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Discovery of novel and potent benzhydryl-tropane trypanocides highly selective for *Trypanosoma cruzi*

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ABSTRACT

A benzhydryl tropinone oxime that is potently toxic to *Trypanosoma cruzi* has been previously identified. An SAR investigation determined that no part of the original compound was superfluous and all early SAR probes led to significant drops in activity. The only alteration that could be achieved without loss of activity was replacement of the aryl chloride substituent with chloro homologues. This led to the discovery of a trifluoromethyl-containing analogue with an EC_{50} against *T. cruzi* of 30 nM and a cytotoxicity selectivity index of over 1000 relative to rat skeletal myoblast L-6 cells.

The protozoan parasite *Trypanosoma cruzi* is the causative agent of Chagas disease which is found in 18 countries in Latin America. In the 2004 World Health Report[1] Chagas disease was estimated to cause 14,000 deaths and a disease burden of 0.7 million DALYs annually. There are currently only two significant therapies for Chagas disease, nifurtimox and benznidazole (Fig. 1).[2] These drug treatments are ineffective at preventing the development and treatment of chronic Chagas disease[3] and induce a number of adverse effects. Hence, there is an urgent need for the development of new cost effective therapy against *T. cruzi* with minimal side effects.

We have previously reported on a high-throughput screening campaign conducted to identify inhibitors of trypanothione reductase (TR) that might consequently be toxic to trypanosomatids.[4,5] Amongst the identified hits was a benzhydryl tropinone oxime that was found to be potently toxic towards *T. cruzi* with an EC_{50} of 110 nM and highly selective for this organism relative to other trypanosomatids such as *Leishmania donovani* and *Trypanosoma brucei*.[5] These data are summarised in Figure 2.

For several reasons, significantly amongst these being the very weak trypanothione reductase inhibitory activity of compound **1** (EC_{50} 35 μM, Fig. 2), we have previously argued that the observed potent *T. cruzi*-selective trypanocidal activity could likely be due to

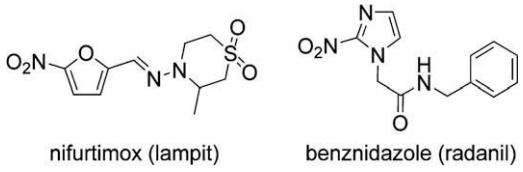
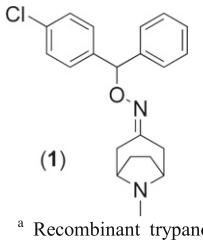


Figure 1. Current *T. cruzi* therapies.



Assay	EC_{50} (μM)
TR (*T.cruzi*)	35[a]
P.falciparum	7.5[b]
L.donovani	>30[c]
T.cruzi	0.11[d]
T.brucei	3[e]
Cytotoxicity	36[f]

[a] Recombinant trypanothione reductase, *Trypanosoma cruzi* Brazilian Silvio strain, X10/1.
[b] *Plasmodium falciparum* 3D7 strain, erythrocylic stage, chloroquine was used as a control, EC_{50} 9.4 nM.
[c] *Leishmania donovani* MHOM/ET/67/L82 strain, amastigote stage, miltefosine was used as a control, EC_{50} 0.27 μM.
[d] *Trypanosoma cruzi* Tulahaen C2C4 strain, amastigote stage, benznidazole was used as a control, EC_{50} 1.8 μM.
[e] *Trypanosoma brucei rhodesiense* strain STIB 900, bloodstream form. Melarsoprol (EC_{50} 17 nM) was used as a control.
[f] Rat skeletal myoblast cell L-6 strain, podophyllotoxin was used as a control, EC_{50} 14 nM.

Figure 2. Structure and antiparasitic activity of benzhydryl tropinone oxime **1**.

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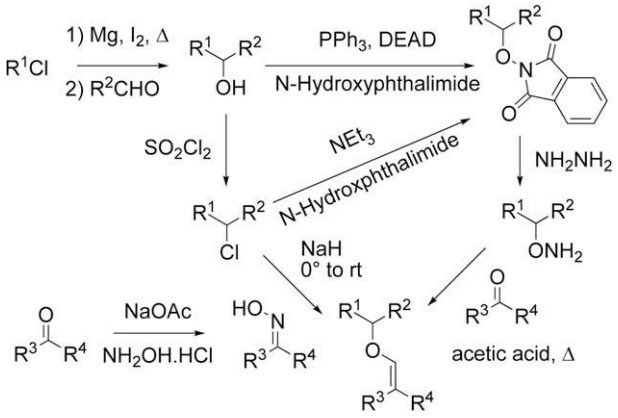
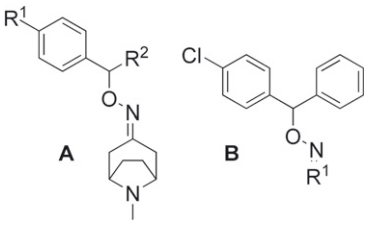


Figure 3. Benzhydryl tropane oxime synthesis. See Supplementary data.

off-target activity.[5] Subsequently, we gained access to a higher throughput parasite assay and it was principally for this reason that we initiated a hit optimisation campaign around compound **1**, using a whole parasite primary assay. This communication will disclose the results of our SAR investigation into this compound.

Two main synthetic methods were utilised (Fig. 3). One method enabled rapid analogue production of the tropane portion of **1** by reaction of a biaryl oxylamine with an appropriate ketone. The biaryl oxylamine was synthesised via deprotection of the corresponding benzhydryloxyphthalimide[6] which in turn was synthesised from the biaryl alcohol, either directly through a Mitsunobu reaction[6] or via its corresponding chloride.[7,8] The second method enabled rapid analogue production of the benzhydryl portion, through reaction of the tropinone oxime with an appropriate arylmethyl chloride. Results from the whole cell *T. cruzi* assay of the first set of compounds (Fig. 4) confirmed that two aryl groups were essential for activity and removal of one of the phenyl rings (**2** and **3**) resulted in loss of activity. It was also determined that the basic nitrogen atom in the tropane ring was important, as replacement with cycloalkyl (**4**) or alkyl (**5**) resulted in complete loss of activity. Even removal of the *N*-methyl group (**6**) or the tropane bridge (**7**) led to significant loss of activity.

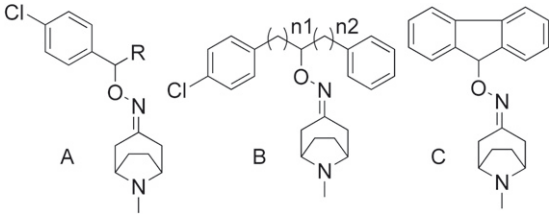
Having established that the tropane ring and two aryl groups are important, a second set of compounds was assembled to investigate changes in aryl ring connectivity. Replacing the unsubstitut-



Cmpds	Struct	R^1	R^2	EC_{50} (μM)	
				T.cruzi[a]	Cytotox[b]
1	A	Cl	Ph	0.07	15
2	A	Cl	H	>10	>100
3	A	H	H	>10	>100
4	B	cyclohexyl	-	>10	>100
5	B	CMe_2	-	>10	>100
6	B	des-Me tropane	-	2.0	9.8
7	B	4-(N-Me piperidine)	-	3.7	50

[a] *Trypanosoma cruzi* Tulahaen C2C4 strain, amastigote stage, benznidazole was used as a control, EC_{50} 1.8 μM.
[b] Rat skeletal myoblast cell L-6 strain, podophyllotoxin was used as a control, EC_{50} 14 nM.

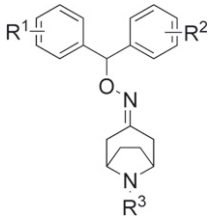
Figure 4. Benzhydryl tropane oxime SAR: deletion analogues.



Cmpds	Struct	R		EC_{50} (μM)	
				T.cruzi[a]	Cytotox[b]
8	A	2-pyridine		2.9	>100
9	A	3-pyridine		0.5	>100
10	A	4-pyridine		6.2	>100
11	A	2-thiazole		2.3	>100
12	A	4-thiazole		0.8	71
13	A	5-thiazole		6.0	>100
		n1	n2		
14	B	0	1	2.1	40
15	B	0	2	6.5	20
16	B	0	3	4.3	17
17	B	1	0	1.9	49
18	B	2	0	0.8	16
19	C	-	-	>10	68

[a] *Trypanosoma cruzi* Tulahaen C2C4 strain, amastigote stage, benznidazole was used as a control, EC_{50} 1.8 μM.
[b] Rat skeletal myoblast cell L-6 strain, podophyllotoxin was used as a control, EC_{50} 14 nM.

Figure 5. SAR probing of the benzhydryl group.



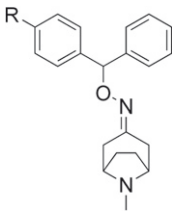
Cmpds	R^1	R^2	R^3	EC_{50} (μM)	
				T.cruzi[a]	Cytotox[b]
1	4-Cl	H	Me	0.07	15
20	3-Cl	H	Me	2.1	19
21	2-Cl	H	Me	6.8	15
22	4-Cl	4-Cl	Me	6.0	16
23	H	H	Me	1.3	>100
24	4-OMe	H	Me	0.3	>100
25	3-OMe	H	Me	2.0	48
26	2-OMe	H	Me	>10	>100
27	4-Cl	H	CH_2CH_2OH	7.8	47
28	4-Cl	H	$CH_2CH_2NMe_2$	6.9	15

[a] *Trypanosoma cruzi* Tulahaen C2C4 strain, amastigote stage, benznidazole was used as a control, EC_{50} 1.8 μM.
[b] Rat skeletal myoblast cell L-6 strain, podophyllotoxin was used as a control, EC_{50} 14 nM.

Figure 6. Benzhydryl and tropane substituent SAR.

ed phenyl ring with a pyridine or thiazole led to a significant loss in activity (**8**–**13**, Fig. 5), though to greatly varying extents, as the 4-pyridyl analogue **10** was 12 times weaker again than the 3-pyridyl analogue **9**. Similarly, introducing a carbon chain between the two phenyl rings on either side led to variable losses in activity (**14**–**18**), and constraining the rings (**19**) led to complete loss of activity.

This tight SAR suggested that the benzhydryl group should be retained for optimum activity. Therefore, by way of finer SAR probing, a series of compounds with electron withdrawing and donating groups in different positions on the benzhydryl phenyl rings were synthesised (**20**–**28**, Fig. 6). These compounds all lost significant



Cmpds	R	EC_{50} (μM)	
		T.cruzi[a]	Cytotox[b]
1	Cl	0.07	15
29	F	0.8	50
30	Br	0.04	40
31	I	0.04	39
32	Me	0.3	51
33	CN	0.4	53
34	CF_3	0.03	35

[a] *Trypanosoma cruzi* Tulahaen C2C4 strain, amastigote stage, benznidazole was used as a control, EC_{50} 1.8 μM.
[b] Rat skeletal myoblast cell L-6 strain, podophyllotoxin was used as a control, EC_{50} 14 nM.

Figure 7. Chloro homologue SAR.

activity, except the *p*-methoxy compound (**24**) which retained reasonable activity with an EC_{50} of 300 nM. We similarly investigated fine alterations of the tropane ring, namely by extension of the *N*-alkyl substituent. As shown for compounds **27** and **28**, significant loss of activity was observed for the tropane *N*-hydroxyethyl and *N*-(dimethylamino)ethyl substituents, respectively.

Thus far, no improvement in the activity of the initial hit **1** had been obtained, suggesting that all structural aspects of this compound were important for biological activity. With this information in hand, a final set of very fine SAR probes was assembled, where the chlorine atom was replaced with homologues. These results are shown in Figure 7. Gratifyingly, good activity was consistently observed and in three cases was better than the original hit. Thus the bromo (**30**), iodo (**31**) and trifluoromethyl compounds (**34**) displayed outstanding activity with whole organism EC_{50} values of 40, 40 and 30 nM, respectively. The weaker activity for the fluoro, methyl and cyano-containing analogues suggests a combination of electronegativity but principally hydrophobicity is important for most potent activity.

In summary, we have described a series of potent trypanocides, benzhydryl tropinone oximes, which do not tolerate variation to the structure apart from substitution of the chloro-aryl substituent by other hydrophobic electronegative substituents. Compound **34**, where the chlorine atom is replaced by a trifluoromethyl group, has an EC_{50} against *T. cruzi* of 30 nM with cytotoxicity to mammalian cells more than 1000-fold weaker.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.022.

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