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Urea Derivatives of 2-Aryl-benzothiazol-5-amines: A New Class of Potential Drugs for Human African Trypanosomiasis

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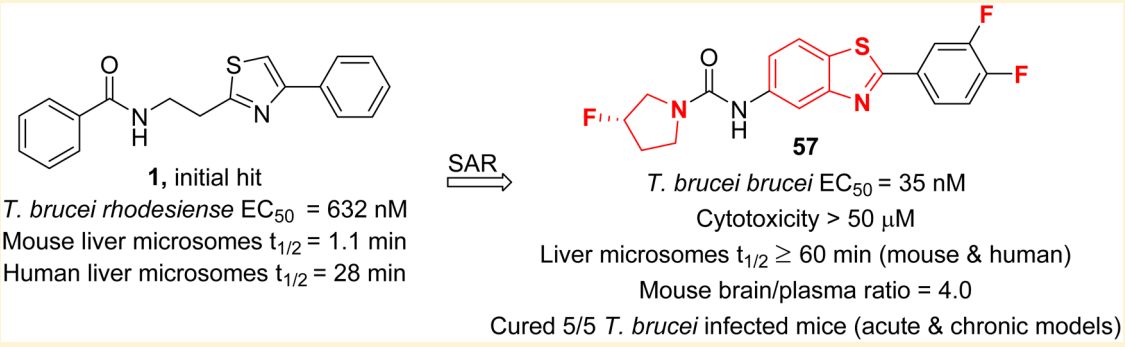
Supporting Information



ABSTRACT: A previous publication from this lab (Patrick, et al. *Bioorg. Med. Chem.* **2016**, *24*, 2451−2465) explored the antitrypanosomal activities of novel derivatives of 2-(2-benzamido)ethyl-4-phenylthiazole (**1**), which had been identified as a hit against *Trypanosoma brucei*, the causative agent of human African trypanosomiasis. While a number of these compounds, particularly the urea analogues, were quite potent, these molecules as a whole exhibited poor metabolic stability. The present work describes the synthesis of 65 new analogues arising from medicinal chemistry optimization at different sites on the molecule. The most promising compounds were the urea derivatives of 2-aryl-benzothiazol-5-amines. One such analogue, (*S*)-2-(3,4-difluorophenyl)-5-(3-fluoro-*N*-pyrrolidylamido)benzothiazole (**57**) was chosen for in vivo efficacy studies based upon in vitro activity, metabolic stability, and brain penetration. This compound attained 5/5 cures in murine models of both early and late stage human African trypanosomiasis, representing a new lead for the development of drugs to combat this neglected disease.

INTRODUCTION

Human African trypanosomiasis (HAT) is an insect vectored disease caused by infection of either *Trypanosoma brucei gambiense* or *Trypanosoma brucei rhodesiense*. Despite the decreased incidence of the disease during the first decade of this century,[1] the World Health Organization (WHO) currently estimates 20000 actual cases with 65 million people at risk and that over 98% of cases are due to *T. b. gambiense* [http://www.who.int/mediacentre/factsheets/fs259/en/]. The disease progresses from a first stage (hemolymphatic) infection to second stage (CNS) infection after the parasites have crossed the blood−brain barrier (BBB). The *T. b. gambiense* infection is characterized by a slow progression of months or even years from the early to late stage infection, while the *T. b. rhodesiense* infection progresses more rapidly. Late stage HAT is always fatal in untreated patients regardless of the causative parasite.[2,3]

The need for new anti-HAT drugs persists, as current drugs are few, antiquated, toxic, prone to resistance, and require parenteral administration. Treatments for *T. b. rhodesiense* infections are limited to suramin (a polysulfonated naphthylurea) for early stage and melarsoprol (an organoarsenical) for late stage disease. First line treatments for *T. b. gambiense* infections include pentamidine (an aromatic diamidine) for early stage and nifurtimox−eflornithine combination therapy (NECT) for late stage disease. Neither drug currently used against early stage disease is able to cross the BBB.[2,4,5]

Pafuramidine,[6,7] an oral prodrug of 2,5-bis(4-amidinophenyl)furan (furamidine),[8,9] advanced to phase III clinical trials against early stage HAT but was abandoned after the observation of nephrotoxicity in an expanded phase I trial.[10] The nitrated imidazole derivative fexinidazole[11] and the benzoxaborole SCYX-7158[12] have also entered clinical trials in recent years. The high attrition rate of clinical trial candidates (around 90% of those entering phase I)[13] underscores the need for a pipeline of new trypanocidal compounds.

Following the failure of pafuramidine in clinical trials, the emphasis of this lab has shifted from amidines to other classes

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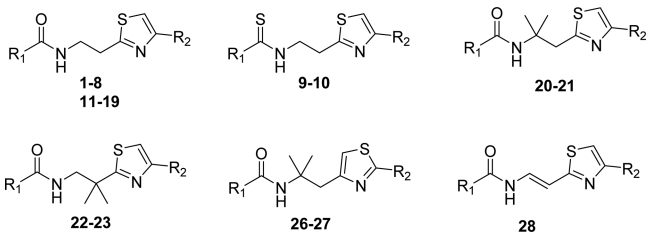
Table 1. Antitrypanosomal Activities of Thiazole Derivatives 1−28



compd	R_1	R_2	*T. brucei* EC_{50} (μM)[a,b]	*T. brucei* EC_{90} (μM)[c,d]
1	phenyl	phenyl	0.191 (0.632)	0.340
2	pyrrolidin-1-yl	phenyl	0.0848 (0.125)	0.188
3	pyrrolidin-1-yl	2-fluorophenyl	0.0322 (0.0495)	0.0868
4	piperidin-1-yl	phenyl	0.0194 (0.0204)	0.0336
5	piperidin-1-yl	3-fluorophenyl	0.0113 (0.0090)	0.0163
6	piperidin-1-yl	2,4-difluorophenyl	0.0094 (0.0099)	0.0117
7	piperidin-1-yl	3,4-difluorophenyl	0.0105 (0.0097)	0.0203
8	azepan-1-yl	phenyl	0.110 (0.0516)	0.236
9	phenyl	phenyl	1.44	2.63
10	piperidin-1-yl	phenyl	0.0243	0.0424
11	2-methylpiperidin-1-yl	3-fluorophenyl	0.0370	0.0586
12	*cis*-2,6-dimethylpiperidin-1-yl	3-fluorophenyl	0.399	0.564
13	4-oxopiperidin-1-yl	3-fluorophenyl	0.667	0.813
14	3-fluoropiperidin-1-yl	3-fluorophenyl	0.0285	0.0557
15	3,3-difluoropiperidin-1-yl	3-fluorophenyl	0.108	0.168
16	4-fluoropiperidin-1-yl	3-fluorophenyl	0.0366	0.0575
17	4,4-difluoropiperidin-1-yl	3-fluorophenyl	0.164	0.230
18	1,2,3,4-tetrahydroquinolin-1-yl	3-fluorophenyl	5.95	14.0
19	1,2,3,4-tetrahydroisoquinolin-2-yl	3-fluorophenyl	9.76	13.8
20	phenyl	phenyl	>10	>10
21	phenyl	3-fluorophenyl	>10	>10
22	phenyl	phenyl	>10	>10
23	phenyl	phenyl	1.30	2.4
24	phenyl	phenyl	>10	>10
25	pyrrolidin-1-yl	phenyl	>10	>10
26	piperidin-1-yl	phenyl	7.84	9.9
27	azepan-1-yl	phenyl	>10	>10
28	piperidin-1-yl	3-fluorophenyl	0.0946	0.216

[a]Concentration of compound required to inhibit growth by 50% (EC_{50}) against *T. b. brucei* BF427 or *T. b. rhodesiense* STIB900 (numbers in parentheses, data reported previously[14]). [b]Control for *T. b. brucei* BF427 EC_{50} assay average ± standard error of the mean (SEM): pentamidine (1.05 ± 0.12 nM, n = 30). [c]Concentration of compound required to inhibit growth by 90% (EC_{90}) against *T. b. brucei* BF427. [d]Control for *T. b. brucei* BF427 EC_{90} average ± SEM: pentamidine (3.54 ± 0.36 nM (n = 30).

of compounds. A phenotypic high-throughput screen (HTS) of a 700000 compound library performed by the Genomics Institute of the Novartis Research Foundation (GNF)[3] led to the identification of over 1000 compounds (grouped into over 100 distinct scaffolds) that inhibited growth of *T. brucei* in vitro at concentrations below 3.6 μM and were nontoxic to mammalian cells (Huh7). Promising results based on the synthesis and in vitro activity of analogues of one of these scaffolds, an oxazolopyridine derivative, have been reported.[3] Another hit from this HTS was 2-(2-benzamido)ethyl-4-phenylthiazole (**1**, Table 1). A number of analogues of this compound were highly potent against the parasite but had poor metabolic stability.[14] This paper describes continued work on the latter scaffold.

RESULTS AND DISCUSSION

Chemistry. The previous report describes the syntheses of the initial hit (**1**, Table 1) and over 70 analogues (including **2**−**8**).[14] Traditional medicinal chemistry approaches for hit to lead optimization were followed as the biochemical targets of these compounds were unknown. These derivatives had alterations primarily at either of the two terminal rings, but the SAR of the internal portion of the molecule remained largely unexplored. The present work explores modification of other sites of the molecule with the syntheses of 65 novel compounds. The new compounds **9** and **10** are thiocarbonyl analogues of existing compounds **1** and **4**. Analogues **11**−**19** are derivatives of the highly potent urea **5** bearing various substituents on the piperidine ring. Modification of the ethylene linker, either by alkylation or unsaturation, gave rise to compounds **20**−**28**. Further alteration of the internal portion of the molecule led to fused ring analogues including benzimidazole **29**, benzoxazoles **30**−**32**, and benzothiazoles (**33**−**73**, Table 2). In all cases except analogues **71**−**73**, the three-carbon bridge between the amide and thiazole nitrogen atoms of **1** is retained.

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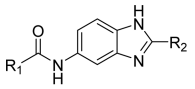
Table 2. Antitrypanosomal Activities of Fused Ring Derivatives 29−73[a]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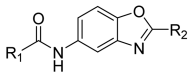
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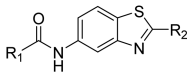
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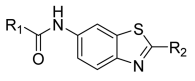
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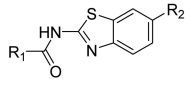
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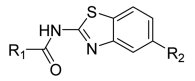
73

compd	R_1	R_2	*T. b. brucei* EC_{50} (μM)[a,b]	*T. b. brucei* EC_{90} (μM)[c,d]
29	phenyl	phenyl	>10	>10
30	phenyl	phenyl	>10	>10
31	pyrrolidin-1-yl	phenyl	5.25	>10
32	piperidin-1-yl	phenyl	2.80	9.30
33	phenyl	phenyl	2.38	7.74
34	thiophen-2-yl	phenyl	0.882	2.13
35	thiophen-3-yl	phenyl	1.57	2.49
36	thiazol-2-yl	phenyl	5.86	>10
37	thiazol-4-yl	phenyl	>10	>10
38	pyrrolidin-1-yl	phenyl	0.325	0.817
39	thiazolidin-3-yl	phenyl	1.24	2.38
40	piperidin-1-yl	phenyl	0.366	0.809
41	1,2,3,6-tetrahydropyridin-1-yl	phenyl	1.12	1.35
42	phenyl	4-fluorophenyl	1.92	3.00
43	pyrroldin-1-yl	4-fluorophenyl	0.375	0.895
44	piperidin-1-yl	4-fluorophenyl	0.427	0.737
45	phenyl	3-fluorophenyl	1.78	3.62
46	pyrrolidin-1-yl	3-fluorophenyl	0.158	0.391
47	piperidin-1-yl	3-fluorophenyl	0.174	0.602
48	4-methylpiperazin1-yl	3-fluorophenyl	3.15	7.55
49	piperazin-1,4-diyl	3-fluorophenyl	>20	>20
50	pyrrolidin-1-yl	2-fluorophenyl	0.983	1.24
51	piperidin-1-yl	2-fluorophenyl	0.935	1.14
52	pyrrolidin-1-yl	2,3-difluorophenyl	0.183	0.394
53	pyrrolidin-1-yl	2,4-difluorophenyl	0.929	1.48
54	pyrrolidin-1-yl	3,4-difluorophenyl	0.0918	0.181
55	3-fluoropyrrolidin-1-yl	3,4-difluorophenyl	0.0519	0.0919
56	(*R*)-3-fluoropyrrolidin-1-yl	3,4-difluorophenyl	0.326	0.491
57	(*S*)-3-fluoropyrrolidin-1-yl	3,4-difluorophenyl	0.0348	0.0548
58	3,3-difluoropyrrolidin-1-yl	3,4-difluorophenyl	0.934	1.66
59	(3*R*,4*R*)-3,4-difluoropyrrolidin-1-yl	3,4-difluorophenyl	0.114	0.747
60	3-(trifluoromethyl)pyrrolidin-1-yl	3,4-difluorophenyl	1.69	4.88
61	3-cyanopyrrolidin-1-yl	3,4-difluorophenyl	2.85	4.67
62	3-aminopyrrolidin-1-yl	3,4-difluorophenyl	2.32	2.72
63	3-(dimethylamino)pyrrolidin-1-yl	3,4-difluorophenyl	1.70	1.95
64	3-hydroxypyrrolidin-1-yl	3,4-difluorophenyl	7.68	13.6
65	3-methoxypyrrolidin-1-yl	3,4-difluorophenyl	3.05	4.59
66	piperidin-1-yl	3,4-difluorophenyl	0.223	0.282
67	4-fluoropiperidin-1-yl	3,4-difluorophenyl	0.341	0.596
68	4,4-difluoropiperidin-1-yl	3,4-difluorophenyl	2.98	4.17
69	pyrrolidin-1-yl	H	>20	>20
70	pyrrolidin-1-yl	Me	>20	>20
71	3-fluoropyrrolidin-1-yl	3,4-difluorophenyl	>20	>20
72	3-fluoropyrrolidin-1-yl	3,4-difluorophenyl	2.23	>20
73	3-fluoropyrrolidin-1-yl	3,4-difluorophenyl	2.64	5.28

[a]Concentration of compound required to inhibit growth by 50% (EC_{50}) against *T. b. brucei* BF427 or *T. b. rhodesiense* STIB900 (numbers in parentheses, data reported previously[14]). [b]Control for *T. b. brucei* BF427 EC_{50} assay average ± standard error of the mean (SEM): pentamidine (1.05 ± 0.12 nM, n = 30). [c]Concentration of compound required to inhibit growth by 90% (EC_{90}) against *T. b. brucei* BF427. [d]Control for *T. b. brucei* BF427 EC_{90} average ± SEM: pentamidine (3.54 ± 0.36 nM (n = 30).

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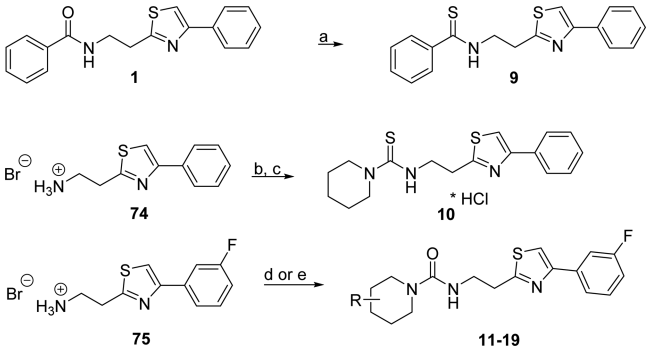
The treatment of amide **1** with Lawesson's reagent in THF gave thioamide **9** (Scheme 1). The reaction of amine **74**,[14]

Scheme 1. Synthesis of Thiazole Derivatives 11−19[a]



[a]Reagents and conditions: (a) Lawesson's reagent, THF; (b) CS_2, Et_3N, DCM, rt, and then Boc_2O, DMAP, 0 °C; (c) piperidine, Et_3N, DCM; (d) carbonyl 1,1′-diimidazole, Et_3N, DCM, 0 °C and then appropriate 2° amine, 0 °C to rt; (e) appropriate 2° amine, triphosgene, Et_3N, DCM, 0 °C and then **69** (for **14** and **16**). Structures **9**−**17** are defined in Table 1.

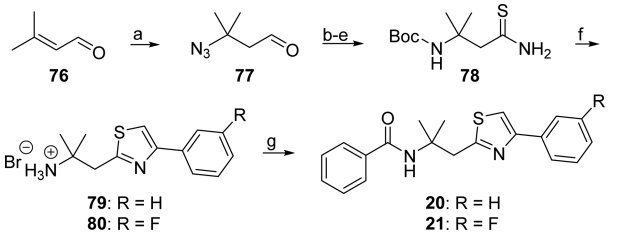
carbon disulfide, and trimethylamine in DCM overnight, followed by the addition of di-*tert*-butyl dicarbonate and DMAP at 0 °C,[15] give the crude isocyanate derivative, which was reacted with piperidine to give thiourea **10**, isolated as its hydrochloride salt.

While piperidyl urea **5** was readily prepared by the reaction of the primary amine **75** and piperidine-1-carbonyl chloride,[14] alternate strategy was required for substituted piperidine ureas **11**−**19** where the corresponding *N*-carbonyl chlorides were not readily available. The use of triphosgene to generate these derivatives from substituted piperidines was generally unreliable as the reactions proceeded very slowly and were difficult to monitor in the absence of UV chromophores. For example, 4-fluoropiperidine was reacted with triphosgene for 4 days before the addition of **75** to afford urea **16**. Attempts at preparing the isocyanate derivative of **75** using triphosgene resulted in varying amounts of the undesired symmetric urea, regardless of the reaction conditions employed, due to the high reactivity of the primary amine. The reaction of **69** with 1,1′-carbonyldiimidazole (CDI) in DCM in the presence of Et_3N at 0 °C resulted in the selective formation of the desired carbonylimidazolide derivative in situ. Addition of the appropriate secondary amine to the reaction mixture gave piperidine ureas **11**−**15**, **17**, and tetrahydroisoquinoline urea **19**. However, this method was not amenable to analogue **18** (presumably due to steric hindrance), which was successfully prepared from of **75** and the *N*-carbonyl chloride of 1,2,3,4-tetrahydroquinoline generated in situ using triphosgene.

The analogues with modified linkers include thiazole analogues having geminal dimethyl groups on the carbon adjacent to the amide nitrogen (**20**−**21**) or on the carbon attached to the thiazole ring (**22**−**23**). Analogues **24**−**27** have the methyl groups on the carbon next to the amide nitrogen as well as a reversed substitution pattern on the thiazole ring. Compound **28** incorporates a vinyl linker.

The syntheses of compounds **20** and **21** (Scheme 2) began with the conjugate addition of hydrazoic acid (NaN_3, concd HCl, DCM)[16] to 3-methylbut-2-enal (**76**) to obtain 3-azido-3-methylbutanal (**77**).[17] The aldehyde was reacted with

Scheme 2. Synthesis of Compounds 20 and 21[a]



[a]Reagents and conditions: (a) NaN_3, concd HCl, Et_3N, DCM; (b) $NH_2OH{\cdot}HCl$, aq $NaHCO_3$; (c) Ac_2O, reflux; (d) H_2, 10% Pd/C, Boc_2O, MeOH; (e) $NaHS{\cdot}xH2O$, $MgCl_2$, 15-crown-5, DMF, appropriate bromomethyl ketone, EtOH, reflux; (g) benzoyl chloride, Et_3N, THF.

hydroxylamine, and the resulting oxime was dehydrated to give 3-azido-3-methylbutanenitrile using acetic anhydride. The azide underwent catalytic hydrogenation (60 psi, 10% Pd/C, MeOH) in the presence of di-*tert*-butyl dicarbonate to afford 3-boc-amino-3-methylbutanenitrile. The nitrile was converted to thioamide **78** using sodium hydrosulfide hydrate, magnesium chloride, and 15-crown-5 in DMF.[14,18] This intermediate was reacted with 2-bromoacetophenone or 2-bromo-3′-fluoroacetophenone in refluxing ethanol (effecting thiazole ring closure and nitrogen deprotection) to give amines **79** and **80**, which precipitated from the reaction mixture as their hydrobromide salts. Benzoylation of the amines (benzoyl chloride, Et_3N, THF) gave target compounds **20** and **21**.

Ethyl cyanoacetate (**81**, Scheme 3) underwent methylation (CH_3I, Ce_2CO_3, DMF) to its 2,2-dimethyl derivative[19] followed by treatment with ammonia in methanol to give cyano-amide **82**.[20] Catalytic hydrogenation of the nitrile (H_2, 60 psi, 5% Rh/Al$_2$O3, concd NH_4OH, EtOH) to the amine (avoiding the use of Raney nickel[21] or circuitous pathways[22]) followed by protection with di-*tert*-butyl dicarbonate gave the amido-carbamate **83**, which was reacted with Lawesson's reagent to afford thioamide **84**. This intermediate was reacted with 2-bromoacetophenone as above to give amine **85** as the hydrobromide salt, which underwent acylation as above to target compounds **22** and **23**.

The synthesis of target compounds **24**−**27** (Scheme 4) began with the conjugate addition of hydrazoic acid (NaN_3, concd HCl, DCM)[16] to mesityl oxide (**86**) to give 4-azido-4-methylpentan-2-one.[23] Bromination of the α-methyl carbon followed by a Hantzsch thiazole synthesis involving thiobenzamide and then catalytic hydrogenation of the azido group gave amine **87**, isolated as its HCl salt. The reaction of **87** with the appropriate acyl halide as above gave benzamide **24**, and ureas **25**−**26**. The decreased reactivity of the sterically hindered amine **87** allowed the use of triphosgene for selective formation of its isocyanate derivative, which was then quenched with azepane to prepare urea **27** in the absence of the less readily available azepane-1-carbonyl chloride.

The preparation of compound **28** (Scheme 5) began with a Hantzsch thiazole synthesis involving thioamide **88** (prepared from the corresponding amide and P_2S_5)[24] and α-bromo ketone **89** followed by acidic hydrolysis of the acetal. The resulting aldehyde was reacted with triethyl phosphonoacetate and excess sodium hydride in THF by a modification of a known procedure[25] to give the vinyl acid **90**. The acid was treated with ethyl chloroformate in acetone in the presence of Et_3N followed by the addition of sodium azide[26] to give acyl

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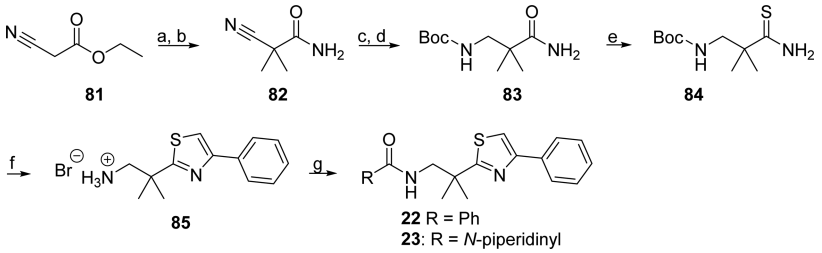
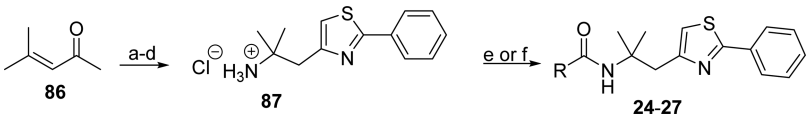
Scheme 3. Synthesis of Compounds 22 and 23[a]



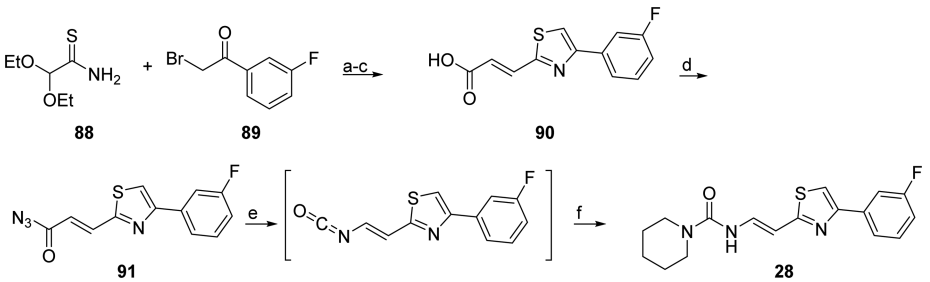
[a]Reagents and conditions: (a) CH_3I, Cs_2CO_3, DMF; (b) NH_3, MeOH; (c) H_2, 60 psi, 5% Rh/Al_2O_3, concd NH_4OH, EtOH; (d) Boc_2O, montmorillonite K10 (from base) or aq NaOH, DCM (from HCl salt); (e) Lawesson's reagent, THF; (f) 2-bromoacetophenone, EtOH, reflux; (g) appropriate acyl chloride, Et_3N, DCM

Scheme 4. Synthesis of Compounds 24–27[a]



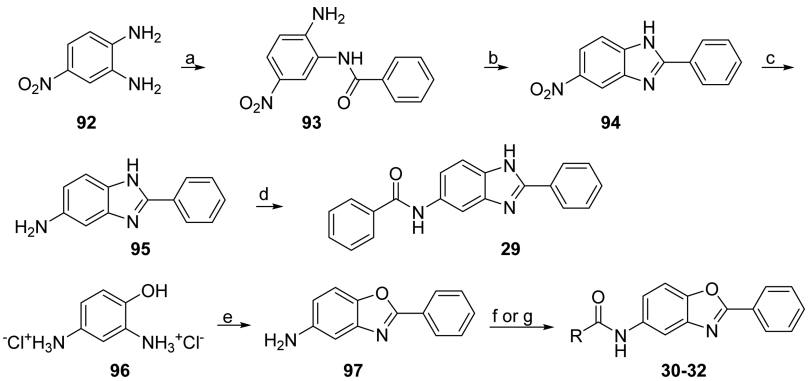
[a]Reagents and conditions: (a) NaN_3, concd HCl, Et_3N, DCM; (b) Br_2, MeOH; (c) thiobenzamide, EtOH; (d) H_2, 10% Pd/C, EtOH and then EtOH/HCl; (e) appropriate acyl halide, Et_3N, THF or DCM; (f) triphosgene, Et_3N, DCE, −5 °C to rt and then azepane (for **27**). Structures **24–27** are defined in Table 1.

Scheme 5. Synthesis of Compound 28[a]



[a]Reagents and conditions: (a) EtOH, rt, overnight; (b) aq HCl, acetone, reflux 1.5 h; (c) triethyl phosphonoacetate, NaH (excess), THF; (d) $ClCO_2Et$, Et_3N, acetone, 0 °C and then NaN3; (e) toluene, reflux 1.5 h and then piperidine, Et_3N, rt.

Scheme 6. Synthesis of Benzimidazole and Benzoxazole Derivatives 29–32[a]



[a]Reagents and conditions: (a) benzoyl chloride, Et_3N, THF, −10 °C; (b) $BF_3{\cdot}Et_2O$, dioxane, reflux, 3 h; (c) $SnCl_2{\cdot}2H_2O$, concd HCl, reflux, 2 h; (d) benzoyl chloride, Et_3N, DCM, rt, overnight; (e) benzoic acid, PPA, 110–180 °C, 4 h; (f) benzoyl chloride, Et_3N, DCM, rt, overnight; (g) triphosgene, Et_3N, DCM, 0 °C and then appropriate 2° amine, 0 °C to rt, overnight. Structures **28–30** are defined in Table 2.

azide **91**. A Curtius rearrangement of the azide to the vinyl isocyanate was effected in refluxing toluene (in place of benzene)[26] followed by the addition of piperidine to give the vinyl urea **28**.

The syntheses of the benzimidazole and benzoxazole derivatives are depicted in Scheme 6. The reaction of 4-nitrobenzene-1,2-diamine (**92**) with benzoyl chloride (THF at −10 °C) resulted in the selective formation of amide **93**, which underwent ring closure to benzimidazole **94** in the presence of boron trifluoride etherate in refluxing dioxane. Reduction of the nitro group using tin(II) dichloride in refluxing concentrated HCl gave amine **95**, which underwent benzoylation in DCM to

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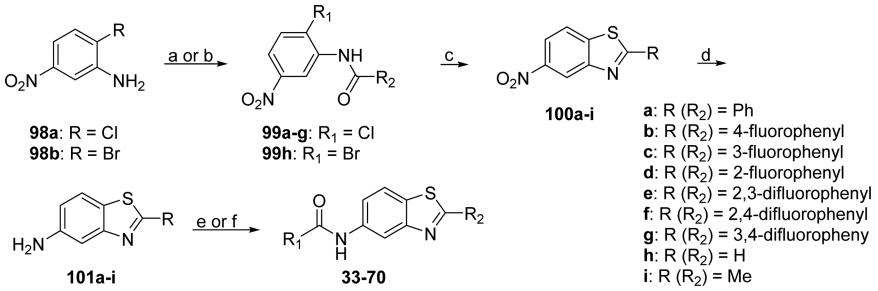
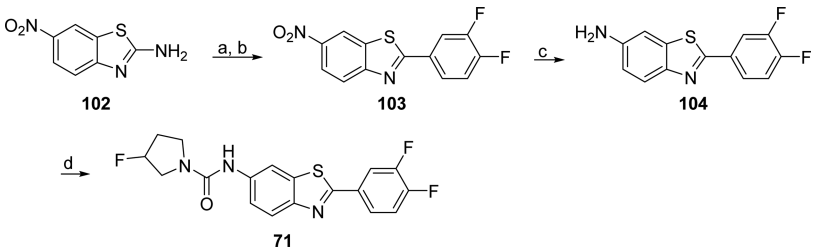
Scheme 7. Synthesis of Benzothiazole Derivatives 33−70[a]



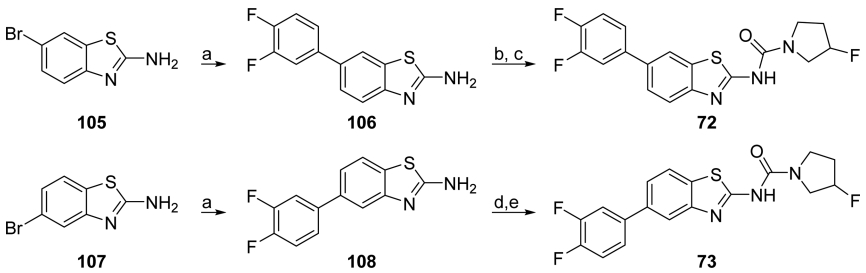
[a]Reagents and conditions: (a) appropriate benzoyl chloride, Py, rt, overnight; (b) HCO_2H, reflux (for **99h**); (c) $Na_2S{\cdot}9H_2O$, S_8, EtOH, reflux; (d) Fe, NH_4Cl, aq EtOH, reflux; (e) appropriate acyl halide or active ester, Et_3N, DCM, rt, overnight (for amides); (f) triphosgene, Et_3N, DCM, −5 °C and then appropriate 2° amine, 0 °C to rt, overnight (for ureas).

Scheme 8. Synthesis of Compound 71[a]



[a]Reagents and conditions: (a) $NaNO_2$, CuBr, aq HBr, 0 °C to rt; (b) 3,4-difluorophenylboronic acid, $PdCl_2(PPh_3)_2$, PPh_3, aq Na_2CO_3, DME, reflux 1.5 h; (c) Fe, NH_4Cl, aq EtOH, reflux; (d) triphosgene, Et_3N, DCM, −5 °C and then appropriate 2° amine, 0 °C to rt, overnight).

Scheme 9. Synthesis of Compounds 72 and 73[a]



[a]Reagents and conditions: (a) 3,4-difluorophenylboronic acid, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, aq K_2CO_3, dioxane, reflux; (b) carbonyl 1,1′-diimidazole, CH_3CN rt; (c) 3-fluoropyrrolidine HCl, Et_3N, DCM, rt; (d) phenyl chloroformate, Py, THF, 0 °C to rt; (e) 3-fluoropyrrolidine HCl, Py, reflux.

target compound **29**, the HCl salt of the known free base.[27] 4-Hydroxybenzene-1,3-diamine dihydrochloride (**96**) was reacted with benzoic acid in PPA to give 2-phenyl-5-aminobenzoxazole (**97**). Amine **97** underwent benzoylation to give amide **30** or reaction with triphosgene and triethylamine in DCM followed by addition of the appropriate secondary amine to obtain ureas **31** and **32**.

The syntheses of benzothiazoles **33−70** (Scheme 7) began with the acylation of 2-chloro-5-nitroaniline (**98a**) with the appropriate benzoyl chloride in pyridine to obtain *N*-phenylbenzamides **99a−g**. Formamide **99h**[28,29] was prepared from bromoaniline **98b** in refluxing formic acid. Amides **99a−h** were reacted with sodium sulfide nonahydrate and sulfur in refluxing ethanol[30] to give 5-nitrobenzothiazoles **100a−h**. These intermediates plus commercially available **100i** were reduced to the corresponding amines **101a−i** using iron powder and ammonium chloride in refluxing aqueous ethanol[30] after the failure to obtain **101a** either by catalytic hydrogenation or stannous chloride reduction of **100a**. The target benzothiazole amides **33−35**, **37**, **42**, and **45** were prepared from amines **101a−c** and the appropriate acyl chloride in the presence of Et_3N in DCM. Thiazole 2-carboxylic acid was treated with 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]-pyridinium 3-oxide hexafluorophosphate) (HATU) in the presence of Et_3N in THF followed by the addition of amine **101a** to obtain amide **36**. Urea analogues **38−41**, **43−44**, and **46−70** were prepared by reaction the isocyanate derivatives of primary amines **101a−i** (generated in situ using triphosgene) with the appropriate secondary amine. Initially (for **38**, **40**, **41**, and **43**), the isocyantes were prepared by dropwise addition of a solution of triphosgene (in DCM) to a solution of the primary amine and triethylamine in DCM at −5 °C in order to avoid formation of the undesired symmetric urea. In subsequent reactions, solid triphosgene was added to the reaction mixture in a single portion, producing the same result provided the concentration of the primary amine was below 20 mM. The 3-aminopyrrolidinyl urea **62** was prepared from 3-Boc-aminopyrrolidine and **101g**, followed by amine deprotection using TFA. The hydrochloride salt of (3*R*,4*R*)-3,4-difluoropyrrolidine,[31] the secondary amine precursor to

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analogue **59**, was prepared in five steps from L-tartaric acid by modification of known procedures.[32−37]

Compound **71** (Scheme 8) is a regioisomer of **55** in which the urea function is attached to the 6-position of the benzothiazole system. A pathway to intermediate **103** analogous to that of **100g** beginning with 2-chloro-4-nitroanaline was unsuccessful with difficulties encountered at the ring closure step. Intermediate **103** was prepared by diazotization−bromination of 2-amino-6-nitro-benzothiazole (**102**) followed by a Suzuki coupling with 3,4-difluorophenylboronic acid catalyzed by tetrakis(triphenylphosphine)palladium(0). The two remaining steps in the pathway are analogous to the preparation of **55**.

Compounds **72** and **73** (Scheme 9) are regioisomers of **71** and **55**, respectively, in which the substitution patterns on the benzothiazole system are reversed. A Suzuki coupling between 2-amino-6-bromobenzothiazole (**105**) and 3.4-difluorophenylboronic acid catalyzed by $Pd(dppf)Cl_2$ gave intermediate **106**, after the failure of reaction conditions using $Pd(PPh_3)_4$ analogous to those employed to prepare **103**. Amine **106** was reacted with CDI, followed by treatment of the resulting intermediate with 3-fluoropyrrolidine hydrochloride and trimethylamine in DCM to give urea **72**. While the preparation of intermediate **108** from **107** was analogous to that of **106** from **105**, different conditions were required for the formation of urea **73**. Amine **108** was treated with phenyl chloroformate, and the resulting carbamate was reacted with 3-fluoropyrrolidine hydrochloride in refluxing pyridine to give the desired product **73**.

In Vitro Antitrypanosomal Activity. Compound **1** (Table 1) and over 70 analogues (including **2**−**8**) were tested previously against *T. b. rhodesiense*.[14] The potency of **1** was enhanced 30-fold by replacement of the aromatic ring attached to the carbonyl group (R_1) with piperidine (urea analogue **4**). Smaller enhancements of activity were observed upon fluorination of the opposite aromatic ring (R_2) at any position or in the 2,4- and 3,4-difluorophenyl derivatives. Incremental enhancements were achieved by combining favorable modifications at both sites (e.g., **5**, the most potent overall). Little change in activity resulted when the substitution pattern on the thiazole ring was reversed, as observed in multiple pairs of regioisomers.

The new compounds were tested against *T. b. brucei* (a different parasite). To provide a more accurate comparison of activities of the new and existing compounds, a small number of the existing compounds (**1**−**8**) were also tested against *T. b. brucei*. A greater than 3-fold disparity between the EC_{50} values against the two different parasites was observed for **1**, but these differences were less than 2-fold for **2**−**8**.

None of the new thiazoles **9**−**28** showed enhanced potencies relative to their existing counterparts. The replacement of the carbonyl oxygen with sulfur resulted in an 8-fold loss of activity (**1** vs **9**) or similar potency (**4** vs **10**). The potency of **5** was not enhanced by the introduction of substituents on the piperidine ring (**11**−**19**). Small losses of activity (around 3-fold) were observed in compounds having of 2-methyl (**11**), 3-fluoro (**14**), and 4-fluoro substituents (**16**) on the piperidine ring, all having EC_{50} values below 50 nM. Greater losses of activity were observed with the 2,6-dimethyl (**12**), 4-oxo (**13**), 3,3- and 4,4-difluoro (**15** and **17**) analogues, with EC_{50} values between 100 and 700 nM. The tetrahydroquinoline (**18**) and -isoquinoline (**19**) derivatives were the least active of this subset, with EC_{50} values above 5 μM. The presence of geminal dimethyl groups on the ethylene linker (**20**−**27**) generally resulted in no detectable activity, regardless of the position of the methyl groups or thiazole geometry. The introduction of a *trans*-double bond in the linker resulted in an 8-fold loss of potency (**5** vs **28**).

Activities of the fused ring analogues **29**−**73** are shown in Table 2. Neither the 5-benzamidobenzimidazole (**29**) nor -benzoxazole (**30**) derivatives showed detectable activity, while the corresponding benzothiazole **33** exhibited an EC_{50} value of 2.38 μM. The benzothiazole urea derivatives **38** (EC_{50} = 325 nM) and **40** (EC_{50} = 366 nM) were around seven times more potent than amide **33** as well as 8−16 times more potent than the corresponding benzoxazoles **31** and **32** ($EC_{50} \geq 2.8$ μM). After these findings, all further efforts were focused on the benzothiazole derivatives. The thiophenyl amides **34** and **35** were slightly more potent than benzamide **33**, but the thiazolyl amides **36** and **37** were less potent, Of the amides **33**−**37**, only **34** exhibited submicromolar potency but was still less potent than urea **38**. Insertion of a sulfur atom into the pyrrolidine ring or a double bond into the piperidine ring resulted in 3−4-fold losses of potency (**38** vs **39**, **40** vs **41**).

The effect of fluorination of the aromatic ring attached to the 2-position of benzothiazole (R_2) upon activity depended upon the position of the fluorine atom(s). The potency of amide **33** was only slightly enhanced (less than 2-fold) by introduction of either a 3-fluoro (**42**) or 4-fluoro (**45**) substituent. The potency of pyrrolidyl urea **38** was either slightly diminished, enhanced 2-fold, or diminished 3-fold by introduction of a 4-, 3-, or 2-fluoro substituent (**43**, **46** (EC_{50} = 158 nM), and **50**, respectively). A similar pattern was observed for piperidyl urea **40**, as seen in analogues **44**, **47**, and **51**. The potency of pyrrolidyl urea **46** bearing a 3-fluorophenyl group was enhanced by the addition of a second fluorine atom at the 4-position (**54**, EC_{50} = 91.8 nM). However, the 2,3-difluoro analogue **52** was similar in potency to **46**, while the 2,4-difluoro isomer **53** was nearly six times less potent.

Further modifications were made to the urea portion of the molecule. The replacement of piperidine with *N*-methylpiperazine resulted in an 18-fold loss of activity (**47** vs **48**). No activity was detected with the piperazine-1,4-dicarboxamide derivative **49**.

The potency of pyrrolidyl urea **54** was slightly enhanced by the introduction of a 3-fluoro substituent on the pyrrolidine ring (**55**, EC_{50} = 51.9 nM). The (*S*)-enantiomer **57** (EC_{50} = 34.8 nM) proved to be the active stereoisomer, being nearly 10 times more potent than (*R*)-enantiomer **56** and the most potent overall in this study. The 3,3-difluoro analogue **58** (EC_{50} = 934 nM was over 25 times less active than **57** and over 10 times less active than **54**. The *trans*-3,4-difluoro derivative **59** (in which either of the two aliphatic fluorines has the same spatial orientation as the aliphatic fluorine of **57**) had an EC_{50} value of 114 nM, being about three times less potent than **57** but about eight times more potent than **58**.

Replacement of the 3-pyrrolidyl fluorine atom of **55** with other substituents led to decreased activity in all cases. The trifluoromethyl analogue **60** was over 30 times less active. The electron withdrawing cyano derivative **61** as well as the electron donating amino, dimethylamino, hydroxyl, and methoxy analogues (**62**−**65**) were all substantially less potent than **55**, with EC_{50} values between 1.5 and 8 μM.

The activity of piperidyl urea **66** was slightly diminished by the introduction of a fluorine atom at the 4-position of the piperidine ring (**67**), in contrast to the results noted above with

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Table 3. Cytotoxicity and Selectivity of Select Compounds

	T. b. brucei	CRL-8155[a,b]			HepG2[c,d]		
compd	EC_{50} (μM)	EC_{50} (μM)[e]	EC_{90} (μM)[f]	SI[g]	EC_{50} (μM)[e]	EC_{90} (μM)[f]	SI[g]
10	0.0243	>50	>50	>2058	>50	>50	>2058
11	0.037	>50	>50	>1351	>50	>50	>1351
12	0.399	>50	>50	>125	>50	>50	>125
13	0.667	>50	>50	>75	>50	>50	>75
14	0.0285	>50	>50	>1754	>50	>50	>1754
15	0.108	>50	>50	>463	>50	>50	>463
16	0.0366	>50	>50	>1366	>50	>50	>1366
17	0.164	>50	>50	>305	>50	>50	>305
28	0.0946	>50	>50	>529	>50	>50	>529
34	0.882	>50	>50	>57	>50	>50	>57
38	0.325	>50	>50	>154	>50	>50	>154
40	0.366	>50	>50	>137	>50	>50	>137
43	0.375	>50	>50	>133	>50	>50	>133
44	0.427	>50	>50	>117	>50	>50	>117
46	0.158	>50	>50	>316	>50	>50	>316
47	0.174	>50	>50	>287	>50	>50	>287
50	0.983	>50	>50	>51	>50	>50	>51
51	0.935	>50	>50	>53	>50	>50	>53
52	0.183	>50	>50	>273	>50	>50	>273
54	0.0918	33.9	>50	369	>50	>50	>545
55	0.0519	>50	>50	>963	>50	>50	>963
56	0.326	>50	>50	>153	>50	>50	>153
57	0.0348	>50	>50	>1437	>50	>50	>1437
58	0.934	>50	>50	>54	>50	>50	>54
59	0.114	27.1[h]	>50	>238	>50	>50	>439
66	0.223	>50	>50	>224	>50	>50	>224
67	0.341	>50	>50	>147	>50	>50	>147
pent[i]	0.00105	45.4	>100	>43200	>100	>100	>93000

[a]Human lymphoblasts (CRL-8155). [b]Control for CRL-8155 EC_{50} assay average ± SEM: quinacrine (4.23 ± 0.97 μM (n = 7); EC_{90} assay average ± SEM: quinacrine (9.94 ± 2.41 μM (n = 7). [c]Human hepatocytes (HepG2). [d]Control for HepG2 EC_{50} assay average ± SEM: quinacrine (10.44 ± 1.39 μM (n = 7); EC_{90} assay average ± SEM: quinacrine (18.23 ± 2.34 μM (n = 7). . [e]Concentration of compound required to inhibit growth by 50% (EC_{50}) of mammalian cell lines. [f]Concentration of compound required to inhibit growth by 90% (EC_{90}) of mammalian cell lines. [g]Selectivity index expressed as the ratio EC_{50} (cell line)/EC_{50} (*T. b. brucei*), rounded to the nearest integer. [h]Average of three assays. [i]Pentamidine.

the pyrrolidyl ureas (**54** vs **55**). The 4,4-difluoro derivative **68** was over 10 times less active than **66**, similar to the disparity between pyrrolidyl urea **54** and its 3,3-difluoro analogue **58**.

The presence of an aromatic substituent on the benzothiazole system, as well as the spatial orientation of both substituents, proved to be essential to activity. Removal of the aromatic ring from the 2-position (**69**) or replacement by a methyl group (**70**) resulted in loss of activity. The relocation of the urea moiety from the 5- to the 6-position also resulted in no detectable activity (**55** vs **71**). The reversal of the substitution patterns on the benzothiazole system (**71** vs **72**, **55** vs **73**) resulted in weak activity (EC_{50} > 2 μM). Thus, the spatial orientation of the thiazole and amide nitrogens proved to be essential to activity.

Similarities and differences existed between the SAR of the thiazole[14] and benzothiazole derivatives. In both groups, the urea derivatives were more potent than the corresponding benzamides. Piperidyl ureas were more potent than the corresponding pyrrolidyl ureas among the thiazoles, but the opposite was true for the benzothiazoles. Monofluorination of the aromatic ring at any position was generally beneficial in the thiazoles, but monofluorination was beneficial only at the 3-position for the benzothiazoles. Aromatic 3,4-difluorination was beneficial in both groups, while 2,4-difluorination was beneficial only to the thiazoles. The introduction of any substituents on the piperidine ring resulted in losses of activity regardless of the internal core of the molecule.

Cytotoxicity and Selectivity. Cytotoxicity data for compounds **1**−**8** were reported previously.[14] All new compounds with EC_{50} values below 1 μM (except **53**) were tested for toxicity against human lymphocytes CRL-8155 and human hepatocytes HepG2 (Table 3). Most of the compounds (including **57**) exhibited no detectable toxicity to either cell line. Only compounds **54** and **59** had detectable toxicity to the CRL-8155 cells (EC_{50} = 33.9 and 27.1 μM respectively), and none had detectable toxicity to the HepG2 cells. The selectivity of each compound for the parasite over each of the two cell lines was calculated. Compounds **10**, **11**, **14**, **16**, and **57** exhibited selectivity indices above 1000 against either cell line. Seventeen analogues (**12**, **15**, **17**, **28**, **38**, **40**, **43**, **44**, **46**, **47**, **52**, **54**, **55**, **56**, **59**, **66**, and **67**) had selectivity indices above 100 against either cell line. Within this group, compound **28** had a selectivity index above 500 in the CRL-8155 assay, while **54** and **55** had selectivity indices above 500 in the HepG2 assay.

Metabolic Stability. Select compounds were assayed for stability to mouse and human liver microsomes (Table 4). The short half-lives of compounds **4** and **5** are consistent with previous results obtained using a different protocol.[14] The introduction of an additional aromatic fluorine atom (**5** vs **6**, **5** vs **7**) offered no advantage regarding metabolic stability.

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Table 4. Stability of Select Compounds to Mouse and Human Liver Microsomes

compd	*T. b. brucei* EC_{50} (μM)	mouse microsomes $t_{1/2}$ (min)[a,b]	human microsomes $t_{1/2}$ (min)[a,c]
4	0.0194	<5 (2.4%)	7.6
5	0.0113	<5 (0%)[d]	5.3
6	0.0094	<5 (0%)[d]	<5 (46%)
7	0.0105	<5 (0%)[d]	<5 (22%)
9	1.44	<5 (0%)[d]	
10	0.0243	<5 (1.8%)	8.2
11	0.0370	<5 (21%)	<5 (3.9%)
14	0.0285	<5 (18%)	10
15	0.108	<5 (14%)	5.1
16	0.0366	<5 (5.5%)	5.6
17	0.164	<5 (20%)	5.8
23	1.30	<5 (0%)[d]	
26	7.84	<5 (0%)[d]	
28	0.0946	13	<5 (28%)
30	>10	17	60
32	2.80	11	>60 (77%)
33	2.38	50	>60 (53%)
38	0.325	18	30
40	0.366	17	>60 (62%)
46	0.158	12	37
47	0.174	11	8.9
54	0.0918	14	>60 (66%)
55	0.0519	>60 (52%)	>60 (99%)
57	0.0348	>60 (73%)	>60 (53%)
59	0.114	>60 (67%)	>60 (96%)

[a]Microsome reactions were incubated at 37 °C with KH_2P0_4 (0.16 M), NADPH (1 mM), of microsomes (0.5 mg/mL), and test compounds (1.5 μM). Numbers in parentheses are percentages of compound remaining after 5 min (where $t_{1/2}$ < 5 min) or after 60 min (where $t_{1/2}$ > 60 min). [b]Control for mouse microsome assay average ± SEM: dextromethorphan (7.79 ± 1.40 min, n = 7) and testosterone (5.26 min. ± 0.637 min, n = 7). [c]Control for human microsome assay average ± SEM: dextromethorphan (39.15 ± 5.13 min, n = 7) and testosterone (22.7 ± 5.89 min, n = 6). [d]Below limit of detection.

None of the new thiazole derivatives (**9**–**28**) that were tested were metabolically stable. Only analogue **28** ($t_{1/2}$ = 13 min) had a half-life above 10 min in the mouse microsomes, and all others in this group had half-lives below 5 min with 0–21% of the test compounds remaining after the 5 min time point. These compounds were also unstable to the human microsomes, where only analogue **14** had a half-life as high as 10 min. All four compounds in this group having potencies below 50 nM (**10**, **11**, **14**, and **16**) were unstable to both mouse and human microsomes. No modifications of the urea portion of the molecule or of the ethylene bridge were found to improve metabolic stability. Thus, none of the compounds **9**–**28** offered any advantage regarding either potency or metabolic stability.

The metabolic stabilities of the fused ring analogues **29**–**73** were more promising. All compounds in this group that were tested had half-lives greater than 10 min in the mouse microsomes and at least 30 min (except for **47**) in the human microsomes. The inactive benzoxazole amide **30** and its weakly active piperidyl urea analogue **32** had half-lives of between 10 and 20 min in mouse microsomes and at least 60 min in human microsomes. The benzothiazole amide **33**, although only weakly active against the parasite, had half-lives of 50 and >60 min in the mouse and human microsomes, respectively. The corresponding pyrrolidyl and piperidyl ureas **38** and **40**, although more potent had **33**, had shorter half-lives (>20 min) in in mouse microsomes, and **38** had a shorter half-live (30 min) in human microsomes. Fluorination of the phenyl ring at the 3-position (**38** vs **46**, **40** vs **47**) led to enhanced potency of both ureas but not enhanced stability, as both fluorinated derivatives **46** and **47** were less stable to mouse microsomes and only **46** was at least as stable to human microsomes. The pyrrolidyl urea **46** (R_2 = 3-fluorophenyl, $t_{1/2}$ = 37 min) was four times more stable to human microsomes than corresponding piperidyl urea **47**. Urea **54**, the (more highly potent) 3,4-difluorophenyl analogue of **46**, showed similar stability ($t_{1/2}$ = 14 min) to mouse microsomes but enhanced stability ($t_{1/2}$ > 60 min) to human microsomes. The addition of a 3-fluoro substituent on the pyrrolidine ring (**55**) resulted in greater potency, greater than 4-fold increased stability to mouse microsomes, and similar stability to human microsomes compared to **54**. High metabolic stability ($t_{1/2}$ > 60 min in both mouse and human microsomes) was also observed in the (*S*)-enantiomer **57** and the *trans*-3,4-difluoropyrrolidine derivative **59**.

Mouse Oral PK. Compounds **54**, **55**, and **57**, and **59** were administered to groups of three mice in single doses of 50 mg/kg by oral gavage in a dosing vehicle consisting of Tween 80 (7%), EtOH (3%), and DMSO (5%) in 0.9% sodium chloride solution (Table 5). Compound **57** (the most potent in vitro)

Table 5. Oral Pharmacokinetics of Compounds 54, 55, 57, and 59 in Mice

compd	C_{max} (μM)[a]	$AUC_{0\ min-\infty}$ (min × μM)[a]
54[b]	2.01 ± 0.46	443.3 ± 43.07
55[b]	10.04 ± 0.65	6459.4 ± 481.8
57[b]	23.34 ± 3.02	13254 ± 1237
57[c]	18.55 ± 5.54	14673 ± 2833
57[d]	8.54 ± 0.41	6352 ± 469
59[b]	10.96 ± 0.58	13515 ± 224.6

[a]Average values ± SEM of three mice each given a single dose at 50 mg/kg by oral gavage. [b]Dosing vehicle consisted of Tween 80 (7%), EtOH (3%), and DMSO (5%) in 0.9% sodium chloride solution. [c]Dosing vehicle consisted of Phosal 53 MCT(60%), PEG400 (30%), and EtOH (10%). [d]Dosing vehicle consisted of methylcellulose cP 400 (0.5%) and Tween 80 (0.5%) in water.

clearly showed the highest blood levels of the four compounds tested in the same vehicle, with a maximum blood concentration of 23.3 μM and an AUC of 13216 min·μM. Compound **59** had a lower C_{max} but a similar AUC compared to **57**. Less than 1% of the C_{max} of **57** remained at the 24 h time point compared to 34% of the C_{max} of **59**. More than the 17% of the C_{max} of **59** remained at the 32 h time point. Compound **57** gave a PK profile similar to that obtained using the original vehicle when administered in a vehicle consisting of Phosal 53 MCT (60%), PEG400 (30%), and EtOH (10%); however, the use of a vehicle consisting of methylcellulose cP 400 (0.5%) and Tween 80 (0.5%) in water resulted in a lower C_{max} and AUC values (lines 3–5 of Table 5).

Brain Penetration. Brain penetration studies (Table 6) were initially performed upon compounds **54**, **55**, and **57** but were expanded to include other compounds in order to explore the SAR of brain penetration. Groups of three mice were given single 5 mg/kg ip doses of the test compounds and were sacrificed 1 h postdose. The benzothiazole pyrrolidyl urea **38** exhibited a mean brain concentration of 4.2 μM (over 10 times

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Table 6. Brain Penetration of Select Compounds in Mice at One Hour Post-Dose

compd	$[compd]_{plasma}$ (μM)[a]	$[compd]_{brain}$ (μM)[a]	brain/plasma ratio
1	0.507 ± 0.087	0.153 ± 0.033	0.300 ± 0.040
4	0.533 ± 0.328	0.244 ± 0.109	0.613 ± 0.128
10	2.00 ± 0.885	0.187 ± 0.089	0.089 ± 0.006
31	0.270 ± 0.036	0.397 ± 0.077	1.58 ± 0.484
38	3.403 ± 0.328	4.24 ± 0.698	1.24 ± 0.157
46	2.22 ± 0.461	2.28 ± 0.532	1.01 ± 0.037
52	1.3 ± 0.406	2.31 ± 0.746	1.76 ± 0.032
54	2.61 ± 0.727	4.25 ± 0.693	1.81 ± 0.458
55	3.26 ± 0.630[b]	5.48 ± 0.683[b]	2.11 ± 0.669
56	1.20 ± 0.270	6.35 ± 2.257	4.86 ± 1.055
57	2.27 ± 0.888	9.07 ± 3.973	4.00 ± 0.361
58	2.81 ± 0.452	21.7 ± 1.96	7.92 ± 0.693
59	2.19 ± 0.424	16.2 ± 2.88	7.71 ± 1.63
60	4.15 ± 1.368	1.97 ± 0.612	0.477 ± 0.052
63	0.780 ± 0.202	3.00 ± 0.796	3.80 ± 0.331
66	2.52 ± 0.185	1.29 ± 0.156	0.510 ± 0.023
67	2.51 ± 0.203	2.52 ± 0.248	1.01 ± 0.081

[a]Average values ± SEM of three mice each given a single ip dose at 5 mg/kg in vehicle consisting of Tween 80 (7%), EtOH (3%), and DMSO (5%) in 0.9% sodium chloride solution. [b]Average values ± SEM of six mice.

greater than that of the corresponding benzoxazole analogue **31**) and a mean brain to plasma ratio (BPR) of 1.2. Fluorination of the terminal aromatic ring had relatively little effect as **38**, and the 3,4-difluorophenyl derivative **54** had nearly identical mean brain concentrations, although the latter did have a slightly higher BPR. By contrast, the mean brain concentrations of the 3-fluoro (**46**) and 2,3-difluoro (**52**) analogues were somewhat lower than that of **38**. The brain levels of **54** were enhanced by the introduction of a 3-fluoro substituent on the pyrrolidine ring (**55**–**57**). Of these compounds, the greatest enhancement was seen in the (*S*)-enantiomer **57**, with a mean brain concentration of 9.1 μM and a BPR of 4.0. The (*R*)-enantiomer **56** also exhibited the ability to cross the BBB. The highest brain levels were seen in the difluoropyrrolidyl derivatives **58** and **59**, each having a mean brain concentration exceeding 16 μM and a BPR above 7.5. Replacement of the fluorine atom with a trifluoromethyl group (**55** vs **60**) resulted in lower brain levels. Similar decreases also resulted when the pyrrolidine ring was replaced with piperidine (**54** vs **66**). Introduction of a 4-fluoro substituent on the piperidine ring also resulted in enhanced brain levels (**66** vs **67**), consistent with the results of fluorination of the pyrrolidine ring.

The brain and plasma concentrations of compound **57** were studied in a time-course experiment following oral dosing at 50 mg/kg (Table 7). The data show that **57** partitions to the brain compartment with BPRs of 1.48, 4.43, and 5.48 at 1, 4, and 8 h postdose, respectively. At 24 h, the plasma concentrations are undetectable and brain levels are down to submicromolar concentrations. Compared to the previous experiment (Table 6), the BPR ratio for **57** is lower at 1 h postdose (1.48 vs 4.0), probably due to the oral route of administration (as opposed to the ip route used before), leading to slower systemic absorption and distribution. The time-course experiment demonstrates that brain concentrations of **57** are sustained at high levels for at least 8 h postdose and help account for the successful results in the late-stage efficacy model discussed below.

Table 7. Brain Time-Course Study of Compound 57

time (h)	$[compd]_{plasma}$ (μM)[a]	$[compd]_{brain}$ (μM)[a]	brain/plasma ratio
1	27.14 ± 9.91	38.23 ± 13.51	1.48 ± 0.16
4	10.25 ± 2.21	45.2 ± 10.4	4.43 ± 0.3
8	8.97 ± 1.05	48.08 ± 2.47	5.48 ± 0.6
24	<LLQ[b]	0.28 ± 0.13	
C_{max} (μM)	27.39 ± 9.67	54.84 ± 4.64	
$AUC_{0\ min-\infty}$ (min × μM)	10798 ± 1975	43116.8 ± 2304	

[a]Average concentration ± SEM of 3 mice at the stated time point after each mouse received a single oral dose at 50 mg/kg in vehicle consisting of Tween 80 (7%), EtOH (3%), and DMSO (5%) in 0.9% sodium chloride solution. [b]LLQ (lower limit of quantitation) is 0.010 μM.

In Vivo Efficacy. Upon the basis of its promising in vitro activity, cytotoxicity, metabolic stability, PK, and brain penetration data, compound **57** was selected as a candidate for in vivo efficacy studies. An acute model (requiring 60 days to complete) was employed, followed by a chronic model (requiring 180 days to complete).

In a model of the acute phase of HAT, five mice infected with *T. b. rhodesiense* STIB900 were given the test compound at 50 mg/kg po b.i.d. × 4 days, beginning 2 days postinfection. Efficacy PK blood samples were collected from three of the five mice prior to dose 7, and at 1 and 6 h postdose. Average plasma levels of the test compound were 9.1 ± 7.6 μM (predose), 18.6 ± 7.2 μM (1 h), and 15.0 ± 4.2 μM (6 h). Compound **57** attained 5/5 cures as determined by the absence of detectable parasitemia in any of the treated mice 60 days postinfection (Figure 1). All mice receiving only vehicle showed high parasitemia on the last day of dosing, with all concentrations >1.5 × 10^7 parasites/mL of blood, and were euthanized.

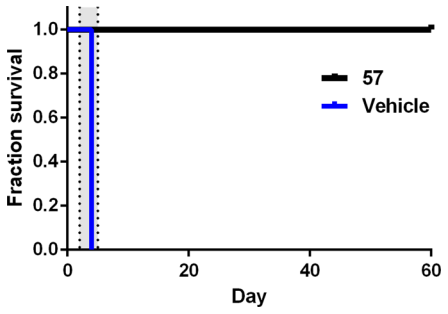

Figure 1. Mouse efficacy model of acute *T. brucei* infection. All mice were infected with *T. b. rhodesiense* STIB900 on day 0. Groups of five mice were treated with compound **57** (50 mg/kg by oral gavage b.i.d.) or vehicle from day 2–5 (gray-shaded area). Mice were monitored for parasitemia in tail blood samples through day 60 postinfection.

In a model of the chronic phase of HAT, compound **57** was administered to five mice infected with *T. b. brucei* TREU667 at 50 mg/kg/po b.i.d. × 10 days, beginning 21 days postinfection. This dose was chosen because earlier PK studies demonstrated good plasma exposure at 50 mg/kg (Table 5). Efficacy PK blood samples were collected from three of the five mice prior to dose 15, and at 1 and 6 h postdose. Average plasma levels of the test compound were 15.8 ± 8.7 μM (predose), 23.1 ± 603 μM (1 h), and 26.7 ± 7.1 μM (6 h). Compound **57** attained 5/5 cures as determined by the absence of detectable parasitemia in any of the treated mice through 180 days postinfection

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(Figure 2). No mice receiving vehicle alone had spontaneous cures. The mice receiving diminazene aceturate (which does not cross the BBB)[38] had temporary clearance of parasitemia but subsequently relapsed, most likely from parasites leaving the brain and returning to the hemolymphatic system. By observing mice for 180 days, more than ample time was allowed for mice to show signs of illness or parasites to become visible on blood films (as was observed with the diminazene-treated mice), thus providing assurance that the **57**-treated mice were cured. Experiments evaluating lower doses of **57** are underway and will be published at a later time.

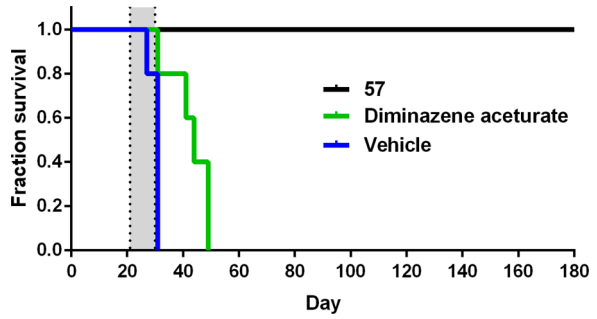

Figure 2. Mouse efficacy model of chronic *T. brucei* infection. All mice were infected with *T. b. brucei* TREU667 strain on day 0. Groups of five mice were treated with compound **57** (50 mg/kg by oral gavage b.i.d.) or vehicle from day 21−30 (gray-shaded area). A control group received a single dose of diminazene on day 21. Mice were monitored for parasitemia in tail blood samples through day 180 postinfection.

CONCLUSIONS

While a number of the initial analogues[14] were highly potent against the parasite in vitro, they exhibited poor metabolic stability, which precluded their further advancement as drug candidates. The goal of the present study was to improve metabolic stability while maintaining potency. Neither replacing the carbonyl oxygen with sulfur, the introduction of various substituents at the urea end of the molecule, nor the alkylation or unsaturation of the ethylene linker provided any advantage regarding either antitrypanosomal activity or metabolic stability. Enhanced metabolic stability was observed only in the fused ring analogues where thiazol-2-ethylamino fragment was replaced with a benzoxazol- or benzothiazol-5-amino moiety, but antitrypanosomal activity was retained only in the benzothiazole derivatives. These results strongly suggest that the ethylene linker played a role in the poor metabolic stability of the initial thiazole analogues. Brain penetration studies demonstrated the ability of the benzoxazole and benzothiazole derivatives, as a whole, to cross the BBB and even concentrate in the brain.

The most significant findings in this paper were the promising biological data for compound **57**. This molecule exhibited good activity in vitro (EC_{50} = 34.8 nM), no detectable toxicity to two human cell lines (CRL-8155 and HepG2), and half-lives of at least 60 min in both mouse and human liver microsomes. It also demonstrated promising oral PK (C_{max} = 23.3 μM and AUC = 13216 min × μM) and excellent brain penetration (brain concentration = 9.07 μM and BPR = 4.00) at 1 h after administration of a 5 mg/kg ip dose in mice. These results led to the selection of compound **57** for in vivo efficacy studies. Oral dosing of this molecule achieved 5/5 cures in both acute and chronic murine models of HAT. The efficacy PK experiments in both studies demonstrated that the compound accumulated in plasma after dosing. Synthesis of additional analogues, as well as further testing of compound **57** (and **59**), including dose optimization and comprehensive pharmacokinetic studies, is either planned or currently in progress. Experiments to identify the biochemical target(s) of action in *T. brucei* are also underway.

EXPERIMENTAL SECTION

***T. brucei* Growth Inhibition Assay.** Compounds were tested for antitrypanosomal activity against *T. b. brucei* (strain BF427) in HMI-9 media as previously described.[39] Cells were tested in triplicate against serial dilutions of compounds and quantified with Alamar Blue (ThermoFisher Scientific, Waltham, MA) at 48 h.[40] EC_{50} and EC_{90} values were calculated by nonlinear regression using software by the Collaborative Drug Database (Burlingame, CA. www.collaborativedrug.com). Growth assays for *T. b. brucei* included the control compound, pentamidine isethionate (Sigma-Aldrich, St. Louis, MO.

Cytotoxicity in Mammalian Cells. Compounds were assayed for cytotoxicity against CRL-8155 (human lymphoblasts) and HepG2 cells (human hepatocellular carcinoma) as previously described.[41] Cells were exposed to serial dilutions of compounds for 48 h, and cytotoxicity was quantified using Alamar Blue[41] (ThermoFisher Scientific, Waltham, MA). Compounds were assayed in quadruplicate, and CC_{50} and CC_{90} values were calculated by nonlinear regression using software by the Collaborative Drug Database (Burlingame, CA. www.collaborativedrug.com). Growth assays for CRL-8155 and HepG2 included the control compound, quinacrine (Sigma-Aldrich.

In Vitro Liver Microsome Assays. Pooled liver microsomes from mouse and human sources were purchased from BD Biosciences (San Jose, CA). Microsome reactions were preincubated for 5 min at 37 °C with 0.16 M KH_2PO_4, 1 mM NADPH, and 0.5 mg/mL of microsomes. At t = 0, 1.5 μM of test compounds were added and the t = 0 time point was immediately quenched with the addition of 4× the sample volume of 100% acetonitrile. Subsequent time points were quenched at 5, 10, 15, 30, and 60 min post addition of test compounds, then processed using liquid chromatography/tandem mass spectrometry analysis. Assays were performed with control compounds, testosterone, and dextromethorphan (purchased from Sigma-Aldrich).

Mouse Oral Pharmacokinetics. Compounds were administered orally to mice at a concentration of 50 mg/kg in a vehicle consisting of either: (1) DMSO (5%), Tween 80 (7%), and EtOH (3%) in physiological saline (0.9%) solution, (2) Phosal 53 MCT (60%), PEG400 (30%), and EtOH (10%), or (3) methylcellulose cP400 (0.5%) and Tween 80 (0.5%) in water. Phosal 53 MCT is a lecithin emulsifier purchased from American Lecithin Co. Three mice were used per group, and 40 μL of blood was collected from the tail at time points extending out to as much as 32 h postdose into heparinized capillary tubes. Whole blood was then spotted onto FTA DMPK-C cards (GE Healthcare, Boston, MA) and was measured by liquid chromatography/tandem mass spectrometry. For the efficacy PK experiments, samples were collected from experimental mice (n = 3) before the stated day of dose, then at 1 and 6 h postdose using the same collection method as above.

Brain: Plasma Compound Concentration Measurements in Mice. Test compounds were administered at 5 mg/kg ip to three mice in a vehicle consisting of DMSO (5%), Tween 80 (7%), and EtOH (3%) in physiological saline (0.9%) solution.[3] At 1 h post injection, blood was collected, plasma was separated by brief centrifugation, and the brain was removed and homogenized in acetonitrile. Levels of compound in the plasma and brain were determined via liquid chromatography/tandem mass spectrometry. Calculations of brain concentrations accounted for 3% volume/weight of blood in the brain. For the brain time-course experiment of compound **57**, compounds were administered at 50 mg/kg po in the same vehicle listed above. Mice were sacrificed in groups of three at 1, 4, 8, and 24 h postdosing for collection of brains and plasma for analysis as mentioned above.

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Acute Efficacy Studies in Mice. Experiments were carried out using the standard operating procedure used by WHO screening centers[42] and done in compliance with the University of Washington Institutional Animal Care and Use Committee (IACUC) approved protocol. Groups of five female Swiss-Webster mice (ND4 outbred, ages 6−8 weeks) were infected on day 0 with 1×10^4 *T. b. rhodesiense* (strain STIB900) parasites. The test compound was administered by oral gavage at 50 mg/kg every 12 h from day 2 to day 5, for a total of eight doses in a 200 μL volume of a vehicle consisting of DMSO (5%), Tween 80 (7%), and EtOH (3%) in physiological saline (0.9%) solution. Parasitemia was monitored via microscopic analysis of tail blood for 60 d postinfection or until parasites were detected. Mice were removed from the experiment once parasites were detected in the blood, at which point the mouse was euthanized.

Chronic Efficacy. Again, experiments were done in compliance with the University of Washington IACUC approved protocol. According to published procedures,[38] groups of five mice were infected with 1×10^4 *T. b. brucei* (strain TREU667) at day 0 to establish a chronic infection. Treatment began on day 21 postinfection, and mice received 50 mg/kg test compound orally b.i.d. for 10 d (total of 20 doses) in a 200 μL volume of a vehicle composed of Phosal 53 MCT (60%), PEG400 (30%), and EtOH (10%). A control group received vehicle with no compound and another control group received a single intraperitoneal dose of diminazene aceturate at 10 mg/kg in water on day 21. The diminazene aceturate temporarily clears parasites from the blood, but because it does not cross the BBB, the blood is later repopulated from parasites in the CNS.[38] Post dosing, parasitemia was monitored via microscopic examination of tail blood slides until 180 days postinfection. Mice were removed from the experiment once parasites were detected in the blood.

General Chemistry Experimental. Uncorrected melting points were measured on a Thermo Scientific 9200 melting point apparatus. ^{1}H NMR spectra were recorded on a Varian Inova 400 MHz, a Bruker AVANCE 400 MHz, or a Varian Inova 600 MHz spectrometer. Anhydrous solvents were purchased from Aldrich Chemical Co., Milwaukee, WI, or from Fisher Scientific, Waltham, MA, in Sure-Seal or AcroSeal containers and were used without further purification. Organic starting materials were purchased from the same sources or were prepared by published procedures as noted. Reaction mixtures were monitored by TLC on silica gel or by reverse phase HPLC. Organic layers of extraction mixtures were neutralized as necessary with acidic or basic washes, washed with saturated NaCl solution, and dried over $MgSO_4$ before being evaporated under reduced pressure. Normal phase flash column chromatography was performed using Davisil grade 633, type 60A silica gel (200−425 mesh). Analytical HPLC chromatograms were recorded on an Agilent 1100 or 1200 series chromatograph using a Zorbax Rx C8 column (4.6 mm × 75 mm, 3.5 μm) maintained at 40 °C and UV photodiode array detection at 230, 254, 265, 290, and 320 nm. Area % values are reported at the wavelengths where the strongest signals of the products were observed. Mobile phases consisted of mixtures of MeOH (0−95%) in water containing formic acid (80 mM), ammonium formate (20 mM), and Et_3N (15 mM). Samples were eluted at appropriate gradients at a flow rate of 1.5 mL/min. Low resolution ESI mass spectra were recorded on an Agilent Technologies 1100 series LC/MSD trap mass spectrometer or at the North Carolina State University Mass Spectrometry Facility located in the Department of Chemistry. In cases of hydrochloride salts, the *m/z* values reported are those of the free bases. Elemental analyses were measured by Atlantic Microlab, Norcross, GA, and unless stated otherwise, were within ±0.4% of calculated values. All target compounds are judged to be >95% pure by elemental analysis and analytical HPLC. Representative syntheses for all target compounds as well as for intermediates of benzothiazoles **33**−**70** are given herein. Synthetic details for all compounds are given in Supporting Information.

2-(2-Benzthioamido)ethyl-4-phenylthiazole (9). Lawesson's reagent (493 mg, 1.22 mmol) was added to a solution of 2-(2-benzamido)ethyl-4-phenylthiazole (**1**,[14] 353 mg, 1.15 mmol) in THF (20 mL). The mixture was stirred overnight before being diluted with saturated $NaHCO_3$ solution and extracted into DCM. The oily residue was recrystallized from MeOH as light-yellow crystals (184 mg, 50%): mp 124−126 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (t, *J* = 5.5 Hz, 1H), 8.00 (d, *J* = 0.7 Hz, 1H), 7.99−7.92 (m, 2H), 7.80−7.72 (m, 2H), 7.54−7.46 (m, 1H), 7.46−7.37 (m, 4H), 7.37−7.29 (m, 1H), 4.12 (td, *J* = 6.9, 5.2 Hz, 2H), 3.49 (t, *J* = 6.9 Hz, 2H). EIMS *m/z* 324.9 $(M + 1)^+$. HPLC 100 area % (265 nm). Anal. Calcd for $C_{18}H_{16}N_2S_2$: C, 66.63; H, 4.97; N, 8.63; S, 10.40. Found: C, 66.36; H, 5.16; N, 8.38.

2-(2-Piperidin-1-yl-thioamido)ethyl-4-phenylthiazole Hydrochloride (10). A suspension of 4-phenylthiazol-2-ethylamine hydrobromide (**74**,[14] 500 mg, 1.75 mL) in DCM (8 mL) was treated with Et_3N (0.6 mL, 4.30 mmol) to give a homogeneous solution. Carbon disulfide (1.2 mL, 19.3 mmol) was added, and the mixture was stirred overnight. After the mixture cooled in an ice bath, DMAP (10 mg, 4.8 mol %) and a solution of di-*tert*-butyl dicarbonate (383 mg, 1.75 mmol) in DCM (1 mL) were added.[15] The ice bath was removed, and the mixture was stirred for 2 h. The mixture was concentrated under reduced pressure, diluted with ether, and filtered. The filtrate was evaporated to give 2-(2-isothiocyanatoethyl)-4-phenylthiazole as an oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (s, 1H), 8.02−7.91 (m, 2H), 7.49−7.39 (m, 2H), 7.42−7.29 (m, 1H), 4.11 (dd, *J* = 6.7, 5.9 Hz, 2H), 3.44 (dd, *J* = 6.7, 5.8 Hz, 2H).

A solution of the 2-(2-isothiocyanatoethyl)-4-phenylthiazole, piperidine (200 μL, 2.02 mmol), and Et_3N (300 μL, 2.15 mmol) in DCM (10 mL) was stirred overnight at room temperature. After aqueous workup, the crude product was converted to the HCl salt using EtOH and concentrated HCl, followed by recrystallization from EtOH/ether to give white crystals (249 mg, 39%): mp 138−140 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, *J* = 0.6 Hz, 1H), 7.96−7.92 (m, 2H), 7.80 (s, 1H), 7.48−7.39 (m, 2H), 7.37−7.30 (m, 1H), 3.88 (td, *J* = 6.8, 4.3 Hz, 2H), 3.79−3.72 (m, 4H), 3.34 (t, *J* = 7.0 Hz, 2H), 1.63−1.55 (m, 2H), 1.52−1.41 (m, 4H). EIMS *m/z* 332.0 $(M + 1)^+$. HPLC 96.9 area % (254 nm). Anal. Calcd for $C_{17}H_{21}N_3S_2$·HCl: C, 55.49; H, 6.03; N, 11.42. Found: C, 55.59; H, 6.12; N, 11.31.

General Procedure for Compounds 11−19. A mixture of 4-(3-fluorophenyl)thiazol-2-ethylamine hydrobromide (**75**,[14] 0.4−0.7 mmol) and Et_3N (min of 3 equiv) in DCM was cooled to −5 °C before the addition of 1,1′-carbonyldiimidazole (CDI, 1.2−1.3 equiv). After 1 h, the secondary amine or its hydrochloride salt was added. The mixture was stirred at room temperature overnight or until complete by HPLC before being diluted with water and extracted into DCM. The crude products were then recrystallized from appropriate solvents

*2-[2-(2-Methyl)piperidin1-yl-amido]ethyl-4-(3-fluorophenyl)-thiazole (**11**).* **11** was prepared by the general procedure from **75** (153 mg, 0.506 mmol) and 2-methylpiperidine (100 μL, 0.851 mmol). The crude product was recrystallized from DCM/hexanes as a white solid (75.1 mg, 43%): mp 81−83 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 7.80 (ddd, *J* = 7.8, 1.6, 0.9 Hz, 1H), 7.74 (ddd, *J* = 10.7, 2.6, 1.5 Hz, 1H), 7.48 (td, *J* = 8.0, 6.2 Hz, 1H), 7.16 (dddd, *J* = 9.0, 8.3, 2.7, 0.9 Hz, 1H), 6.57 (t, *J* = 5.4 Hz, 1H), 4.23 (d, *J* = 4.3 Hz, 1H), 3.79−3.70 (m, 1H), 3.41 (dt, *J* = 12.6, 6.5 Hz, 2H), 3.17 (t, *J* = 6.9 Hz, 2H), 2.73 (td, *J* = 13.1, 2.8 Hz, 1H), 1.60−1.40 (m, 5H), 1.30−1.10 (m, 1H), 1.04 (d, *J* = 6.8 Hz, 3H). EIMS *m/z* 348.0 $(M + 1)^+$. HPLC 100 area % (254 nm). Anal. Calcd for $C_{18}H_{22}FN_3OS$·0.25H_2O: C, 61.43; H, 6.44; N, 11.94. Found: C, 61.22; H, 6.69; N, 11.82.

Representative Synthesis of Compounds 20−27. *2-(2-Benzamido-2-methylpropyl)-4-phenylthiazole (**20**).* Benzoyl chloride (200 μL, 1.72 mmol) was added to a stirred mixture of 2-(2-amino-2-methylpropyl)-4-phenylthiazole hydrobromide (**79**, 258 mg, 0.824 mmol) and Et_3N (500 μL, 3.59 mmol) in THF (10 mL). The mixture was stirred overnight before being diluted with H_2O and extracted into EtOAc. The product was recrystallized from EtOAc/hexanes as white crystals (191 mg, 69%): mp 107−108 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (s, 1H), 7.93−7.88 (m, 3H), 7.83−7.79 (m, 2H), 7.54−7.48 (m, 1H), 7.46−7.37 (m, 4H), 7.34−7.28 (m, 1H), 3.60 (s, 2H), 1.45 (s, 6H). EIMS *m/z* 337.1 $(M + 1)^+$. HPLC 98.4 area % (254 nm). Anal. Calcd for $C_{20}H_{20}N_2OS$: C, 71.40; H, 5.99; N, 8.33. Found: C, 71.49; H, 6.04; N, 8.22.

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(*E*)-*N*-[2-(4-[3-Fluorophenyl]thiazol-2-yl)vinyl]piperidine-1-carboxamide (28). A solution of (*E*)-3-[4-(3-fluorophenyl)thiazol-2-yl]acryloyl azide (**91**, 140 mg, 0.514 mmol) in toluene (10 mL) was refluxed for 1.5 h, resulting in a Curtius rearrangement to the vinyl isocyanate.[26] After the reaction mixture cooled to rt, piperidine (80 μL, 0.810 mmol) and Et_3N (80 μL, 0.810 mmol) were added and the mixture was stirred for 2 h before being diluted with water and extracted into EtOAc. The product was purified on a column of silica eluting with hexanes/EtOAc (2:1) followed by recrystallization from EtOAc/hexanes to give a pale-yellow solid (56.1 mg, 33%): mp 101.5−103 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (d, *J* = 10.5 Hz, 1H), 8.04 (s, 1H), 7.75 (dt, *J* = 7.7, 1.2 Hz, 1H), 7.71 (ddd, *J* = 10.5, 2.6, 1.5 Hz, 1H), 7.52 (td, *J* = 8.0, 6.1 Hz, 1H), 7.29 (dd, *J* = 10.5, 8.7 Hz, 1H), 7.26−7.17 (m, 1H), 5.91 (d, *J* = 8.7 Hz, 1H), 3.50 (t, *J* = 5.4 Hz, 4H), 1.67−1.52 (m, 6H). EIMS *m*/*z* 332.1 $(M + 1)^+$. HPLC 100 area % (290 nm). Anal. Calcd for $C_{17}H_{18}FN_3OS$: C, 61.61; H, 5.47; N, 12.68. Found: C, 61.37; H, 5.48; N, 12.40.

Representative Synthesis of Fused Ring Amides 29−30, 33−37, 42, and 45. *5-Benzamido-2-phenylbenzothiazole (**33**).* Et_3N (0.5 mL, 3.59 mmol) was added to a solution of 5-amino-2-phenylbenzothiazole (**101a**, 340.3 mg, 1.50 mmol) and benzoyl chloride (250 μL, 2.16 mmol) in DCM. The mixture was stirred at rt overnight before being diluted with water and extracted into DCM. Recrystallization of the product from EtOH gave white crystals (406 mg, 82%): mp 221−222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.62 (d, *J* = 2.0 Hz, 1H), 8.18−8.07 (m, 3H), 8.06−7.97 (m, 2H), 7.85 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.68−7.52 (m, 6H). EIMS *m*/*z* 331.0 $(M + 1)^+$. HPLC 99.2 area % (290 nm). Anal. Calcd for $C_{20}H_{14}N_2OS$: C, 72.69; H, 4.27; N, 8.48. Found: C, 72.45; H, 4.50; N, 8.42.

General Procedure for 5- and 6-Aminobenzothiazole Urea Derivatives 38−41, 43−44, and 46−71. A stirred solution of the appropriate primary amine (0.5−2 mmol) and Et_3N (min of 2 equiv) in DCM was chilled to maximum internal temperature of −5 °C (ice−salt bath). Triphosgene (0.35−0.55 equiv) was added either dropwise as a solution in DCM (for **38**, **40**, **41**, and **43**) or all at once in solid form for all others. The method of introduction was noncritical provided the final concentration of the primary amine was below 20 mM. The mixture was maintained at −5 °C until the starting material was no longer detectable by HPLC (typically 1 h or less). The secondary amine (min of 1 equiv) or its HCl salt (in which cases an additional equivalent of Et_3N was employed) was added, and the solution was allowed to warm to room temperature overnight. The reaction mixture was diluted with water and extracted into DCM. Unless stated otherwise, the product was directly recrystallized from the appropriate solvent(s).

*(S)-2-(3,4-Difluorophenyl)-5-(3-fluoro-N-pyrrolidylamido)-benzothiazole (**57**).* **57** was prepared from 5-amino-2-(3,4-difluorophenyl)benzothiazole (**101g**, 0.63 mmol) and (*S*)-3-fluorpyrrolidine hydrochloride. The product was purified on a silica gel column eluting with hexanes/EtOAc (1:2) and recrystallized from EtOAc/hexanes as white crystals (86 mg, 36%): mp 208−209 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (s, 1H), 8.33 (d, *J* = 2.0 Hz, 1H), 8.13 (ddd, *J* = 11.3, 7.7, 2.2 Hz, 1H), 7.99 (d, *J* = 8.8 Hz, 1H), 7.96−7.89 (m, 1H), 7.69−7.58 (m, 2H), 5.39 (dm, *J* = 53.3 Hz, 1H), 3.80−3.52 (m, 3H), 3.47 (td, *J* = 10.3, 6.9 Hz, 1H), 2.27−2.00 (m, 2H). EIMS *m*/*z* 378.0 $(M + 1)^+$. HPLC 97.0 area % (254 nm). Anal. Calcd for $C_{18}H_{14}F_3N_3OS{\cdot}0.3H_2O$: C, 56.48; H, 3.84; N, 10.98. Found: C, 56.21; H, 3.77; N, 10.89.

A scale-up synthesis from a total of 6.6 mmol of **101g** was performed in three batches. The combined product was chromatographed as above and recrystallized from EtOH as white crystals (1.59 g, 64%): mp 210−211.55 °C. HPLC 97.8% (254 nm). Anal. Calcd for $C_{18}H_{14}F_3N_3OS$: C, 57.29; H, 3.74; N, 11.13. Found: C, 57.32; H, 3.81; N, 11.02.

6-(3,4-Difluorophenyl)-2-(3-fluoro-*N*-pyrrolidylamido)-benzothiazole (72). 1,1′-Carbonyldiimidazole (163 mg, 1.01 mmol) was added to a solution of 2-amino-6-(3,4-difluorophenyl)-benzothiazole (**106**, 200 mg, 0.761 mmol) in CH_3CN (10 mL). The solution was stirred overnight with the formation of a precipitate, which was filtered off and rinsed with acetone. This material was added to a solution of 3-fluoropyrrolidine hydrochloride (87.0 mg, 0.693 mmol) and Et_3N (250 μL, 1.79 mmol) in DCM (10 mL). The suspension was stirred overnight with the dissolution of the newly formed product. The resulting solution was diluted with water and extracted into DCM. The crude product was recrystallized from EtOH/H_2O as a beige powder (177 mg, 62%): mp 171.5−173 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.36 (s, 1H), 8.26−8.20 (m, 1H), 7.81 (ddd, *J* = 12.4, 7.8, 2.2 Hz, 1H), 7.70 (dd, *J* = 8.4, 1.9 Hz, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.57 (dddd, *J* = 7.7, 4.7, 2.3, 0.9 Hz, 1H), 7.52 (dt, *J* = 10.6, 8.3 Hz, 1H), 5.39 (d, *J* = 53.1 Hz, 1H), 3.87−3.42 (m, 4H), 2.28−2.00 (m, 2H). EIMS *m*/*z* 378.1 $(M + 1)^+$. HPLC 96.0 area % (290 nm). Anal. Calcd for $C_{18}H_{14}F_3N_3OS{\cdot}0.4H_2O$: C, 56.21; H, 3.88; N, 10.93. Found: C, 56.17; H, 4.00; N, 10.75.

5-(3,4-Difluorophenyl)-2-(3-fluoro-*N*-pyrrolidylamido)-benzothiazole (73). Phenyl chloroformate (200 μL, 1.59 mmol) was added dropwise by syringe to a solution of 2-amino-5-(3,4-difluorophenyl)benzothiazole (108, 132 mg, 0.505 mmol) and pyridine (200 μL, 2.47 mmol) in THF (10 mL) maintained at −5 °C, accompanied by the formation of a precipitate. The reaction mixture was diluted with water and extracted with EtOAc. The extract was evaporated to near dryness followed by dilution with hexane to give crude phenyl (5-(3,4-difluorophenyl)benzothiazol-2-yl)carbamate (174 mg, 90%), which was used in the next step without further purification.

3-Fluoropyrrolidine hydrochloride (56.8 mg, 0.524 mmol) was added to a solution of crude (5-(3,4-difluorophenyl)benzothiazol-2-yl)carbamate (153 mg, 0.401 mmol) in pyridine (10 mL). The mixture was refluxed for 1 h. The cooled mixture was diluted with H_2O (10 mL), and the resulting suspension was stored overnight at 4 °C. The precipitate was filtered off and dried to give an off-white powder (95.7 mg, 57% overall): mp >250 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (s, 1H), 7.97 (d, *J* = 8.2 Hz, 1H), 7.91−7.73 (m, 2H), 7.64−7.45 (m, 3H), 5.39 (d, *J* = 53.0 Hz, 1H), 3.91−3.40 (m, 4H), 2.29−1.99 (m, 2H)). EIMS *m*/*z* 378.1 $(M + 1)^+$. HPLC 96.0 area % (290 nm). Anal. Calcd for $C_{18}H_{14}F_3N_3OS$: C, 57.29; H, 3.74; N, 11.13. Found: C, 57.13; H, 3.83; N, 11.15.

***N*-(2-Chloro-5-nitrophenyl)benzamides 99a−g.** *N-(2-Chloro-5-nitrophenyl)benzamide (**99a**)..*[23,43,44] Benzoyl chloride (2.63 g, 18.71 mmol) was added dropwise by syringe to a solution of 2-chloro-5-nitroaniline (**98a**, 3.06 g, 17.72 mmol) in dry pyridine (7.5 mL) under argon, resulting in the formation of a precipitate. More pyridine (7.5 mL) was added, and the mixture was stirred overnight. The reaction mixture was poured into water. The precipitated product was recrystallized from EtOH as white crystals (4.62 g, 95%): mp 171−172 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.35 (s, 1H), 8.58 (d, *J* = 2.8 Hz, 1H), 8.13 (dd, *J* = 8.9, 2.8 Hz, 1H), 8.08−7.97 (m, 2H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.69−7.61 (m, 1H), 7.61−7.52 (m, 2H). HPLC 100 area % (265 nm). Anal. Calcd for $C_{13}H_9ClN_2O_3$: C, 56.44; H, 3.28; N, 10.13. Found: C, 56.43; H, 3.19; N, 10.15.

*N-(2-Chloro-5-nitrophenyl)-3,4-difluorobenzamide (**99g**).* **99g** was prepared analogously to **99a** from 3,4-difluorobenzoyl chloride (2.77 g, 15.69 mmol) and 2-chloro-5-nitroaniline (**98a**, 2.60 g, 15.07 mmol). The product was recrystallized from EtOH as white crystals (3.84 g, 82%): mp 149−51 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.50 (s, 1H), 8.53 (d, *J* = 2.7 Hz, 1H), 8.15 (dd, *J* = 8.9, 2.8 Hz, 1H), 8.06 (ddd, *J* = 11.4, 7.8, 2.2 Hz, 1H), 7.95−7.89 (m, 1H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.67 (dt, *J* = 10.5, 8.3 Hz, 1H). HPLC 99.1 area % (265 nm). Anal. Calcd for $C_{13}H_7ClF_7N_2O_3$: C, 49.94; H, 2.26; N, 8.96. Found: C, 49.87; H, 2.29; N, 8.98.

2-Aryl-5-nitrobenzothiazoles 100a−g. *5-Nitro-2-phenylbenzothiazole (**100a**).* A mixture of *N*-(2-chloro-5-nitrophenyl)benzamide (**99a**, 3.00 g, 10.85 mmol), sodium sulfide nonahydrate (2.96 g, 12.34 mmol), and sulfur (397 mg, 12.37 mmol) in EtOH (100 mL) was refluxed for 2.5 h. The cooled reaction mixture was evaporated under reduced pressure, diluted with 1 M HCl (100 mL), and extracted into EtOAc. The product was purified on a column of silica gel eluting in DCM, followed by recrystallization from EtOH to give white needles (1.57 g, 57%): mp 195−196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (d, *J* = 2.3 Hz, 1H), 8.45 (d, *J* = 8.8 Hz, 1H), 8.30 (dd, *J* = 8.8, 2.3

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Hz, 1H), 8.15 (ddt, J = 8.4, 3.1, 1.9 Hz, 2H), 7.74−7.57 (m, 3H). HPLC 100 area % (290 nm). Anal. Calcd for $C_{13}H_8N_2O_2S$: C, 60.93; H, 3.15; N, 10.93. Found: C, 90.95; H, 2.98; N, 11.03.

*5-Nitro-2-(3,4-difluorophenyl)benzothiazole (**100g**).* **100g** was prepared analogously to **100a** from *N*-(2-chloro-5-nitrophenyl)-3,4-difluorobenzamide (**99g**, 3.78 g, 12.09 mmol), but the crude product was directly recrystallized from EtOH as a white solid (3.00 g, 85%): mp 175−196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84−8.79 (m, 1H), 8.48 (dt, J = 8.8, 0.4 Hz, 1H), 8.31 (dd, J = 8.8, 2.2 Hz, 1H), 8.20 (ddd, J = 11.3, 7.6, 2.3 Hz, 1H), 8.02 (dddd, J = 8.7, 4.3, 2.3, 1.4 Hz, 1H), 7.69 (dt, J = 10.5, 8.4 Hz, 1H). HPLC 97.6 area % (290 nm). Anal. Calcd for $C_{13}H_6F_2N_2O_2S$: C, 53.43; H, 2.07; N, 9.59. Found: C, 53.18; H, 2.09; N, 9.59.

5-Amino-2-aryl-benzothiazoles 101a−g. *5-Amino-2-phenylbenzothiazole (**101a**).*[30] A mechanically stirred mixture of 5-nitro-2-phenylbenzothiazole (**100a**, 1.87 g, 7.30 mmol), iron powder (2.21 g, 39.57 mmol), and ammonium chloride (818 mg, 15.30 mmol) in EtOH (100 mL) and water (50 mL) was stirred at reflux for 2 h until the reaction was complete by HPLC. The hot mixture was filtered through Celite, and the filtrate was evaporated to near dryness under reduced pressure. The residue was diluted with water and extracted into DCM. The product was recrystallized from EtOH as yellow crystals (1.47 g, 89%): mp 205−206 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08−7.97 (m, 2H), 7.71 (dd, J = 8.6, 0.5 Hz, 1H), 7.61−7.49 (m, 3H), 7.17 (dd, J = 2.2, 0.5 Hz, 1H), 6.79 (dd, J = 8.6, 2.2 Hz, 1H), 5.33 (s, 2H). HPLC 100 area % (254 nm). Anal. Calcd for $C_{13}H_{10}N_2S$: C, 69.00; H, 4.45; N, 12.38. Found: C, 69.07; H, 4.59; N, 12.19.

*5-Amino-2-(3,4-difluorophenyl)benzothiazole (**101g**).* **101g** was prepared analogously to **101a** from 5-nitro-2-(3,4-difluorophenyl)-benzothiazole (**100g**, 2.96 g, 10.1 mmol) as a yellow solid (1.60 g, 60%): mp >174 °C dec. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (ddd, J = 11.4, 7.7, 2.2 Hz, 1H), 7.87 (dddd, J = 8.6, 4.3, 2.2, 1.4 Hz, 1H), 7.73 (dd, J = 8.6, 0.4 Hz, 1H), 7.61 (dt, J = 10.5, 8.4 Hz, 1H), 7.18 (dd, J = 2.2, 0.5 Hz, 1H), 6.81 (dd, J = 8.6, 2.2 Hz, 1H), 5.37 (s, 2H). HPLC 100 area % (254 nm). Anal. Calcd for $C_{13}H_8F_2N_2S$: C, 59.53; H, 3.07; N, 10.68. Found: C, 59.29; H, 2.94; N, 10.62.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b01163.

General experimental procedures, synthesis and characterization of target compounds and intermediates (PDF)
Molecular formula strings (CSV)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

BPR, brain to plasma ratio; CDI, 1,1′-carbonyldiimidazole; HAT, human African trypanosomiasis; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate); IACUC, Institutional Animal Care and Use Committee; LLQ, lower limit of quantitation; SEM, standard error of the mean; SI, selectivity index; WHO, World Health Organization

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