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Synthesis and Evaluation of 9,9-Dimethylxanthene Tricyclics Against Trypanothione Reductase, *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania donovani*

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Abstract—Derivatives of 9,9-dimethylxanthene were synthesised and evaluated against trypanothione reductase (TR) and in vitro against parasitic trypanosomes and leishmania. High in vitro antiparasitic activity was observed for some derivatives with one compound showing high activity against all three parasites (ED_{50} values of 0.02, 0.48 and 0.32 μM, for *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania donovani*, respectively). The lack of correlation between inhibitory activity against TR and ED_{50} values suggests that TR is not the target.

Introduction

Tricyclic compounds exemplified by acridines, benzoazepines, phenothiazines, and pyridoquinolines have provided drug leads in the area of chemotherapy of trypanosomiasis and leishmaniasis.[1–4] Inhibition of the parasite enzyme trypanothione reductase (TR) is one possible mechanism of action of these compounds. TR is a vulnerable target for drugs that disrupt the natural redox defence systems of the parasitic protozoa *Trypanosoma* and *Leishmania*, the causative agents of human African trypanosomiasis (*T. brucei* sspp), Chagas disease (*T. cruzi*), and leishmaniasis (*Leishmania* sspp).[5]

A literature search revealed that tricyclics based on the 9,9-dimethylxanthene moiety have not been previously investigated as potential TR inhibitors. Thus we decided to conduct a preliminary investigation into the potential of the subject compounds exemplified by **1**–**3** (Fig. 1). The rationale behind the choice of the subject compounds was as follows: First, being an aromatic hydrophobic tricyclic moiety, the 9,9-dimethylxanthene moiety bears resemblance to the aromatic hydrophobic tricyclic moieties found in other tricyclic compounds already reported as competitive inhibitors of TR, where the tricyclic moiety binds in the hydrophobic pocket involved in the recognition of the spermidine moiety of trypanothione disulfide, the substrate for TR.[6,7] Second, the chemically reactive 2,7 and 4,5 positions of the xanthene moiety provide potential multiple sites for introducing chemical diversity.[8,9] This would ultimately aid analogue synthesis and exploration of structure–activity relationships within this class of compounds. Third, a terminal tertiary amino group (exemplified by the dimethylamino group) was incorporated into compounds **1**–**3** to provide a positive charge which has been shown to favor TR over glutathione reductase (GR), the closest related host enzyme.[10] Intermediary functional groups (amides, amines and thioureas) were incorporated to improve solubility properties.

Chemistry

The synthesis of derivatives **1**–**3** is depicted in Schemes 1 and 2. The starting materials **4b** and **5** are commercially available while **4a** was synthesised from **5** by adapting a literature method.[11] Compound **1a** was synthesised in moderate yield in a one-pot reaction from **4a** while **1c** was prepared via the corresponding acid chloride derivative of **4b** (Scheme 1).

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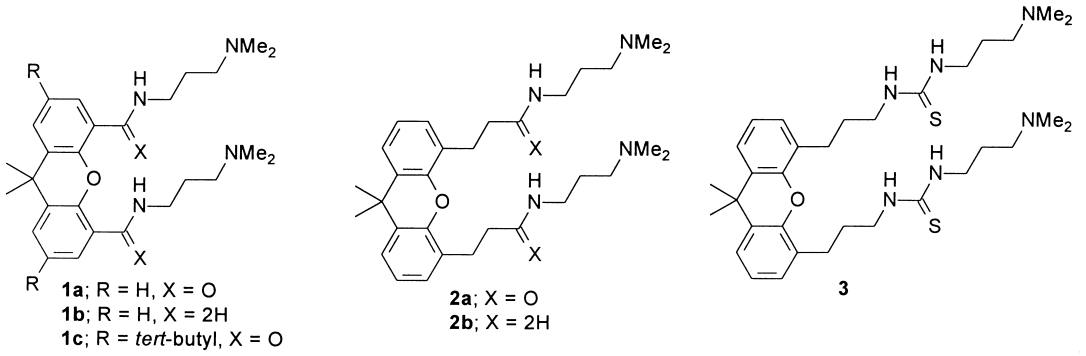
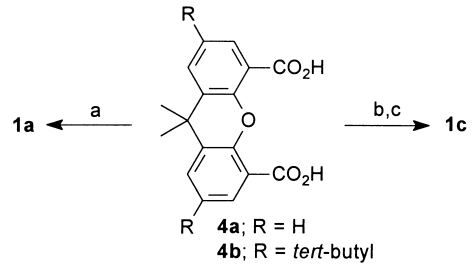


Figure 1. Structures of 9,9-dimethylxanthene tricyclics with two side chains.

For the synthesis of compounds **1b**, **2a**, **2b**, and **3**, the key intermediates were dialdehyde **6** and α,β-unsaturated diester **7**. Compound **6** was obtained via *ortho* lithiation while Wittig two-carbon homologation of **6** gave **7**. Compound **1b** was synthesised in a one-pot reaction from dialdehyde **6** and subsequent in situ reduction of the imine formed using polymer-supported borohydride while compound **2a** was obtained in four steps involving catalytic hydrogenation (Pd-C), hydrolysis, activation of the acid via acid chloride formation and coupling to the appropriate amine. Compounds **2b** and **3** were synthesised from a common intermediate **9** as shown. While the two-step sequence to **2b** proceeded in satisfactory yield with the exception of the final step, low yields were a feature of reactions leading to **3** (Scheme 2). All new compounds gave ^{1}H NMR and FABMS consistent with their structures.

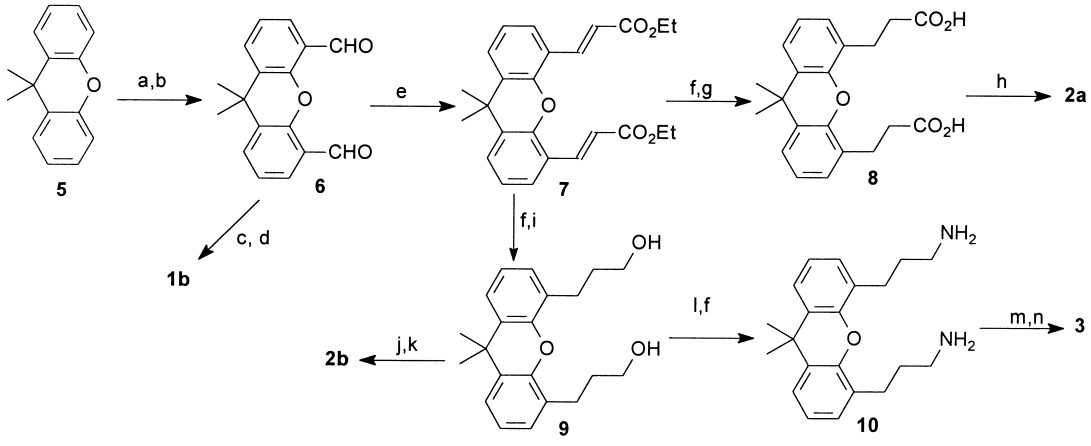


Scheme 1. Reagents and conditions: (a) 2.5 equiv of $(PhO)_2P(O)N_3$, 2.0 equiv of Et_3N, 2.0 equiv of $H_2N(CH_2)_3NMe_2$, PhMe, 85 °C, 18 h, 65%; (b) 3.0 equiv of $(COCl)_2$, DMF (cat.), CH_2Cl_2, reflux, 3 h; then (c) 2.0 equiv of $H_2N(CH_2)_3NMe_2$, 2.0 equiv Et_3N, CH_2Cl_2, 25 °C, 12 h, 91%.

Results and Discussion

The data in Table 1 shows the activity of derivatives **1**–**3** against TR, *L. donovani* amastigotes, *T. cruzi* amastigotes as well as *T. brucei* bloodstream form trypomastigotes.[12] The data for standard control drugs is included for comparison purposes. As far as the inhibition of TR is concerned compounds **1a**–**1c** bearing either one (**1b**) or no methylene spacer (**1a** and **1c**) between the tricyclic moiety and the secondary nitrogen atom generally show weaker inhibition of TR compared to derivatives with a two or three carbon methylene spacer (**2a**, **2b**, and **3**).

For in vitro antiparasitic activity, the rapidly dividing extracellular *T. brucei* bloodstream form trypomastigotes showed the highest in vitro sensitivity to derivatives **1**–**3** with ED_{50} values below 5 μg/mL in all cases while the



Scheme 2. Reagents and conditions: (a) 3.0 equiv of BuLi, 3.0 equiv of TMEDA; then (b) 3.0 equiv of DMF, Et_2O, 25 °C, 18 h, 72%; (c) 1.8 equiv of $H_2N(CH_2)_3NMe_2$, MeOH, 25 °C, 15 h; (d) 4.0 equiv of Amberlite IRA–400 borohydride resin, 25 °C, 18 h, 100%; (e) 3.0 equiv of $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, reflux, 1 h, 93%; (f) H_2, Pd-C, EtOAc, 25 °C, 18 h, 100%; (g) 3.0 equiv of $LiOH.H_2O$, THF:H_2O (10:1), 25 °C, 12 h, 93%; (h) 3.0 equiv of $(COCl)_2$, DMF (cat.), CH_2Cl_2, reflux 3 h, then 2.0 equiv of $H_2N(CH_2)_3NMe_2$, 2.0 equiv Et_3N, CH_2Cl_2, 25 °C, 12 h, 65%; (i) 2.0 equiv of $LiAlH_4$, Et_2O, 0 °C, 0.5 h, 97%; (j) 3.0 equiv of I_2, 3.0 equiv of Ph_3P, 3.0 equiv of imidazole, Et_2O:CH_3CN (3:1), 25 °C, 5 h, 72%; (k) 16.0 equiv of $H_2N(CH_2)_3NMe_2$, THF, reflux, 1 h, 68%.; (l) 2.0 equiv of Ph_3P, 2.0 equiv of DIAD, 2.0 equiv of $(PhO)_2P(O)N_3$, THF, −15 to 25 °C, 4 h, 57%; (m) 14.0 equiv of carbon CS_2, 2.0 equiv of DCC, THF, 25 °C, 18 h, 20%; (n) 2.6 equiv of $H_2N(CH_2)_3NMe_2$, CH_2Cl_2, 25 °C, 18 h, 100%.

Table 1. Inhibition of TR and in vitro antiprotozoal activity of control drugs and compounds **1**–**3**[12]

Compound	% Inhibition of TR	ED_{50} (μg/mL) (μM)			Cytotoxicity[a] MIC (μg/mL)
		L. donovani	*T. cruzi*	*T. brucei*	
Pentostam	—	12.5 (102.7)[b]	—	—	
Benznidazole	—	—	8.5 (32.7)	—	
Pentamidine	—	—	—	0.01 (0.03)	
1a	40	>30	>30	4.5 (9.66)	>300
1b	13.8	>30	>30	3.32 (7.58)	>300
1c	28.4	**0.32 (0.55)**	**0.28 (0.48)**	**0.01 (0.02)**	>30
2a	58	17.03 (32.6)	>30	4.3 (8.24)	>300
2b	63	>30	>30	**0.07 (0.14)**	>30
3	52	>30	>30	**0.06 (0.098)**	>30

[a]Cytotoxicity is derived from the host macrophages used to culture the amastigote stages of *T. cruzi* and *L. donovani*.
[b]Pentostam is sodium stibogluconate and the ED_{50} value is expressed as μg Sb^v/mL (mM Sb^v).

intracellular slow dividing amastigote stages of *L. donovani* and *T. cruzi* were the least sensitive. Compounds **1c**, **2b**, and **3** showed the highest in vitro potency against *T. brucei*. However, the most notable compound is **1c**, which on a molar basis, showed high activity against all three parasites with ED_{50} values either comparable (*T. brucei*) or superior (*L. donovani* and *T. cruzi*) to the standard control drugs.

Although compound **2a** shows comparable activity against TR to **2b** and **3** it does not possess comparable high in vitro activity against *T. brucei*. Coupled with the lack of activity of **2b** and **3** against *L. donovani* and *T. cruzi* but high activity against *T. brucei,* this suggests that TR inhibition is not totally responsible for the observed in vitro activities of these compounds. Other factors such as cell penetration and metabolism, etc., may be playing a crucial role. It is noteworthy that for the intracellular *L. donovani* and *T. cruzi* amastigotes, the drug needs to pass through the macrophage to reach the amastigote. Hence achieving selective toxicity is a greater challenge in *L. donovani* and *T. cruzi* than in *T. brucei*. For compound **1c**, which showed high activity against all three parasites but even weaker inhibitory activity against TR, it is apparent that TR is not the target. Nevertheless it is encouraging that the more potent compounds **1c**, **2b** and **3** are not overtly toxic to the mammalian macrophages at or below a concentration of 30 μg/mL (estimated microscopically).

In conclusion within this series of compounds: (i) there is no clear correlation between potency as inhibitors of TR and the in vitro antiparasitic activities, and (ii) there is no apparent single structural feature controlling in vitro antiparasitic activities. These observations are consistent with conclusions of previous findings.[2,16,17] In the case of compound **1c** which shows pronounced activity against all three parasites, TR is clearly not the target. The mechanism of action of the promising derivatives **1c**, **2b**, and **3** needs investigation if they are to serve as useful leads for rational drug design.

Acknowledgements

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References and Notes

1. Benson, T. J.; McKie, J. H.; Garforth, J.; Borges, A.; Fairlamb, A. H.; Douglas, K. T. *Biochem. J.* **1992**, *286*, 9.
2. Chan, C.; Yin, H.; Garforth, J.; McKie, J. H.; Jaouhari, R.; Speers, P.; Douglas, K. T.; Rock, P. J.; Yardley, V.; Croft, S. L.; Fairlamb, A. H. *J. Med. Chem.* **1998**, *41*, 148.
3. Krauth-Siegel, R. L.; Lohrer, H.; Bucheler, U. S. In *Biochemica Protozoology*; Coombs, G. H; North, M. J. (Eds.); London: Taylor and Francis, 1991, pp 493–503.
4. Krauth-Siegel, R. L.; Jacoby, E. M.; Jockers-Scherubl, M. C.; Schlichting, I.; Barbe, J. T. In *Flavins and Flavoproteins*; Stevenson, K. J., Massey, V.; Williams, C. H., Jr. Eds.; University of Calgary: Canada, 1996; pp 35–44.
5. Fairlamb, A. H.; Blackburn, P.; Ulrich, P.; Chait, B. T.; Cerami, A. *Science* **1985**, *227*, 1485.
6. Jacoby, E. M.; Schlichting, I.; Lantwin, C. B.; Kabsch, W.; Krauth-Siegel, R. L. *Proteins* **1996**, *24*, 73.
7. Bond, C. S.; Zhang, Y.; Berriman, M.; Cunningham, M. L.; Fairlamb, A. H.; Hunter, W. N. *Structure* **1999**, *7*, 81.
8. Carell, T.; Wintner, E. A.; Rebek, J. *J. Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2061.
9. Carell, T.; Wintner, E. A.; Bashir-Hashemi, A.; Rebek, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2059.
10. Faerman, C. H.; Savvides, S. N.; Strickland, C.; Breidenbach, M. A.; Ponasik, J. A.; Ganem, B.; Ripoll, D.; Krauth-Siegel, R. L.; Karplus, P. A. *Bioorg. Med. Chem.* **1996**, *4*, 1247.
11. Nowick, J. S.; Ballester, P.; Ebmeyer, F.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 8902.
12. TR assays: These were performed essentially as described before[13] by equilibrating a mixture of the assay buffer (40 mM Hepes, pH 7.5, 1 mM EDTA), 150 μM NADPH and 100 μM test compound at 26.5 °C for 15–20 min. After addition of TR (*T. cruzi* recombinant purified from *Escherichia coli*) the mixture was pre-incubated for 2 min at 26.5 °C. To initiate the reaction 100 μM trypanothione disulfide (Bachem) was added. Triplicate assays were performed. In vitro antiparasitic activity:

L. donovani (MHOM/ET/67/L82) and *T. cruzi* (MHOM/BR/00/Y): Peritoneal macrophages were harvested from female CD1 mice (Charles River Ltd., Margate, UK) by peritoneal lavage 24 h after starch (Merk Ltd., Leics., UK) induced recruitment. After two washes in medium the exudate cells were dispensed into 16-well Lab-tek™ tissue culture slides (Nunc Inc., IL, USA) at 4×10^4/well in a volume of 200 μL of RPMI-1640 medium (Sigma-Aldrich Company Ltd., Dorset, UK) and 10% heat inactivated foetal calf serum (Harlan-Sea-Lab Ltd, Crawley, UK). After 24 h, macrophages were infected at a ratio of 10:1 (4×10^5/well) with *L. donovani* amastigotes freshly isolated from hamster spleen or at a ratio of 5:1 (2×10^5/well) with *T. cruzi* trypomastigotes derived from the overlay of MDCK fibroblasts. Infected macrophages were then maintained in the presence of drug in a 3-fold dilution series, with quadruplicate cultures at each concentration, for 5 days for *L. donovani* cultures and 3 days for *T. cruzi* cultures. After these periods of drug exposure slides were methanol fixed and Giemsa stained and drug activity determined by counting the percentage of macrophages cleared of amastigotes in treated cultures in comparison to untreated cultures[14] Sodium stibogluconate ($NaSb^v$) (Glaxo-Wellcome, Dartford, UK) and nifurtimox (Bayer, UK) or benznidazole were used as the respective control drugs. *T. brucei* (S427): Compounds were tested in triplicate in a 3-fold dilution series from a top concentration of 30 μM. Parasites were diluted to 2×10^5/mL and added in equal volumes to the test compounds in 96-well, flat bottom Microtest III tissue culture plates (Becton Dickinson and Company, NJ, USA). Appropriate controls with pentamidine isethionate (Rhone-Poulonc-Rorer) as the standard were set up in parallel. Plates were maintained for 3 days at 37 °C in a 5% CO_2/air mixture. Compound activity was determined by the use of a tetrazolium salt colorimetric assay[15] on day 3.

13. Borges, A.; Cunningham, M. L.; Tovar, J.; Fairlamb, A. H. *Eur. J. Biochem.* **1995**, *228*, 745.
14. Neal, R. A.; Croft, S. L. *J. Antimicrob. Chemother.* **1984**, *14*, 312.
15. Ellis, J. A.; Fish, W. R.; Sileghem, M.; McOdimba, F. *Vet. Parasitol.* **1993**, *50*, 143.
16. Evans, A. T.; Croft, S. L. *Biochem. Pharmacol.* **1994**, *48*, 613.
17. Hammond, D. J.; Hogg, J.; Gutteridge, W. E. *Exp. Parasitol.* **1985**, *60*, 32.