Volume 1 | Number 1 | Jan 2013 | Pages 1–100

MedChemComm

www.rsc.org/medchemcomm

pDOS

pIT3
IT3

ROYAL SOCIETY OF CHEMISTRY

EFMC
European Federation for Medicinal Chemistry

ROYAL SOCIETY
OF CHEMISTRY

www.rsc.org/medchemcomm

Journal Name

Dynamic Article Links ▶

ARTICLE TYPE

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

Gautam Patel,[a] Norma E. Roncal,[b] Patricia J. Lee,[b] Susan E. Leed,[b] Jessey Erath,[d] Ana Rodriguez,[c,d] Richard J. Sciotti,[b] and Michael P. Pollastri[*a]

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 10 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 15 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 20 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 and malaria (caused by *Plasmodium sp.*), these diseases have a significant and adverse effect on length of quality 25 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.

30 One such method is target repurposing,[2] where essential parasitic targets (such as kinases,[3-7] phosphodiesterases,[8, 9] and histone deacetylases[10]) are matched with homologous and druggable targets in humans that have been the subject of previous drug discovery programs. Assessment of chemical 35 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 40 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.* 45 *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]

Table 1 Benchmark screening of human Aurora inhibitors.[a]
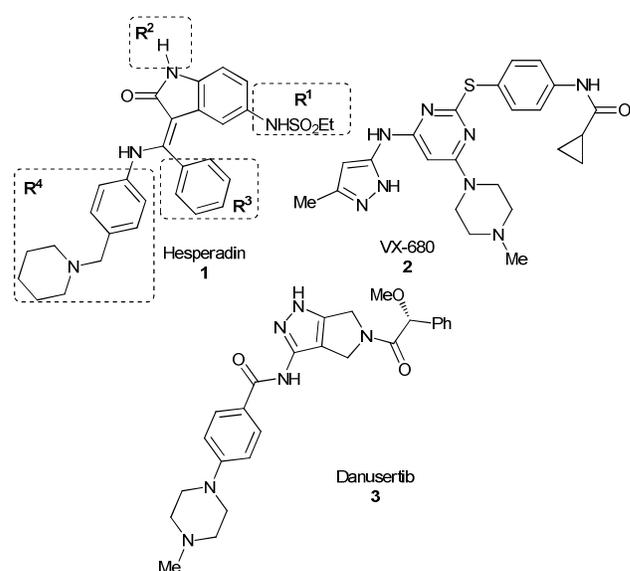
Compound	EC$_{50}$ (μM)					TC$_{50}$ (μM)
	T brucei	*T. cruzi*	*L. major* Promastig	*L. major* Amastig	*P. falciparum*	HepG2
1	0.06[13]	39	0.12	2.37	0.01	< 0.2
2	10[14]	nt	>21	nt	0.838	3.3
3	0.6[4]	nt	>21	nt	0.857	4

[a] nt=not tested; all EC$_{50}$ and TC$_{50}$ values data are averages of three replicate experiments.

Encouraged by the preliminary results for *T. brucei* described 55 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 60 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 65 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 R^1-R^4 as shown in Table 1, modelled after the synthetic route described in a patent.[15] Synthesis initiated with indolone **4,** which was nitrated[15] 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 R^1 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 R^4) to obtain access the analogs **15** following deprotection. Finally, inclusion of various orthoester reagents in the sequence afforded R^3 variations (compound **17**).
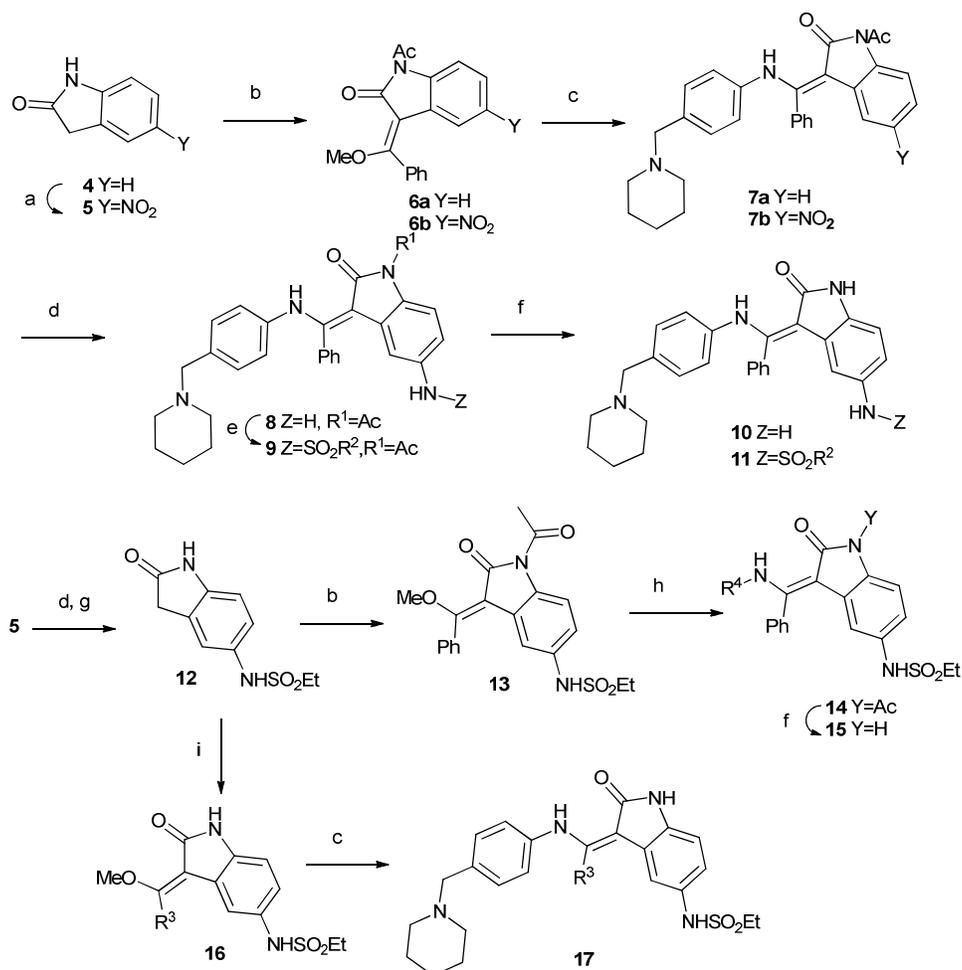
Scheme Synthesis of analogs of hesperadin (**1**). *Reagents and conditions.* (a) HNO_3, H_2SO_4 -15 ºC; (b) $PhC(OCH_3)_3$, Ac_2O; (c) 4-(piperidin-1-ylmethyl)aniline, DMF; (d) Zn, NH_4Cl, MeOH, H_2O; (e) RSO_2Cl, Et_3N, DMF; (f) .NaOH, MeOH; (g) $EtSO_2Cl$, Et_3N, DMF; (h) $R-NH_2$, DMF;(i) $RC(OCH_3)_3$, Ac_2O.

Screening results and discussion

The analogs were tested in parasite cultures, and the results are summarized in Table 2. First, variation of the R^1 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 R^1 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 R^4 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 R^3 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
5 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
10 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 EC_{50}. Remarkably, the compounds
15 show highly consistent potency values across strains; this is also observed for the W2 and C2B strains (R^2=0.95 and 0.95, respectively).

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

Table 2. Potency of analogs of **1** against three protozoan parasites.

Cmpd	R¹	R²	R³	R⁴	EC₅₀ (µM)					TC₅₀ (µM)
					T brucei	*T. cruzi*	*L. major* Promastig	*L. major* Amastig	*P. falciparum*	HepG2
1	NHSO₂Et	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	NO₂	Ac	Ph		0.13	3	1.08	2.04	0.275	> 6.0
8	NH₂	Ac	Ph		3.25	45	>4	>4	0.218	1.81
10	NH₂	H	Ph		1.16	>50	0.79	>4	0.199	> 6.0
11a	NHSO₂Me	H	Ph		0.02	>50	0.39	>4	0.052	0.2
11b	NHSO₂Ph	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	NHSO₂Et	H	H		1.32	>50	2.77	>4	0.241	5
17b	NHSO₂Et	H	Me		3.36	>50	>4	>4	1.364	> 6.0
17c	NHSO₂Et	H	Et		1.79	>50	2.8	>4	0.233	> 6.0
17d	NHSO₂Et	H	*n*-Bu		1.67	>50	2.6	>4	0.167	> 6.0
15a	NHSO₂Et	H	Ph		>50	>50	>4	>4	>15	>10.0
15b	NHSO₂Et	H	Ph	Ph	1.44	>50	>4	>4	0.109	4.7
15c	NHSO₂Et	H	Ph	p-Tol	0.37	>50	2.6	>4	1.607	3.1
15d	NHSO₂Et	H	Ph		0.1	>50	0.94	1.68	0.054	1.31
15e	NHSO₂Et	H	Ph		1.53	>50	0.62	>4	0.595	> 6.0

25 **Figure** Plot of EC_{50} values of *P. falciparum* C235 versus D6 strains.
R^2=0.99
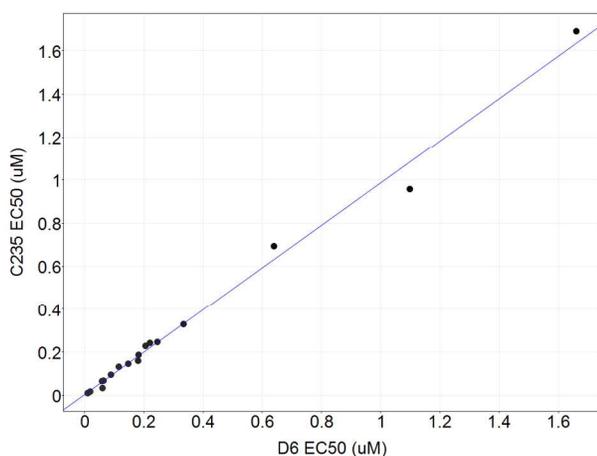
Conclusions

30 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
35 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
40 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.

Acknowledgements

Notes and references

5 *ᵃ Northeastern University Department of Chemistry & Chemical Biology, 360 Huntington Avenue, Boston, MA USA. Tel: 617-373-2703; E-mail: m.pollastri@neu.edu; ᵇ Experimental Therapeutics, Walter Reed Army Institute for Research, 2460 Linden Lane, Silver Spring, MD, 20910; ᶜNew York University School of Medicine, Department of Microbiology,* 10 *Division of Parasitology, 341 E. 25ᵗʰ St. New York, NY USA; ᵈAnti-Infectives Screening Core, New York University School of Medicine, New York, NY 10010.*

15 † Electronic Supplementary Information (ESI) available: Synthetic preparations and assay details are described in the ESI. See DOI: 10.1039/b000000x/Compound data is available as a searchable shared data set at http://collaborativedrug.com.

20 1. Metrics: Disability-Adjusted Live Year (DALY). http://www.who.int/healthinfo/global_burden_disease/metrics _daly/en/, Accessed 01/23/2012, 2011.

2. M. P. Pollastri and R. K. Campbell, *Future Med. Chem.*, 2011, **3**, 1307-1315.

25 3. R. Diaz-Gonzalez, F. M. Kuhlmann, C. Galan-Rodriguez, L. Madeira da Silva, M. Saldivia, C. E. Karver, A. Rodriguez, S. M. Beverley, M. Navarro and M. P. Pollastri, *PLoS Negl. Trop. Dis.*, 2011, **5**, e1297.

4. S. O. Ochiana, V. Pandarinath, Z. Wang, R. Kapoor, M. J. Ondrechen, 30 L. Ruben and M. P. Pollastri, *Eur. J. Med. Chem.*, 2013, **62**, 777-784.

5. G. Patel, C. E. Karver, R. Behera, P. J. Guyett, C. Sullenberger, P. Edwards, N. E. Roncal, K. Mensa-Wilmot and M. P. Pollastri, *J. Med. Chem.*, 2013, **56**, 3820-3832.

35 6. A. Woodland, R. Grimaldi, T. Luksch, L. A. T. Cleghorn, K. K. Ojo, W. C. Van Voorhis, R. Brenk, J. A. Frearson, I. H. Gilbert and P. G. Wyatt, *ChemMedChem*, 2013, **8**, 1127-1137.

7. K. K. Ojo, J. R. Gillespie, A. J. Riechers, A. J. Napuli, C. L. M. J. Verlinde, F. S. Buckner, M. H. Gelb, M. M. Domostoj, S. J. 40 Wells, A. Scheer, T. N. C. Wells and W. C. Van Voorhis, *Antimicrob. Agents Chemother.*, 2008, **52**, 3710-3717.

8. N. D. Bland, C. Wang, C. Tallman, A. E. Gustafson, Z. Wang, T. D. Ashton, S. O. Ochiana, G. McAllister, K. Cotter, A. P. Fang, L. Gechijian, N. Garceau, R. Gangurde, R. Ortenberg, M. J. 45 Ondrechen, R. K. Campbell and M. P. Pollastri, *J. Med. Chem.*, 2011, **54**, 8188-8194.

9. H. P. de Koning, M. K. Gould, G. J. Sterk, H. Tenor, S. Kunz, E. Luginbuehl and T. Seebeck, *J. Infect. Dis.*, 2012, **206**, 229-237.

50 10. J. M. Kelly, M. C. Taylor, D. Horn, E. Loza, I. Kalvinsh and F. Björkling, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 1886-1890.

11. M. M. Siman-Tov, A. C. Ivens and C. L. Jaffe, *Biochim. Biophys. Acta, Gene Struct. Expression*, 2001, **1519**, 241-245.

12. L. Reininger, J. M. Wilkes, H. Bourgade, D. Miranda-55 Saavedra and C. Doerig, *Mol. Microbiol.*, 2011, **79**, 205-221.

13. N. Jetton, K. G. Rothberg, J. G. Hubbard, J. Wise, Y. Li, H. L. Ball and L. Ruben, *Mol. Microbiol.*, 2009, **72**, 442-458.

14. Z. Li, T. Umeyama and C. C. Wang, *PLoS Pathog.*, 2009, **5**, e1000575.

60 15. R. Walter, A. Heckel, G. J. Roth, J. Kley, G. Schnapp, M. Lenter, M. J. C. A. Van, W. Spevak and U. Weyer-Czernilofsky, WO2002036564A1, 2002.

16. G. De Muylder, K. K. H. Ang, S. Chen, M. R. Arkin, J. C. Engel and J. H. McKerrow, *PLoS Negl. Trop. Dis.*, 2011, **5**, 65 e1253.

Hesperadin (1)
T. brucei EC$_{50}$: 0.06μM
L. major EC$_{50}$: 0.12 μM
P. falciparum EC$_{50}$: 0.01μM
HepG2 TC$_{50}$: <0.2 μM

11b: R=SO$_2$Ph
T. brucei EC$_{50}$: 0.01 μM
L. major EC$_{50}$: 0.85 μM
P. falciparum EC$_{50}$: 0.107μM
HepG2 TC$_{50}$: 3.29 μM

11c: R=Ac
T. brucei EC$_{50}$: 0.15 μM
L. major EC$_{50}$: 1.52 μM
P. falciparum EC$_{50}$: 0.068 μM
HepG2 TC$_{50}$: >6 μM