Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 22, pp. 2765-2770, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00510-0

STRUCTURAL COMPARISON OF ALKYLPOLYAMINE ANALOGUES WITH POTENT IN VITRO ANTITUMOR OR ANTIPARASITIC ACTIVITY

Frank H. Bellevue III, Michelle Boahbedason, Ronghui Wu, and Patrick M. Woster*
Department of Pharmaceutical Sciences, Wayne State University, Detroit, MI 48202

Robert A. Casero, Jr.
The Johns Hopkins Oncology Center, 424 N. Bond St., Baltimore, MD 21231

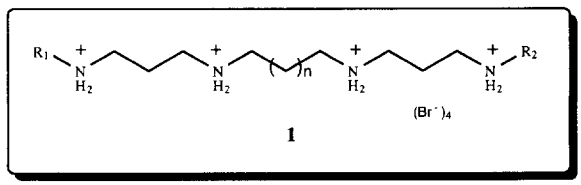
Donna Rattendi, Schennella Lane and Cyrus J. Bacchi
Haskins Laboratories, Pace University, New York, NY 10038

Abstract: A series of symmetrically or unsymmetrically alkyl-substituted polyamine analogues were synthesized containing either a 3-3-3 or 3-7-3 polyamine backbone. These analogues were evaluated in vitro as antitumor agents in the NCI H157 and NCI H82 lung carcinoma cell lines, and as antitrypanosomal agents against four strains of *Trypanosoma brucei brucei* or *Trypanosoma brucei rhodesiense*. The resulting biological data suggest that it is possible to specifically target tumor cells or parasitic organisms with alkylpolyamine analogues based on structure.

The enzyme spermidine/spermine-N^1-acetyltransferase (SSAT), the rate-limiting step in the polyamine back-conversion pathway, has been shown to be an important modulator of intracellular polyamine levels.[1] In addition, SSAT has become an important target for the design of novel antitumor agents, since a number of symmetrically substituted alkylpolyamine analogues have recently been discovered which exhibit potent antitumor effects in vitro and in vivo.[2-6] We recently described a synthetic route leading to two unsymmetrically substituted norspermine analogues, 1-(N-ethyl)amino-11-(N-propargyl)amino-4,8-diazaundecane (PENSpm, **1a**) and 1-(N-ethyl)amino-11-[N-[cyclopropyl)methyl]amino]-4,8-diazaundecane (CPENSpm, **1b**), both of which act as potent tumor cell growth inhibitors in vitro.[7] The cytotoxicity observed following treatment with these and other alkylpolyamine analogues in two cell lines, the NCI H157 non-small cell lung carcinoma and the NCI H82 small cell lung carcinoma, has been correlated to their ability to superinduce SSAT, often to levels as high as 1000 times the baseline value.[7-9] In addition to inducing SSAT, alkylpolyamines such as **1a** and **1b** appear to downregulate ornithine decarboxylase (ODC) and *S*-adenosyl-methionine decarboxylase (AdoMet-DC) in some tumor cell lines, but they do not substitute for the cellular function of the natural polyamines.[1] We have recently demonstrated that **1b** initiates programmed cell death in six breast tumor cell lines,[10] and in the H157 lung carcinoma line.[11] A combination of these cellular events ultimately lead to cytotoxicity in sensitive cell lines such as the H157 non-small cell lung carcinoma. The interesting activity of **1a** and **1b** in the H157 and H82 cell lines led us to design and synthesize a series of bis(alkyl)substituted polyamine analogues with general structure **1** (Figure 1). These analogues were then evaluated for antitumor activity against the H157 and H82 cell lines in vitro.

A series of bis(benzyl)polyamine analogues has been described by Bitonti et al.[12] which exhibit potent effects on cell growth and intracellular polyamine levels in cultured rat hepatoma (HTC) cells.[13] However, unlike alkyl-substituted polyamine analogues, these aromatic-substituted polyamines were originally developed for their marked

antimalarial activity.[12,14] These analogues, and in particular MDL 27695 (**1d**, Figure 1), appear to act by regulation of the polyamine biosynthetic pathway. The success of **1d** as an antimalarial agent prompted the evaluation of the compound for activity against *Leishmania donovani*. The analogue shows excellent activity against both antimony-susceptible and antimony-resistant strains of the organism in vitro and in vivo.[15,16] Thus, **1d** has emerged as a promising lead in the search for novel antiparasitic agents. As part of an ongoing program aimed at the discovery of novel antitrypanosomal agents, **1d** and the related analogues in Figure 1 were evaluated for their ability to inhibit growth in vitro against one strain of *Trypanosoma brucei brucei* (LAB EATRO 110) and three strains of *Trypanosoma brucei rhodesiense* (KETRI 243, KETRI 243 As-10-3 and KETRI 269). We now report the synthetic routes leading to alkylpolyamines of general structure **1**, as well as the results of our preliminary biological studies.



1

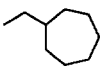
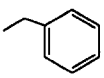
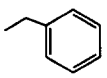
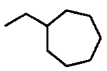
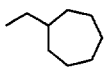
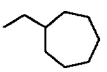
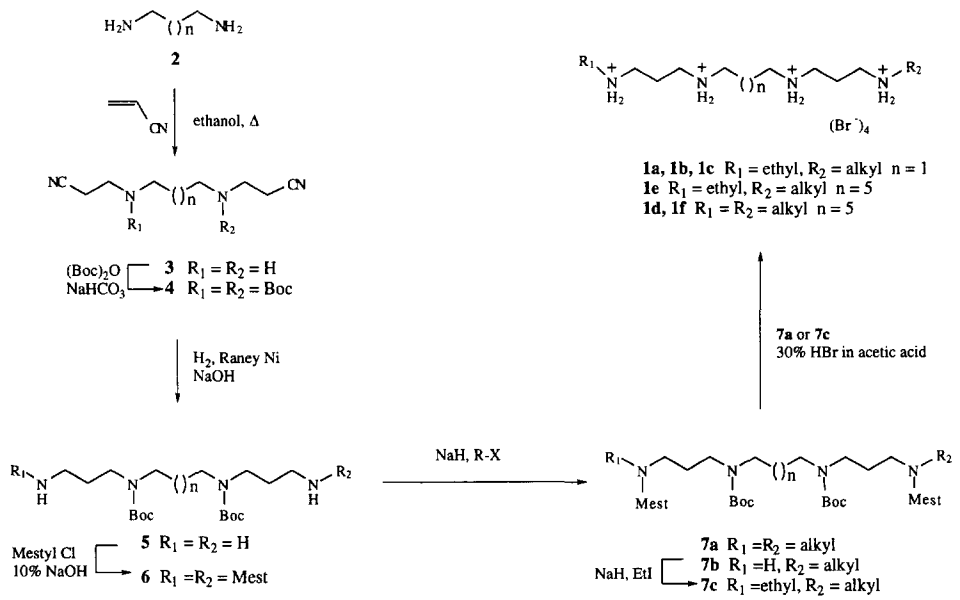
n	R_1	R_2		
1	CH_3CH_2-		**1a**	PENSpm
1	CH_3CH_2-		**1b**	CPENSpm
1	CH_3CH_2-		**1c**	CHENSpm
5			**1d**	bis-benzyl-3-7-3 (MDL 27695)
5	CH_3CH_2-		**1e**	CHE-3-7-3
5			**1f**	bis-CH-3-7-3

Figure 1. Bis(alkyl)-substituted polyamine analogues.

The synthesis of the unsymmetrical, bis(alkyl)substituted polyamines **1a-1c** was accomplished using a pathway that we previously described.[7] However, a more facile route was used to access the symmetrically substituted analogues **1d** and **1f**, which was ultimately adapted to afford the unsymmetrically substituted analogue **1e**. This synthetic route, which is now used routinely in our laboratories to synthesize both symmetrically and unsymmetrically alkyl-substituted polyamines, is shown in Scheme 1. Thus, the appropriate diamine **2** was bis-

cyanoethylated (acrylonitrile in refluxing ethanol)[14] to afford the corresponding bis-nitrile **3**. Compound **3** was then N-Boc protected[17] to form intermediate **4**, which was subjected to Raney nickel reduction (H_2, Raney nickel, NaOH, 50 psi)[18] to produce the free amine **5**. Subsequent dimesitylation of this diamine[6,7,19] resulted in the corresponding fully protected tetramine **6**.

Scheme 1



Alkylations of intermediate **6** were conducted in DMF in the presence of 2.2 equivalents of sodium hydride. Manipulation of the number of equivalents of alkyl halide or alkyl tosylate used in the alkylation allowed for the formation of the dialkylated product **7a** and the monoalkylated product **7b** in varying ratios; a 2.2-fold excess of alkyl halide resulted in the almost exclusive formation of **7a**, while a 1.5-fold excess of alkyl halide resulted in an approximately 50:50 mixture of **7a** and **7b**. Compounds **7a** and **7b** could be readily separated by silica gel chromatography, and ethylation of **7b** then afforded the unsymmetrically alkylated precursor **7c**. Simultaneous deprotection of the N-Boc and N-mesityl protecting groups[20] (30% HBr in AcOH, RT, 24 h) then afforded the corresponding symmetrically or unsymmetrically alkyl-substituted polyamines of general structure **1**. Although none are described in this manuscript, deprotection of **7b** provides a route to monosubstituted polyamines.

Cultured Cell Experiments. The NCI H157 (LCLC) and NCI H82 (SCLC) cell lines were maintained in culture as previously described.[21] Each cell line was re-fed with fresh medium (RPMI 1640 with 9% fetal calf serum, 100 units/mL of penicillin, 100 units/mL of streptomycin) every 3 days to maintain log phase growth. In experiments where the natural polyamines were added to cultures, aminoguanidine was included in the medium at a concentration of 1 mM to inhibit production of the toxic by-products of the amine oxidases present in the fetal calf serum. Treated cells were exposed to analogues **1a-1f** in concentrations of either 0.1, 1.0, 5.0, or 10.0 μM. After a

96 hour treatment period, cells were harvested and assayed for cell growth as previously described.[7] Cell viability was measured by standard trypan blue exclusion. The results of these experiments are shown in Table I. The known antitumor agent bis(ethyl)spermine (BESpm) was included for comparison. The antitumor effects of **1d** and its analogues in L1210 leukemia cells have been previously reported.[13,22]

Table I. In Vitro IC_{50} Values (μM) for Alkylpolyamine Analogues Against H157 Non-Small Cell Lung Carcinoma and H82 Small Cell Lung Carcinoma.

Compound	H157	H82
BESpm	2.9	---
1a	0.7	5.2
1b	0.4	0.7
1c	0.5	1.7
1e	>10*	9.0
1f	2.1	1.5

*No significant effects were seen at any concentration tested up to 10 μM.
Each data point is the average of two determinations which in each case differed by less than 5%

Antitrypanosomal Assay Procedure. Assays for inhibition of trypanosomal growth following treatment with **1a-1f** were conducted as previously described.[23] Activity was compared to that of melarsen oxide, a known trypanocidal agent, as a positive control. Trypanosomes were grown in modified IMDM +20% horse serum in 24 well microplates at 37 °C. Wells were inoculated with 1 x 10^5 trypanosomes and compounds were solubilized in medium. One half the volume of each well was changed daily. Cell counts (Coulter counter) were made at 24 and 48 h. Control cells grew to 5 x 10^6/mL. IC_{50} values were determined from semi-log plots. *T.b. brucei* Lab 110 EATRO is a pentamidine and melarsen-sensitive strain and *T.b. rhodesiense* KETRI 243 As-10-3 is a melarsen and pentamidine resistant clone of a clinical isolate, KETRI 243. KETRI 269 is also melarsen-resistant.

Table II. In Vitro IC_{50} Values (μM) for Growth Inhibition of Four Strains *of Trypanosoma brucei* by bis-Substituted Polyamine Analogues

Compound	LAB 110	K 243	K 269	K 243 As-10-3
1a	>100*	>100	>100	>100
1b	>100	>100	>100	>100
1c	>100	>100	>100	>100
1d	14.5	15.1	12.3	13.0
1e	18.0	18.4	21.0	26.2
1f	0.125	0.98	0.69	0.78
Melarsen Oxide	0.001	0.04	0.	----

*No significant effects were seen at any concentration tested up to 100 μM.
Each data point is the average of two determinations which in each case differed by less than 5%

Although the previously described analogue **1d** has been evaluated for antitumor, antimalarial and antileishmanial activity, its usefulness as a trypanocide has not been previously investigated. The data presented above suggests that **1d** is an effective antitrypanosomal agent against the four strains used in our assay, with IC_{50} values ranging from 12.3 to 15.1 μM. Since all of the previously reported polyamines with antiparasitic activity contained terminal bis(aralkyl) substituents,[14] we wished to determine whether analogues with various alkyl substituents could demonstrate similar activity. The preliminary biological studies described above suggest that this is the case, and they have begun to suggest structure/activity trends within the broad class of bis-alkylated polyamine analogues. Interestingly, analogues with a 3-3-3 carbon skeleton (**1a**, **1b** and **1c**) were potent antitumor agents in vitro (IC_{50} against NCI H157 0.4 and 0.5 μM, respectively), but had little antitrypanosomal activity in in vitro screens, as shown in Table II. By contrast, agents with a 3-7-3 carbon skeleton (**1e**, **1f**) showed potent antiparasitic activity (Table II), but only moderate to weak antitumor activity (IC_{50} against NCI H157 2.1 and >10.0 μM, respectively). The analogue **1f** showed potency against all organisms tested which was superior to that of **1d**, and which approached the potency of the known trypanocide melarsen oxide. This data strongly suggests that it is possible to synthesize alkylpolyamine analogues of high potency which are specifically targeted to parasitic cells. Additional analogues in this series are being synthesized and evaluated to test this hypothesis. The most active analogue described above, **1f**, is currently undergoing in vivo testing, and is also being evaluated against other parasitic organisms such as *Leishmania donovanii* and *Plasmodiun falciparum*. It has been suggested that the antiproliferative and antiparasitic activities of **1d** are due to the ability of the analogue to be rapidly metabolized by polyamine oxidase.[13] The metabolism of the alkylpolyamine analogue bis(ethyl)norspermine, which involves N-dealkylation followed by SSAT acetylation and oxidation by polyamine oxidase, has also been described.[24] We have not determined whether our analogues undergo similar metabolism, or whether any putative metabolism plays a role in the biological activity of these analogues. For this reason, the metabolism of the alkylpolyamine analogues described in this manuscript will also be investigated. The results of these continuing studies are forthcoming.

Acknowledgements. This research was supported by National Institutes of Health grants CA63552 (PMW), CA51085 (RAC) and CA58184 (RAC). In addition, this investigation received financial support from the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (PMW, CJB). The excellent technical assistance of Ms. Alexis Mesa is gratefully acknowledged.

REFERENCES

1. Casero, R. A.; Pegg, A. E. *FASEB J.* **1993**, *7*, 653.

2. Bergeron, R. J.; Neims, A. H.; McManis, J. S.; Hawthorne, T. R.; Vinson, J. R. T.; Bortell, R.; Ingeno, M. J. *J. Med. Chem.* **1988**, *31*, 1183.

3. Porter, C. W.; Bernacki, R. J.; Miller, J.; Bergeron, R. J. *Cancer Res.* **1993**, *53*, 581.

4. Chang, B. K.; Bergeron, R. J.; Porter, C. W.; Vinson, J. R. V.; Liang, Y.; Libby, P. R. *Cancer Chem. Pharm.* **1992**, *30*, 183.

5. Chang, B. K.; Bergeron, R. J.; Porter, C. W.; Liang, Y. *Cancer Chem. Pharm.* **1992**, *30*, 179.

6. Bergeron, R. J.; McManis, J. S.; Liu, C. Z.; Feng, Y.; Weimar, W. R.; Luchetta, G. R.; Wu, Q.; Ortiz-Ocasio, J.; Vinson, J. R. T.; Kramer, D.; Porter, C. *J. Med. Chem.* **1994**, *37*, 3464.

7. Saab, N. H.; West, E. E.; Bieszk, N. C.; Preuss, C. V.; Mank, A. R.; Casero, R. A.; Woster, P. M. *J. Med. Chem.* **1993**, *36*, 2998.

8. Casero, R. A.; Celano, P.; Ervin, S. J.; Porter, C. W.; Bergeron, R. J.; Libby, P. R. *Cancer Res.* **1989**, *49*, 3829.

9. Casero, R. A.; Ervin, S. J.; Celano, P.; Baylin, S. B.; Bergeron, R. J. *Cancer Res.* **1989**, *49*, 639.

10. McCloskey, D. E.; Casero, R. A.; Woster, P. M.; Davidson, N. E. *Cancer Res.* **1995**, *55*, 3233.

11. McClosky, D. E.; Yang, J.; Woster, P. M.; Davidson, N. E.; Casero, R. A. *Clinical Cancer Res.* **1996**, *2*, 441.

12. Bitonti, A. J.; Dumont, J. A.; Bush, T. L.; Edwards, M. L.; Stemerick, D. M.; McCann, P. P.; Sjoerdsma, A. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 651.

13. Bitonti, A. J.; Bush, T. L.; McCann, P. P. *Biochem. J.* **1989**, *257*, 769.

14. Edwards, M. L.; Stemerick, D. M.; Bitonti, A. J.; Dumont, J. A.; McCann, P. P.; Bey, P.; Sjoerdsma, A. *J. Med. Chem.* **1991**, *34*, 569.

15. Baumann, R. J.; Hanson, W. L.; McCann, P. P.; Sjoerdsma, A.; Bitonti, A. J. *Antimicrob. Agents Chemother.* **1990**, 722.

16. Baumann, R. J.; McCann, P. P.; Bitonti, A. J. *Antimicrob. Agents Chemother.* **1991**, 1403.

17. Wunsch, E.; Graf, W.; Keller, O.; Keller, W.; Wersin, G. *Synthesis* **1986**, 958.

18. Bergeron, R. J.; Garlich, J. R.; Stolowich, N. J. *J. Org. Chem.* **1984**, *49*, 2997.

19. Yajima, H.; Takeyama, M.; Kanaki, J.; Nishimura, O.; Fujino, M. *Chem. Pharm. Bull.* **1978**, *26*, 3752.

20. Roemmele, R. C.; Rapoport, H. *J. Org. Chem.* **1988**, *53*, 2367.

21. Casero, R. A.; Go, B.; Theiss, H. W.; Smith, J.; Baylin, S. B.; Luk, G. D. *Cancer Res.* **1987**, *47*, 3964.

22. Edwards, M. L.; Prakash, N. J.; Stemerick, D. M.; Sunkara, S. P.; Bitonti, A. J.; Davis, G. F.; Dumont, J. A.; Bey, P. *J. Med. Chem.* **1990**, *33*, 1369.

23. Guo, J. Q.; Wu, Y. Q.; Rattendi, D.; Bacchi, C. J.; Woster, P. M. *J. Med. Chem.* **1995**, *38*, 1770.

24. Bergeron, R. J.; Weimar, W. R.; Luchetta, G.; Streiff, R. R.; Wiegand, J.; Perrin, J.; Schreier, K. M.; Porter, C.; Yao, G. W.; Dimova, H. *Drug. Metab. Disp.* **1995**, *23*, 1117.

(*Received in USA* 28 *August* 1996; *accepted* 18 *October* 1996)