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Bioorganic & Medicinal Chemistry Letters 16 (2006) 3229–3232
Bioorganic & Medicinal Chemistry Letters

Novel alkylpolyaminoguanidines and alkylpolyaminobiguanides with potent antitrypanosomal activity

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Received 3 February 2006; revised 13 March 2006; accepted 14 March 2006
Available online 17 April 2006

Abstract—A series of polyaminoguanidines and polyaminobiguanides were synthesized and evaluated as potential antitrypanosomal agents. These analogues inhibit trypanothione reductase (TR) with IC_{50} values as low as 0.95 μM, but do not inhibit the closely related human enzyme glutathione reductase (GR). The most effective analogues, **7a**, **7b** and **8d**, inhibited parasitic growth in vitro with IC_{50} values of 0.18, 0.09 and 0.18 μM, respectively. These agents represent a promising new class of potential antitrypanosomal agents.
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Human African trypanosomiasis (HAT), caused by the protozoan parasites *Trypanosoma brucei gambiense* (West African trypanosomiasis) and *Trypanosoma brucei rhodesiense* (East African trypanosomiasis),[1] is a daily threat to more than 60 million people in 36 countries of sub-Saharan Africa. Between 300,000 and 500,000 are thought to have the disease, with more than 50,000 deaths occurring in 2002.[2] Early stage disease is usually treated effectively with suramin or pentamidine, but field diagnosis is difficult in rural areas, and many patients progress to late-stage disease before seeking treatment. There are only two effective treatments for late-stage HAT, as shown in Figure 1. The ornithine decarboxylase inhibitor eflornithine, **1**, has been shown to be curative in end-stage infections caused by *T. b. gambiense*, but ineffective against *T. b. rhodesiense*.[3–6] Eflornithine is expensive to produce, and thus its availability in impoverished nations is limited. End-stage HAT is most often treated with melarsoprol, **2**, which is converted to the active metabolite melarsen oxide, **3**.[7–9] Serious side effects of melarsoprol include a 10% incidence of encephalopathy which is fatal in 3–5% of patients. The situation is further complicated by the emergence of arsenic-resistant strains of *T. b. gambiense* and *T. b. rhodesiense*.

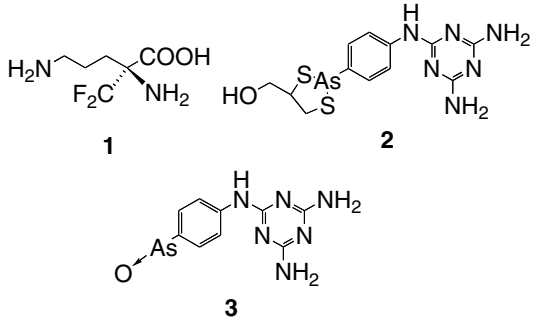

Figure 1. The structures of eflornithine (**1**), melarsoprol (**2**) and melarsen oxide (**3**).

Trypanosomes possess a number of parasite-specific targets for drug design.[9] We, and others, have focused on the discovery of agents that can interrupt polyamine metabolism in trypanosomes. *T. brucei* produces the polyamines putrescine and spermidine from ornithine in a manner analogous to the human pathway, but does not produce the tetraamine spermine. Instead, these parasites convert spermidine and two molecules of host-derived glutathione into trypanothione (**4**, Fig. 2), which is used to protect the organism against oxidative stress.[10,11] The formation of **4** depends on two ATP-de-

Keywords: Polyamine; Trypanothione; Trypanothione reductase; Trypanosome; Trypanosomiasis; Inhibitor; Antiparasitic; Guanidine; Biguanide.
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doi:10.1016/j.bmcl.2006.03.048

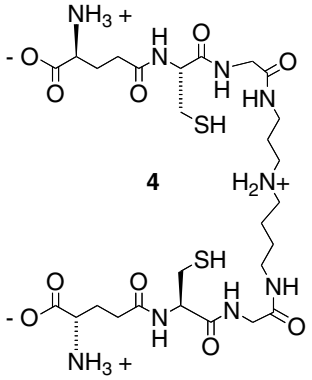


Figure 2. The structure of trypanothione, **4**.

pendent enzymes, glutathionylspermidine synthetase (GSPS) and trypanothione synthetase (TS).[12,13] In the presence of oxidative species, trypanothione is oxidized to the disulfide, and must be recycled to the reduced form **4** by the enzyme trypanothione reductase (TR). The arsenical drug melarsoprol acts by forming a covalent complex with trypanothione, thus inactivating it and exposing the organism to oxidative damage.

The crystal structure of TR from *Trypanosoma cruzi* has been solved,[14] but relatively few inhibitors of this enzyme have been described.[15–22] We previously reported a series of alkylpolyamine analogues that exhibit potent antitrypanosomal activity in vitro.[23–25] These analogues disrupt polyamine metabolism through changes in pK_a values that alter their degree of protonation at physiological pH.[26] They enter proliferating cells using the polyamine transport system and downregulate polyamine biosynthesis, but do not substitute for the natural polyamines in terms of cell growth and survival functions,[25] leading to polyamine depletion and cell death. We identified a structurally distinct subset of polyamine analogues with a 3-7-3 carbon skeleton that produce potent and selective antitrypanosomal effects, and have shown that these analogues are potent inhibitors of TR (unpublished results). We reasoned that polyamine analogues containing substituted terminal guanidines or biguanides would produce similar cellular effects, since these moieties would produce even greater charge perturbations when compared to the natural polyamines. Substituted guanidines and biguanides, with pK_a values in the range of 13.5[27] and 13.0,[28] respectively, are more basic than secondary amines (pK_a values near 11). In addition, the biguanide group appears in a number of important therapeutic agents,[29] including chlorhexidine and the antimalarial chlorguanide. It has recently been shown that non-polyamine amidines and guanidines possess potent antitrypanosomal activity.[30] Also, the biguanide chlorhexidine acts as an inhibitor of TR from *T. cruzi*.[22] In order to test the hypothesis described above, we designed and synthesized a limited series of substituted polyaminoguanidines and biguanides with general structures **5**–**10** (Fig. 3).

The synthetic route to alkylpolyaminoguanidines **5**, **6** and **7** is shown in Scheme 1. Amines **11a**–**d** were added to cyanogen bromide in refluxing ethanol to afford the

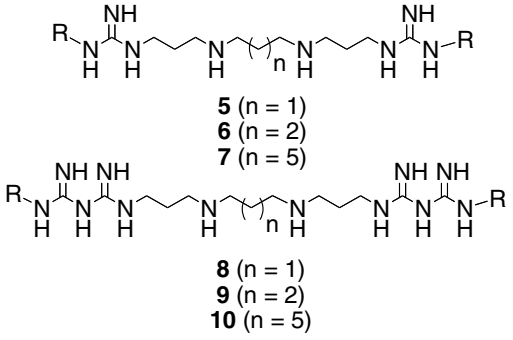
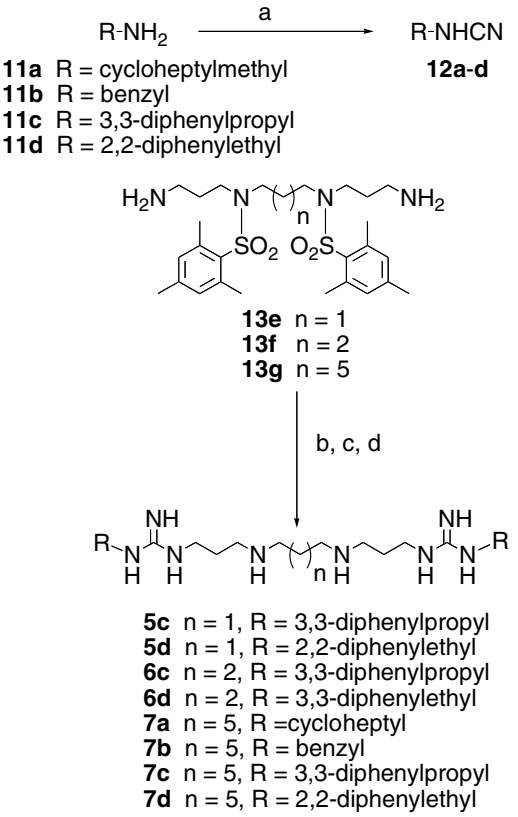
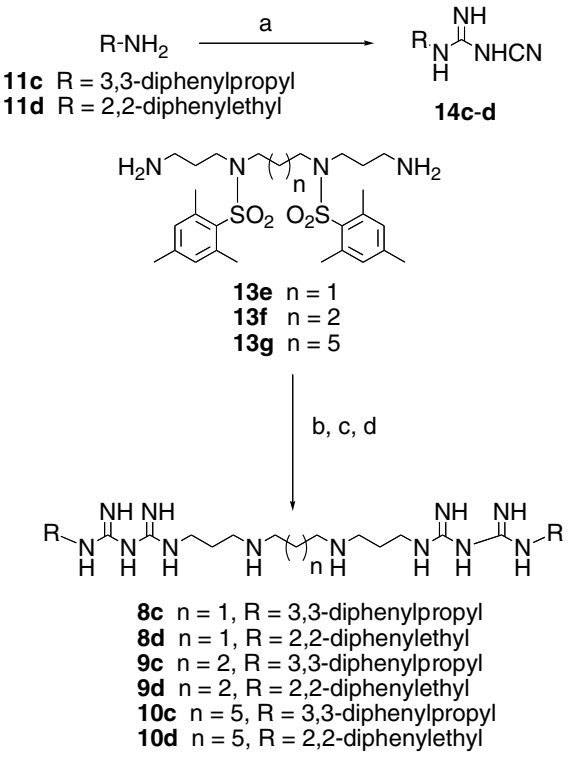


Figure 3. Structures of alkyl- and aralkylpolyaminoguanidines **5**–**7** and polyaminobiguanides **8**–**10** with potent antitrypanosomal activity in vitro.



Scheme 1. Reagents and conditions: (a) cyanogen bromide, EtOH, reflux; (b) HCl, CH_2Cl_2; (c) **12a**–**d**, chlorobenzene, reflux; (d) 30% HBr in AcOH, phenol, CH_2Cl_2.

corresponding N-cyano intermediates **12a**–**d** (85% av yield).[31] The bis-protected tetraamines **13e**–**g** were then prepared as previously described,[23,24] converted to their dihydrochloride salts (HCl, CH_2Cl_2) and appended to **12a**–**d** (chlorobenzene, reflux, 75% av yield). The mesityl-protecting groups were removed (30% HBr in AcOH, phenol, CH_2Cl_2) to afford the desired guanidines **5c**–**d**, **6c**–**d** and **7a**–**d** (89% av yield). These syntheses were also conducted using versions of **13e**, **13f** and **13g** that were N-Boc-rather than N-mesityl-protected (not shown), allowing for deprotection in the final step under milder conditions where appropriate (5% (v/v) trifluoroacetic acid in CH_2Cl_2).



Scheme 2. Reagents and conditions: (a) $NaN(CN)_2$, $BuOH/H_2O$ EtOH, reflux; (b) HCl, CH_2Cl_2; (c) **14c–d**, chlorobenzene, reflux; (d) 30% HBr in AcOH, phenol, CH_2Cl_2.

A similar strategy was employed for the synthesis of alkylpolyaminobiguanides **8**, **9** and **10**, as outlined in Scheme 2. Amines **11c** and **11d** were converted to the corresponding cyanoguanidines **14c** and **14d** ($NaN(CN)_2$, $BuOH/H_2O$, 74% av yield),[32] which were combined with **13e–g** as previously described (76% av yield) to afford the mesityl-protected target molecules. Deprotection as described above then provided the substituted biguanides **8c–d**, **9c–d** and **10c–d** (69% av yield). An analogous route (not shown) utilizing the N-Boc protection group was also employed, as above.

In order to act as parasite-specific antitrypanosomal agents, active molecules must inhibit TR without affecting the closely related human enzyme glutathione reductase (GR). It has been suggested that charge density is a major factor in the ability of an inhibitor to discriminate between TR and GR.[33] Thus, all target molecules of general structure **5–10** were evaluated as inhibitors of TR and GR, and for the ability to kill cultured bloodforms of *Trypanosoma brucei brucei* EATRO 110. TR and GR activity were measured by monitoring the disappearance in UV absorption at 340 nm resulting from the reduction of oxidized trypanothione or glutathione in the presence of NADPH.[15] Antitrypanosomal activity was measured in vitro as previously described.[23,24] The results of these studies are summarized in Table 1. These data indicate that all of the target molecules, except for **7a** and **7b**, are effective inhibitors of TR, with IC_{50} values ranging from 0.95 to 5.07 μM. None of the analogues tested produced any significant inhibition of GR at concentrations of up to 100 μM, indicating that they are highly selective for the parasitic enzyme. Each analogue was a potent growth inhibitor, with IC_{50} values against *T. b. brucei* between 0.09 and 3.35 μM. In the guanidine series, compounds with a 3-7-3 carbon backbone (**7a–d**) were the most effective agents, consistent with our earlier findings with substituted polyamines.[23–25]

Table 1. IC_{50} values for TR and GR inhibition, and for growth inhibition of *Trypanosoma brucei brucei* for compounds **5–10**

Compound	TR inhibition IC_{50} (μM)	GR inhibition IC_{50} (μM)	Inhibition of *T. b. brucei* IC_{50} (μM)
5c	5.07	>100	1.95
5d	3.60	>100	2.05
6c	2.97	>100	2.4
6d	4.56	>100	3.35
7a	17.68	>100	0.18
7b	69.47	>100	0.09
7c	2.24	>100	0.61
7d	4.72	>100	0.5
8c	3.68	>100	1.76
8d	4.16	>100	0.18
9c	2.96	>100	0.19
9d	2.74	>100	1.05
10c	0.95	>100	0.62
10d	1.66	>100	0.45

All IC_{50} values were derived from dose–response curves in which each data point was the average of three determinations with <5% variability.

Although biguanide therapeutic agents are known, only chlorhexidine has been reported to inhibit TR, and it was not evaluated as a growth inhibitor.[22] Thus, **8–10** are the first biguanides reported that possess antitrypanosomal activity. The most potent biguanides, **8d** and **9c**, had a 3-3-3 or 3-4-3 carbon backbone, respectively, although compounds **10c** and **10d** (3-7-3 backbone) were also reasonably potent. Data from additional members of the biguanide series will be required to make meaningful SAR correlations. It is interesting that TR inhibition and antitrypanosomal activity did not seem to correlate well in either series, and the weakest TR inhibitors, guanidines **7a** and **7b**, were the most potent antitrypanosomal agents. Oxidation of substituted polyamines to toxic metabolites can contribute to their activity,[34,35] but polyamine oxidase cannot be detected in *T. brucei*. It is more likely that differences in potency can be attributed to differential uptake by the P2 aminopurine transporter that is known to import pentamidine.[36,37] This transporter selectively concentrates amidines and guanidines, producing lethal effects mediated through accumulation in the mitochondrion and disruption of kinetoplast function.[37] Experiments are underway to test this hypothesis, and we are currently evaluating selected analogues in a murine model of trypanosomal infection. These results, as well as the synthesis of additional analogues, will be described in subsequent publications.

Acknowledgments

This research was supported by NIH Grant RO1CA85509. We gratefully acknowledge Prof. Alan

Fairlamb of the University of Dundee, for providing purified trypanothione reductase for this study.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2006.03.048.

References and notes

1. Woster, P. M. *Annu. Rep. Med. Chem.* **2001**, *36*, 99.
2. From the WHO Special Program for Research and Training in Tropical Diseases <http://www.who.int/tdr/>.
3. Sjoerdsma, A.; Schechter, P. J. *Clin. Pharmacol. Ther.* **1984**, *35*, 287.
4. Schechter, P. J.; Sjoerdsma, A. *Parasitol. Today* **1986**, *2*, 223.
5. Sjoerdsma, A.; Schechter, P. J. *Lancet* **1999**, *354*, 254.
6. Burri, C.; Brun, R. *Parasitol. Res.* **2003**(Suppl. 1), 49.
7. Pecoul, B.; Gastellu, M. *Lancet* **1999**, *354*, 955.
8. Pepin, J.; Milord, F.; Khonde, A.; Niyonsenga, T.; Loko, L.; Mpia, B. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88*, 447.
9. Werbovitz, K. A. *Curr. Med. Chem.* **2000**, *7*, 835.
10. Bacchi, C. J.; Yarlett, N.; Goldberg, B.; Bitonti, A. J.; McCann, P. P. In *Biochemical Protozoology*; Coombs, G. H., North, M. J., Eds.; Taylor and Francis: Washington, DC, 1991; pp 469–481.
11. Bacchi, C. J.; Yarlett, N. *Acta Trop.* **1993**, *54*, 225.
12. Kwon, D. S.; Lin, C. H.; Chen, S.; Coward, J. K.; Walsh, C. T.; Bollinger, J. M. *J. Biol. Chem.* **1997**, *272*, 2429.
13. Chen, S.; Lin, C. H.; Walsh, C. T.; Coward, J. K. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 505.
14. (a) Krauth-Siegel, R. L.; Inhoff, O. *J. Mol. Biol.* **2003**, *90*, 77; (b) Bond, C. S.; Zhang, Y.; Berriman, M.; Cunningham, M. L.; Fairlamb, A. H.; Hunter, W. N. *Structure* **1999**, *7*, 81.
15. Li, Z.; Fennie, M. W.; Ganem, B.; Hancock, M. T.; Kobaslija, M.; Rattendi, D.; Bacchi, C. J.; O'Sullivan, M. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 251.
16. Bonse, S.; Richards, J. M.; Ross, S. A.; Lowe, G.; Krauth-Siegel, R. L. *J. Med. Chem.* **2000**, *43*, 4812.
17. Hamilton, C. J.; Saravanamuthu, A.; Poupat, C.; Fairlamb, A. H.; Eggleston, I. M. *Bioorg. Med. Chem.* **2006**, *14*, 2266.
18. Lee, B.; Bauer, H.; Melchers, J.; Ruppert, T.; Rattray, L.; Yardley, V.; Davioud-Charvet, E.; Krauth-Siegel, R. L. *J. Med. Chem.* **2005**, *48*, 7400.
19. Parveen, S.; Khan, M. O.; Austin, S. E.; Croft, S. L.; Yardley, V.; Rock, P.; Douglas, K. T. *J. Med. Chem.* **2005**, *48*, 8087.
20. Dixon, M. J.; Maurer, R. I.; Biggi, C.; Oyarzabal, J.; Essex, J. W.; Bradley, M. *Bioorg. Med. Chem.* **2005**, *13*, 4513.
21. Aguirre, G.; Cabrera, E.; Cerecetto, H.; Di Maio, R.; Gonzalez, M.; Seoane, G.; Duffaut, A.; Denicola, A.; Gil, M. J.; Martinez-Merino, V. *Eur. J. Med. Chem.* **2004**, *39*, 421.
22. Meiering, S.; Inhoff, O.; Mies, J.; Vincek, A.; Garcia, G.; Kramer, B.; Dormeyer, M.; Krauth-Siegel, R. L. *J. Med. Chem.* **2005**, *48*, 4793.
23. Bellevue, F. H.; Boahbedason, M. L.; Wu, R. H.; Casero, R. A., Jr.; Rattendi, D.; Lane, S.; Bacchi, C. J.; Woster, P. M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2765.
24. Zou, Y.; Wu, Z.; Sirisoma, N.; Woster, P. M.; Casero, R. A.; Weiss, L. M.; Rattendi, D.; Lane, S.; Bacchi, C. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1613.
25. Casero, R. A., Jr.; Woster, P. M. *J. Med. Chem.* **2001**, *44*, 1.
26. Bergeron, R. J.; McManis, J. S.; Weimar, W. R.; Schreier, K.; Gao, F.; Wu, Q.; Ortiz-Ocasio, J.; Luchetta, G. R.; Porter, C.; Vinson, J. R. T. *J. Med. Chem.* **1995**, *38*, 2278.
27. Angyal, S. J.; Warburton, W. K. *J. Chem. Soc.* **1951**, 2492.
28. Kaljurand, I.; Rodima, T.; Leito, I.; Koppel, I. A.; Schwesinger, R. *J. Org. Chem.* **2000**, *65*, 6202; Goizman, M. S.; Sarkisyan, S. O.; Sarkisyan, A. A.; Persianova, I. V. *Khim. Farm. Zh.* **1985**, *19*, 884.
29. Bharatam, P. V.; Patel, D. S.; Iqbal, P. *J. Med. Chem.* **2005**, *48*, 7615.
30. Arafa, R. K.; Brun, R.; Wenzler, T.; Tanious, F. A.; Wilson, W. D.; Stephens, C. E.; Boykin, D. W. *J. Med. Chem.* **2005**, *48*, 5480.
31. Goldin, S. M.; Fischer, J. B.; Knapp, A. G.; Reddy, N. L.; Berlove, D.; Durant, G. J.; Katragadda, S.; Hu, L. Y.; Magar, S.; Fan, W. H.; Yost, E.; Guo, J. Q. U.S. Patent 6288123, 2001; *Chem. Abstr.* **2001**, 668349.;
32. Gerhard, R.; Heinz, B.; Herbert, F. *J. Prakt. Chem. (Leipzig)* **1964**, *26*, 414.
33. Faerman, C. H.; Savvides, S. N.; Strickland, C.; Breidenbach, M. A.; Ponasik, J. A.; Ganem, B.; Ripoll, D.; Krauth-Siegel, R. L. *Bioorg. Med. Chem.* **1996**, *4*, 1247.
34. Bitonti, A. J.; Dumont, J. A.; Bush, T. L.; Stemerick, D. M.; Edwards, M. L.; McCann, P. P. *J. Biol. Chem.* **1990**, *265*, 382.
35. Lawson, K. R.; Marek, S.; Woster, P. M.; Casero, R. A.; Gerner, E. W. *Clin. Cancer Res.* **2002**, *5*, 1241.
36. Carter, N. S.; Berger, B. J.; Fairlamb, A. H. *J. Biol. Chem.* **1995**, *270*, 28153.
37. Bray, P. G.; Barrett, M. P.; Ward, S. A.; deKoning, H. P. *Trends Parasitol.* **2003**, *19*, 232.