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Repurposing human Aurora kinase inhibitors as leads for anti-protozoan drug discovery

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Abstract

Hesperadin, an established human Aurora B inhibitor, was tested against cultures of *Trypanosoma brucei*, *Leishmania major*, and *Plasmodium falciparum*, and was identified to be a potent proliferation inhibitor. A series of analogs was designed and tested to establish the initial structure-activity relationships for each parasite. In this study, we identified multiple non-toxic compounds with high potency against *T. brucei* and *P. falciparum* with good selectivity. These compounds may represent an opportunity for continued optimization.

Introduction

Neglected tropical diseases (NTDs) are a collection of debilitating infectious diseases that typically affect the poorest populations in the world, such as human African trypanosomiasis (caused by *Trypanosoma brucei*), leishmaniasis (caused by *Leishmania sp.*), and Chagas disease (*T. cruzi*). When taken together with malaria (caused by *Plasmodium sp.*), these diseases have a significant and adverse effect on length of quality of life, estimated in 2011 to be over 60 million disability-adjusted life-years.¹ Drug discovery for these diseases is cost limited, given that the vast majority of patients cannot pay for treatment. Therefore, pragmatic and cost effective methods for drug discovery are needed.

One such method is target repurposing,² where essential parasitic targets (such as kinases,^{3–7} phosphodiesterases,^{8,9} and histone deacetylases¹⁰) are matched with homologous and druggable targets in humans that have been the subject of previous drug

discovery programs. Assessment of chemical matter identified in those programs against the infectious pathogen can often result in identification of new leads for the infectious disease. Furthermore, the medicinal chemistry optimization against the pathogen can be facilitated and accelerated by considering the previous SAR developed for the chemotype.

Aurora kinases are central enzymes in cellular division, and have been the focus of drug discovery efforts in oncology, resulting in multiple inhibitors advanced into clinical trials. Both *L. major*¹¹ (which causes cutaneous leishmaniasis) and *P. falciparum*¹² express Aurora kinases, and established inhibitors of Aurora have been shown to be effective cell proliferation inhibitors for *Trypanosoma brucei* (including hesperadin (**1**),¹³ VX-680 (**2**)¹⁴ and danusertib (**3**).⁴

Encouraged by the preliminary results for *T. brucei* described above, we assessed these three human Aurora kinase inhibitors against other trypanosomatid pathogens *L. major* (promastigote and intracellular amastigote forms), and the D6 strain of *P. falciparum* (Table 1). We also tested **1** against the intracellular amastigotes form of *T. cruzi*, the causative agent of Chagas disease. We counter-screened against the hepatic cancer cell line (HepG2) as a general surrogate for host cell toxicity. We observed a range of potencies, and note that **1** displayed a potent growth inhibitory phenotype against *P. falciparum* and *L. major* parasites, though host cell toxicity was apparent. In light of these results, we opted to focus on further exploration of the SAR of this chemotype as a potential antiparasitic agent.

Synthetic strategy

We designed a synthetic strategy to access three regions of the hesperadin molecule, labelled R¹-R⁴ as shown in Table 1, modelled after the synthetic route described in a patent.¹⁵ Synthesis initiated with indolone **4**, which was nitrated¹⁵ and condensed with methyl orthobenzoate to provide **6**. Displacement of the vinylogous ester, nitro group reduction, and sulfonylation with a small set of sulfonyl chlorides afforded the R¹ analogs **11** following N-deprotection (Table 2). The nitroindolone **5** could be converted to the sulphonamide **12**, which was subjected to a similar sequence as above with varied amine nucleophiles (to vary R⁴) to obtain access the analogs **15** following deprotection. Finally, inclusion of various orthoester reagents in the sequence afforded R³ variations (compound **17**).

Screening results and discussion

The analogs were tested in parasite cultures, and the results are summarized in Table 2. First, variation of the R¹ position (Table 2) revealed a preference for the ethyl sulphonamide moiety present in **1** over the methyl (**11a**) or phenyl sulphonamide (**11b**) or replacement with an acetamide (**11c**). However, **11c** afforded reduced potency against HepG2 cells, providing improvement in cellular selectivity over the other analogs. Complete removal of the R¹ functionality (**7a**) led to a significant reduction in antimalarial and anti-leishmanial activity, though **7a** was equipotent to **1** against *T. brucei* and selective over HepG2 cells. The free amine (**10**) showed marked reduction in activity across pathogens.

In variation of the R⁴ substituent, substitution of oxygen for carbon of the piperidine ring of **1** provides a slight reduction in antiprotozoan activity (**15d**), though activity was also

reduced in HepG2 cells, maintaining some selectivity. The results in our data set suggest the requirement for a basic nitrogen, noting also that reduction in basicity (*e.g.* morpholino group) results in loss of potency. Removal of the methylene spacer (**15e**) caused a strongly adverse effect on potency against all pathogens, as does removal of the piperidinyl moiety altogether (**15c**). Though the phenyl analog (**15b**) shows diminished potency against *P. falciparum*, the selectivity over HepG2 cells is in excess of 50-fold. Finally, removal of the intervening aromatic group (**15a**) results in complete loss of activity across parasites.

Finally, survey of the R³ phenyl of **1** confirms that replacements with H (**17a**) or small alkyl chains (**17b–d**) has a detrimental effect on activities compared to **1**. Interestingly, the methyl analog (**17b**) is far less potent than the others, which are all in a range of ~20 fold less potent than **1**, with diminished HepG2 activity. Notably, none of the compounds tested showed appreciable activity against *T. cruzi*.

Drug resistance is a significant issue for malaria. We therefore opted to test our analogs across three additional strains of *P. falciparum* besides the drug-sensitive D6 strain: W2 (chloroquine resistant), C235 (chloroquine, mefloquine and pyrimethamine resistant), and C2B (multidrug resistance with atovaquone resistance). The data is tabulated in the Electronic Supplementary Information (ESI), but is presented in Figure 2 as a scatter plot showing D6 versus C235 EC₅₀. Remarkably, the compounds show highly consistent potency values across strains; this is also observed for the W2 and C2B strains (R²=0.95 and 0.95, respectively).

We also note a complete lack of correlation between the compounds' activities against promastigote and axenic amastigote form of *L. major*, which is consistent with previous reports.¹⁶

Conclusions

In summary, we have identified analogs of **1**, an established human Aurora kinase inhibitor, that display modest-to-excellent potency against the protozoan pathogens that cause African sleeping sickness, malaria, and leishmaniasis. Importantly, these compounds are not acting as general cell toxins, as we have observed varying margins of selectivity. Notably, we have also found that these compounds show broad utility as equipotent inhibitors of a range of drug resistant strains of malaria. Though these compounds have not yet been tested against Aurora kinase homologs in the respective parasites, we expect that this will be a useful place to begin to identify molecular mechanisms of action.^{11–13} This, along with our ongoing optimization of this chemotype against these pathogens will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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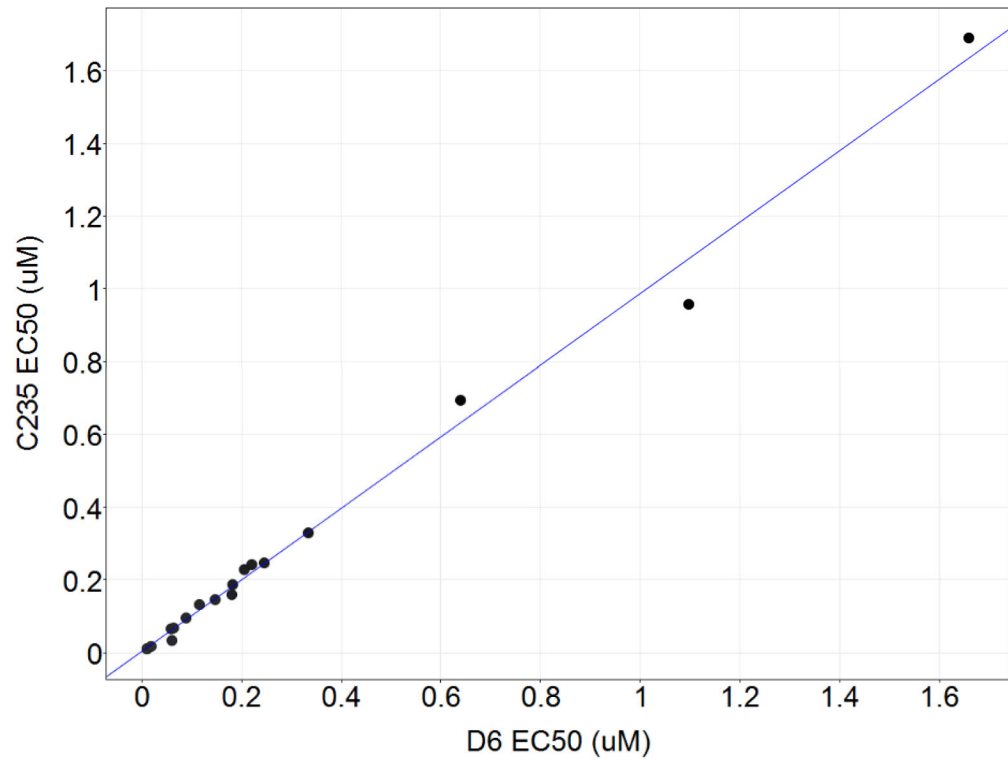
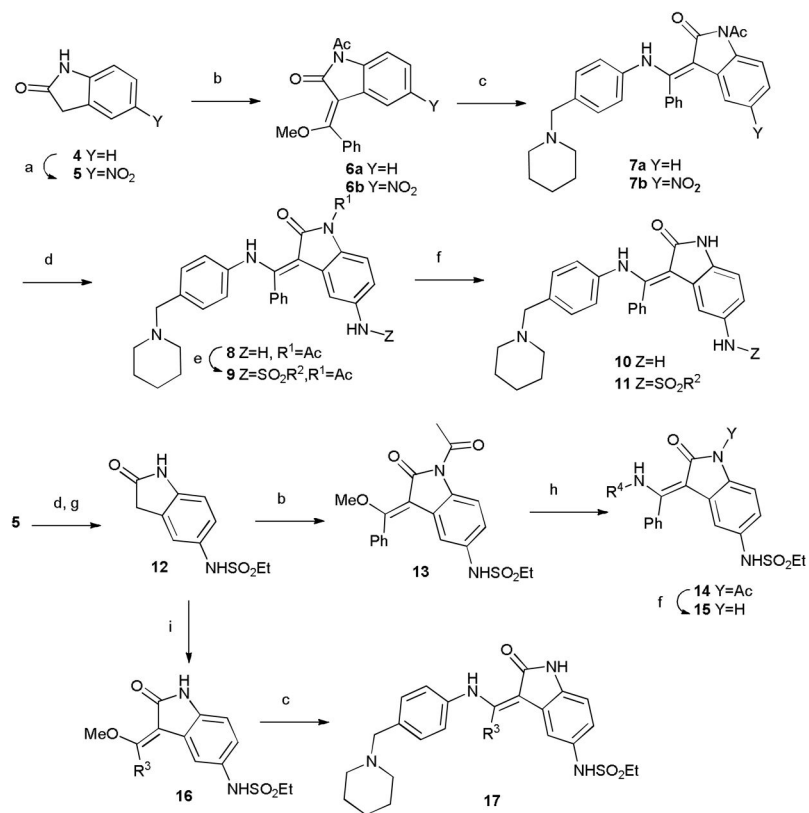


Figure.
Plot of EC₅₀ values of *P. falciparum* C235 versus D6 strains. R²=0.99

**Scheme.**

Synthesis of analogs of hesperadin (**1**). *Reagents and conditions.* (a) HNO_3 , H_2SO_4 -15°C ; (b) $\text{PhC}(\text{OCH}_3)_3$, Ac_2O ; (c) 4-(piperidin-1-ylmethyl)aniline, DMF; (d) Zn, NH_4Cl , MeOH, H_2O ; (e) RSO_2Cl , Et_3N , DMF; (f). NaOH, MeOH; (g) EtSO_2Cl , Et_3N , DMF; (h) R-NH_2 , DMF; (i) $\text{RC}(\text{OCH}_3)_3$, Ac_2O .

Table 1

Benchmark screening of human Aurora inhibitors.^a

Compound	EC ₅₀ (μM)				TC ₅₀ (μM)	
	<i>T. cruzi</i>	<i>L. major</i>	<i>Promastig</i>	<i>L. major</i>	<i>P. falciparum</i>	HepG2
1	0.06 ¹³	39	0.12	2.37	0.01	<0.2
2	10 ¹⁴	nt	>21	nt	0.838	3.3
3	0.6 ⁴	nt	>21	nt	0.857	4

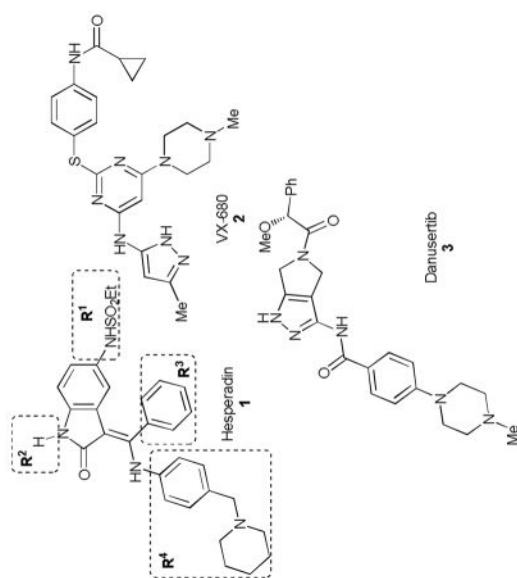
^a nt=not tested; all EC₅₀ and TC₅₀ values data are averages of three replicate experiments.

Table 2

Potency of analogs of **1** against three protozoan parasites.

Cmpd	R ¹	R ²	R ³	R ⁴	EC ₅₀ (μM)						TC ₅₀ (μM) HepG2
					<i>T. brucei</i>	<i>T. cruzi</i>	<i>L. major</i> Promastig	<i>L. major</i> Amastig	<i>P. falciparum</i>		
1	NHSO ₂ Et	H	Ph		0.06	39	0.12	2.37	0.01	<0.2	
7a	H	H	Ph		0.07	25	0.77	>4	0.230	> 6.0	
7b	NO ₂	Ac	Ph		0.13	3	1.08	2.04	0.275	> 6.0	
8	NH ₂	Ac	Ph		3.25	45	>4	>4	0.218	1.81	
10	NH ₂	H	Ph		1.16	>50	0.79	>4	0.199	> 6.0	
11a	NHSO ₂ Me	H	Ph		0.02	>50	0.39	>4	0.052	0.2	
11b	NHSO ₂ Ph	H	Ph		0.01	16	0.85	1.91	0.107	3.29	
11c	NHAc	H	Ph		0.15	>50	1.52	>4	0.068	> 6.0	
17a	NHSO ₂ Et	H	H		1.32	>50	2.77	>4	0.241	5	
17b	NHSO ₂ Et	H	Me		3.36	>50	>4	>4	1.364	> 6.0	
17c	NHSO ₂ Et	H	Et		1.79	>50	2.8	>4	0.233	> 6.0	
17d	NHSO ₂ Et	H	<i>n</i> -Bu		1.67	>50	2.6	>4	0.167	> 6.0	
15a	NHSO ₂ Et	H	Ph		>50	>50	>4	>4	>15	>10.0	
15b	NHSO ₂ Et	H	Ph	Ph	1.44	>50	>4	>4	0.109	4.7	
15c	NHSO ₂ Et	H	Ph	p-Tol	0.37	>50	2.6	>4	1.607	3.1	
15d	NHSO ₂ Et	H	Ph		0.1	>50	0.94	1.68	0.054	1.31	
15e	NHSO ₂ Et	H	Ph		1.53	>50	0.62	>4	0.595	> 6.0	