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Synthesis and anti-protozoal activity of C2-substituted polyazamacrocycles

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Abstract—A focused library of C2-substituted-1,4,7,10-tetraazacyclododecanes was synthesised and the compounds were tested for their ability to kill trypanosome and malaria parasites. Several compounds showed significant in vitro activity and were selectively active against the parasites over human embryonic kidney cells used as a counter screen.

Human African trypanosomiasis, also known as sleeping sickness in the late stage of the disease, threatened to run out of control in the final years of the twentieth century when a series of epidemics swept across Africa.[1] The disease is caused by protozoan parasites, transmitted between people by tsetse fly vectors. Two drugs, suramin and pentamidine are registered to treat the disease during the haemolymphatic stage and a further two, melarsoprol and eflornithine are licensed to treat the late stage disease. However, a number of problems are associated with the administration and use of these compounds.[2] For example, all are administered by injection, adverse events are common (in the case of melarsoprol some 5% of patients taking the drug die from a reactive encephalopathy) and they are expensive. For these reasons, there is an urgent need for new drugs to treat human African trypanosomiasis.

A mode of action is known for only one of the currently registered drugs, eflornithine **1** (difluoromethylornithine, Fig. 1),[3] a suicide inhibitor of the enzyme ornithine decarboxylase that plays a key role in polyamine biosynthesis.[4] Since administration of high levels of extracellular spermidine protects trypanosomes against the toxic effect of eflornithine,[5] polyamine metabolism is considered as a validated drug target in these cells.[6] We recently reported the synthesis of carbamate-derived polyazamacrocycles **2**, which are compounds that we believed may interfere with polyamine biosynthesis in parasites.[7] While biological testing of these compounds revealed significant anti-parasitic activity against bloodstream form African trypanosomes, the structural requirements for their mode of action were unclear.

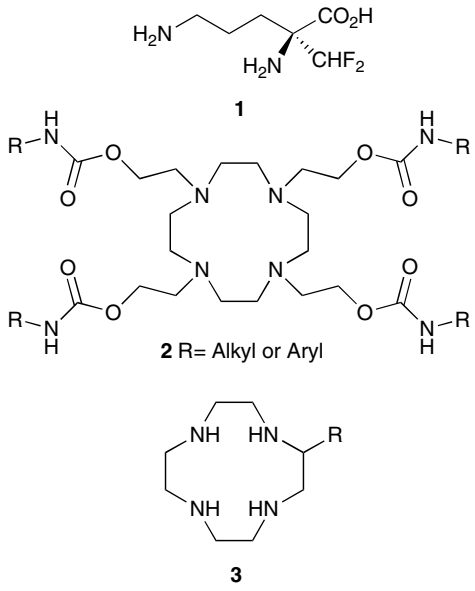

Figure 1.

Keywords: Macrocycles; Anti-protozoal; Trypanosomes; Malaria.
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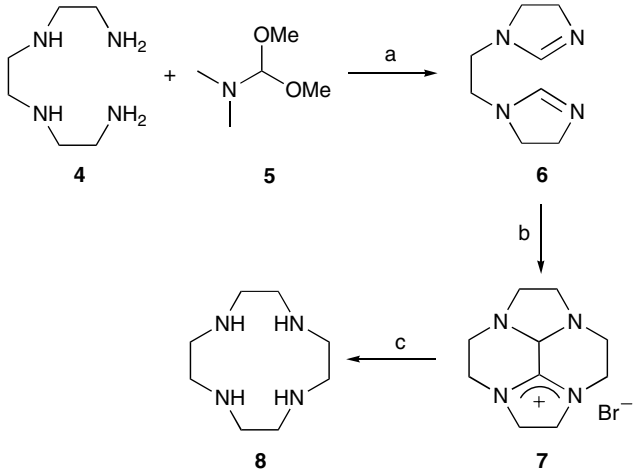
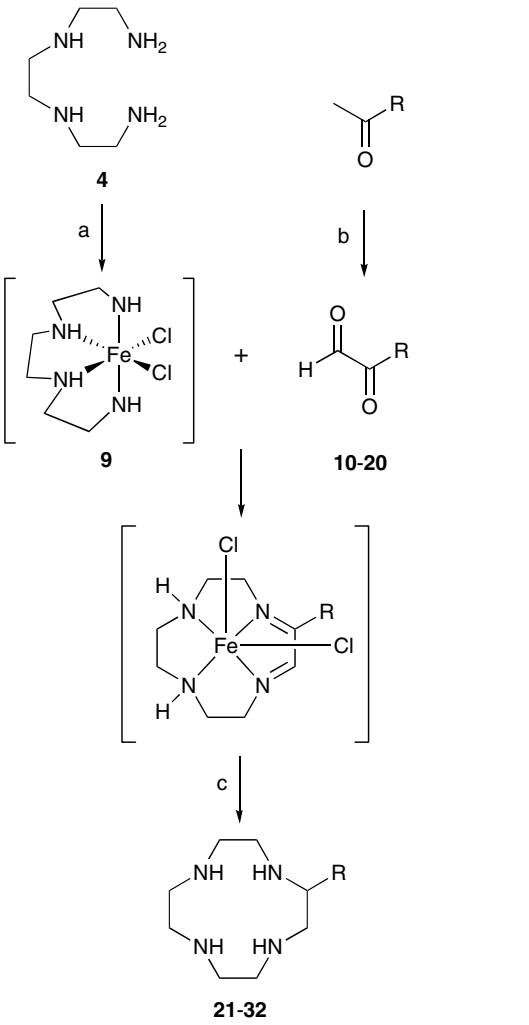
In order to probe further the anti-protozoal activity of 1,4,7,10-tetraazacyclododecanes, we now report the synthesis and biological testing of a focused library of C2-substituted polyazamacrocycles **3**, which are compounds that lack reactive carbamate side-chains but still retain significant in vivo activity against bloodstream form African trypanosomes. The toxicity of these compounds against malaria parasites from *Plasmodium falciparum* is also described.

Our first aim in this study was the synthesis of the parent, unsubstituted 1,4,7,10-tetraazacyclododecane, cyclen **8**. Many different approaches for the synthesis of cyclen have been reported due to its importance as an intermediate for the preparation of diagnostic and therapeutic pharmaceutical agents.[8–10] These methods include high dilution conditions,[11] Richman–Atkins cyclisations,[12] as well as phase transfer conditions.[13] All these processes involve the condensation of terminal halides with bis-sulfonamide sodium salts. We recently used the phase transfer approach for the synthesis of our carbamate-derived polyazamacrocycles.[7] However, for the preparation of cyclen **8** on a large scale with reproducibly high yields, a three-step approach involving bis-imidazoline **6** proved the most efficient.[14] Reaction of triethylenetetraamine **4** with *N*,*N*-dimethylformamide dimethyl acetal **5** under neat conditions gave bis-imidazoline **6** in quantitative yield (Scheme 1). Macrocyclisation of **6** with 1,2-dibromoethane in the presence of potassium carbonate gave the imidazolinium compound **7** in quantitative yield. Hydrolysis of **7** using potassium hydroxide gave cyclen **8** in 71% overall yield in only 3 steps.

Having synthesised the parent 1,4,7,10-tetraazacyclododecane, a series of C2-substituted analogues were then prepared using a metal-templated approach involving condensation of *cis*-iron dichloride complex **9** with a series of glyoxals **10**–**20**, followed by the reductive removal of the metal template (Scheme 2).[15] Glyoxals **10**–**20** were easily prepared in one step by selenium dioxide oxidation of the corresponding methyl ketone.[16] This gave glyoxals **10**–**20** in generally high yield and the majority



Scheme 1. Reagents and conditions: (a) neat, Δ, 100%; (b) 1,2-dibromoethane, K_2CO_3, MeCN, Δ, 100%; (c) KOH, H_2O, Δ, 71%.



Scheme 2. Reagents and conditions: (a) $FeCl_3$, MeOH; (b) SeO_2, 1,4-dioxane, H_2O, R = ^{t}Bu (**10**), 100%; 4-NO_2–Ph (**11**), 80%; 4-MeO–Ph (**12**), 100%; 4-Cl–Ph (**13**), 100%; 4-Br–Ph (**14**), 100%; 4-CF_3–Ph (**15**), 100%; thiophen-2-yl (**16**), 100%; naphthalen-2-yl (**17**), 83%; 3,5-bis(trifluoromethyl)phenyl (**18**), 100%; 2-fluorenyl (**19**), 42%; phenanthren-2-yl (**20**), 99%; (c) i—$NaBH_4$, MeOH, Δ, ii—HCl then NaOH, R = ^{t}Bu (**21**), 63%; Ph (**22**), 84%; 4-NO_2–Ph (**23**), 67%; 4-MeO–Ph (**24**), 83%; 4-Cl–Ph (**25**), 78%; 4-Br–Ph (**26**), 71%; 4-CF_3–Ph (**27**), 64%; thiophen-2-yl (**28**), 79%; naphthalen-2-yl (**29**), 77%; 3,5-bis(trifluoromethyl)phenyl (**30**), 54%; 2-fluorenyl (**31**), 100%; phenanthren-2-yl (**32**), 79%.

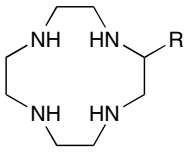
of these were stored as their hydrates prior to the condensation reaction. Attempts were made to prepare these glyoxals using solvent-free microwave conditions.[17] However, conversions were generally poor, and thus the longer solvent-based approach was preferred. The glyoxals were then treated with *cis*-iron dichloride complex **9** which was prepared using triethylenetetraamine **4** and iron(III) chloride. Reduction of the resulting diimine with sodium borohydride gave the desired C2-substituted-1,4,7,10-tetraazacyclododecanes **21**–**32** in good overall yield (Scheme 2).[18]

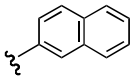
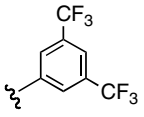
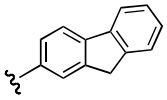
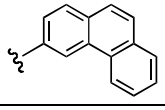
The library of C2-substituted polyazamacrocycles was tested for anti-protozoal activity against bloodstream form *Trypanosoma brucei* using a derivative of the Alamar

blue assay.[19] The compounds were also tested for their ability to kill asexual erythrocytic stages of *P. falciparum*. The successful development of useful anti-parasitic agents requires the compounds to have low toxicity to human cells, and thus, the C2-substituted polyazamacrocycles were also tested against human embryonic kidney (HEK) cells. The results of the biological testing are shown in Table 1 (also shown are two positive standards, pentamidine for *T. brucei* and chloroquine for *P. falciparum*). Several of these compounds including cyclen **8** and aromatic analogues **22**–**24** show little or no toxicity towards either trypanosome or malaria parasites tested. However, a number of other aromatic derivatives (**25**, **27** and **29**–**32**) show significant anti-protozoal activity, with less activity against the mammalian HEK cells. In particular, the *p*-Cl-phenyl **25** and naphthalene **29** are the most selective for the parasites and thus, these compounds provide some scope for the future development of more potent and selective analogues. The subtle effect of the structural activity of these compounds should also be noted. For example, changing the *para*-substituent on the phenyl group (e.g., **23** or **24** vs **25**) has a dramatic effect on the ability of the compound to kill trypanosomes. The exact mode of action of these compounds is still unknown and thus, we are unable to explain these subtle structural effects. Future work will focus on the preparation of structural analogues to probe the mechanism of cytotoxicity, including a targeted analysis of metabolites of the polyamine pathway in these cells.

In summary, we have designed and synthesised a small library of C2-substituted-1,4,7,10-tetraazacyclododecanes with the aim of interfering with polyamine biosynthesis in parasites. On testing, a number of compounds showed significant, selective toxicity to both trypanosome and malaria parasites. This second generation of 1,4,7,10-tetraazacyclododecanes contains more stable compounds than in our first generation (carbamate derived analogues) but the new compounds still retain significant activity against these parasites. These C-2 substituted derivatives should have greater bioavailability judging from their estimated log*P* values (Table 1), and thus they possess greater potential in leading to a useful drug to treat parasitic infection. Work is currently underway on the development of more potent and selective analogues of C2-substituted-1,4,7,10-tetraazacyclododecanes including the preparation of derivatives tagged with moieties that are recognised by the P2 aminopurine and other transporters.[20]

Table 1. Anti-protozoal activity of cyclic polyamines **8**, **21**–**32**



Compound	R	Estimated log*P*	*T. brucei* EC_{50} (μM)	*P. falciparum* EC_{50} (μM)	HEK EC_{50} (μM)
Pentamidine	—	—	0.006	—	—
Chloroquine	—	—	—	0.008	—
8	H	−1.8	>200	>100	5.0
21	^{t}Bu	−0.1	133.0	>100	>200
22	Ph	−0.2	>200	26.0	>200
23	4-NO_2–Ph	−0.4	>200	13.0	>200
24	4-MeO–Ph	−0.1	>200	26.0	>200
25	4-Cl–Ph	0.8	2.8	2.3	181.2
26	4-Br–Ph	0.7	21.8	5.8	>200
27	4-CF_3–Ph	0.8	6.8	5.0	73
28		−0.4	72.3	46.7	>200
29		1.0	6.9	1.7	>200
30		1.7	6.8	4.8	61.0
31		1.8	2.6	1.3	26.0
32		2.2	1.3	<1.5	24.9

Acknowledgments

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Supplementary data

Experimental procedures and spectroscopic data for all compounds synthesised. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.02.037.

References and notes

1. Barrett, M. P.; Burchmore, R. J.; Stich, A.; Lazzari, J. O.; Frasch, A. C.; Cazzulo, J. J.; Krishna, S. *Lancet* **2003**, *362*, 1469.
2. Barrett, M. P.; Gilbert, I. H. *Adv. Parasitol.* **2006**, *63*, 125.
3. Burri, C.; Brun, R. *Parasitol. Res.* **2003**, *90*, S49.
4. Grishin, N. V.; Osterman, A. L.; Brooks, H. B.; Phillips, M. A.; Goldsmith, E. J. *Biochemistry* **1999**, *38*, 15174.
5. Li, F.; Hua, S. B.; Wang, C. C.; Gottesdiener, K. M. *Exp. Parasitol.* **1998**, *88*, 255.
6. Gerner, E. W.; Meyskens, F. L., Jr. *Nat. Rev. Cancer* **2004**, *4*, 781.
7. Wilson, J. M.; Giordani, F.; Farrugia, L. J.; Barrett, M. P.; Robins, D. J.; Sutherland, A. *Org. Biomol. Chem.* **2007**, *5*, 3651.
8. Bradshaw, J. S.; Krakoviak, K. E.; Izatt, R. M. *Aza-Crown Macrocycles: The Chemistry of Heterocyclic Compounds*; Wiley and Sons: New York, 1993.
9. Kumar, K.; Tweedle, M. *Pure Appl. Chem.* **1993**, *65*, 51.
10. Caravan, P.; Ellison, J. J.; McMurray, T. J.; Lauffer, R. B. *Chem. Rev.* **1999**, *99*, 2293.
11. Stetter, H.; Roos, E. E. *Chem. Ber.* **1954**, *87*, 566; Stetter, H.; Roos, E. E. *Chem. Ber.* **1955**, *88*, 1390.
12. Richman, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268.
13. Lukyanenko, N. G.; Basok, S. S.; Filonova, L. K. *J. Chem. Soc., Perkin Trans. 1* **1988**, 3141.
14. Athey, P. S.; Kiefer, G. E. *J. Org. Chem.* **2002**, *67*, 4081.
15. Edlin, C. D.; Faulkner, S.; Parker, D.; Wilkinson, M. P. *Chem. Commun.* **1996**, 1249.
16. Marchand, N. J.; Grée, D. M.; Martelli, J. T.; Grée, R. L. *J. Org. Chem.* **1996**, *61*, 6063.
17. Paul, S.; Gupta, M.; Gupta, R.; Loupy, A. *Synthesis* **2002**, 75.
18. The synthesis of C-2 substituted 1,4,7,10-tetraazacyclododecanes with nitrogen-containing side-chains was also attempted. However, selenium dioxide mediated oxidation of methyl ketones with nitrogen-containing side-chains (e.g., 4-aminophenyl, 2-pyridyl, 3-pyridyl and 2-pyrrolyl), returned only complex mixtures of compounds, thus preventing access to these compounds using this approach.
19. Raz, B.; Iten, M.; Grether-Buhler, Y.; Kaminsky, R.; Brun, R. *Acta Trop.* **1997**, *68*, 139.
20. Carter, N. S.; Fairlamb, A. H. *Nature* **1993**, *361*, 173; Carter, N. S.; Berger, B. J.; Fairlamb, A. H. *J. Biol. Chem.* **1995**, *270*, 28153.