Triazolopyrimidines and Imidazopyridines: Structure–Activity Relationships and in Vivo Efficacy for Trypanosomiasis

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Supporting Information

ABSTRACT: Better therapeutics are greatly needed to treat patients infected with trypanosomatid parasites such as *Trypanosoma cruzi* or *Trypanosoma brucei*. This report describes 28 new imidazopyridines and triazolopyrimidines with potent and selective antitrypanosomal activity. Drug-like properties were demonstrated in a number of in vitro assays. In vivo efficacy was observed for **19** and **20** in acute mouse models of *T. cruzi* infection. Compounds **19** and **20** represent potential leads for new anti-Chagas disease drugs.

KEYWORDS: Trypanosomiasis, Chagas disease, imidazopyridine, triazolopyrimidine, drug discovery

Trypanosomatid parasites that effect humans include *Trypanosoma brucei* and *Trypanosoma cruzi*. Human African trypanosomiasis, or sleeping sickness, is caused by *Trypanosoma brucei* parasites in sub-Saharan Africa and is transmitted by the tsetse fly. American trypanosomiasis, or Chagas disease, is caused by *Trypanosoma cruzi* parasites in Latin America and is transmitted by the triatomine or “kissing” bug. Both diseases primarily afflict people living in low-resource settings, which lead to delayed diagnosis and treatment. In addition, the existing antitrypanosome medicines are far from optimal due to toxicity, emerging resistance, the need for parenteral administration in some cases, and rigorous treatment and monitoring regimens leading to high costs. New more effective, safe, and affordable drugs are greatly needed to help control these neglected diseases.

This research focused on optimizing compounds containing imidazopyridine and triazolopyrimidine core scaffolds. The work builds on previously reported imidazopyridines¹ and triazolopyrimidines,² both demonstrated to have drug-like properties and potent antitrypanosomal activity in murine models. In exploring these new analogs, we are looking to improve solubility and to reduce protein binding; thus, we are paying careful attention to changes in the cLogP. It was discovered that the activity of the triazolopyrimidines was mediated by selective inhibition of the trypanosomatid proteasome.² As will be discussed, the structurally similar imidazopyridines compounds act by the same mechanism. The objectives of this work were to optimize the pharmacological and antiparasitic properties of the compounds to demonstrate efficacy in a stringent animal infection model.

Changes to the molecules focused on modifications to the R₁ and R₂ groups (Tables 1 and 2). Synthetic methods were reported in the previous publications, and full experimental details are provided in the Supporting Information. Compounds 1–16 are substituted imidazopyridines, and compounds 17–28 are substituted triazolopyrimidines. Table 1 shows the imidazopyridine compounds that are derived from the previously published HB175 (compound **64** in ref 1) and their inhibitory activity on *T. brucei*, *T. cruzi*, and mammalian cells in vitro. HB175 has an EC₅₀ on *T. cruzi* of 36 nM. Replacement of phenyl (R₂) with trifluoromethyl (**1**) leads to a 1.5-fold higher (worsening) *T. cruzi* EC₅₀, whereas introduction of fluorophenyl variants at the R₂ position (**2–4**) results in 3–4-fold improvement in potency. The difluorophenyl variants (3,4-, 3,5-, 2,4-, 2,5-, 2,3-) at R₂ (compounds **5–7**, **10**, and **11**) resulted in improved potency of 1.6–2.3-fold compared to that of HB175. The disubstituted fluorophenyl analogs are slightly more potent than the monosubstituted fluorophenyl analogs. A major loss of activity, leading to *T. cruzi* EC₅₀ values above 2 μM, was observed with analog 4-hydroxyphenyl (**8**). The addition of fluorine at varying positions on the pyridine (**9**, **12**, and **13**) resulted in *T. cruzi* EC₅₀s around 100 nM (~2-fold higher than HB175). Modifications to the urea portion of HB175 led to the 3-fluoro substituted pyrrolidine analogs **14–16** that had considerably diminished potency (12-, 28-, 93-fold, respectively) when compared to the corresponding pyrrolidyl urea analogs.

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Table 1. In Vitro Activity and Cytotoxicity of Imidazopyridines^a

compound	R ₁	R ₂	<i>T. cruzi</i> EC ₅₀ (nM) ^b	<i>T. brucei</i> EC ₅₀ (nM) ^c	CLR-8155 EC ₅₀ (nM) ^d	Hep G2 EC ₅₀ (nM) ^e
HB175	pyrrolidin-1-yl	phenyl	36	4.0	>1563	10 880
1	pyrrolidin-1-yl	trifluoromethyl	65	24	>50 000	>50 000
2	pyrrolidin-1-yl	3-fluorophenyl	11	7.8	518.0	28 200
3	pyrrolidin-1-yl	4-fluorophenyl	14	8.4	>50 000	>50 000
4	pyrrolidin-1-yl	2-fluorophenyl	11	8.0	4715	>50 000
5	pyrrolidin-1-yl	3,4-difluorophenyl	25	17	>50 000	>50 000
6	pyrrolidin-1-yl	3,5-difluorophenyl	19	20	1797	>50 000
7	pyrrolidin-1-yl	2,4-difluorophenyl	27	18	7335	32 400
8	pyrrolidin-1-yl	4-hydroxyphenyl	2200	14	5209	>50 000
9	pyrrolidin-1-yl	2-fluoropyridin-3-yl	96	12	23 350	>50 000
10	pyrrolidin-1-yl	2,5-difluorophenyl	20	9.9	24 730	>50 000
11	pyrrolidin-1-yl	2,3-difluorophenyl	28	10	>50 000	>50 000
12	pyrrolidin-1-yl	5-fluoropyridin-3-yl	45	5.1	>50 000	>50 000
13	pyrrolidin-1-yl	6-fluoropyridin-3-yl	160	11	27 100	>50 000
14	(<i>R</i>)-3-fluoropyrrolidin-1-yl	2-fluoropyridin-3-yl	530	6.7	>50 000	>50 000
15	(<i>R</i>)-3-fluoropyrrolidin-1-yl	phenyl	1300	6.5	1840	29 600
16	(<i>R</i>)-3-fluoropyrrolidin-1-yl	pyridin-3-yl	4100	17	>50 000	>50 000

^aHB175 is the parent compound for comparison. ^bEC₅₀ values are averages of triplicate. Benznidazole was included as a control in each assay, with average EC₅₀ values ± SEM of 685.07 ± 119.84 μM (*n* = 14). ^cEC₅₀ values are averages of triplicates. Pentamidine was included as a control in each assay, with average EC₅₀ values ± SEM of 1.42 ± 0.17 μM (*n* = 21). ^dEC₅₀ values are averages of quadruplicates. Quinacrine was included as a control in each assay, with average EC₅₀ values ± SEM of 2974.1 ± 542.1 μM (*n* = 11). ^eEC₅₀ values are averages of quadruplicates. Quinacrine was included as a control in each assay, with average EC₅₀ values ± SEM of 7635.7 ± 534.7 μM (*n* = 8).

Table 2. In Vitro Activity and Cytotoxicity of Triazolopyrimidines^a

compound	R ₁	R ₂	<i>T. cruzi</i> EC ₅₀ (nM) ^b	<i>T. brucei</i> EC ₅₀ (nM) ^c	CLR-8155 EC ₅₀ (nM) ^d	Hep G2 EC ₅₀ (nM) ^e
GNF6702	2,4-dimethyl-1,3-oxazole	pyridin-2-yl	19.3	16.6	>2500	>2500
17	2,5-dimethyl-1,3-oxazole	pyridin-2-yl	14.4	9.10	>5000	>5000
18	(<i>R</i>)-3-fluoropyrrolidin-1-yl	2-fluoropyridin-3-yl	580	11.4	>50 000	>50 000
19	3,3-difluoropyrrolidin-1-yl	pyridin-2-yl	61.2	18.7	>50 000	>50 000
20	2,4-dimethyl-1,3-oxazole	2-chlorophenyl	20.0	20.8	6500	>50 000
21	3,3-difluoropyrrolidin-1-yl	2-chlorophenyl	79.7	82.8	34 090	>50 000
22	3,3-difluoropyrrolidin-1-yl	phenyl	81.0	32.0	39 740	>50 000
23	3,3-difluoropyrrolidin-1-yl	2-fluorophenyl	70.7	53.0	47 090	>50 000
24	3,3-difluoropyrrolidin-1-yl	3-fluorophenyl	83.8	35.8	>50 000	>50 000
25	3,3-difluoropyrrolidin-1-yl	4-fluorophenyl	81.2	50.8	>50 000	>50 000
26	3,3-difluoropyrrolidin-1-yl	4-chlorophenyl	83.7	165	>50 000	>50 000
27	3,3-difluoropyrrolidin-1-yl	3-chlorophenyl	59.3	166	>50 000	>50 000
28	2,4-dimethyl-1,3-oxazole	2-chloropyridin-3-yl	172	39.2	>50 000	>50 000

^aGNF6702 is the parent compound for comparison. ^bEC₅₀ values are averages of triplicates. Benznidazole was included as a control in each assay, with average EC₅₀ values ± SEM of 586.6 ± 51.1 μM (*n* = 10). ^cEC₅₀ values are averages of triplicate. Pentamidine was included as a control in each assay, with average EC₅₀ values ± SEM of 2.18 ± 0.32 μM (*n* = 10). ^dEC₅₀ values are averages of quadruplicates. Quinacrine was included as a control in each assay, with average EC₅₀ values ± SEM of 2757.5 ± 528.7 μM (*n* = 7). ^eEC₅₀ values are averages of quadruplicates. Quinacrine was included as a control in each assay, with average EC₅₀ values ± SEM of 8683.9 ± 729.8 μM (*n* = 7).

The mechanism of action for the imidazopyridine series of compounds has not been previously elucidated.¹ Because of the similarities to the triazolopyrimidines, it seems likely that the imidazopyridines would also inhibit the trypanosome proteasome. This hypothesis was tested by measuring the EC₅₀ values of *T. cruzi* cells overexpressing the proteasome β 4 subunit containing the F24L mutation that was shown to confer resistance to GNF6702.² The imidazopyridine compound, HB175, showed a 23.7-fold shift in EC₅₀ similar to what was previously observed with GNF6702 (Figure S1). In addition, two new triazolopyrimidines (**19** and **20**) were also observed to give large shifts in EC₅₀. The shifted EC₅₀ was only observed when the mutant enzyme was induced in cells with tetracycline as opposed to the cells without induction. It was previously shown that no shift was observed by overexpression of the wild-type proteasome β 4 subunit.² These findings are consistent with the imidazopyridines mediating their anti-trypanosomal action by targeting the proteasome. It was also previously shown that GNF6702 functionally led to the buildup of ubiquitinated proteins in trypanosomes further establishing the mechanism of proteasome inhibition.²

A series of new triazolopyrimidine compounds, structurally related to GNF6702, were also synthesized (Table 2). As an alternative at R₁, the 2,5-dimethyl-1,3-oxazole was introduced at this position (**17**) and showed a similar potency to GNF6702 on both trypanosomes. The 3-fluoropyrrolidine (**18**) triazolopyrimidine analog led to ~30-fold diminished potency compared to GNF6702. The 3,3-difluoro pyrrolidin-1-yl substitution at R₁ in triazolopyrimidines (**19**, **21–27**) resulted in ~4-fold decrease in the potency. Introduction of the 2-chlorophenyl substitution at R₂ and 2,4-dimethyl-1,3-oxazole at R₁ resulted in compound (**20**) with an EC₅₀ of 20 nM against *T. cruzi*, a similar potency to GNF6702. Interestingly, the 2-chlorophenyl substitution at R₂ with the 3,3-difluoropyrrolidin-1-yl at R₁ (**21**) had diminished activity, with *T. cruzi* EC₅₀ of 80 nM. Varying the position of the chlorine in the phenyl ring (R₂) to meta (**27**) and para (**26**) resulted in similar activities. The 2-chloropyridin-3-yl analog (**28**) showed nine-fold reduced activity compared to GNF6702.

For imidazopyridine and triazolopyrimidine analogs, some differences were seen for the SAR of *T. brucei* versus *T. cruzi*. More potency enhancement was observed for *T. brucei* than *T. cruzi* upon fluorination of the aromatic ring (R₂) at any position 2–4 or difluorophenyl analogs 5–7 and 10–11. Furthermore, pyrrolidyl urea based analogs with trifluoromethyl substitution (R₂) **1**, 4-hydroxyphenyl (R₂) **8** appeared to substantially lose potency against *T. cruzi* compared to *T. brucei*. (R)-Enantiomer analogs **14–16** were more active on *T. brucei* than on *T. cruzi*. For fluoro substituted pyridin-3-yl analogs (**9**, **12**, and **13**), the *T. brucei* potency order was 5-fluoro-pyridn-3-yl > 6-fluoro-pyridn-3-yl > 2-fluoro-pyridn-3-yl, whereas the *T. cruzi* potency order was 6-fluoro-pyridn-3-yl > 2-fluoro-pyridn-3-yl > 5-fluoro-pyridn-3-yl. In the imidazopyridine series, compound **12** exhibited an EC₅₀ value of 5 nM on *T. brucei* (17-fold more active than it was against *T. cruzi*). In both series of compounds, it was observed that the potency tended to be greater against *T. brucei* than *T. cruzi*, which may be due to the added requirement that compounds penetrate into the intracellular environment of the mammalian host cells to exert activity on *T. cruzi*, whereas *T. brucei* are cultured without host cells.

Additionally, compound **20** had outstanding results in a washout experiment compared to posaconazole (Table 3). In

Table 3. In Vitro Washout Experiment of *T. cruzi* Infected 3T3 Fibroblasts^a

compound	concentration (X EC ₅₀)	relapse day (after washout)
benznidazole	50	^b
	25	>60
	12.5	>60
posaconazole	50	30
	25	18
	12.5	14
20	50	>60
	25	>60
	12.5	>60

^aCells were treated with the indicated compounds for 16 days (refreshed every 4 days), then observed for 60-days post-washout.

^bIndicates the 3T3 monolayer died before the end of the experiment.

this experiment, *T. cruzi* infected 3T3 fibroblasts were exposed to compounds at varying concentrations (12.5X, 25X, and 50X the TcEC₅₀) for 16 days followed by washout and observation of outgrowth for another 60 days.³ Compound **20** remained clear of parasites for >60 days at all concentrations, whereas posaconazole had free swimming trypomastigotes at 14, 18, and 30 days postwashout. In comparison, benznidazole remained clear of parasites at 60 days post washout at 12.5X and 25X the TcEC₅₀, although toxicity to the host-cell fibroblasts was observed at the highest concentration of 50X the TcEC₅₀. These results indicate that the triazolopyrimidine (**20**) appears to have a cytotoxic effect on *T. cruzi* similar to benznidazole, which differentiates it from posaconazole, which has a cytostatic effect.³

A number of compounds in the imidazopyridine series exhibited mild to moderate cytotoxicity on mammalian cells, particularly against the lymphocytic cell line, CRL-8155 (Table 1). Although the selectivity index (antiparasitic EC₅₀/mammalian cell EC₅₀) is >100 in the majority of cases, the potential for these compounds to produce in vivo toxicity needs to be monitored. However, it is worth noting that previous investigation with HB175 administered to mice at a dose of 50 mg/kg twice per day for 5 days (resulting in cures of acute *T. brucei* infection) showed no weight change compared to vehicle-treated mice and no signs of clinical toxicity.¹

Most of the triazolopyrimidine compounds (Table 2) demonstrated no cytotoxicity on mammalian cells up to the maximum concentrations tested (i.e., 50 μ M or the solubility limit in culture medium). Exceptions included **20–23** on the lymphocytic cell line, CRL-8155. In these cases, the selectivity index still was close to 1000.

Selected compounds were assayed for stability to human and mouse liver microsomes (Table 4). The half-lives of compounds **2–6** and **16** were relatively fast (<30 min). Interestingly, compound **9** demonstrated microsome half-lives of >90 min. This stability is probably attributable to the presence of 2-fluoropyridn-3-yl (R₂) substitution. The metabolic stability of pyrrolidine imidazopyridines was improved by fluorination on pyrrolidine⁴ (e.g., **14**, **15**, and **16**), which delayed microsome metabolism to >90 min. By adding another nitrogen atom into the core-ring (giving the triazolopyrimidines), excellent microsome stability was demonstrated as exemplified by **17** and **19–28**. To find an

Table 4. In Vitro ADME and Pharmacokinetic Characterization of Selected Molecules^a

compound	microsomes $t_{1/2}$ (min)		solubility pH @ 7.4, 6.5, and 2.0 (μ M)	cLogP	brain/plasma @ 60 min ($n = 3$) (μ M) (average $\pm$ SEM)	oral pharmacokinetics		FU%
	human	mouse				$C_{\max}$ (μ M) (average $\pm$ SEM)	AUC (min μ mol) (average $\pm$ SEM) ($n = 3$)	
HB175	30.6	10.1	6.9, 5.2, 35.3	3.94	0.04 $\pm$ 0.01	4.33 $\pm$ 0.77	1240 $\pm$ 132	2.70
1				3.17	0.08 $\pm$ 0.00	1.08 $\pm$ 0.15	74.6 $\pm$ 12.0	
2	11.1	5		4.08		7 $\pm$ 1.2	2150 $\pm$ 267	
3	12.2	5.7		4.08	0.04 $\pm$ 0.02	6.27 $\pm$ 0.76	1460 $\pm$ 194	
4	17.1	10.3		4.08	0.02 $\pm$ 0.00	2.23 $\pm$ 0.09	696 $\pm$ 14.8	
5	16.9	4.8		4.22	0.02 $\pm$ 0.01	6.67 $\pm$ 1.66	1650 $\pm$ 325	
6	10.5	7.7		4.22		4.8 $\pm$ 0.53	1290 $\pm$ 125	
7				4.22	0.01 $\pm$ 0.00	9.5 $\pm$ 0.92	2140 $\pm$ 129	
8				3.64	0.0 $\pm$ 0.00	0.13 $\pm$ 0.03	19.7 $\pm$ 1.50	
9	>90	>90	9.3, 5.9, 43.1	3.26	0.0 $\pm$ 0.00	25.6 $\pm$ 4.1	4410 $\pm$ 1033	4.93
10				4.22		2.8 $\pm$ 0.4	771 $\pm$ 54.0	
11				4.22	0.0 $\pm$ 0.00	1.83 $\pm$ 0.2	651 $\pm$ 69.0	
12				2.86		1.07 $\pm$ 0.2	222 $\pm$ 40.0	
13				3.26				
14	>90	38.1		3.00				
15	>90	28.2		3.68				
16	>90	19.6		2.46				
GNF6702	>90	>90	1.8, 2.2, 4.3	3.05	0.12 $\pm$ 0.00	54.4 $\pm$ 3.3	45923 $\pm$ 2047	12.7
17	>90	28.7	3.3, 2.6, 2.7	3.51				
18			39.6, 38.4, 40.4	3.54				14.6
19	>90	>90	12.4, 16.6, 44.3	3.96	0.02 $\pm$ 0.00	31.13 $\pm$ 2.4	15738 $\pm$ 982.7	11.0
20	>90	>90	1.98, 2.4, 1.8	4.46	0.23 $\pm$ 0.04	39.3 $\pm$ 1.7	40010 $\pm$ 2302	1.50
21	>90	>90	14.4, 13.9, 15.3	5.37				3.60
22	>90	>90	4.6, 6.6, 8.0	4.81				4.00
23	>90	>90	4.4, 4.6, 5.3	4.96				4.03
24	>90	>90	2.4, 2.77, 2.75	4.96				3.30
25	>90	>90	0.65, 1.52, 1.47	4.96				3.43
26	>90	>90	1.43, 1.11, 0.64	5.37				1.37
27	>90	>90	3.11, 4.69, 4	5.37				1.00
28	>90	>90	3.84, 2.59, 4.08	3.52				13.5

^a $C_{\max}$ = maximum blood concentration; AUC = area under curve; FU% = fraction unbound (%).

alternative group for R_1 in GNF6702, the 2,5-dimethyl-1,3-oxazole-4-carboxamide analog (**17**) was synthesized, but it demonstrated relatively poor microsome stability.

Initial pharmacokinetic studies for selected compounds were performed by administering the compounds to groups of three mice at a single dose of 50 mg/kg by oral gavage. A pattern can be observed in that plasma exposure (both $C_{\max}$ and AUC) was generally higher for the triazolopyrimidine compounds (GNF6702, **19** and **20**) than for the imidazopyridine compounds (HB175 and **1–12**) (Table 4). An exception was compound **9**, which demonstrated a high exposure ($C_{\max}$ of 25.6 μ M and an AUC of 4414 min- μ M). In vitro studies of liver microsome stability were also conducted (Table 4). As noted previously, nearly all of the triazolopyrimidine compounds had mouse microsome half-lives of >90 min, whereas most of the imidazopyridine compounds had shorter (<30 min) half-lives. Thus, the lower in vivo exposures of the imidazopyridines are likely explained by more rapid liver metabolism. The high exposure of the imidazopyridine (**9**) is consistent with this analysis that longer microsome half-lives (>90 min) are correlated with higher plasma levels.

The pharmacokinetic properties in mice of compounds **19** and **20** were studied in greater detail by comparing the plasma profiles following iv and oral administration. Parameters such as clearance, plasma half-life, volume of distribution, and

bioavailability are documented (Table S1). Note that we were unable to perform a parallel study with GNF6702 due to its poor solubility, which hindered the ability to formulate a clear solution at the necessary concentration for IV administration. Most noteworthy were the long plasma half-life (avg. 770 min) of **20** compared to 93 min for **19** and the low clearance (avg. 0.98 mL/min/kg) for **20** compared to 5.3 mL/min/kg for **19**. Both compounds had relatively small volume of distribution 0.7–1.1 L/kg and relatively good oral bioavailabilities of ~70%.

Solubility plays a role for formulation and bioavailability; thus, solubility studies at three pH values (7.4, 6.5, and 2.0) were performed (Table 4). Because of better metabolic stability, the triazolopyrimidines were the main focus of these studies. Solubility was low for the reference compound GNF6702, less than 5 μ M at all pH values, and this finding was mirrored for most compounds in the series. However, compound **19** was an exception with improved solubility in the 16–50 μ M range.

Plasma protein-binding experiments (in 50% buffered mouse plasma) were carried out with a subset of compounds. As reference, GNF6702 was found to have a free fraction of 12.7%. Compound **19** was comparable with a free fraction of 11.0%, whereas **20** showed a lower free fraction of 1.5%. A

Figure 1. Efficacy of selected triazolopyrimidine compounds in *T. cruzi* acute efficacy model in mice. Compounds were administered for 5 days (days 6–10 postinfection). GNF6702 and 19 were given at 50 mg/kg twice per day. 20 was given at 50 mg/kg once per day because of its longer half-life. Benznidazole (BNZ) was given at 100 mg/kg once per day per standard dosing in mice for this drug.^{5,6}

representative compound from the imidazopyridine series (HB175) was found to have a free fraction of 2.9%.

Permeability into the brain was also measured on a subset of compounds by comparing the concentration in brain tissue versus plasma in mice at 1 h post dose (5 mg/kg ip). The purpose of these experiments was to assess which compounds could potentially be suitable for African trypanosomiasis, where the drug needs to cross the blood–brain barrier to be effective in late-stage disease. Compounds in the imidazopyridine series (HB175, 1, 3, 4, 5, 7, 8, 9, 11) all had very low free fractions in the brain (all <8%) (Table 4). The triazolopyrimidine, GNF6702, was slightly better with brain to plasma ratio of 12%. The best compound was 20 with a mean brain concentration of 1.9 μM compared to plasma concentration of 8.8 μM for a percentage of 23.1%. Although this may achieve the necessary threshold for efficacy testing, the simultaneous finding of high plasma protein binding (98.5%) indicates that it is unlikely to be effective against a chronic *T. brucei* infection model. As a result, the attention was focused on efficacy testing against *T. cruzi* where CNS activity is not considered necessary. On the basis of in vitro activity, cytotoxicity, metabolic stability, protein binding, and pharmacokinetic properties, compounds 19 and 20 were selected for in vivo efficacy testing in a *T. cruzi* infection model and were compared to the parent compound GNF6702 and to the clinical drug, benznidazole.

The efficacy experiment was conducted using the “acute-established” infection model with luciferase expressing *T. cruzi*.⁷ In this model, mice were infected with 2×10^4 Tulahuen strain (DTU VI) on day 0, a dose that is >95% lethal by 20 days postinfection. The infection was allowed to become established for 7 days before treatment was given from day 7–11 postinfection. GNF6702, 19, 20, and benznidazole were administered by oral gavage to groups of mice ($n = 5$) at the indicated doses (Figure 1). The in vivo imaging system (IVIS) was used to serially monitor the infection and demonstrated a high level of parasitism of all mice at the time of the first dose (day 6). The vehicle-treated mice had uncontrolled infection (see day 13), evidence of lassitude weight loss, and were euthanized on day 13 (after images were taken). In contrast, the other groups of mice had dramatic reduction in the levels of infection at day 13. Mice treated with GNF6702 and 20 were below the limit of detection at this same time point. The benznidazole-treated mice all appear to have infection at day 13 by IVIS, which continued to rise

through day 24. At that point, with the additional control of the immune response, the uncured mice went into the “chronic” infection phase and demonstrated low-grade *T. cruzi* infection thereafter (see day 42). The mice in the 20 group had undetectable parasites until day 24 when they appeared to return in all individuals. Mice treated with 19 had a similar pattern (although some infection was apparent on day 17). The compound giving the longest period of suppression was GNF6702 with the infection not becoming clearly evident until day 42. Imaging at day 65 confirmed that none of the mice treated with GNF6702 or 20 was cured (data not shown). However, all mice treated with GNF6702, 19, 20, and benznidazole had 100% survival out to day 65 when the experiment was terminated, whereas 100% of the vehicle treated mice died due to the infection. In addition, there were no apparent side effects (such as significant weight loss) associated with any of the administered compounds.

The in vivo efficacy studies lead to the following conclusions and speculation. First, the acute-established infection model with the Tulahuen strain in Swiss Webster mice is a high bar to clear with respect to demonstrating cure. As shown here and by others,⁸ even the clinical drug, benznidazole, does not result in cures using the acute infection model. Therefore, we are encouraged to see that the degree of parasite suppression is greater and more sustained for the compounds under investigation than for benznidazole. This is particularly true for GNF6702 and 20. Note that the latter compound is administered once per day at 50 mg/kg and the former is given twice per day at the 50 mg/kg dose. These compounds are similar in structure except at the R2 position where 20 has a 2-chlorophenyl. We attribute the strong in vivo activity of these compounds to the high potency against *T. cruzi* (EC_{50} of ~ 20 nM) combined with excellent plasma exposure. Compound 19 is attractive because of the somewhat higher solubility (a potential liability for GNF6702 and 20), but it appears to be slightly inferior to GNF6702 and 20 in the efficacy study probably because of its three-times higher EC_{50} and higher plasma clearance.

In summary, 28 new compounds are described in this paper (16 imidazopyridines and 12 new triazolopyrimidines). Most compounds are potent against both *T. brucei* and *T. cruzi* with EC_{50} values <100 nM and are highly selective when compared to mammalian cells. Studies of brain permeability indicate that none of the compounds achieves desirable levels (brain/plasma ratio of greater than 0.3); thus, they are not further

investigated for *T. brucei*, but only for *T. cruzi* where brain permeability is not required. The triazolopyrimidine compounds in general have greater stability than the imidazopyridines when incubated with liver microsomes, and this translates to higher blood exposures in mice ($C_{\max}$ and AUC). The lead compound **20** was shown to be cytotoxic against *T. cruzi* in a stringent in vitro assay. On the basis of the optimal combination of properties (primarily considering *T. cruzi* EC_{50} and the plasma pharmacokinetic profile), compounds **19** and **20** were compared head-to-head with GNF6702 and benznidazole in the acute-established *T. cruzi* infection model in mice. The experiment showed that the three triazolopyrimidines produced greater parasite suppression than the clinical drug benznidazole. None of the compounds completely cured the mice, but this is expected with only 5 days of treatment during the acute phase when parasite levels are extremely high. GNF6702 was previously shown to cure mice with chronic *T. cruzi* infection.² Therefore, studies are planned to test the efficacy **19** and **20** in chronic *T. cruzi* model as well as to investigate their safety.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00498.

Mouse iv pharmacokinetics; detailed experimental procedures including chemical syntheses (PDF)

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Notes

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■ ABBREVIATIONS

ADME, absorption, distribution, metabolism, and excretion; AUC, area under the curve; cLogP, calculated log of the partition coefficient; $C_{\max}$, maximum blood/plasma concentration; DTU, discrete typing unit; EC_{50} , effective concentration to inhibit growth by 50%; EC_{90} , effective concentration to inhibit growth by 90%; %FU, percent fraction unbound; IVIS, in vivo imaging system; PBS, phosphate buffered saline; PSMB4, proteasome $\beta 4$ subunit; q.d., once a day (quaque die)

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