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Design of polyamine-based therapeutic agents: new targets and new directions

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Abstract

Enzymes in the biosynthetic and catabolic polyamine pathway have long been considered targets for drug development, and early drug discovery efforts in the polyamine area focused on the design and development of specific inhibitors of the biosynthetic pathway, or polyamine analogues that specifically bind DNA. More recently, it has become clear that the natural polyamines are involved in numerous known and unknown cellular processes, and disruption of polyamine functions at their effector sites can potentially produce beneficial therapeutic effects. As new targets for polyamine drug discovery continue to evolve, the rational design of polyamine analogues will result in more structurally diverse agents. In addition, the physical linkage of polyamine-like structures to putative drug molecules can have beneficial effects resulting from increases in DNA affinity and selective cellular uptake. The present chapter will summarize recent advances in the development of alkylpolyamine analogues as antitumour agents, and describe subsequent advances that have resulted from incorporating polyamine character into more diverse drug molecules. Specifically, new polyamine analogues, and the role of polyamine fragments in the design of antiparasitic agents, antitumour metal complexes, histone deacetylase inhibitors and lysine-specific demethylase 1 inhibitors, will be described.

Introduction: biosynthesis inhibitors and alkylpolyamine analogues

The polyamine pathway is an important target for drug design, since alteration of cellular polyamine levels results in the disruption of a variety of cellular functions [1]. Inhibitors of the polyamine pathway have traditionally been developed as potential antitumour and/or antiparasitic agents [1,2]. Specific inhibitors of polyamine biosynthesis have been useful as research tools to elucidate the cellular functions of the naturally occurring polyamines, but their success as therapeutic agents has been limited. This failure has been, in part, due to the ability of mammalian cells to compensate for inhibition of a single enzyme in the pathway by up-regulating other enzymes or by modulating polyamine transport. Specific inhibitors have now been developed for the enzymes in the biosynthetic pathway, ODC (ornithine decarboxylase), AdoMetDC [AdoMet (*S*-adenosylmethionine) decarboxylase] and the

aminopropyltransferases spermidine synthase and spermine synthase. The structures of these classical polyamine biosynthesis inhibitors are shown in Figure 1. The compound DFMO (α -difluoromethylornithine; **1**), also known as eflornithine, is a mechanism-based inhibitor of ODC that was originally synthesized as an antitumour agent [3,4]. Clinical trials for DFMO as an antitumour agent were discontinued owing to lack of efficacy. However, there are some indications that it can be used for the treatment of glioblastoma [5,6] and prostate cancer [7], and it has gained recent attention as a chemopreventative agent [8,9]. DFMO is also approved for use in the treatment of West African trypanosomiasis caused by *Trypanosoma brucei gambiense*, but is ineffective against infections caused by *Trypanosoma brucei rhodesiense* (East African trypanosomiasis) [10–13]. It is currently marketed as a depilatory agent in the US. A number of effective inhibitors of AdoMetDC have been developed, but none have been marketed. The antileukaemic MGBG [methylglyoxal bis(guanyldrazone); **2**], is a potent competitive inhibitor of mammalian AdoMetDC, with a K_i value of less than 1 μ M [14]. However, MGBG is of limited use as a chemotherapeutic agent owing to a wide variety of other effects on cells, including induction of severe mitochondrial damage. More recently, conformationally restricted MGBG analogues such as the Ciba–Geigy compound CGP 39937 (**3**) have been shown to have similar antitumour effects, but with reduced toxicity. One of the analogues in this series, CGP 48664, has advanced to Phase I and II human clinical trials. A number of structural analogues of AdoMet have also been synthesized. The most promising of these, MDL-73,811 or AbeAdo (**4**) is a potent enzyme-activated inhibitor of AdoMetDC [15]. AbeAdo has shown promise as an antitrypanosomal agent [16], but has not been developed for clinical use. Potent and specific inhibitors for the aminopropyltransferases spermidine synthase (AdoDATO; **5**) [17] and spermine synthase (AdoDATAD; **6**) [18,19] have been synthesized, but their pharmacokinetic properties precluded their development as drugs. A number of amine analogues have been reported to inhibit the polyamine oxidases SMO (spermine oxidase) and APAO (N^1 -acetylpolyamine oxidase), including the well-established inhibitor MDL 72527 (**7**) [20]. To date, inhibitors specific for one of the two polyamine oxidases have not been identified.

In addition to enzyme inhibitors, a series of (bis)ethylpolyamines have been extensively studied as antitumour agents. Design of these analogues was based on the finding that natural polyamines utilize several feedback mechanisms which autoregulate their synthesis [1], and that they can be taken into cells by the polyamine transport system [21]. These analogues specifically down-regulate the synthesis of polyamines, but cannot substitute for the natural polyamines in their cell growth and survival functions [1,21]. The analogue MDL 27695 (**8**, Figure 1) is an early example of this type of analogue and possesses both antitumour and antiparasitic activity [22,23]. Among the most successful of these analogues (Figure 1) are BENSpm [bis(ethyl)norspermine; **9**], BESpm [bis(ethyl)spermine; **10**], BEHSpm [bis(ethyl)homospermine; **11**] and BE-4 $\times$ 4 [1,20-(ethylamino)-5,10,15-triazanodecane; **12**]. The N,N' -bis(ethyl)polyamines are readily transported into mammalian cells [24], where they deplete cellular polyamines, decrease ODC and AdoMetDC activity, and can ultimately produce cytotoxicity, depending on the cell lines used [1,25,26]. These compounds have been shown to possess a wide variety of therapeutic effects that vary widely with surprisingly small structural changes [1]. More recent discovery efforts involving bis(ethyl)polyamines have resulted in a series of analogues with conformationally restricted central chains, and these analogues possess significant antitumour and antiparasitic activity [27–32]. Along similar lines, a series of bis(ethyl)oligamine analogues have been described that show promise as chemotherapeutic agents [33–35]. The first examples of unsymmetrically substituted alkylpolyamines were described in 1993 [36], and subsequent studies reveal that agents typified by CPENSpm (**13**), CHENSpm (**14**) and IPENSpm (**15**) (Figure 2) are also potent antitumour agents [1,37–42]. The library of terminally alkylated

polyamine analogues has been extended to include more than 120 alkylpolyamines designed with varying polyamine backbone structures (3-4, 3-3-3, 3-4-4 and 3-7-3), and variation of the terminal alkyl substituents has been attempted to determine the optimal overall structure for antitumour effects. These agents produce a variety of cellular effects, and possess anti-tumour and/or antiparasitic activity *in vitro* [36,39–45]. Compounds **16–24** are representative of more than 25 unsymmetrically substituted alkylpolyamine analogues with 96 h IC₅₀ values of less than 4 µM against the H157 non-small-cell lung tumour line. These analogues contain more structural diversity than previously seen, including aromatic moieties, unsaturations, stereochemistry and heteroatoms, indicating that exploration of the chemical space surrounding the terminal substituents will yield additional promising antitumour agents. Compounds **22**, **23** and **24** (also known as PN11400, PN11401 and PN11402 respectively), are currently in development as antitumour agents.

Polyamine-based antiparasitic agents

A number of alkylpolyamine analogues have been shown to possess impressive antiparasitic activity *in vitro*, and in some cases *in vivo* (Figure 3). Compounds **25** and **26** inhibited the growth of *Trypanosoma brucei brucei* (Lab110 EATRO strain) with 48 h IC₅₀ values of 0.061 µM and 1.6 µM respectively, and maintained nearly identical submicromolar activity against the KETRI 243 and arsenic resistant KETRI 234 AS-10-3 clinical isolates of *T. brucei rhodesiense*. The analogue **27**, known as BW-1, also inhibited growth of the Lab 110 EATRO strain (48 h IC₅₀=0.24 µM), with equivalent activity against KETRI 243 (0.19 µM), KETRI 269 (0.75 µM) and KETRI 243 As-10-3 (0.20 µM) [1]. Compound **27** also inhibited the growth of the microsporidian *Enterocytozoon cuniculi* with a 48 h IC₅₀ of 0.47 µM, and was curative in a mouse model for microsporidiosis [46]. It has also been shown that **27** is a competitive inhibitor and substrate for the microsporidial form of polyamine oxidase, and that, like the parent compound **8** [1], may act through activation by oxidation within the parasite [47]. Compound **28** is also an effective trypanocide, with a 48 h IC₅₀ of 0.031 µM against Lab 110 EATRO, and 0.04 and 0.165 µM against KETRI 243 and KETRI 243 As-10-3 respectively. Interestingly, the 48 h IC₅₀ value for the related analogue **29** (0.31 µM) is 10-fold higher than **28**, suggesting that bis substitution is optimal for antiparasitic activity *in vitro*. Finally, guanidines and biguanides such as compounds **30–32** have potent antitrypanosomal activity (Lab 110 EATRO 48 h IC₅₀=0.18, 0.09 and 0.18 µM respectively). These analogues are potent inhibitors of the parasitic enzyme trypanothione reductase, but have no activity against the human form of glutathione reductase [44]. The related analogue **33** (also known as **2d**) inhibited Lab110 EATRO growth (48 h IC₅₀=0.62 µM), and has recently been shown to inhibit the growth of *Leishmania donovani* (48 h IC₅₀=6 µM, R. Madhubala and P. Woster, unpublished work).

Polyamine–metal complexes

Incorporation of polyamine side-chain residues into the structure of known antitumour agents that target DNA can lead to increases in antitumour activity. Dinuclear bis(platinum) complexes in which the metal centres were separated by diaminoalkanes are more potent than cisplatin against murine and human tumour cells *in vitro*, including cisplatin-resistant cell lines [48]. The trinuclear Pt (platinum) compound known as BBR 3464 (**34**) (Figure 4), in which three Pt centres are separated by diaminohexyl spacers [49,50] was shown to produce cytotoxicity in L1210 mouse leukaemia cells that was 30 times greater than cisplatin *in vitro* and to bind to DNA in a time-dependent manner [49] that was distinct from the binding pattern of cisplatin [50]. Recently, compound **35** (BBR3610) was shown to produce cytotoxicity superior to **34** through a caspase 8-dependent mechanism [51]. To date, the structure–activity relationships of polyamine–Pt complexes remain unexplored, and polyamine complexes with other transition metals, such as Re (rhenium), Rh (rhodium) and

Ru (ruthenium), have not been synthesized and evaluated for antitumour activity. We postulated that transition metals complexed to polyamines with known affinity for DNA and established antitumour effects may be of value in the treatment of breast cancer, and other tumour types where cisplatin produces a poor therapeutic response. From an initial library of 25 polyamine–metal complexes containing Pt, Re, Rh or Ru centres, 11 analogues exhibited 96 h IC₅₀ values of <10.0 µM in an H157 non-small-cell lung cancer MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay, with three of the 11 having 96 h IC₅₀ values of <1.0 µM. Four analogues, Pt compound **36**, Ru compound **37** and Re compounds **38** and **39** (Figure 4), were selected for dose–response studies using an MTT assay in the MCF7 breast tumour cell line, in which they exhibited 96 h IC₅₀ values of 6.6, 3.8, 9.3 and 6.9 µM respectively. By contrast, the 96 h IC₅₀ value for cisplatin was >250 µM under the same conditions. These results demonstrate that polyamine complexes containing three distinct (Pt, Ru and Re) metal centres possess antitumour activity far superior to cisplatin against MCF7 breast tumour cells *in vitro*. Polyamine–metal complexes **36–39** formed more extensive cross-links with plasmid DNA than cisplatin in a time- (6 h) and concentration- (3.0–300 µM) dependent manner. These results clearly demonstrate that polyamine–metal complexes are more potent than the cisplatin in breast tumour cells *in vitro*, and show a greater ability to bind to DNA.

Polyamine-based inhibitors of HDAC (histone deacetylase)

It is widely known that normal mammalian cells exhibit an exquisite level of control of chromatin architecture by maintaining a balance between HAT (histone acetyltransferase) and HDAC activity. In some tumour cells, hypoacetylation of histones results in aberrant gene silencing leading to the underexpression of growth regulatory proteins, and contributing to the development of cancer. HDAC inhibitors such as TSA (trichostatin A), MS-275 {*N*-(2-aminophenyl)-4-[*N*-(pyridin-3-ylmethoxycarbonyl)-aminomethyl]benzamide} and SAHA (suberoylanilide hydroxamic acid) can cause growth arrest in a wide range of transformed cells, and can inhibit the growth of human tumour xenografts [52]. Clinical studies indicate that HDAC inhibitors, such as SAHA and MS-275, are effective therapies for human cancer, but dose-limiting toxicity from the HDAC inhibitor remains a problem [53]. Traditional class I, IIa, IIb and IV HDAC inhibitors possess three structural features that are required for optimal activity: an aromatic cap group, an aliphatic chain and a metal-binding functional group (Figure 5). We hypothesized that addition of a polyamine side chain would increase the affinity of the agent for chromatin and facilitate import into cells via the polyamine transport system. We also felt that it would be possible to vary the structure of the terminal substituent on the polyamine side chain so as to target each of the 11 class I, IIa, IIb and IV HDAC isoforms [52] specifically. An initial library of 16 PAHA (polyaminohydroxamic acid) analogues was thus synthesized and screened for inhibitory activity against a global mixture of HDACs derived from HeLa cell lysates [54]. Compounds **40** and **41** (Figure 5) inhibited HDAC in this assay by 75% or greater at 1.0 µM. Compared with MS-275, **41** produced significantly greater induction of acetylated histone H3 and H4, and promoted greater re-expression of the cyclin-dependent kinase inhibitor p21^{Waf1}, in the ML-1 mouse leukaemia cell line. In addition, 1.0 µM **42** inhibited the HDAC mixture by 51.5%, but caused a 253-fold induction of acetylated α-tubulin, accompanied by minimal increases in acetylated histones H3, H4 and p21^{Waf1} [55]. These results strongly suggest that **42** shows a marked selectivity towards HDAC6, which is known to deacetylate α-tubulin. Although **40–42** obviously were able to enter mammalian cells, they did not serve as substrates for the polyamine transport system, presumably because of the negative charge of the hydroxamic acid moiety under the assay conditions.

To explore additional metal-binding moieties that could facilitate cellular transport, a series of 40 PABAs (polyaminobenzamides) and their homologues were synthesized and evaluated

as inhibitors of global HDAC [55]. Representative structures for this class are shown in Figure 5. Compound **43** exhibited an IC₅₀ value against global HDAC of 4.9 µM, which is comparable with the reported IC₅₀ value for MS-275 (4.8 µM). Analogues in the PABA series were evaluated against four HDAC isoforms representing Class I (HDAC 1, 3 and 8) and Class II (HDAC6). In these analogues, structural modifications were made in the linker chain length, in the polyamine substituent and in some cases in the metal-binding moiety. Isoform selectivity among the four HDACs evaluated varied significantly, demonstrating that the global percentage of HDAC inhibition is a composite of strong and weak inhibition at different isoforms and suggesting that the observed HDAC selectivity with PAHAs such as **42**, and PABAs such as **43** may be in part due to structural variations in their polyamine side chains. As mentioned above, one of the potential advantages of incorporating polyamine side chains into PAHA and PABA HDAC inhibitors was that the resulting molecules could utilize the polyamine transport system. Our preliminary data demonstrates that PABAs such as **43** are effectively imported using the polyamine transport system, as verified by [¹⁴C]spermidine uptake competition assays [55].

Three of the most active global HDAC inhibitors in the PABA series [52], compounds **44**, **45** and **46**, were markedly selective for HDAC1, and were evaluated against MCF7 wild-type tumour cells *in vitro*. Over a range of concentrations between 0.3 and 30 mM, PABA **44** was inactive, whereas **45** was cytostatic. However, PABA **46** was cytotoxic in the MCF7 cell line, with a 96 h IC₅₀ of 0.9 µM. In the MCF10A non-tumorigenic breast epithelial cell line, **46** exhibited a 96 h IC₅₀ of 24 µM, thus demonstrating selectivity for tumour cells. Under the same conditions, SAHA exhibited 96 h IC₅₀ values of 8.5 mM (MCF7) and 31 mM (MCF10A), and thus **46** compares favourably with known HDAC inhibitors that are currently in use in the clinic. Importantly, **44–46** were efficiently imported by the polyamine transporter, as determined by [¹⁴C]spermidine-uptake competition assays. Microscopic examination of treated cells revealed that cytotoxicity was mediated by apoptosis in MCF7 cells treated with **46**. Subsequent experiments showed that **46**, but not **44** or **45**, promoted the induction of ANXA1 (annexin A1). HDAC inhibitors have been shown to promote apoptosis through induction of ANXA1 [56], and recent studies suggest that breast tumours expressing high levels of ANXA1 are more likely to respond to chemotherapy than cells with low levels of ANXA1 [57]. Additional research is required to determine whether there is a functional relationship between inhibition of a specific HDAC isoform and induction of ANXA1.

Polyamine-based inhibitors of LSD1 (lysine-specific demethylase 1)

The potential role of polyamine analogues as inhibitors of LSD1, as well as the epigenetic effects such analogues may have on the re-expression of aberrantly silenced genes, has been described in detail elsewhere in this *Essays in Biochemistry* volume (chapter 7 by Casero). LSD1 was identified in part because its C-terminal domain shares significant sequence homology with the polyamine oxidases APAO and SMO [58,59]. Several groups have identified polyamine analogues that act as inhibitors of these two polyamine oxidases. MGBG (**2**) (Figure 1), a classical inhibitor of polyamine metabolism, was a potent inducer of APAO, but not SMO, in rat liver [60]. MDL 72,527 (**7**) (Figure 1), is a potent inhibitor of murine polyamine oxidase [61], as well as human APAO and SMO, but does not inhibit MAO [59,62]. It has previously been demonstrated that the polyaminoguanidine guazatine is a non-competitive inhibitor of maize polyamine oxidase [63]. Taken together, these results suggest that potent and selective inhibitors for the homologous flavin-dependent amine oxidase LSD1 can also be designed and synthesized that contain the structural motifs described above.

We have reported the synthesis of a novel series of polyamino (bis)guanidines and polyaminobiguanides [44] that are highly effective inhibitors of the parasitic enzyme trypanothione reductase, but that do not affect the human counterpart enzyme glutathione reductase. These compounds act as potent antitrypanosomal agents *in vitro*, with 48 h IC₅₀ values against *T. brucei* as low as 90 nM. Based on the observation that polyaminoguanidines such as guazatine are potent inhibitors of amine oxidases, we also evaluated these compounds for the ability to inhibit the closely related enzyme LSD1. These studies established that polyaminoguanidines and polyaminobiguanides such as **47** and **33** respectively (Figure 6), act as non-competitive inhibitors of LSD1 [64]. Based on the inhibitory activity of **47**, five additional guanidine analogues, **48–52** (Figure 6), were synthesized and evaluated for the ability to increase H3K4me2 (histone 3 dimethyl-lysine 4), a direct indicator of LSD1 inhibition. After a 48-h exposure, 5 µM compound **49** produced a 2.3-fold increase in the level of H3K4me2 in the KG1a haematopoietic cell line, but not in HL60 human promyelocytic leukaemia cells. Compound **50** caused a 1.6-fold increase in H3K4me2 in the KG1a line, but only at 10 µM, and did not affect H3K4me2 levels in the HL60 line. Compounds **48**, **51** and **52** had no effect in either cell line. These results suggest that replacement of the four methyl substituents in **47** with more bulky groups leads to a drastic reduction in LSD1 inhibition, and subsequently there is little effect on H3K4me2 levels. The synthesis of additional analogues in the polyaminoguanidine and polyaminobiguanide series is ongoing.

Conclusions and future directions

Prior to 1990, antitumour drug discovery research in the polyamine area was characterized by the design, synthesis and development of polyamine biosynthesis inhibitors. Despite a great deal of research, development of specific inhibitors as antitumour agents has only resulted in advancing one compound to the market. Drug discovery efforts in the polyamine field have begun to move away from the design of inhibitors for specific enzymes in the polyamine pathway, and to some degree away from the synthesis of analogues of the natural polyamines. However, the inclusion of polyamine-like structures in putative drug molecules allows them to take advantage of polyamine transport, and increases their affinity for chromatin. Thus agents with known therapeutic effects can be made more specific for target cells by virtue of mimicking the properties of the natural polyamines. Analogues of this type will capitalize on their polyamine character to target them to specific sites, but the activity of these derivatives will not depend on the polyamine structure within the molecule.

Terminally alkylated polyamine analogues continue to hold promise as therapies for a range of human cancers. A relatively large number of symmetrically substituted alkylpolyamines have been synthesized, and a number of these have been advanced to pre-clinical trials, and in some cases human clinical trials. In human clinical trials, some adverse effects have been noted, especially neurological symptoms (unilateral weakness, dysphagia, dysarthria, numbness, paresthesias, and ataxia [65], and aphasia, dizziness, vertigo and slurred speech [66]). Other Phase I studies in patients with non-small-cell lung cancer indicate that bis(ethyl)polyamines can be administered safely, and dose-limiting toxicities are mainly gastrointestinal [67]. Because the natural polyamines are ubiquitous in human cells, are strong cations and perform a variety of functions, it is not surprising that these analogues produce off-target effects. Through the use of biochemical and molecular biological techniques, combined with microarray and proteomics data, it will be possible to associate specific molecular targets with each cellular effect of the natural polyamines. As new effector sites for the natural polyamines are uncovered, a collaborative effort between chemists and biologists will facilitate optimization of analogue structure for each of these sites, thus reducing off-target effects and affording more specific therapeutic agents.

Rational drug design principles can ultimately be used to identify a wide variety of novel therapeutic agents.

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Summary

- Classical polyamine analogues, including biosynthesis inhibitors and linear analogues of the natural polyamines, have been extensively studied as antitumour and/or antiparasitic agents. Despite this intensive level of study, only a single agent, DFMO, has advanced to the clinic. However, 'second generation' alkylpolyamines containing greater structural diversity show great promise as chemotherapeutic agents.
- Selected polyamine analogues with substitutions on the terminal nitrogens, such as BW-1, have excellent antiparasitic activity in multiple organisms. In addition, guanidine- and biguanide-based analogues that are structurally related to alkylpolyamines have potent antiparasitic activity.
- Polyamines complexed to the transition metal platinum have been developed that produce significant antineoplastic effects. More recently, polyamine complexes with platinum, and with other transition metals such as rhenium, rhodium or ruthenium, are more potent than cisplatin, and may be useful in the treatment of breast cancer.
- Incorporation of a polyamine side chain into the structure of known HDAC inhibitors produced analogues that are as potent or more potent than existing HDAC inhibitors. In addition, these analogues are targeted to chromatin, can utilize the polyamine transport system to enter cells, and in some cases show good selectivity for individual HDAC isoforms. The effects of these analogues may be mediated through induction of the pro-apoptotic protein factor ANXA1.
- Polyaminoguanidines and polyaminobiguanides previously identified as antitrypanosomal agents are structurally similar to guanidines that inhibit amine oxidases. As such, these analogues were evaluated as inhibitors of the recently discovered enzyme LSD1. These compounds produce significant epigenetic changes in tumour cells that lead to the re-expression of tumour suppressor factors that are important in human cancer.

Figure 1.
Structures of the classical inhibitors of polyamine biosynthesis

Figure 2. Structures and 96 h IC_{50} values against the NCI H157 lung tumour cell line for selected alkylnonanes with antitumour activity

Figure 3. Structures of alkyldiamine analogues with antiparasitic activity *in vitro* and/or *in vivo*.

Figure 4.
Polyamine-transition metal complexes with antitumour activity

Figure 5. General structure for polyamine-based HDAC inhibitors, and selected PAHA and PABA analogues

Figure 6.
Structures of compound 47 (1c), 33 (2d) and 1c analogues 48–52