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Letter

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	Chemical Biology

Scaffold and Parasite Hopping: Discovery of New Protozoal Proliferation Inhibitors

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Abstract

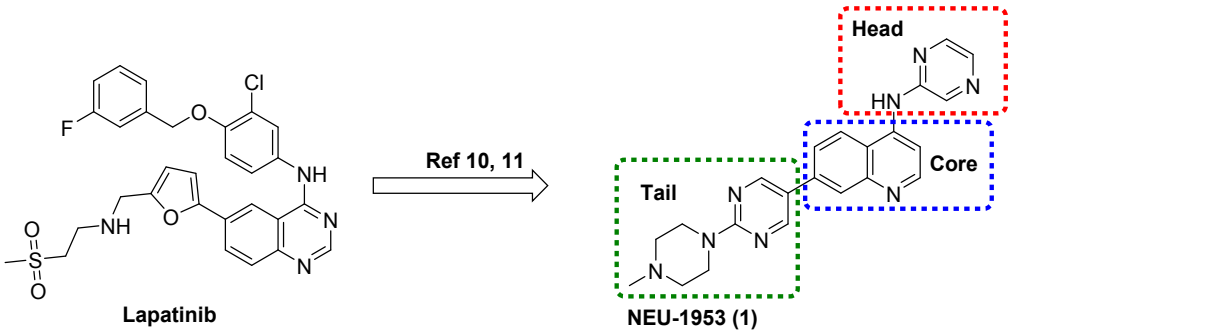
Utilizing a target repurposing and parasite-hopping approach, we tested a previously-reported library of compounds that were active against *Trypanosoma brucei*, plus 31 new compounds, against a variety of protozoan parasites including *Trypanosoma cruzi*, *Leishmania major, Leishmania donovani* and *Plasmodium falciparum*. This led to the discovery of several compounds with submicromolar activities and improved physicochemical properties that are early leads towards the development of chemotherapeutic agents against kinetoplastid diseases and malaria.

Keywords: Kinetoplastids, leishmaniasis, Chagas disease, malaria, protozoan parasite inhibitors, parasite-hopping

Neglected tropical diseases (NTDs) are a group of life-threatening diseases affecting a significant population of the world. These diseases are especially devastating in developing and economically disadvantaged countries. Most of the treatments available to treat NTDs have serious adverse effects. Further, the issues of emerging resistance and a lack of affordable drugs is driving the search for new treatments or vaccines; this search is a priority for the World Health Organization (WHO). Chagas disease and leishmaniasis, caused by *Trypanosoma cruzi* and *Leishmania* spp., respectively, are among the 20 NTDs highlighted by the WHO as urgently needing new therapies.[1-3]

Chagas disease, also known as American trypanosomiasis, is a life-threatening illness endemic mostly in Latin American countries that is transmitted primarily by the kissing bug (*Reduviidae* family). According to the WHO, 8 million people worldwide are estimated to be infected by *T. cruzi*. It is responsible for 41% of heart failure cases in Latin America[4, 5] killing more than 10,000 people per year. More than 25 million people are at risk of acquiring Chagas disease.[6] Leishmaniasis is a group of diseases caused by more than 20 different species of the *Leishmania* parasite transmitted by the sandfly and affecting humans in 98 countries. Approximately 1.3 million new cases and 20,000 to 30,000 deaths occur annually due to leishmaniases.[7] Though not considered an NTD, malaria is a life-threatening tropical disease caused by five species of protozoan *Plasmodium* parasites (mainly *Plasmodium falciparum* and *P. vivax*) that are transmitted by the bite of infected female *Anopheles* mosquitoes. In 2016, an estimated 216 million cases of malaria occurred worldwide leading to an approximately 445,000 malaria-related deaths worldwide.[8]

As part of our drug discovery program for tropical diseases, we have employed a target class repurposing approach[9] wherein we identified lapatinib as an antitrypanosomal agent.[9] The synthetic re-optimization of lapatinib and its related analogs for activity against *T. brucei* resulted in the discovery of **NEU-1953**[10, 11] (**1**, Figure 1) which was further optimized to improve aqueous solubility as an anti-HAT (human African trypanosomiasis) agent.[12] Here we report the assessment of this family of compounds against *P. falciparum, Leishmania* spp., and *T. cruzi,* along with some newly prepared analogs.



T. brucei EC_{50} = 1.5 μM
T. cruzi EC_{50} = 1.5 μM
P. falciparum D6 EC_{50} = 0.53 μM
L. major EC_{50} = >15 μM

T. brucei EC_{50} = 0.43 μM
T. cruzi EC_{50} = 6 μM
P. falciparum D6 EC_{50} = 0.03 μM
L. major EC_{50} = >15 μM

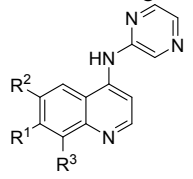
Aq. sol. = 44 μM
Human PPB% = 87
Human liver microsome CL_{int} = 179 μL/min/mg protein
Rat hepatocytes CL_{int} = 127 μL/min/10^6 cells

Figure 1. Optimization of lapatinib leading to the identification of **NEU-1953** (**1**)[10, 11] as a multi-parasite inhibitor

Synthesis. The compounds **17**, **18**, **22-27**, **33-35**, **39**, **42-45**, **S21-S23**, **S25-S27**, **S31-S35** and **S38-S41** were synthesized using previously reported protocols[10, 12, 13] with slight modifications as described in the **Supporting Information** (**Schemes S1-S7**). All other compounds (**1-16**, **28-32**, **36**, **38**, **40**, **41**, **S1-S20**, **S28-S30** and **S36-S37**) were procured from our internally-available *T. brucei* compound library.[11, 12]

Trypanosoma cruzi. Screening the compounds against intracellular *T. cruzi* amastigotes revealed 12 compounds with submicromolar activity and an additional 25 compounds with low micromolar activity (≤10 μM) (**Tables 1-3** and **Supporting Information Tables S1-S3**). The hit selection criteria against *T. cruzi* is EC_{50} <10 μM with a 10-fold selectivity over host cells.[14] Considering **1** as a starting point (*T. cruzi* EC_{50} = 6.0 μM),[11] several modifications were made to understand the complete structure-activity relationships (SAR) profile. Removal of the *N*-methyl from the piperazine (**8**, EC_{50} >50 μM) resulted in a complete loss of activity; the *N*-methyl is slightly favored over *N*-ethyl (**11**, EC_{50}= 7.7 μM) or *N*-propyl (**12**, EC_{50}= 7.6 μM). Further, **11** and **12** were found to be toxic against the host cell line (C2C12; **Supporting Information Table S7**). The insertion of a bridging carbon in the piperazine ring (**10**) or increasing sp^3 content by replacing the piperazine ring with the homopiperazine (**9**, EC_{50}= 1.2 μM) and spirocyclic amines (**13**, EC_{50}= 0.52 μM and **14**, EC_{50}= 0.22 μM) led an increase in solubility, better ADME properties and equipotent or improved potency against *T. cruzi* compared to **1**. The *N*-atoms of the pyrimidine were not necessary for potency (c.f. **2** and **18**) but play an important role in the aqueous solubility (**2**, aq. sol.= 1.7 μM and **18**, aq. sol.= 4.1 μM). The insertion of a hydrogen bond donor (-NH) in the tail region (**6**) abrogated the potency. The potency and selectivity data of tail group modification and *ortho*-methyl substituted compounds are listed in **Table 1**.

Table 1. Inhibition profile of quinolines **1-18** with modified tail regions against *T. cruzi*, *L. major* and *P. falciparum* D6



1-18

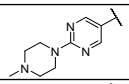
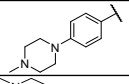
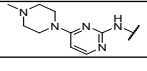
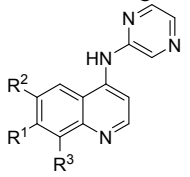
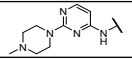
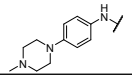
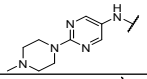
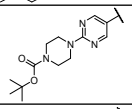
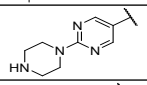
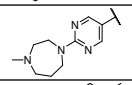
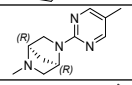
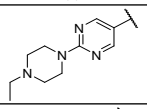
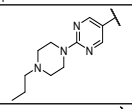
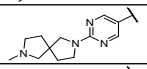
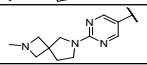
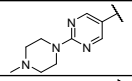
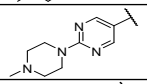
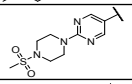
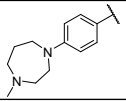
Entry	R^1	R^2	R^3	*T. cruzi* EC_{50} (μM)[a] / SI[b]	*L. major*[c] EC_{50} (μM) / SI[d]	*P. falciparum D6* EC_{50} (μM)[e] / SI[f]
1		-H	-H	6.0 / 5.7	>15	0.026 / >1461
2		-H	-H	6.1 / 6.7	>23	0.074 / >513
3		-H	-H	>20	5.3 / 4.2	0.054 /402

Table 1. Inhibition profile of quinolines **1-18** with modified tail regions against *T. cruzi*, *L. major* and *P. falciparum* D6



1-18

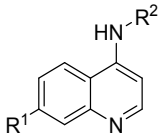
Entry	R^1	R^2	R^3	*T. cruzi* EC_{50} (μM)[a] / SI[b]	*L. major*[c] EC_{50} (μM) / SI[d]	*P. falciparum D6* EC_{50} (μM)[e] / SI[f]
4		-H	-H	>50	3.3 / 6.1	0.18 / 111
5		-H	-H	17	7.9 / 2.2	0.13 / 131
6		-H	-H	47	1.7 / 14	0.077 / 312
7		-H	-H	>50	0.35 / >89	0.15 / >207
8		-H	-H	>50	>5.2	0.13 / >300
9		-H	-H	1.2 / 1.6	24 / 1.4	0.024 / 1375
10		-H	-H	6.1 / >1.6	2.6 / >14	0.052 / >712
11		-H	-H	7.7 / 0.05	24 / 1.0	0.029 / 862
12		-H	-H	7.6 / 0.0075	4.3 / >8.4	0.023 / >1565
13		-H	-H	0.52 / 19	>23	0.030 / 533
14		-H	-H	0.22 / 1.9	>24	0.058 / >414
15		$-CH_3$	-H	22	1.8 / 3.3	0.18 / 33
16		-H	$-CH_3$	25	>24	0.069 / 139
17		-H	-H	45	0.73 / >45	>4.3
18		-H	-H	0.37 / 1.0	5.5 / 1.7	0.010 / 960

nd = not determined; [a]All SEM values within 25%; [b]SI=C2C12 or NIH3T3 TC_{50}/*T. cruzi* EC_{50}; [c]All r^2 values are >0.8 unless noted otherwise; [d]SI=HepG2 TC_{50}/*L. major* EC_{50}; [e]All r^2 values are >0.9 unless noted otherwise; [f]SI= HepG2 TC_{50}/*P. falciparum* D6 EC_{50}

Further modifications were performed on the head group, and some cross-over compounds (**19-32** and **S24-S30**) with different head and tail combinations were prepared (**Table 2 Supporting Information**

Table S2). Replacement of the pyrazine with saturated rings led to the identification of **28** and **29** with EC_{50} values of 2.1 and 1.2 μM, respectively, against *T. cruzi*. In general, substituted anilines such as **22** (EC_{50}= 0.060 μM) and **23** (EC_{50}= 0.080 μM) were preferred over heterocyclic head groups but a decrease in aqueous solubility was observed (**Supporting Information, Table S8**).

Table 2. Inhibition profile of quinolines **19-32** with cross-over head and tail combinations against *T. cruzi*, *L. major*, *L. donovani* and *P. falciparum* D6



19-32

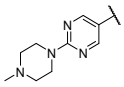
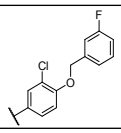
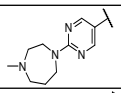
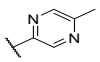
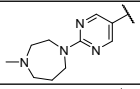
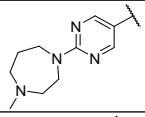
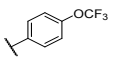
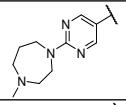
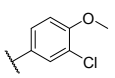
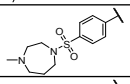
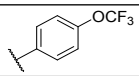
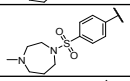
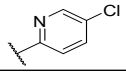
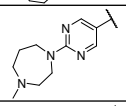
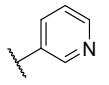
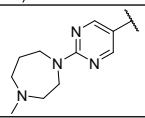
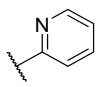
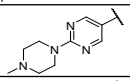
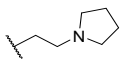
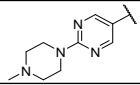
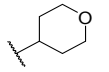
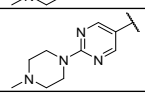
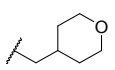
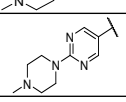
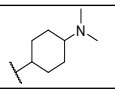
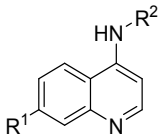
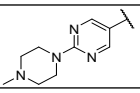
Entry	R^1	R^2	*T. cruzi* EC_{50} (μM)[a] / SI[b]	*L. major*[c] EC_{50} (μM) / SI[d]	*L. donovani* EC_{50} (μM)[e] / SI[f]	*P. falciparum D6* EC_{50} (μM)[g] / SI[h]
19			0.030 / 290	>1.5	0.023 / 1739	0.018 / 167
20			nd	>23	nd	0.024 / >1500
21			nd	9.2 / 2.0	nd	0.019 / 947
22			0.060 / 38	8.2 / 0.8	0.085 / 7.8	0.0060 / 233
23			0.080 / 12	1.6 / 4.3	>10	0.0060 / 1133
24			0.13 / 40	0.22 / 7.3	>10	0.14 / 11
25			0.16 / 21	1.3 / 1.4	>10	0.051 / 35
26			>10	>22	>10	0.025 / >1320
27			1.3 / 7.5	>24	>10	0.039 / 487
28			2.1 / 1.9	>24	>10	0.024 / 125
29			>10	>25	4.0	0.025 / 600
30			1.2 / 3.2	>24	8.7	0.026 / 615
31			> 10	>2.2	>10	0.053 / 47

Table 2. Inhibition profile of quinolines **19-32** with cross-over head and tail combinations against *T. cruzi*, *L. major*, *L. donovani* and *P. falciparum* D6



19-32

Entry	R¹	R²	*T. cruzi* EC_{50} (μM)[a] / SI[b]	*L. major*[c] EC_{50} (μM) / SI[d]	*L. donovani* EC_{50} (μM)[e] / SI[f]	*P. falciparum D6* EC_{50} (μM)[g] / SI[h]
32			> 10	>2.4	>10	0.037 / >97

nd = not determined; [a]All SEM values within 25%; [b]SI=C2C12 TC_{50}/*T. cruzi* EC_{50}; [c]All r^2 values are >0.8 unless noted otherwise; [d]SI=HepG2 TC_{50}/*L. major* EC_{50}; [e]All SEM values within 25%; [f]SI=B10R TC_{50}/*L. donovani* EC_{50}; [g]All r^2 values are >0.9 unless noted otherwise; [h]SI= HepG2 TC_{50}/*P. falciparum* D6 EC_{50}

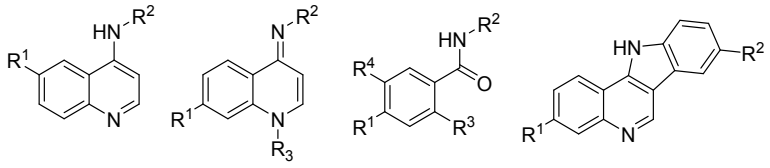
In an attempt to explore the SAR around the scaffold, a range of quinolinimines, open chain compounds, isocryptolepines and C-6 substituted quinolines were synthesized and the potency and selectivity data are listed in **Table 3** and **Supporting Information Table S3**. Compound **33** (EC_{50} = 0.33 μM) was the most potent compound with improved and acceptable physicochemical and ADME properties.

The quinolinimine analog of **1** (**39**) possessed better aqueous solubility and *in vitro* ADME properties but led to a complete loss in potency. These quinolinimine modifications led to the identification of two low micromolar compounds (**37**, EC_{50}= 3.0 μM and **38**, EC_{50}= 2.1 μM).

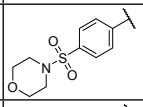
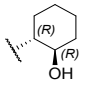
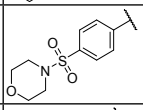
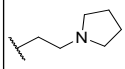
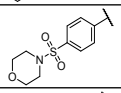
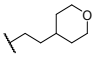
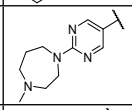
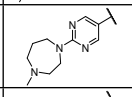
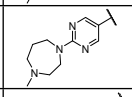
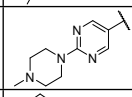
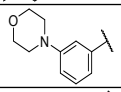
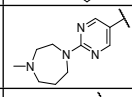
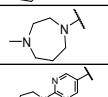
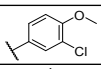
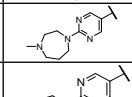
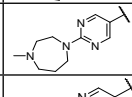
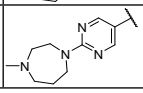
In an attempt to increase the conformational flexibility of the quinoline series, we synthesized a series of open chain analogs (**75-83**) by breaking one of the central core rings. Only two compounds (**42** and **43**) from the open chain series were found to have micromolar activity against *T. cruzi*. Alternatively, isocryptolepine analogs (**84-86**) were designed and synthesized to lock the free rotation of the head group. This strategy identified compounds **44** (EC_{50}= 0.24 μM) and **45** (EC_{50}= 0.21 μM) as potent *T. cruzi* inhibitors but the aqueous solubility was decreased.

The general SAR trends observed for this series against *T. cruzi* and a representative best compound with favorable ADME properties are summarized in **Figure 2**. The selectivity index (SI) of all compounds tested against *T. cruzi* was determined with reference to their toxicity against host cells (C2C12 or NIH3T3). Whilst there was no particular trend observed for toxicity against host cells several active compounds (**13**, **19**, **22**, **23**, **24**, **25** and **33**) have SI values above our targeted threshold (SI > 10).

Table 3. Inhibition profile of C-6 substituted quinolines (**33-35**), quinolinimines (**36-39**), pseudoring/open ring analogs (**40-43**) and isocryptolepines (**44-45**) against *T. cruzi*, *L. major*, *L. donovani* and *P. falciparum* D6



33-35 **36-39** **40-43** **44-45**

Entry	R^1	R^2	R^3	R^4	*T. cruzi* EC_{50} (μM)[a] / SI[b]	*L. major*[c] EC_{50} (μM) / SI[d]	*L. donovani* EC_{50} (μM)[e] / SI[f]	*P. falciparum D6* EC_{50} (μM)[g] / SI[h]
33			--	--	0.33 / >30	>22	nd	0.40 / >80
34			--	--	1.9 / 0.89	>21	nd	0.030 / 307
35			--	--	4.7 / 1.3	>21	>10	0.075 / >280
7136			$-CH_3$	--	10 / 0.81	2.3 / 16	0.020 / 265	0.12 / 300
37			$-CH_2CH_3$	--	3.0 / 1.0	23 / >1.5	>10	0.17 / >200
38			$-CH_2CH_2CH_3$	--	2.1 / 1.4	1.5 / >22	>10	0.087 / >379
39			$-CH_3$	--	>10	2.3 / >10	>10	0.18 / 133
40			-OH	-H	>10	0.36 / >97	>10	>23
41			-OH	-H	>10	>4.3	>10	0.088 / >74
42			-F	-F	5.5 / 1.6	>24	nd	4.6 / >5.2
43			-OH	-H	3.9 / 1.6	>2.2	nd	0.071 / >310
44		-H	--	--	0.24 / 6.7	>5.0	nd	0.046 / >161
45		$-CH_3$	--	--	0.21 / 2.4	0.44 / 10	0.64 / 3.6	0.037 / 127

nd = not determined; [a]All SEM values within 25%; [b]SI=C2C12 TC_{50}/*T. cruzi* EC_{50}; [c]All r^2 values are >0.8 unless noted otherwise; [d]SI=HepG2 TC_{50}/*L. major* EC_{50}; [e]All SEM values within 25%; [f]SI=B10R TC_{50}/*L. donovani* EC_{50}; [g]All r^2 values are >0.9 unless noted otherwise; [h]SI= HepG2 TC_{50}/*P. falciparum* D6 EC_{50}

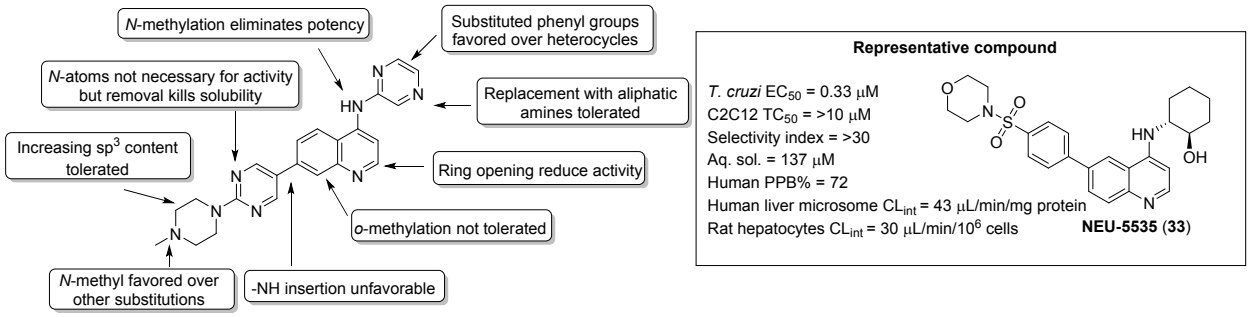


Figure 2. SAR trends for *T. cruzi* summarized around **1** and representative best compound from the series (**33**).

Leishmania major. Screening against *L. major* intracellular amastigotes resulted in several compounds with potency ≤10 μM, the potency and selectivity data of all compounds tested are listed in **Tables 1-3** and **Supporting Information Tables S1-S3**. The SAR trends observed for *L. major* were different from that of *T. cruzi*. Starting with modifications around **1** (*L. major* EC_{50} >15 μM), the insertion of a hydrogen bond donor (-NH) in the tail region (**3**, **4** and **6**) was favored and resulted in improved potency against *L. major* with **6** showing improved aqueous solubility (129 μM) as well as other *in vitro* ADME properties.[11] The removal of nitrogen atoms from the pyrimidine of **6** (EC_{50}= 1.7 μM**)** resulted in a reduction in potency (**5**, EC_{50}= 7.9 μM).[11] *Ortho*-methylation of the aromatic core resulted in entirely different responses, methylation at C-6 resulted in improved potency (**15**, EC_{50}= 1.8 μM), whereas methylation at C-8 resulted in a loss of potency (**16**, EC_{50} >24 μM). The replacement of *N*-methyl in the tail region with the carbamate (**7**, EC_{50}= 0.35 μM), *N*-propyl (**12**, EC_{50}= 4.3 μM) and *N*-methylsulfonyl (**17**, EC_{50}= 0.73 μM) resulted in an enhancement of potency against *L. major.*

Three quinolinimines were active in the micromolar range; **36** (EC_{50}= 2.3 μM), **38** (EC_{50}= 1.5 μM) and **39** (EC_{50}= 2.3 μM) were most promising. Analogs bearing substituted anilines (**23**, EC_{50}= 1.6 μM and **24**, EC_{50}= 0.22 μM) resulted in improved potency but at the cost of significantly reduced solubility. Replacement of the pyrazine with saturated rings and the variation of 6-substituted quinolines did not result in any active compounds. One compound from the isocryptolepines (**45**, EC_{50}= 0.44 μM) and open chain analogs (**40**, EC_{50}= 0.36 μM) were found to be sub-micromolar inhibitors of *L. major*. The general SAR trends observed against *L. major* and representative best compound with improved ADME properties are summarized in **Figure 3**. The SI of all compounds tested against *L. major* was determined with reference to their toxicity against HepG2 cells.

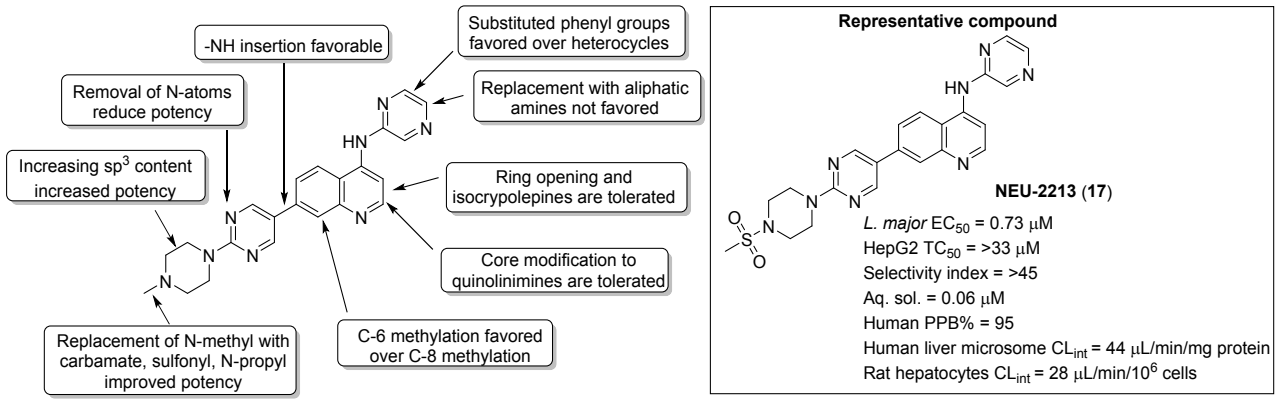


Figure 3. SAR trends for *L. major* around **1** and representative best compound from the series (**17**).

Leishmania donovani. Based on the positive results against *L. major*, we tested selected analogs against *L. donovani,* one of the species that causes visceral leishmaniasis (**Tables 2-3** and **Supporting Information, Tables S2, S3 andS7**). The observed activity trends were not similar across the two species (albeit we note that the host cell lines utilized were different for each species). Screening against *L. donovani* resulted in three compounds with low micromolar inhibition (EC_{50} ≤10 μM) and four compounds with sub-micromolar potency (EC_{50} <1 μM). Replacing the pyrazine of **1** with tetrahydropyran resulted in an active compound against *L. donovani* (**29**, EC_{50}= 4.0 μM). Replacing the piperazine with the homopiperazine and the pyrazine with 4-(trifluoromethoxy)aniline resulted in **22** with improved potency (EC_{50}= 0.085 μM) though this was associated with toxicity against host cells (TC_{50}= 0.66 μM; SI=8). Further, modifying the core to the quinolinimine led to **36** (EC_{50}= 0.02 μM); and different head and tail combinations identified **19** (EC_{50}= 0.023 μM) as the most potent compounds among all those tested. The general SAR trends observed for this series against *L. donovani* and the representative best compound with favorable ADME properties are summarized in **Figure 4**. The SI of all compounds tested against *L. donovani* was determined with reference to their toxicity against host cells (B10R).

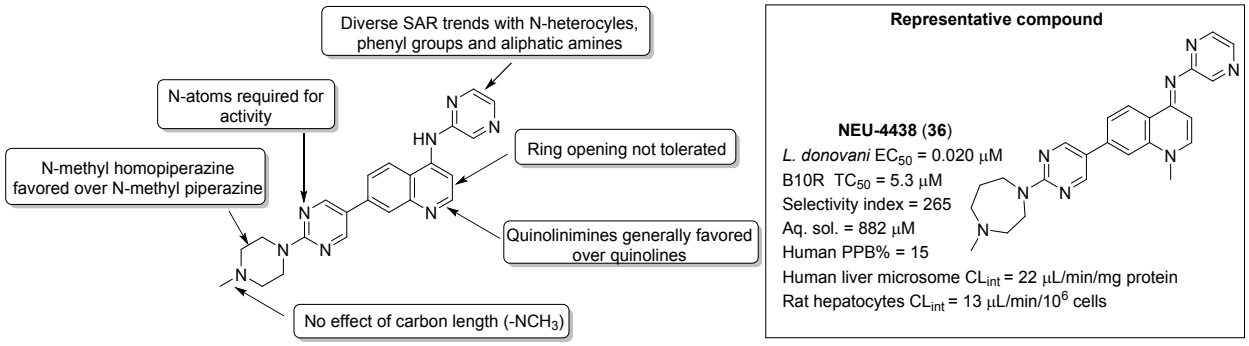


Figure 4. SAR trends for *L. donovani* around **1** and representative best compound from the series (**36**).

Based upon the high potency against *L. donovani*, two compounds (**19** and **36**) were selected for *in vivo* efficacy studies in an acute infection model in both female and male mice at 50 mg/kg b.i.d for 14 days. The results are summarized in **Supporting Information, Figure S1** and **Table S4**. Compound **36** has a better ADME and pharmacokinetic profile[12] over **19** but neither compound showed any significant effect on reducing the parasitemia in mice.

Plasmodium falciparum. We have also recently reported the medicinal chemistry optimization of lapatinib where **1** was found to be a potent inhibitor of *P. falciparum*.[10] Continuing our program on antimalarial drug discovery and based upon our previous observations that 4-anilinoquinolines have activity against *P. falciparum*,[10, 15] this library of compounds was also tested against three different strains of *P. falciparum* D6 (chloroquine sensitive, mefloquine resistant), W2 (chloroquine resistant, mefloquine sensitive), and C235 (chloroquine, mefloquine, and pyrimethamine resistant). The screening against *P. falciparum* resulted in 42 compounds with low nanomolar activity (<0.1 μM), 28 compounds with sub-micromolar activity (0.1-1 μM) and eight compounds with low micromolar activity (1-10 μM). The inhibition profile of all tested compounds against *P. falciparum* D6 and the associated SI values is presented in **Tables 1-3** and **Supporting Information Tables S1-S3**; and the tabulated values against W2 and C235 are provided in the **Supporting Information, Table S5**. As previously reported, **1**[10] was active across all three strains of *P. falciparum* (D6 EC_{50}= 0.026 μM, W2 EC_{50}= 0.062 μM, and C235 EC_{50}= 0.037 μM). The pyrimidine nitrogen atoms were not necessary for potency (**2**, D6 EC_{50}= 0.074 μM). Variations to the *N*-alkyl on the piperazine (*N*-ethyl, **11**, D6 EC_{50}= 0.029 μM; *N*-propyl, **12**, D6 EC_{50}= 0.023 μM) was favored over the free -NH (**8**, D6 EC_{50}= 0.13 μM) in terms of potency with little or no effect on the solubility. Increasing the sp3 content resulted in a significant increase in solubility and maintained the potency (**9**, D6 EC_{50}= 0.03 μM). Further, the replacement of the pyrazine with other amines such as methylated pyrazines (**20** and **21**), pyridines (**26** and **27**), and **s**ubstituted anilines (**22** and **23**) were tolerated and led to the best compound in the series *i.e.* **21** with excellent potency (D6 EC_{50}= 0.02 μM) and favorable ADME properties. *Ortho*-methylation of the aromatic core at C-8 (**16**) was favored over the C-6 position (**15**). Replacing the pyrazine head group with saturated rings (**28**, **29**, **30**, **31** and **32**), C-6 substituted quinolines (**34** and **35**), scaffold modification with quinolinimine (**38**), isocryptolepines (**44** and **45**) and open chain analogs (**41** and **43**) were also successful and resulted in several compounds with low nanomolar to sub-micromolar potency across all three strains of *P. falciparum*. The general SAR trends observed for this series against *P. falciparum* D6 and a representative best compound with favorable ADME properties are summarized in **Figure 5**.

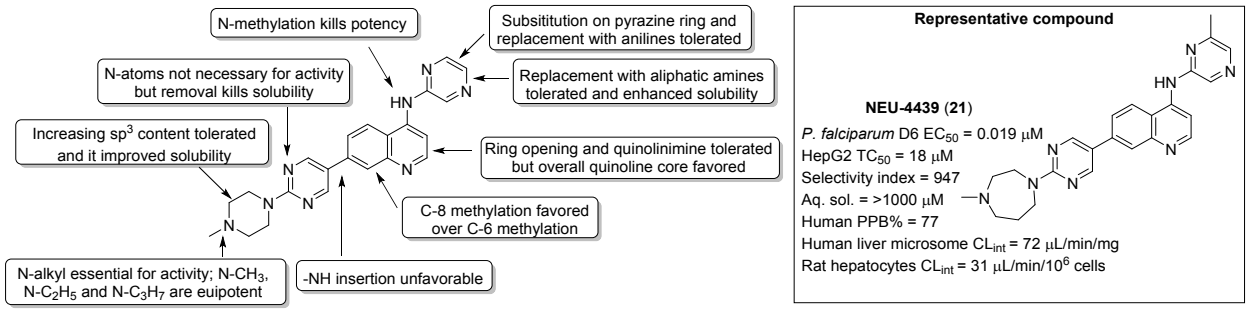


Figure 5. SAR trends for *P. falciparum* D6 around **1** and representative best compound from the series (**21**).

A comparison of activity between *P. falciparum* D6, W2 and C235 (**Figure 6**) revealed most compounds tested had activities within 3-fold of the D6 potency value. Of those tested, 20% of the quinolines (14 of 69 compounds) were less potent against the W2 strain than D6, as was one of the quinolinimines (representing 25% of those tested) though this represents a small sample size and more data is required to understand the trend for this scaffold. Further, 4% of the quinolines (3 of 69 compounds) were less potent against the C235 strain than D6. This suggests a lack of sensitivity to chloroquine resistance for the quinolines presented herein. The open chain and isocryptolepine scaffolds all had potency values within 3-fold against each strain tested.

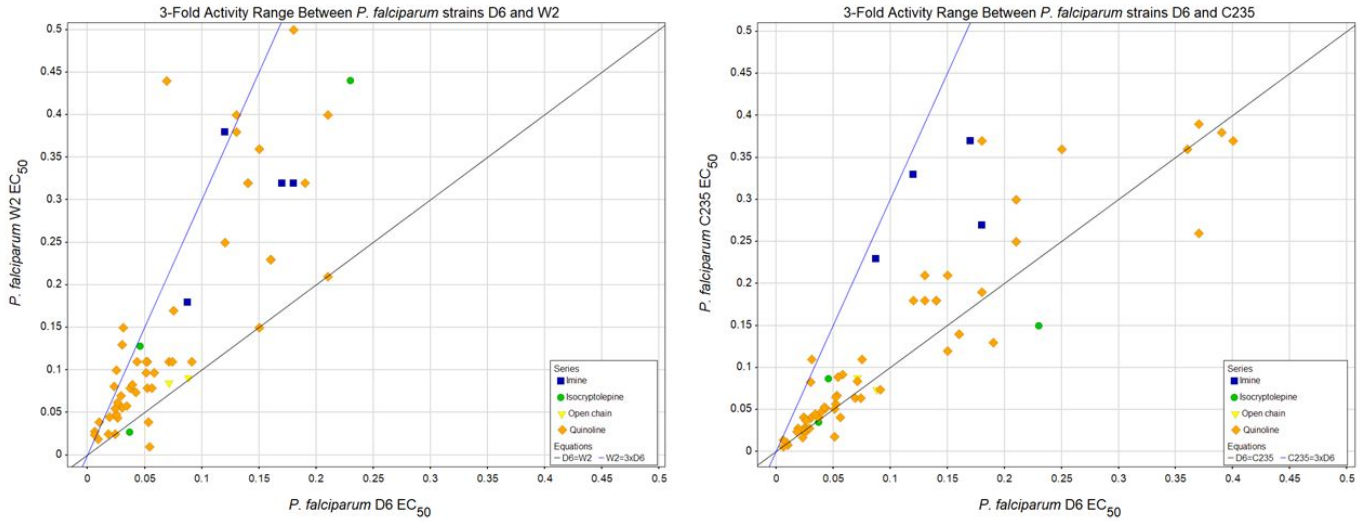


Figure 6. Comparison of potency (compounds with EC_{50} ≤0.5 μM shown) between: (**A**) *P. falciparum* W2 vs D6, and (**B**) *P. falciparum* C235 vs D6. The blue line signifies a 3-fold change in potency between *P. falciparum* D6 and the strain in question, the black line signifies compounds that are equipotent with *P. falciparum* D6 and the strain in question.

Utilizing a parasite-hopping approach, we have screened a previously reported library of *T. brucei* inhibitors along with newly synthesized compounds against *T. cruzi, L. major, L. donovani* and three strains of *P. falciparum*. Assessment of these compounds produced 13 submicromolar active compounds against

T. cruzi (EC_{50} <1μM), five submicromolar active compounds against *L. major* (EC_{50} <1μM), four submicromolar active compounds against *L. donovani* (EC_{50} <1μM) and 42 low nanomolar compounds against *P. falciparum* D6 (EC_{50} <0.1μM). Further, the cross-screening resulted in identification of several potent compounds with good selectivity index and improved ADME profile. Compounds **19** and **36** were tested in an efficacy model of *L. donovani* infection; both compounds failed to translate *in vitro* potency to *in vivo* activity. Work is ongoing to improve compound properties toward discovery of antiparasitic agents that are effective in animal efficacy studies.

Supporting Information

Details of synthetic chemistry, biological assay protocols, biological data of all tested compounds, *in vitro* cell toxicity data, *in vitro* ADME properties and other biological data (annotated with NEU registry numbers) are contained in the Supporting Information.

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Notes

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