Series of Alkynyl-Substituted Thienopyrimidines as Inhibitors of Protozoan Parasite Proliferation

Jennifer L. Woodring,[†] Ranjan Behera,[‡] Amrita Sharma,[‡] Justin Wiedeman,[‡] Gautam Patel,[†] Baljinder Singh,[†] Paul Guyett,[‡] Emanuele Amata,[†] Jessey Erath,^{||,§} Norma Roncal,[⊥] Erica Penn,[⊥] Susan E. Leed,[⊥] Ana Rodriguez,^{||,§} Richard J. Sciotti,[⊥] Kojo Mensa-Wilmot,[‡] and Michael P. Pollastri^{*,†,§}

[†]Department of Chemistry & Chemical Biology, Northeastern University, 360 Huntington Avenue, Boston, Massachusetts 02115, United States

[‡]Department of Cellular Biology, Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia 30602, United States

^{||}Department of Microbiology, New York University School of Medicine, 430 E. 29th Street New York, New York 10010, United States

[⊥]Experimental Therapeutics, Walter Reed Army Institute of Research, 2460 Linden Lane, Silver Spring, Maryland 20910, United States

[§]Anti-Infectives Screening Core, New York University School of Medicine, New York, New York 10010, United States

Supporting Information

ABSTRACT: Discovery of new chemotherapeutic lead agents can be accelerated by optimizing chemotypes proven to be effective in other diseases to act against parasites. One such medicinal chemistry campaign has focused on optimizing the anilinoquinazoline drug lapatinib (**1**) and the alkynyl thieno[3,2-*d*]pyrimidine hit GW837016X (NEU-391, **3**) into leads for antitrypanosome drugs. We now report the structure–activity relationship studies of **3** and its analogs against *Trypanosoma brucei*, which causes human African trypanosomiasis (HAT). The series was also tested against *Trypanosoma cruzi*, *Leishmania major*, and *Plasmodium falciparum*. In each case, potent antiparasitic hits with acceptable toxicity margins over mammalian HepG2 and NIH3T3 cell lines were identified. In a mouse model of HAT, **3** extended life of treated mice by 50%, compared to untreated controls. At the cellular level, **3** inhibited mitosis and cytokinesis in *T. brucei*. Thus, the alkynylthieno[3,2-*d*]pyrimidine chemotype is an advanced hit worthy of further optimization as a potential chemotherapeutic agent for HAT.

KEYWORDS: Trypanosomes, human African trypanosomiasis (HAT), leishmaniasis, Chagas, malaria, protozoan parasite inhibitors, thienopyrimidines

Target class repurposing is a method for discovering new chemical matter against human African trypanosomiasis (HAT),^{1,2} a life-threatening disease caused by the protozoan parasite *Trypanosoma brucei*.³ By taking known human inhibitors of a well-characterized family of targets, inhibitors can be identified and reoptimized for antiparasitic activity. We have applied this approach against *T. brucei*.^{4–9} For example, during a screen of human tyrosine kinase inhibitors, lapatinib (Figure 1, compound **1**), an FDA approved drug for breast cancer tumors,^{10–13} was found to be effective against *T. brucei* growth with an EC₅₀ of 1.4 μM.¹⁴ Optimization of the lapatinib scaffold led to the synthesis of NEU-617 (**2**), which was 40-fold more potent than lapatinib (**1**) and had improved selectivity over human HepG2 cells.⁷ Further optimization of **2** to improve the physicochemical properties led to NEU-1961 (**2a**) with improved LogP and ligand-lipophilicity efficiency (LLE).¹⁵

Figure 1. Optimization of lapatinib.

Received: May 29, 2018

Accepted: September 4, 2018

Published: September 4, 2018

Following our success in identifying lapatinib as a lead for anti-HAT drug discovery,^{15–17} we tested other lapatinib-related compounds synthesized by GlaxoSmithKline¹⁸ for activity against *T. brucei*. Compound GW837016X (NEU-391, Figure 2, compound 3) is a covalent inhibitor of human ErbB-2

Figure 2. Analog designs.

kinase.^{18–21} This compound inhibits proliferation of bloodstream *T. brucei* in vitro (EC₅₀ of 0.4 μM).²² Unlike compound 1, 3 features a thieno[3,2-*d*]pyrimidine scaffold and an alkyne warhead at the 6-position that covalently modifies a cysteine residue of ErbB-2.²¹ We have previously explored bioisosteric replacement of the alkynyl functionality,²² and we herein report a wider range of substituted alkynes and other scaffolds (Figure 2). Besides the thieno[3,2-*d*]pyrimidine chemotype, we assessed matched isomeric thieno[2,3-*d*]pyrimidine and quinazoline scaffolds. Finally, we describe (i) the biological effects of 3 on *T. brucei*, (ii) characterization of the effects on trypanosome cell cycle, and (iii) efficacy of compound 3 in a mouse model of HAT.

The thienopyrimidine (4, 5) and quinazoline (6) templates were synthesized by previously published methods.^{22–25} Each template was treated with a terminal alkyne via a Sonogashira coupling reaction, shown in Scheme 1, to achieve the desired product. Those alkynes that were not commercially available were prepared as outlined in Scheme S1 in the Supporting Information.

Scheme 1. Synthesis of Alkynyl Derivatives^a

^aReagents and conditions: (i) alkyne, PdCl₂(PPh₃)₂, CuI, TEA, DMF, 55 °C, 12 h, 6–52%.

All compounds in series 7, 8, and 9 had different alkyne groups, and they were first tested against *T. brucei* cultures at a single concentration (5 or 10 μM); those inhibiting proliferation >75% were advanced to EC₅₀ determination. Several compounds demonstrated submicromolar potencies. Of the new analogs, 7t, 7j, and 7l were the most potent with EC₅₀ values of 0.23 μM (selectivity index, SI = 43.6), 0.28 μM (SI = 17.5), and 0.76 μM (SI = 40.8), respectively. Compound 7t (EC₅₀ = 0.22 μM) is the

enantiomer of the reference compound 3 (EC₅₀ = 0.4 μM) and is slightly better in terms of potency. Addition of the Boc-protecting group to the pyrrolidine ring in 7s resulted in a 5-fold loss of potency against *T. brucei*. This is likely due to an increase in steric encumbrance of the Boc group or reduction of cellular permeability and not due to the loss of the hydrogen bond donor, given the activity of both 7j and 7l. Furthermore, small aliphatic cyclic rings found in 7t, 7j, and 7l were more potent than both pyridine 7r (EC₅₀ = 1.3 μM) and larger cyclic aliphatic systems such as morpholine 7a (16% inh/5 μM), piperazine 7b (EC₅₀ = 2.5 μM), or thiomorpholine 7k (12% inh/5 μM). This may suggest either unfavorable steric interactions within the putative binding site of the target(s) or unfavorable effects on cellular permeability. Conversely, some steric bulk is needed in this region as evidenced by a loss in activity exhibited by smaller aliphatic substituted compounds such as 7i (EC₅₀ = 3.4 μM) and 7g (52% inh/5 μM) when compared with 3 (EC₅₀ = 0.26 μM). The methylated piperazine tail 8b (EC₅₀ = 1.0 μM) was slightly more potent than the unsubstituted piperazine 8h (EC₅₀ = 1.9 μM), though the analogous morpholine 8a showed little activity (48% inh/5 μM). The quinazoline series (9) generally displayed only modest potencies.

We have had previous success in “parasite hopping”,¹⁷ wherein compounds prepared as potential *T. brucei* inhibitors are tested against other protozoan parasites. Thus, this library was screened against intracellular amastigotes of *T. cruzi* (responsible for Chagas disease) and of *Leishmania major* (leishmaniasis). Based on our previous observation that thienopyrimidines have activity against *Plasmodium falciparum*,²² the library was also tested against different strains of *Plasmodium falciparum* (malaria). From these assays, compounds 8h (EC₅₀ = 0.61 μM) and 9a (EC₅₀ = 0.81 μM) (Table 2) showed submicromolar activity against *T. cruzi*. There does appear to be a preference for the quinazoline core over either thienopyrimidine regioisomer. This is apparent when comparing compounds 9a and 7a, where there is a ~4-fold improvement in potency for the quinazoline core. A similar trend is observed for 7k (EC₅₀ = 5.0 μM) versus 9k (EC₅₀ = 2.5 μM) and 7l (EC₅₀ = > 50 μM) versus 9l (EC₅₀ = 2.0 μM). Interestingly, there appears to be a preference for the morpholine (9a; EC₅₀ = 0.81 μM) and piperazine (8h; EC₅₀ = 0.61 μM) over the thiomorpholine (9k; EC₅₀ = 2.5 μM), indicating a sensitivity to electronegativity differences at this position.

Against *Leishmania major* amastigotes, 15 compounds were active in the micromolar range (<10 μM), but only one compound had submicromolar activity (7r, EC₅₀ value of 0.38 μM, SI = 62.1, Table 2). The *P. falciparum* screen yielded nine analogs with submicromolar activity. The most potent compounds were 8j (EC₅₀ = 0.14 μM, Table 2) and 9b (EC₅₀ = 0.16 μM, Table S2, Supporting Information). Several alkynes exhibited submicromolar activity irrespective of the central scaffold; they included 7b, 8b, 9b, 8f, 8h, 8j, 7t (Table 2), and 9b (Table S2, Supporting Information). These compounds were also screened against the drug-resistant W2 and C235 strains of *P. falciparum* (Table S2, Supporting Information); all compounds were within a 3-fold potency window compared to the drug-sensitive D6 strain, indicating no cross-resistance with chloroquine, mefloquine, or pyrimethamine.

HepG2 cells were employed to screen for overt toxicity and to calculate the selectivity index of the antiparasitic hits. Three compounds had selectivity index of 20 or more against *T. brucei* over HepG2 cells. The selectivity ranged from 43× (7t) to 4.3× (7b). In case of selectivity of compounds against *P. falciparum* D6 over HepG2 cells, there were eight compounds with selectivity of

Table 1. Inhibition Profile of Alkynes against *T. brucei**

A

B

C

Entry	Scaffold ^a	R	<i>T. b. brucei</i> EC ₅₀ ± SEM (μM) or % inh at 5 μM ^b	HepG2 TC ₅₀ (μM)	SI ^c
7a	A		16%	>29	--
8a	B		48%	>29	--
9a	C		27%	>30	--
7b	A		2.5 ± 0.04	11	4.3
8b	B		1.0 ± 0.06	15	15
9b	C		1.0 ± 0.07	8.2	8.2
7c	A		27%	26	--
8c	B		53%	>28	--
9c	C		23%	>28.0	--
7d	A		18%	>27	--
8d	B		0%	21.6	--
7e	A		31%	29.3	--
8e	B		1%	>32	--
7f	A		1%	26.5	--
8f	B		0%	17	--
7g	A		52%	>32	--
8g	B		2.5 ± 0.04	>32	>13
7h	A		16%	>29	--
8h	B		1.9 ± 0	7.7	4.1
7i	A		3.4 ± 0.05	>33	>9.7
8i	B		18%	9.5	--

Entry	Scaffold ^a	R	<i>T. b. brucei</i> EC ₅₀ ± SEM (μM) or % inh at 5 μM ^b	HepG2 TC ₅₀ (μM)	SI ^c
7j	A		0.28 ± 0.07	4.9	18
8j	B		>2.5	>30	--
7k	A		12%	>28	--
9k	C		19%	>29	--
7l	A		0.76 ± 0.07	>31	>41
9l	C		2%	>3.0	--
7m	A		15%	>24	--
7n	A		11%	>32	--
7o	A		33%	>28	--
7p	A		19%	30	--
7q	A		31%	>5.0	--
7r	A		1.3 ± 0.03	24	18
7s	A		1.2 ± 0.03	>26	>22
7t	A		0.22 ± 0.02	9.6	44
Suramin			0.04 ± 0		

* Compare compounds to 3, *T. brucei* EC₅₀ = 0.26/0.4 μM. EC₅₀ = 0.26 μM is obtained from our initial screening program with parasite concentration of 2 × 10³ cells/mL, and for direct comparison with other compounds, 3 was retested in the same assays that its derivatives were tested in at 5 × 10⁴ cells/mL (EC₅₀ = 0.4 μM). ^aAr is 3-chloro-4-((3-fluorobenzyl)oxy)phenyl. ^bCompounds showing >75% growth inhibition at 5 μM for *T. brucei* were tested for EC₅₀ values. *T. brucei* EC₅₀ values are the result of triplicate experiments. ^cSI is selectivity index = HepG2 TC₅₀/Tbb EC₅₀.

20 or more; for *P. falciparum*, 8j had the best selectivity index of more than 200X. We also tested compounds against NIH 3T3 cells, which are the host cell line utilized for culturing the intracellular amastigotes of *T. cruzi*; this data is summarized in Table 2. Three compounds had selectivity index of 20 or more against *T. cruzi* over NIH 3T3 host cells; compound 9a had the best selectivity index of 41.

The dose response curves of selected compounds against *T. brucei*, *P. falciparum*, and *L. major* are provided in Figures S2–S4, respectively, in the Supporting Information. Protocols for *in vitro* proliferation inhibition assays of *T. brucei*, *T. cruzi*, *L. major*, *P. falciparum*, and host cells are also provided in the Supporting Information. These methods have been previously described.^{8,26–28}

In the end, compounds 3, 7j, and 7t were the most promising anti-*T. brucei* agents (Table 1). Noting that compound 3 was previously dosed in mice orally at 100 mg/kg,²¹ we evaluated its pharmacokinetics at 130 mg/kg for possible anti-HAT efficacy studies in mice: its *in vitro* ADME properties were also evaluated. These data, along with an optimized synthetic route for scaling up compound 3, are described in the Supporting Information.

Compound 3 was dosed at 130 mg/kg orally (formulated in 10% NMP and 90% PEG) to female Swiss Webster mice, blood samples were collected at 0.5, 1, 2, 4, 8, and 24 h and analyzed by LC–MS/MS. The results are summarized in Figure S1 and presented in Tables S4 and S5 in the Supporting Information. The drug concentration observed in plasma was above EC₅₀ value for up to 4 h. Given the good *in vitro* potency and acceptable plasma exposure in mice, we performed an efficacy study of 3 in a mouse model of acute phase human African trypanosomiasis.

Mice infected with *T. brucei* (Lister427) were dosed orally with 130 mg/kg once daily for 10 days. The control group was treated with vehicle (10% NMP and 90% PEG). Animals were monitored on alternate days for parasitemia. In untreated (control) mice, parasitemia reached 10⁸/mL of plasma by day 5 (mean parasitemia = 9.30 × 10⁸/mL) (Figure 3b), and the mice were euthanized. The mean survival of compound 3-treated mice was 7.5 days compared to 5 days for untreated mice (Figure 3a). The difference in mouse survival times (2.5 days) was not statistically significant (determined from chi-square analysis of Kaplan–Meier survival data (*p* = 0.127)). Mice treated with compound 3 did not show signs of toxicity. By day 5, the treated group had two animals with 3-fold decrease in parasitemia and

Table 2. Inhibition Profile of Alkynes against *T. cruzi* amastigotes, *L. major* amastigotes, and *P. falciparum* D6

Entry	<i>T. cruzi</i> amastigotes EC ₅₀ ± SEM (μM) ^a	NIH3T3 TC ₅₀ (μM)	SI ^b	<i>L. major</i> amastigotes EC ₅₀ in μM (r ²) ^c	SI ^d	<i>P. falciparum</i> D6 EC ₅₀ in μM (r ²) ^c	SI ^e	HepG2 TC ₅₀ (μM)
7a	3.4 ± 3.4	4.7	1.4	7.6 (0.92)	3.8	3.0 (0.98)	>9.7	>29
8a	nt	nt	--	6.5 (0.94)	4.5	3.0 (0.90)	>9.7	>29
9a	0.8 ± 0.06	33	41	5.5 (0.85)	>5.5	5.9 (0.83)	>5.1	>30
7b	2.1 ± 0.18	4.8	2.3	>15	--	0.64 (0.99)	17	11
8b	3.5 ± 0.11	6.7	1.9	8.1 (0.94)	1.9	0.26 (0.94)	58	15
9b	2.5 ± 0.46	7.2	2.9	10 (0.81)	0.8	0.16 (0.98)	51	8.2
7c	9.6 ± 1.43	52	5.4	4.6 (0.85)	5.9	3.8 (0.91)	7.1	27
8c	>50	>50	--	9.0 (0.87)	3.1	>15	--	>28
9c	8.0 ± 0.30	>50	>6.3	>15	--	>15	--	>28
7d	>50	>50	--	>15	--	>15	--	>27
8d	1.9 ± 0.08	2.5	1.3	11 (0.92)	2.0	4.8 (0.93)	4.6	22
7e	>50	>50	--	>15	--	>15	--	29
8e	30 ± 7.9	>50	>1.7	>15	--	5.7 (0.95)	>5.6	>32
7f	>50	>50	--	6.0 (0.85)	4.5	>15	--	27
8f	1.7 ± 0.11	2.2	1.3	1.4 (0.92)	12	0.80 (0.99)	21	17
7g	8.2 ± 0.27	>50	>6.0	2.5 (0.91)	13	>15	--	>32
8g	2.6 ± 0.07	41	16	5.5 (0.89)	>5.8	1.5 (0.98)	>21	>32
7h	>50	>50	--	4.0 (0.86)	7.3	1.9 (0.97)	>15	>29
8h	0.61 ± 0.16	3.9	6.4	9.1 (0.88)	0.85	0.38 (0.98)	20	7.7
7i	30.7 ± 8.7	>50	>1.6	9.4 (0.77)	3.5	5.9 (0.89)	>5.6	>33
8i	1.5 ± 0.18	1.7	1.1	7.3 (0.93)	1.3	3.02 (0.97)	3.2	9.5
7j	1.8 ± 0.17	6.4	3.6	4.1 (0.77)	1.2	1.3 (0.96)	3.8	4.9
8j	nt	nt	--	4.3 (0.95)	7.0	0.14 (0.99)	>214	>30
7k	5.0 ± 0.53	>50	>10	5.9 (0.81)	4.8	>15	--	>28
9k	2.5 ± 0.21	>50	>20	5.4 (0.93)	>5.4	1.1 (0.96)	>26	>29
7l	>50	>100	--	>15	--	3.6 (0.99)	>8.6	>31
9l	2.0 ± 0.16	>50	>25	>2	--	>15	--	>3.0
7m	13 ± 3.5	47	3.6	6.9 (0.83)	3.5	1.5 (0.99)	>19	>24
7n	>50	>50	--	>15	--	>15	--	>32
7o	>50	>50	--	>15	--	5.7 (0.98)	4.9	>28
7p	19 ± 1.6	47	2.5	>15	--	1.1 (0.97)	27	30
7q	>50	>50	--	>3	--	>3.6	--	>5.0
7r	12 ± 0.45	27	2.3	0.38 (0.94)	63	2.0 (0.90)	12	24
7s	>50	>100	--	1.6 (0.97)	16	1.7 (0.99)	>15	>26
7t	3.7 ± 0.23	10	2.7	4.2 (0.94)	2.3	0.94 (0.97)	10	9.6
Benznidazole	0.79 ± 0.01	--	--					
Amphotericin B				0.035 (0.90)	--	--	--	--
Chloroquine				--	--	0.0089 (0.97)	--	--

^a*T. cruzi* EC₅₀ values are the result of triplicate experiments. ^bSelectivity index = NIH3T3 TC₅₀/Tc EC₅₀. nt: not tested. ^cCompounds screened against *L. major* amastigotes or *P. falciparum* (D6 strain) either in duplicate or quadruplicate. ^dSelectivity index = HepG2 TC₅₀/Lmj EC₅₀. ^eSelectivity index = HepG2 TC₅₀/Pfal EC₅₀.

two animals with 400-fold decrease in parasitemia. One of the latter mice had parasitemia of 4×10^4 /mL on day 9, representing a projected 10^8 -fold decrease of trypanosomes in blood compared to the untreated mice (Figure 3b). Drug dosing was stopped on day 10 after which trypanosome proliferation resumed; reaching 8×10^8 /mL on day 13, all the mice were euthanized (Figure 3b).

Recently, we have reported that lapatinib (**1**) inhibited uptake of transferrin, a growth factor²⁹ in bloodstream trypanosomes. Based upon the structure similarity of **3** to **1**, we hypothesized that these two compounds might inhibit proliferation via the same mechanism. Thus, the possible effect of **3** on transferrin (Tf-647) uptake was tested (Biological Assay Protocols in Supporting Information). We found that compound **3** had no effect on transferrin uptake (data not shown), indicating that **3** inhibits *T. brucei* proliferation through a different mechanism than lapatinib.

Stages in trypanosome division can be classified by enumeration of several single organelles (e.g., kinetoplast (a nucleoid containing mitochondrial DNA termed kinetoplast DNA (kDNA), nucleus, flagellum).^{30,31} Synthesis of mitochondrial and nuclear DNA occurs in S-phase in cells containing one nucleus and one kinetoplast (1K1N) (Figure 4).³² Division of kinetoplasts is completed in G2³² yielding 2K1N trypanosomes (Figure 4) in which nucleus division occurs, producing 2K2N cells (Figure 4). Cytokinesis takes place in 2K2N trypanosomes yielding new progeny with 1K1N genotype that can re-enter S-phase.³³

In "mode of action" studies to learn how compound **3** affected the biology of bloodstream trypanosomes *in vitro*, mid log phase *T. brucei* (5×10^5 cells/mL) were treated with either **3** or DMSO for 3 h, fixed, stained with mCLING^{34,35} and DAPI, and examined with a fluorescence microscope. The number of kinetoplasts (K) and nuclei (N) per cell was enumerated (Figure 4). Compound **3** reduced the fraction of trypanosomes

Figure 3. Evaluation of compound 3 efficacy in mouse model of acute HAT. (A) Mouse ($n = 4$) survival curve postinfection for control and compound 3 treated mice. (B) Parasitemia during compound 3 treatment. Mice (four per group) were infected with 10^5 *T. brucei* Lister427 (day 0). After 24 h, compound 3 (130 mg/kg) was administered by oral gavage once daily for 10 days. Control mice received vehicle (10% NMP and 90% PEG). Parasitemia was determined every 24 h. Different shapes in the graph represent individual mice; DPI = days postinfection. Statistical significance of the difference in mean parasitemia was determined with Student's *t* test.

Figure 4. Compound 3 inhibits mitosis and cytokinesis. Trypanosomes (5×10^5 /mL) were treated with compound 3 (9.5 μ M) or DMSO (0.1%) in HMI-9 medium for 3 h. Cells were incubated with mCLING, and the kinetoplast and nuclear DNA was counterstained with DAPI. The number of kinetoplasts and nuclei per trypanosome ($n > 120$) was quantitated. The mean percentage of trypanosomes with indicated number of nuclei (N) or kinetoplasts (K) is shown. Trypanosomes with two kinetoplasts and one or two nuclei are binned together for clarity of the 3 effect. Error bars represent standard deviation among three biological replicates. The difference in distribution of kinetoplasts and nuclei between control and compound 3-treated trypanosomes was evaluated with Pearson χ^2 analysis ($p = 5.85 \times 10^{-7}$). Inset: Representative images of mCLING and DAPI-stained trypanosomes after treatment with DMSO. Scale bar, 2 μ m.

containing one nucleus and one kinetoplast (1K1N) (i.e., trypanosomes in G1 or S-phase of the cell cycle) but increased the proportion of cells with 2K1N or 2K2N. We conclude that 3 arrests proliferation of trypanosomes after division of the

kinetoplast, that is, after G2 (i.e., leading to a buildup of 2K1N) and before cytokinesis (i.e., causing accumulation of 2K2N trypanosomes).

In conclusion, we have synthesized and assessed the biological activity of alkynyl-substituted thienopyrimidine and quinazoline compounds derived from 3, leading to the discovery of potent antitrypanosome agents, such as 7t ($EC_{50} = 0.22 \mu$ M). The mode of action studies reveals compound 3 arrest proliferation of trypanosomes after G2 phase and before cytokinesis. Assessment of these compounds against other protozoan parasites produced potent compounds viz. 9a (*T. cruzi* $EC_{50} = 0.81 \mu$ M, SI = 40.9), 7r (*L. major* $EC_{50} = 0.38 \mu$ M, SI = 62.1), 8b (*P. falciparum* $EC_{50} = 0.26 \mu$ M, SI = 57.7), 8j (*P. falciparum* $EC_{50} = 0.14 \mu$ M, SI = 214.3), 9b (*P. falciparum* $EC_{50} = 0.16 \mu$ M, SI = 51), and 8h (*P. falciparum* $EC_{50} = 0.16 \mu$ M, SI = 20.3). Work is ongoing to improve compound properties toward discovery of novel antiparasitic agents.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.8b00245.

Details of synthetic chemistry, biological assay protocols, and other data (annotated with NEU registry numbers), and ADME and PK properties of selected analogs (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: m.pollastri@northeastern.edu. Tel: 617-373-2703.

ORCID

Baljinder Singh: 0000-0003-2828-5768

Emanuele Amata: 0000-0002-4750-3479

Michael P. Pollastri: 0000-0001-9943-7197

Author Contributions

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

Funding

Funding from the National Institutes of Health (R01AI124046, R56AI099476 to M.P. and K.M.-W.) is gratefully acknowledged.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to AstraZeneca for performing the *in vitro* ADME experiments tabulated in Table S3 in the Supporting Information.

■ REFERENCES

- (1) Pollastri, M. P.; Campbell, R. K. Target repurposing for neglected diseases. *Future Med. Chem.* **2011**, 3, 1307–1315.
- (2) Klug, D. M.; Gelb, M. H.; Pollastri, M. P. Repurposing strategies for tropical disease drug discovery. *Bioorg. Med. Chem. Lett.* **2016**, 26, 2569–2576.
- (3) World Health Organization. Human African Trypanosomiasis. World Health Organization Fact Sheet No. 259.
- (4) Diaz-Gonzalez, R.; Kuhlmann, F. M.; Galan-Rodriguez, C.; Madeira da Silva, L.; Saldivia, M.; Karver, C. E.; Rodriguez, A.; Beverley, S. M.; Navarro, M.; Pollastri, M. P. The susceptibility of trypanosomatid pathogens to PI3/mTOR kinase inhibitors affords a

new opportunity for drug repurposing. *PLoS Neglected Trop. Dis.* **2011**, *5*, e1297.

(5) Diaz, R.; Luengo-Arratta, S. A.; Seixas, J. D.; Amata, E.; Devine, W.; Cordon-Obras, C.; Rojas-Barros, D. I.; Jimenez, E.; Ortega, F.; Crouch, S.; Colmenarejo, G.; Fiandor, J. M.; Martin, J. J.; Berlanga, M.; Gonzalez, S.; Manzano, P.; Navarro, M.; Pollastri, M. P. Identification and characterization of hundreds of potent and selective inhibitors of Trypanosoma brucei growth from a kinase-targeted library screening campaign. *PLoS Neglected Trop. Dis.* **2014**, *8*, e3253.

(6) Ochiana, S. O.; Pandarinath, V.; Wang, Z.; Kapoor, R.; Ondrechen, M. J.; Ruben, L.; Pollastri, M. P. The human Aurora kinase inhibitor danusertib is a lead compound for anti-trypanosomal drug discovery via target repurposing. *Eur. J. Med. Chem.* **2013**, *62*, 777–784.

(7) Patel, G.; Karver, C. E.; Behera, R.; Guyett, P. J.; Sullenberger, C.; Edwards, P.; Roncal, N. E.; Mensa-Wilmot, K.; Pollastri, M. P. Kinase scaffold repurposing for neglected disease drug discovery: Discovery of an efficacious, lapatanib-derived lead compound for trypanosomiasis. *J. Med. Chem.* **2013**, *56*, 3820–3832.

(8) Patel, G.; Roncal, N. E.; Lee, P. J.; Leed, S. E.; Erath, J.; Rodriguez, A.; Sciotti, R. J.; Pollastri, M. P. Repurposing human Aurora kinase inhibitors as leads for anti-protozoan drug discovery. *MedChemComm* **2014**, *5*, 655–658.

(9) Devine, W.; Woodring, J. L.; Swaminathan, U.; Amata, E.; Patel, G.; Erath, J.; Roncal, N. E.; Lee, P. J.; Leed, S. E.; Rodriguez, A.; Mensa-Wilmot, K.; Sciotti, R. J.; Pollastri, M. P. Protozoan Parasite Growth Inhibitors Discovered by Cross-Screening Yield Potent Scaffolds for Lead Discovery. *J. Med. Chem.* **2015**, *58*, 5522–5537.

(10) Rusnak, D. W.; Lackey, K.; Affleck, K.; Wood, E. R.; Alligood, K. J.; Rhodes, N.; Keith, B. R.; Murray, D. M.; Knight, W. B.; Mullin, R. J.; Gilmer, T. M. The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. *Mol. Cancer Ther.* **2001**, *1*, 85–94.

(11) Xia, W.; Mullin, R. J.; Keith, B. R.; Liu, L. H.; Ma, H.; Rusnak, D. W.; Owens, G.; Alligood, K. J.; Spector, N. L. Anti-tumor activity of GW572016: a dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. *Oncogene* **2002**, *21*, 6255–6263.

(12) Johnston, S. R.; Leary, A. Lapatinib: a novel EGFR/HER2 tyrosine kinase inhibitor for cancer. *Drugs Today* **2006**, *42*, 441–453.

(13) Moy, B.; Kirkpatrick, P.; Kar, S.; Goss, P. Lapatinib. *Nat. Rev. Drug Discovery* **2007**, *6*, 431–432.

(14) Behera, R.; Thomas, S. M.; Mensa-Wilmot, K. New chemical scaffolds for human african trypanosomiasis lead discovery from a screen of tyrosine kinase inhibitor drugs. *Antimicrob. Agents Chemother.* **2014**, *58*, 2202–10.

(15) Woodring, J. L.; Bachovchin, K. A.; Brady, K. G.; Gallerstein, M. F.; Erath, J.; Tanghe, S.; Leed, S. E.; Rodriguez, A.; Mensa-Wilmot, K.; Sciotti, R. J.; Pollastri, M. P. Optimization of physicochemical properties for 4-anilinoquinazoline inhibitors of trypanosome proliferation. *Eur. J. Med. Chem.* **2017**, *141*, 446–459.

(16) Patel, G.; Karver, C. E.; Behera, R.; Guyett, P. J.; Sullenberger, C.; Edwards, P.; Roncal, N. E.; Mensa-Wilmot, K.; Pollastri, M. P. Kinase Scaffold Repurposing for Neglected Disease Drug Discovery: Discovery of an Efficacious, Lapatanib-Derived Lead Compound for Trypanosomiasis. *J. Med. Chem.* **2013**, *56*, 3820–3832.

(17) Devine, W.; Woodring, J. L.; Swaminathan, U.; Amata, E.; Patel, G.; Erath, J.; Roncal, N. E.; Lee, P. J.; Leed, S. E.; Rodriguez, A.; Mensa-Wilmot, K.; Sciotti, R. J.; Pollastri, M. P. Protozoan Parasite Growth Inhibitors Discovered by Cross-Screening Yield Potent Scaffolds for Lead Discovery. *J. Med. Chem.* **2015**, *58*, 5522–5537.

(18) Stevens, K. L.; Alligood, K. J.; Alberti, J. G.; Caferro, T. R.; Chamberlain, S. D.; Dickerson, S. H.; Dickson, H. D.; Emerson, H. K.; Griffin, R. J.; Hubbard, R. D.; Keith, B. R.; Mullin, R. J.; Petrov, K. G.; Gerding, R. M.; Reno, M. J.; Rheault, T. R.; Rusnak, D. W.; Sammond, D. M.; Smith, S. C.; Uehling, D. E.; Waterson, A. G.; Wood, E. R. Synthesis and stereochemical effects of pyrrolidinyl-acetylenic thieno[3,2-d]-pyrimidines as EGFR and ErbB-2 inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 21–26.

(19) Waterson, A. G.; Petrov, K. G.; Hornberger, K. R.; Hubbard, R. D.; Sammond, D. M.; Smith, S. C.; Dickson, H. D.; Caferro, T. R.; Hinkle, K. W.; Stevens, K. L.; Dickerson, S. H.; Rusnak, D. W.; Spehar, G. M.; Wood, E. R.; Griffin, R. J.; Uehling, D. E. Synthesis and evaluation of aniline headgroups for alkynyl thienopyrimidine dual EGFR/ErbB-2 kinase inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1332–1336.

(20) Hubbard, R. D.; Dickerson, S. H.; Emerson, H. K.; Griffin, R. J.; Reno, M. J.; Hornberger, K. R.; Rusnak, D. W.; Wood, E. R.; Uehling, D. E.; Waterson, A. G. Dual EGFR/ErbB-2 inhibitors from novel pyrrolidinyl-acetylenic thieno[3,2-d]pyrimidines. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5738–5740.

(21) Wood, E. R.; Shewchuk, L. M.; Ellis, B.; Brignola, P.; Brashear, R. L.; Caferro, T. R.; Dickerson, S. H.; Dickson, H. D.; Donaldson, K. H.; Gaul, M.; Griffin, R. J.; Hassell, A. M.; Keith, B.; Mullin, R.; Petrov, K. G.; Reno, M. J.; Rusnak, D. W.; Tadepalli, S. M.; Ulrich, J. C.; Wagner, C. D.; Vanderwall, D. E.; Waterson, A. G.; Williams, J. D.; White, W. L.; Uehling, D. E. 6-Ethynylthieno[3,2-d]- and 6-ethynylthieno[2,3-d]pyrimidin-4-anilines as tunable covalent modifiers of ErbB kinases. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 2773–2778.

(22) Woodring, J. L.; Patel, G.; Erath, J.; Behera, R.; Lee, P. J.; Leed, S. E.; Rodriguez, A.; Sciotti, R. J.; Mensa-Wilmot, K.; Pollastri, M. P. Evaluation of Aromatic 6-Substituted Thienopyrimidines as Scaffolds against Parasites That Cause Trypanosomiasis, Leishmaniasis, and Malaria. *MedChemComm* **2015**, *6*, 339–346.

(23) Benish, M. A.; Lawless, M.; Budde, R. J. A. Preparation of thienopyrimidine-based inhibitors of the Src tyrosine kinases family. WO2002057271A2, 2002.

(24) Buchmann, B.; Braeuer, N.; Koppitz, M.; Peters, O.; Ter Laak, A.; Lindenthal, B.; Langer, G.; Wintermantel, T. Preparation of indolylethylthienopyrimidylamines as EP2 receptor modulators. WO2009007421A1, 2009.

(25) Huang, Y.; Wolf, S.; Bista, M.; Meireles, L.; Camacho, C.; Holak, T. A.; Domling, A. 1,4-Thienodiazepine-2,5-diones via MCR (I): synthesis, virtual space and p53-Mdm2 activity. *Chem. Biol. Drug Des.* **2010**, *76*, 116–129.

(26) Devine, W.; Thomas, S. M.; Erath, J.; Bachovchin, K. A.; Lee, P. J.; Leed, S. E.; Rodriguez, A.; Sciotti, R. J.; Mensa-Wilmot, K.; Pollastri, M. P. Antiparasitic Lead Discovery: Toward Optimization of a Chemotype with Activity Against Multiple Protozoan Parasites. *ACS Med. Chem. Lett.* **2017**, *8*, 350–354.

(27) Johnson, J. D.; Denu, R. A.; Gerena, L.; Lopez-Sanchez, M.; Roncal, N. E.; Waters, N. C. Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening. *Antimicrob. Agents Chemother.* **2007**, *51*, 1926–33.

(28) Lecoq, H.; Buffet, P.; Morizot, G.; Goyard, S.; Guigon, G.; Milon, G.; Lang, T. Optimization of topical therapy for Leishmania major localized cutaneous leishmaniasis using a reliable C57BL/6 Model. *PLoS Neglected Trop. Dis.* **2007**, *1*, e34.

(29) Schell, D.; Borowy, N. K.; Overath, P. Transferrin is a growth factor for the bloodstream form of *Trypanosoma brucei*. *Z. Parasitenkd.* **1991**, *77*, 558–560.

(30) Hammarton, T. C. Cell cycle regulation in *Trypanosoma brucei*. *Mol. Biochem. Parasitol.* **2007**, *153*, 1–8.

(31) Siegel, T. N.; Hekstra, D. R.; Cross, G. A. Analysis of the *Trypanosoma brucei* cell cycle by quantitative DAPI imaging. *Mol. Biochem. Parasitol.* **2008**, *160*, 171–174.

(32) Sullenberger, C.; Pique, D.; Ogata, Y.; Mensa-Wilmot, K. AEE788 Inhibits Basal Body Assembly and Blocks DNA Replication in the African Trypanosome. *Mol. Pharmacol.* **2017**, *91*, 482–498.

(33) Hammarton, T. C.; Monnerat, S.; Mottram, J. C. Cytokinesis in trypanosomatids. *Curr. Opin. Microbiol.* **2007**, *10*, 520–527.

(34) Revelo, N. H.; Kamin, D.; Truckenbrodt, S.; Wong, A. B.; Reuter-Jessen, K.; Reisinger, E.; Moser, T.; Rizzoli, S. O. A new probe for super-resolution imaging of membranes elucidates trafficking pathways. *J. Cell Biol.* **2014**, *205*, 591–606.

(35) Wiedeman, J.; Mensa-Wilmot, K. A fixable probe for visualizing flagella and plasma membranes of the African trypanosome. *PLoS One* **2018**, *13*, e0197541.