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# Synthesis of novel benzamidine- and guanidine-derived polyazamacrocycles: Selective anti-protozoal activity for human African trypanosomiasis

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ABSTRACT

Efficient synthetic routes have been developed for the preparation of two new polyazamacrocycles tagged with structural motifs recognised by the *Trypanosoma brucei* P2 aminopurine transporter. Biological testing of these compounds showed highly selective anti-protozoal activity against trypanosomes.



Human African trypanosomiasis (HAT), caused by subspecies of the parasitic protozoan *Trypanosoma brucei*, is endemic in sub-Saharan Africa affecting around 300,000 individuals.[1] A number of drugs including pentamidine, suramin, melarsoprol and eflornithine have been developed to treat specific stages of the disease progression.[1,2] However, these drugs suffer several drawbacks such as host-toxicity, expense and a need for administration by injection and thus, new drugs are urgently required.[3] Novel approaches for the development of new drugs for HAT have investigated compounds which target trypanosome specific plasma membrane transporters.[4] The *T. brucei* P2 aminopurine transporter promotes the uptake of adenosine and the purine base adenine into cells.[5] The P2 transporter also recognises and binds compounds containing benzamidine and melamine motifs, and is responsible for the uptake of the anti-trypanosomal drugs, pentamidine **1** and melarsoprol **2** (Fig. 1).[6] Thus, an effective approach for the development of new selective trypanocidal compounds has involved the targeting of the P2 transporter by attaching P2 recognition motifs to cytotoxic agents.[7]

We recently reported the synthesis of two new classes of polyazamacrocycles, carbamate-derived and C2-substituted 1,4,7,10-tetraazacyclododecanes that were designed to interfere with polyamine biosynthesis in parasites.[8,9] While the most potent of these compounds (e.g. **3** and **4**) showed significant anti-protozoal activity against trypanosome and malaria parasites, they were also relatively toxic to human embryonic kidney cells (Fig. 2). With the aim of developing compounds with more selective toxicity for trypanosomes, we now report the preparation of two new C2-substituted polyazamacrocycles bearing P2 recognition motifs, benzamidine **5** and guanidine **6** (Fig. 2). We also show that, while these compounds are toxic to trypanosomes, they have no activity against malaria or HEK cells.

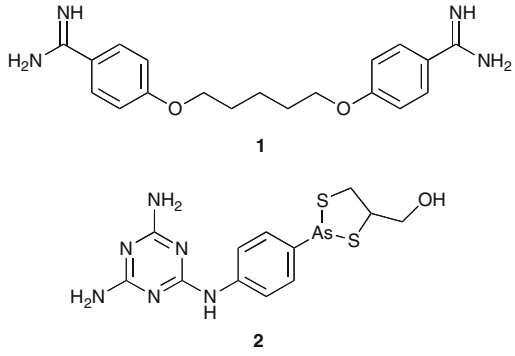


**Figure 1.** Pentamidine and Melarsoprol.

In designing a synthetic route for the preparation of **5** and **6**, it was proposed that both compounds could be prepared from a common intermediate, benzonitrile **11**. This was prepared via a metal-templated approach as shown in scheme 1.[9,10] Thus, *cis*-iron dichloride complex **8** was prepared by reaction of triethylenetetraamine **7** with iron trichloride. In situ condensation of complex **8**

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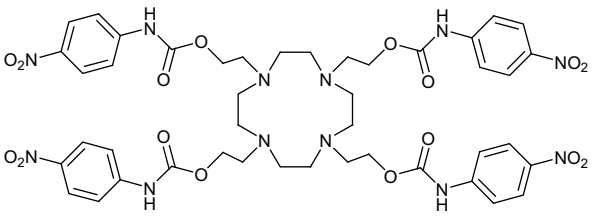
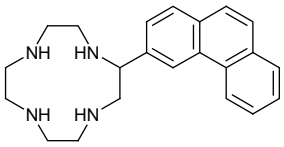


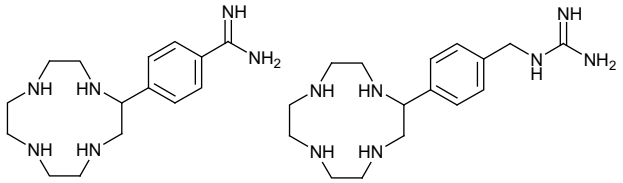
**3**

*T. brucei* $EC_{50}$= 0.5 μM
HEK $EC_{50}$= 1.3 μM



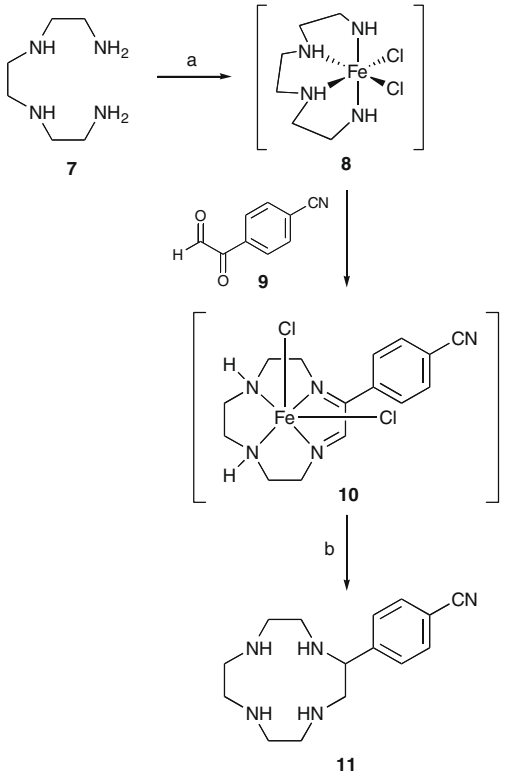
**4**

*T. brucei* $EC_{50}$= 1.3 μM
*P. falciparum* $EC_{50}$= <1.5 μM
HEK $EC_{50}$= 24.9 μM



**5** **6**

**Figure 2.**



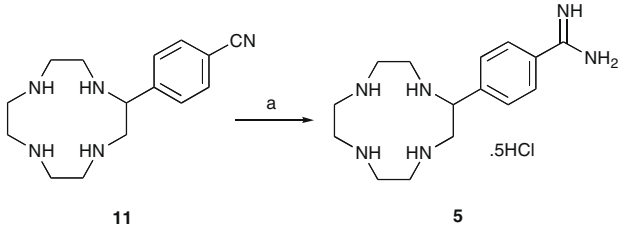
**Scheme 1.** Reagents and conditions: (a) $FeCl_3$, MeOH; (b) i—$NaBH_4$, MeOH, Δ, ii—HCl then NaOH, 72% overall yield.

with 4-oxoacetyl-benzonitrile **9**[11] gave the corresponding diimine **10** and reductive removal of the metal template using sodium borohydride gave benzonitrile **11** in 72% overall yield.

An initial attempt at the synthesis of benzamidine **5** involved a Pinner-type reaction of benzonitrile **11**.[12] While this approach was successful in generating benzamidines with model systems, only starting material was recovered from the reaction with **11**. Amidine-containing compounds have also been prepared from nitriles by reaction with lithium hexamethyldisilazide followed by treatment with hydrogen chloride.[13] This approach proved successful producing benzamidine **5** in an excellent 95% yield (Scheme 2).

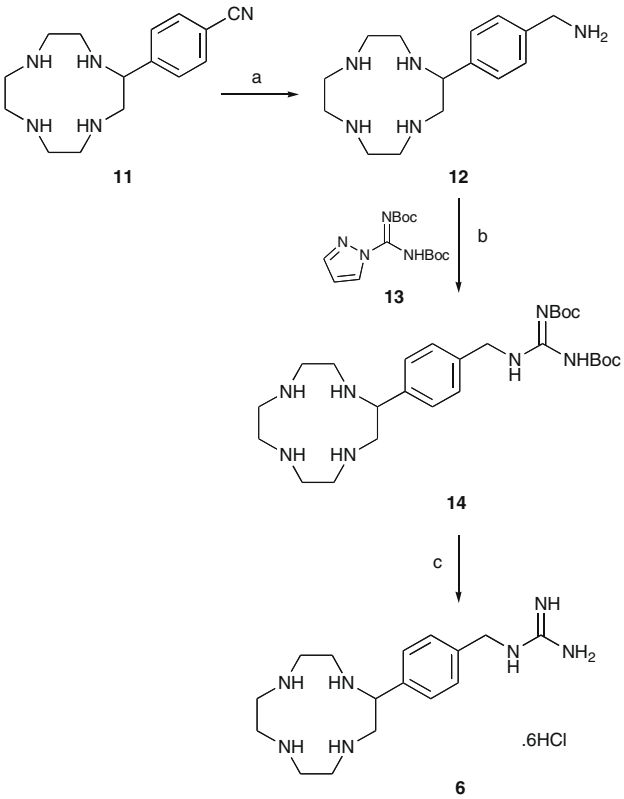
Guanidine analogue **6** was prepared in three steps as shown in scheme 3. Benzonitrile **11** was reduced using borane under mild conditions to give the corresponding amine **12** in 64% yield. Amine **12** was coupled with commercially available *N*,*N*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine **13** in the presence of Hünig's base, which gave the Boc-protected guanidine analogue **14** in quantitative yield.[14] Removal of the Boc-protecting groups was then carried out by treatment of **14** with 6 M hydrochloric acid and this gave guanidine **6** in 99% yield.

The benzamidine and guanidine analogues **5** and **6** were tested for anti-protozoal activity against bloodstream form *T. brucei* using a derivative of the Alamar blue assay (Table 1).[15] To investigate the selectivity of these compounds for trypanosomes, the compounds



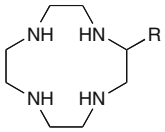
**11** **5**

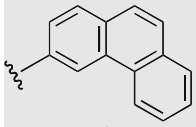
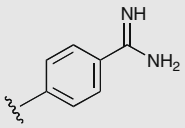
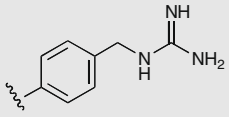
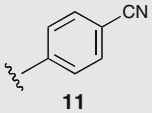
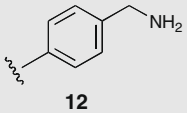
**Scheme 2.** Reagents and conditions: (a) LiHMDS, THF, then HCl in $Et_2O$, 95%.



**Scheme 3.** Reagents and conditions: (a) $BH_3$, THF, 64%; (b) $EtN(iPr)_2$, MeOH, 100%; (c) 6M HCl, 99%.

**Table 1**
Anti-protozoal activity of polyazamacrocycles **4**, **5**, **6**, **11** and **12**




| R | Estimated log*P* | *T. brucei* $EC_{50}$ (μM) | *P. falciparum* $EC_{50}$ (μM) | HEK $EC_{50}$ (μM) |
|---|---|---|---|---|
|  **4**[9] | 2.2 | 1.3 | <1.5 | 24.9 |
|  **5** | −1.44 | 22.0 | >100 | >200 |
|  **6** | −1.64 | 41.2 | >100 | >200 |
|  **11** | −0.65 | >200 | >100 | >200 |
|  **12** | −1.12 | >200 | >100 | >200 |

were also tested for their ability to kill asexual erythrocytic stages of *Plasmodium falciparum* and human embryonic kidney (HEK) cells.

For comparison, the structurally similar polyazamacrocycles, **11** and **12** prepared during the synthesis of the target compounds, **5** and **6** were also tested. From our previous study on the anti-protozoal activity of C2-substituted polyazamacrocycles bearing alkyl or aromatic substituents, a correlation between lipophilicity and anti-protozoal activity was noted.[9] For example, polyazamacrocycles with log P values between 0.5 and 2.5 showed significant activity (e.g. **4**, Table 1) while compounds with log*P* values below 0.5 were relatively inactive. In this current study, similar results were obtained for benzonitrile **11** and benzylamine **12** which have no activity against any of the organisms, confirming that the lipophilicity of these compounds is also out with the optimal range for passive diffusion into cells.[16] However, contrasting results were observed for benzamidine **5** and guanidine **6**. While these compounds still showed no activity against *P. falciparum* and HEK cells, anti-protozoal activity was observed against trypanosomes. Thus, despite possessing low log*P* values, these compounds still accumulate within the trypanosomes resulting in anti-protozoal activity. These results show that benzamidine **5** and guanidine **6** are transported into trypanosomes via an alternative mechanism compared to the other C2-substituted polyazamacrocycles and the presence of the amidine moieties within these compounds strongly suggest transport via the P2 aminopurine transporter.[7d] It should be noted that while the trypanocidal activity of **5** and **6** is only moderate, this approach of tagging C2-substituted polyazamacrocycles with motifs recognised by a specific trypanosome receptor has produced compounds with selective anti-protozoal activity and with low toxicity to human cells (cf. **4**, Table 1). Future variants could be designed that have more potency against trypanosomes, and these new compounds could also be tagged with delivery moieties that could further enhance their selectivity.

In summary, we have developed efficient synthetic routes for the preparation of two novel C2-substituted polyazamacrocycles tagged with amidine motifs recognised by the *T. brucei* P2 aminopurine transporter. Biological testing of these compounds showed that despite their low log*P* values, anti-protozoal activity was still observed against *T. brucei*. The lack of any anti-protozoal activity against *P. falciparum* or HEK indicates that the compounds are selectively accumulated by trypanosomes. Work is currently underway to extend this approach with more potent analogues and also to probe the mechanism of cytotoxicity of these C2-substituted 1,4,7,10-tetraazacyclododecanes.

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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.2008.09.047.

## 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. Pepin, J.; Milord, F. *Adv. Parasitol.* **1994**, *33*, 1.
3. Barrett, M. P.; Gilbert, I. H. *Adv. Parasitol.* **2006**, *63*, 125.
4. Hasne, M.-P.; Barrett, M. P. *J. Appl. Microbiol.* **2000**, *89*, 697.
5. Carter, N. S.; Fairlamb, A. H. *Nature* **1993**, *361*, 173.
6. (a) Barrett, M. P.; Zhang, Z. Q.; Denise, H.; Giroud, C.; Baltz, T. *Mol. Biochem. Parasitol.* **1995**, *73*, 223; (b) Carter, N. S.; Berger, B. J.; Fairlamb, A. H. *J. Biol. Chem.* **1995**, *270*, 28153; (c) de Koning, H. P.; Anderson, L. F.; Stewart, M.; Burchmore, R. J. S.; Wallace, L. J. M.; Barrett, M. P. *Antimicrob. Agents Chemother.* **2004**, *48*, 1515.
7. (a) Klenke, B.; Stewart, M.; Brun, R.; Barrett, M. P.; Gilbert, I. H. *J. Med. Chem.* **2001**, *44*, 3440; (b) Barrett, M. P.; Gilbert, I. H. *Curr. Top. Med. Chem.* **2002**, *2*, 471; (c) Matovu, E.; Stewart, M. L.; Geiser, F.; Brun, R.; Mäser, P.; Wallace, L. M. P.; Burchmore, R. J. S.; Enyaru, K. K. C.; Barrett, M. P.; Kaminsky, R.; Seebeck, T.; de Koning, H. P. *Eukaryot. Cell* **2003**, *2*, 1003; (d) Stewart, M. L.; Bueno, G. J.; Baliani, A.; Klenke, B.; Brun, R.; Brock, J. M.; Gilbert, I. H.; Barrett, M. P. *Antimicrob. Agents Chemother.* **2003**, *48*, 1733.
8. Wilson, J. M.; Giordani, F.; Farrugia, L. J.; Barrett, M. P.; Robins, D. J.; Sutherland, A. *Org. Biomol. Chem* **2007**, *5*, 3651.
9. Reid, C. M.; Ebikeme, C.; Barrett, M. P.; Patzewitz, E.-M.; Müller, S.; Robins, D. J.; Sutherland, A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2455.
10. Edlin, C. D.; Faulkner, S.; Parker, D.; Wilkinson, M. P. *Chem. Commun.* **1996**, 1249.
11. 4-Oxoacetyl-benzonitrile **9** was prepared in one-step by selenium dioxide oxidation of 4-acetyl benzonitrile using a procedure described by Grée and co-workers Marchand, N. J.; Grée, D. M.; Martelli, J. T.; Grée, R. L.; Toupet, L. J. *J. Org. Chem.* **1996**, *61*, 5063. See supplementary data for full details.
12. (a) Pinner, A. *Ber.* **1884**, *17*, 178; (b) Roger, R.; Neilson, D. G. *Chem. Rev.* **1961**, *61*, 179; (c) Stefanic, P.; Simoncic, Z.; Breznik, M.; Plavec, J.; Anderluh, M.; Addicks, E.; Giannis, A.; Kikelj, D. *Org. Biomol. Chem.* **2004**, *2*, 1511.
13. (a) Bacchi, C. J.; Weiss, L. M.; Lane, S.; Frydman, B.; Valasinas, A.; Reddy, V.; Sun, J. S.; Marton, L. J.; Khan, I. A.; Moretto, M.; Yarlett, N.; Wittner, M. *Antimicrob. Agents Chemother.* **2002**, *46*, 55; (b) Boussard, C.; Klimkait, T.; Mahmood, N.; Pritchard, M.; Gilbert, I. H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2673.
14. (a) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *Tetrahedron Lett.* **1993**, *34*, 3389; (b) Hamilton, D. J.; Sutherland, A. *Tetrahedron Lett.* **2004**, *45*, 5739; (c) Bischoff, R.; McDonald, N.; Sutherland, A. *Tetrahedron Lett.* **2005**, *46*, 7147.
15. Raz, B.; Iten, M.; Grether-Buhler, Y.; Kaminsky, R.; Brun, R. *Acta Trop.* **1997**, *68*, 139.
16. The estimated logP value for each compound was calculated using ChemDraw Ultra 11.0 (CambridgeSoft®).