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Discovery and preliminary structure–activity relationship analysis of 1,14-sperminediphenylacetamides as potent and selective antimalarial lead compounds

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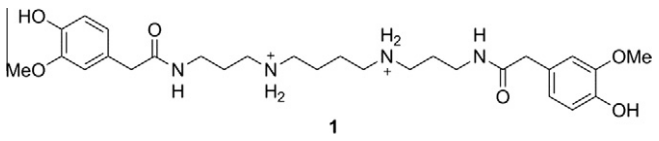
ABSTRACT

Screening of synthesized and isolated marine natural products for in vitro activity against four parasitic protozoa has identified the ascidian metabolite 1,14-sperminedihomovanillamide (orthidine F, **1**) as being a non-toxic, moderate growth inhibitor of *Plasmodium falciparum* (IC_{50} 0.89 μM). Preliminary structure–activity relationship investigation identified essentiality of the spermine polyamine core and the requirement for 1,14-disubstitution for potent activity. One analogue, 1,14-spermine-di-(2-hydroxyphenylacetamide) (**3**), exhibited two orders of magnitude increased anti-*P. f* activity (IC_{50} 8.6 nM) with no detectable in vitro toxicity. The ease of synthesis of phenylacetamido-polyamines, coupled with potent nM levels of activity towards dual drug resistant strains of *P. falciparum* makes this compound class of interest in the development of new antimalarial therapeutics.

Parasitic protozoal infection is a cause of suffering for an estimated one billion people worldwide.[1] Insect bites that transfer protozoa to human hosts lead to the diseases malaria (protozoa: *Plasmodium falciparum*), Chagas Disease (also known as American Trypanosomiasis, *Trypanosoma cruzi*), Leishmaniasis (*Leishmania* spp.) and Sleeping Sickness (also known as Human African Trypanosomiasis, *Trypanosoma brucei rhodesiense*, *Trypanosoma brucei gambiense*). While drugs exist for these diseases, they have undesirable side-effects or are showing reduced efficacy due to the growing prevalence of drug resistant strains.[1]

In the context of our continuing search for new leads for the development of treatments for neglected human diseases,[2] we recently initiated a program of screening a library of synthesized and isolated marine natural products against a panel of four parasitic protozoa: *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum* K1 dual drug-resistant strain, with concurrent counter-screening for toxicity towards the non-malignant L6 rat myoblast cell line. One of the first anti-malarial hits identified was the polyamine diamide orthidine F (**1**), previously reported by us as an anti-inflammatory agent from the New Zealand ascidian *Aplidium orthium*.[3] Herein we report the in vitro screening data for orthidine F, and the results of a preliminary structure–activity relationship study which led to the identification of an analogue exhibiting two-orders of magnitude increased activity towards *Plasmodium falciparum*.



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The anti-protozoal evaluation of **1** (Table 1, entry 1) established the natural product to be a moderately potent in vitro growth inhibitor of *P. falciparum* K1 strain (IC_{50} 0.89 μM) and a modest inhibitor of *Trypanosoma brucei rhodesiense* (IC_{50} 78 μM) while demonstrating no detectable activity towards *T. cruzi* and *Leishmania donovani* and no cytotoxicity against a mammalian cell-line. Polyamines are well recognized as a biologically active scaffold,[4] exhibiting cytotoxicity,[5] receptor binding properties (AMPA,[6] NMDA[7]), enzyme inhibition (trypanothione reductase,[8,9] carbonic anhydrase[10]) and

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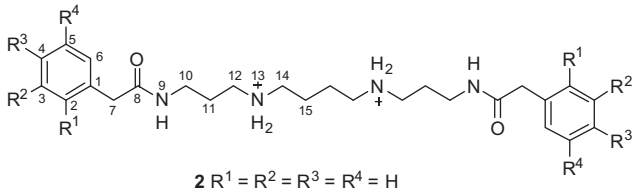
Table 1
Anti-protozoal and cytotoxic activities of **1**–**23**, **24**, **26**

Entry	Compound	IC_{50} (μM)				
		T. b. rhod[a]	*T. cruzi*[b]	*L. don*[c]	*P. falc.* K1[d]	L6[e]
1	**1**	78	>120	>120	0.89	>120
2	**2**	96	>140	90	0.14	>140
3	**3**	51	>130	110	0.0086	>130
4	**4**	87	>130	>130	0.17	>130
5	**5**	86	>130	>130	0.14	130
6	**6**	3.2	>120	>120	4.8	85
7	**7**	38	31	35	0.019	88
8	**8**	120	>120	>120	>6.8	>120
9	**9**	49	>130	>130	0.61	>130
10	**10**	150	>150	>150	>8.5	>150
11	**11**	120	>170	>170	3.6	>170
12	**12**	5.9	>160	>160	7.9	97
13	**13**	110	>160	>160	>8.9	>160
14	**14**	120	>180	>180	>10	>180
15	**15**	7.5	>170	>170	>9.4	140
16	**16**	210	>220	>220	>12	>220
17	**17**	110	>280	>280	>15	>280
18	**18**	3.3	>230	>230	>13	130
19	**19**	110	>160	150	>9.1	>160
20	**20**	63	>200	>200	>11	>200
21	**21**	5.2	>170	>170	>9.6	96
22	**22**	150	>220	>220	>12	210
23	**23**	170	>250	>250	>14	>250
24	**24**	NT[f]	NT	NT	35	94
25	**26**	NT	NT	NT	8.0	110
	Melarsoprol[g]	0.005				
	Benznidazole[g]		1.8			
	Miltefosine[g]			0.53		
	Chloroquine[g]				0.28	
	Podophyllotoxin[g]					0.019

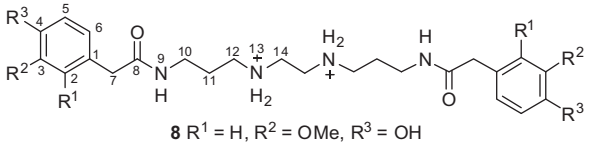
IC_{50} values reported are the average of two independent assays. Assay protocols are described in Ref. 18.

[a] *Trypanosoma brucei rhodesiense*, STIB 900 strain, trypomastigotes stage.
[b] *Trypanosoma cruzi*, Tulahuen C4 strain, amastigotes stage.
[c] *Leishmania donovani*, MHOM-ET-67/L82 strain, amastigote/axenic stage.
[d] *Plasmodium falciparum*, K1 strain, IEF stage.
[e] L6 rat skeletal myoblast cell line.
[f] Not tested.
[g] Melarsoprol, benznidazole, miltefosine, chloroquine and podophyllotoxin were used as positive controls.

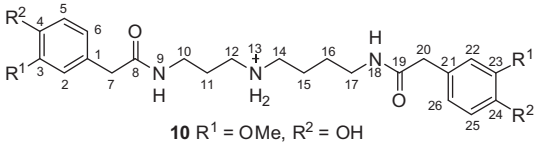
anti-protozoal activity towards *Trypanosoma* sp.[11] and *P. falciparum*.[12,13] Making use of the previously reported facile synthesis of **1**,[3] we undertook a preliminary structure–activity relationship study to explore the relevance of the polyamine core and the terminal 'capping acids'[9] towards the observed anti-protozoal activity. Thus reaction of spermine with six phenylacetic acid derivatives (phenylacetic acid, 2-, 3- and 4-hydroxyphenyl acetic acid, 3,4-dihydroxyphenyl acetic acid and 2,5-dimethoxyphenyl acetic acid) using PyBOP as the coupling reagent (see Supplementary data for details) afforded, after chromatographic purification, analogues **2**[14], **3**, **4**[9], **5**, **6** and **7** in yields of 45%, 73%, 46%, 65%, 62% and 54%, respectively. In a similar fashion, subsets of these capping acids were reacted with *N*,*N*′-bis(3-aminopropyl)ethylenediamine, spermidine, *N*-(2-aminoethyl)-1,3-propanediamine, 1,4-diaminobutane, or 1,13-diamino-4,7,10-trioxa-tridecane to afford diamide analogues **8–16**, **17**[15], **18**–**21**. Reaction of homovanillic acid with 2-morpholinoethanamine or *N*,*N*-dimethylethane-1,2-diamine yielded truncated mono-amides **22** and **23** in 73% and 22% yield, respectively. To explore whether diarylamide substitution of the polyamine core of **2** was a requirement for activity, spermine mono-amide **24** was prepared by a two-step route starting with PyBOP-activated coupling of spermine-trifluoroacetamide (**25**)[16] with phenylacetic acid to yield diamide **26** followed by deprotection using LiOH in THF/H_2O to afford **24**.



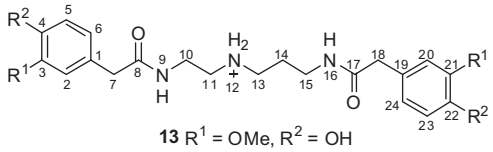
2 $R^1 = R^2 = R^3 = R^4 = H$
3 $R^1 = OH$, $R^2 = R^3 = R^4 = H$
4 $R^1 = R^3 = R^4 = H$, $R^2 = OH$
5 $R^1 = R^2 = R^4 = H$, $R^3 = OH$
6 $R^1 = R^4 = H$, $R^2 = R^3 = OH$
7 $R^1 = R^4 = OMe$, $R^2 = R^3 = H$



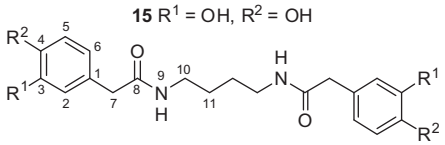
8 $R^1 = H$, $R^2 = OMe$, $R^3 = OH$
9 $R^1 = OH$, $R^2 = R^3 = H$



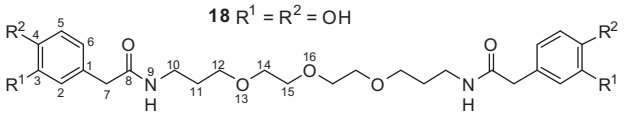
10 $R^1 = OMe$, $R^2 = OH$
11 $R^1 = H$, $R^2 = OH$
12 $R^1 = R^2 = OH$



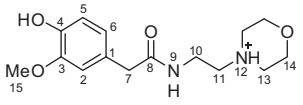
13 $R^1 = OMe$, $R^2 = OH$
14 $R^1 = OH$, $R^2 = H$
15 $R^1 = OH$, $R^2 = OH$



16 $R^1 = OMe$, $R^2 = OH$
17 $R^1 = R^2 = H$
18 $R^1 = R^2 = OH$



19 $R^1 = OMe$, $R^2 = OH$
20 $R^1 = R^2 = H$
21 $R^1 = R^2 = OH$



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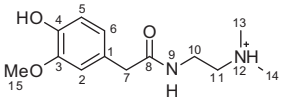

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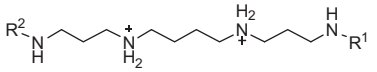

24 $R^1 = H$, $R^2 = PhCH_2CO$
25 $R^1 = COCF_3$, $R^2 = H$
26 $R^1 = COCF_3$, $R^2 = PhCH_2CO$

The library of analogues were screened against a set of four protozoa and for cytotoxicity towards the L6 cell line and the results are summarized in Table 1. A set of spermine analogues that varied in phenylacetic acid substitution (**2**–**7**, entries 2–7) typically exhibited more potent antimalarial activity than orthidine F, with the

2-hydroxyphenylacetamide analogue **3** being the most potent analogue identified in the study. The critical requirement of the hydroxyl group at the 2-position is noted, with the 3- and 4-hydroxy analogues **4** and **5** (entries 4 and 5) being approximately twenty-fold less active than **3**. Also notable in this spermine series was the pronounced activity of the 3,4-dihydroxy analogue **6** (entry 6) towards *T. brucei rhodesiense* (IC_{50} 3.2 μM), tempered somewhat with the observation of increased cytotoxicity towards the L6 cell line. From the same series, only the 2,5-dimethoxy analogue **7** exhibited growth inhibitory activity towards *Trypanosoma cruzi* and *Leishmania donovani*, with all other analogues being considered inactive. In the case of **7** however, pan-panel activity suggests this particular diamide may be exhibiting activity due to a general cytotoxic mechanism. Using a subset of substituted phenylacetic acids, a variety of diamides were prepared (**8**–**23**) that explored the structure–activity requirement of the spermine fragment of orthidine F. As summarized in entries 8–23, incorporation of *N*,*N*′-bis(3-aminopropyl)ethylenediamine (**8**, **9**), spermidine (**10**, **11**, **12**), *N*-(2-aminoethyl)-1,3-propanediamine (**13**, **14**, **15**), 1,4-diaminobutane (**16**, **17**, **18**), 1,13-diamino-4,7,10-trioxatridecane (**19**, **20**, **21**), 2-morpholinoethanamine (**22**) and *N*,*N*-dimethylethane-1,2-diamine (**23**) motifs led to substantially reduced anti-malarial activity. No members of this subset exhibited activity towards *T. cruzi* or *L. donovani*, though the anti-*Trypanosoma brucei rhodesiense* activity associated with the 3,4-dihydroxyphenylacetic acid moiety was again highlighted (**12**, **15**, **18** and **21**, entries 12, 15, 18, 21). Finally, evaluation of the mixed aryl-TFA-diamide **26** and the aryl mono-amide **24** against *P. falciparum* found both to be essentially inactive (entries 24 and 25), indicating the requirement for diarylamide substitution for optimal anti-malarial activity.

In conclusion, this Letter reports our finding of moderate anti-malarial activity for the marine natural product orthidine F and preliminary structure–activity relationship investigation which reveals the requirement of spermine and diamide terminal capping groups for activity, with optimal activity being identified for a 2-hydroxyl substituent. A subset of 3,4-dihydrophenylacetamide-containing analogues also exhibited activity towards *Trypanosoma brucei rhodesiense*, though at the expense of increased cytotoxicity towards the L6 non-malignant cell line. The ease of synthesis of phenylacetamido-polyamines, combined with potent nM levels of activity towards a dual drug-resistant strain of *P. falciparum* and low cytotoxicity[17] is encouraging, prompting future efforts directed towards an expanded SAR study and selection of candidates for in vivo evaluation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.072. These data include MOL files and InChiKeys of the most important compounds described in this article.

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