE ADDED IN PROOF

Just prior to the submission of our manuscript, Baell and co-workers reported on the initial structure−activity relationships centered around compound **1**. This is the result of an independent phenotypic screen to discover anti-*T. brucei* scaffolds.[20]