Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2861–2864
Bioorganic & Medicinal Chemistry Letters

Azinomycin inspired bisepoxides: influence of linker structure on in vitro cytotoxicity and DNA interstrand cross-linking

Rachel C. LePla,[a,†] Cyrille A. S. Landreau,[a] Michael Shipman,[a,*] John A. Hartley[b] and George D. D. Jones[c]

[a]*Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK*
[b]*CR-UK Drug-DNA Interactions Research Group, Department of Oncology, Royal Free and University College Medical School, University College London, 91 Riding House Street, London W1W 7BS, UK*
[c]*Department of Cancer Studies and Molecular Medicine, University of Leicester, University Road, Leicester LE1 7RH, UK*

Received 28 January 2005; revised 22 March 2005; accepted 23 March 2005
Available online 25 April 2005

Abstract—A series of bisepoxides based on the epoxide domain of the naturally occurring azinomycins have been synthesised. The incorporation of an aminomethyl group into the linker between the two alkylating epoxide units is shown to significantly enhance in vitro cytotoxicity against human tumour cell lines and DNA interstand cross-linking efficiency.

Drugs such as cisplatin, chlorambucil and melphalan, which are capable of inducing DNA interstrand cross-links (ISCs), are an extremely important class of clinical cancer chemotherapeutic agents.[1] Further research to find better medicines that act by this mode of action is continuing apace.[2] Azinomycins A (**1**) and B (**2**) are structurally unique natural products that have been isolated from the culture broths of *Streptomyces griseofuscus* S42227 (Fig. 1).[3] These compounds possess potent in vitro cytotoxic activity, significant in vivo antitumour activity and appear to act by disruption of cellular DNA replication by ISC formation.[4,5] Unfortunately, the poor chemical stability of the highly strained and reactive 1-azabicyclo[3.1.0]hexane core of the azinomycins is a major hurdle to their development as anti-cancer therapeutics. Since several studies have established that simplified derivatives based solely upon the epoxide domain of the azinomycins are cytotoxic and are potent DNA alkylating agents,[6] we reasoned that dimeric structures such as **3** might serve as stable yet effective DNA cross-linkers (Fig. 1). Previously, we have demonstrated the validity of this approach using bisepoxides **3a**, **3c** and **3e**, possessing simple flexible hydrocarbon linkers between the epoxide subunits. These compounds were shown to induce ISCs of duplex DNA in an in vitro assay at lower concentrations than some clinically used drugs such as cisplatin.[7] Encouraged by these initial findings, we sought to explore how the nature of the linker itself could impact the biological function of this novel class of DNA ISC agent. In this letter, we reveal that the incorporation of a heteroatom within the linker can be used to enhance both DNA ISC efficiency and in vitro anti-cancer activity.

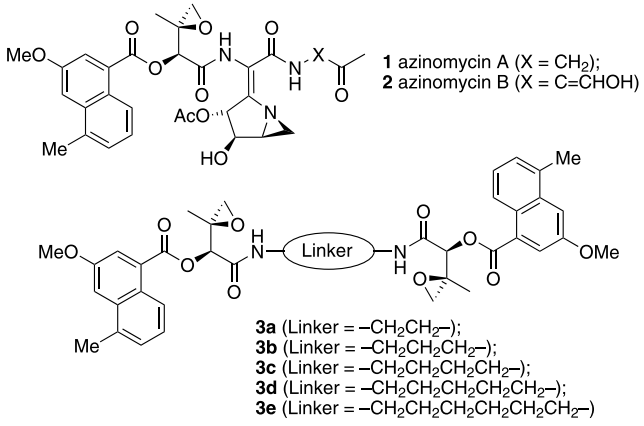


Figure 1. Structures of the naturally occurring azinomycins and synthetic bisepoxides based upon the epoxide domain.

Keywords: Epoxides; DNA alkylation; Interstrand cross-linking; Anti-cancer.
* Corresponding author. Tel.: +44 24765 23186; fax: +44 24765 24429; e-mail: m.shipman@warwick.ac.uk
† Present address: Cancer Biomarkers and Prevention Group, University of Leicester, University Road, Leicester LE1 7RH, UK.

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.03.091

In view of the known propensity of cations to associate with the negatively charged phosphate backbone of DNA, we anticipated that introduction of an amino group into the linker might enhance favourable electrostatic interactions between the bisepoxide with the DNA duplex at physiological pH. In this way, the effective concentration of the bisepoxide would be increased leading, we hoped, to higher levels of ISC formation. Increased water solubility was expected to be an additional benefit arising from the introduction of a polar amino group into the linker.

In designing a simple amino substituted linker, we recognised that a minimum of five atoms would be required to impart hydrolytic stability. For this reason, we selected bisepoxide **3f** as the target. This compound was readily made by selective deprotection of the benzyl ester of (2*S*,3*S*)-**4**[8] by hydrogenolysis followed by coupling of the resultant carboxylic acid with 0.40 molar equivalents of commercially available N^2-(methyl)diethylenetriamine (Scheme 1).[9] For comparison purposes in the biochemical assays, **3a** and **3c** were resynthesised.[7] In addition, two new 'all-carbon' bisepoxides bearing three and five atoms, namely **3b** (82%) and **3d** (49%), were made from 1,3-diaminopropane and 1,5-diaminopentane, respectively, using the same method.

The DNA interstrand cross-linking activities of the bisepoxides were initially determined with an agarose gel assay using the pUC18 linearised plasmid.[10] The ^{32}P 5′-end labelled duplex was incubated with **3** (0.1–100 nM) for 1 h at 37 °C prior to denaturing with alkali and subsequent gel electrophoresis. The extent of cross-linking was determined by quantifying the relative amounts of double stranded and single stranded DNA by storage phosphorimage analysis.

Since **3f** contains five atoms in the linker, initial experiments focused on determining precisely how the length of the linker effects ISC efficiency in the 'all-carbon' series. From our earlier study, we knew that **3e** was appreciably less effective and it was not re-examined herein.[7] Among the dimers bearing purely alkyl linkers, **3b** appears the most efficient, with 50 ± 4% ISCs (mean ± SD) observed at 10 nM (Fig. 2). However, the cross-linking potency is only slightly reduced when the length of the linker is either shortened or increased by just one methylene unit (**3a** and **3c** producing, respectively, 41 ± 2% and 44 ± 11% ISCs at this concentration). Further homologation is more markedly detrimental to ISC formation (14 ± 4% for **3d** at 10 nM).

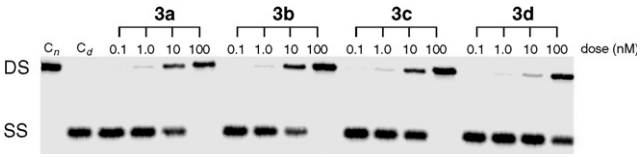
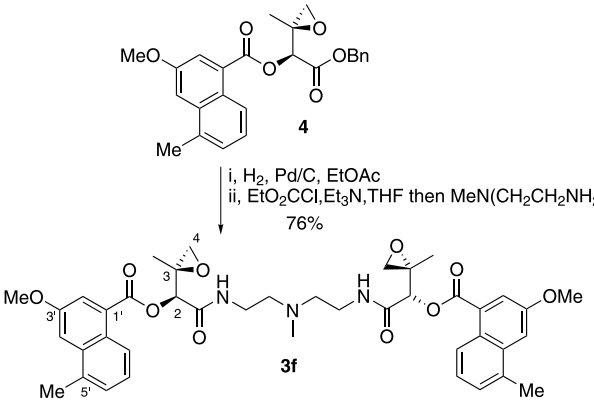


Figure 2. Agarose cross-linking gel for bisepoxides **3a–d**. Linearised plasmid DNA was treated with the agents at the concentrations shown for 1 h prior to alkali denaturation and gel electrophoresis. C_n and C_d are control non-denaturated and denatured samples, respectively. DS and SS indicate the positions of double and single stranded DNA, respectively. Experiments were performed in triplicate.

In additional studies, 'all-carbon' bisepoxides **3b** and **3d** were evaluated against **3f** incorporating the methylamino group (Fig. 3). Gratifyingly, **3f** proves to be the most potent compound, with 99 ± 1% ISCs at 10 nM and 37 ± 2% ISCs at just 1 nM. In contrast, **3b** and **3d** produce 10 ± 1% and 4 ± 1%, respectively, at 1 nM. From these data, we estimate that **3f** is ca. 2–4-fold more efficient at inducing ISCs than **3b** in this assay and ca. 7–9-fold better than **3d**.

In a second series of experiments based upon an assay reported by Coleman et al.,[5e] interstrand cross-linking activities were measured using a 15-mer DNA duplex containing a 5′-d(GCC)-3′ sequence located within an unreactive A–T tract. This triplet is known to readily react with azinomycin B itself producing appreciable levels of ISCs.[5b,e] The cross-linking studies were performed with each strand of the duplex, in turn, 5′-^{32}P end labelled. After gel electrophoresis on 20% polyacrylamide, two new bands of lower mobility were detected (Fig. 4). The first running close to the starting DNA being assigned to monoalkylation, the second, of much lower mobility, to the ISC product. The extent of monoalkylation and ISC for each drug was quantified by phosphorimage analysis and the results are summarised in Table 1. Several aspects of these results merit comment. First, the efficiency of ISC formation follows the order: **3f > 3b > 3a ≈ 3c > 3d**; this is similar to that witnessed in the pUC18 linearised plasmid assay. This reactivity trend is preserved with several other DNA sequences.[11] Second, even though the DNA sequence is not optimised for **3f** (the reacting electrophilic centres are



Scheme 1. Preparation of bisepoxide **3f**.

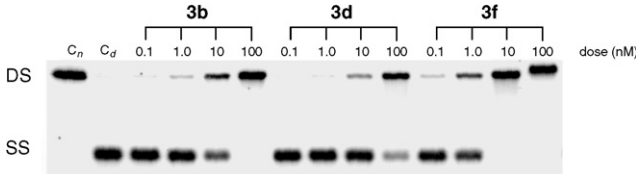


Figure 3. Agarose cross-linking gel for bisepoxides **3b**, **3d** and **3f**. Linearised plasmid DNA was treated with the agents at the concentrations shown for 1 h prior to alkali denaturation and gel electrophoresis. Experiments were performed in triplicate.

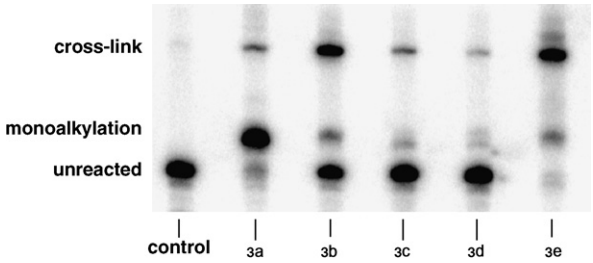


Figure 4. Representative polyacrylamide gel showing the extent of alkylation and ISCs by bisepoxides using ^{32}P-labelled oligonucleotide (**6**). Experimental conditions detailed in Table 1.

Table 1. ISC efficiencies determined using a 15-mer oligonucleotide[a]

Drug	Labelled strand	Cross-link formation (%)	Monoalkylation (%)	Unreacted DNA (%)
3a	**5**	10	4	86
	6	9	79	12
3b	**5**	42	6	52
	6	40	15	45
3c	**5**	10	4	86
	6	9	12	79
3d	**5**	5	4	91
	6	5	12	83
3f	**5**	69	11	20
	6	70	23	7

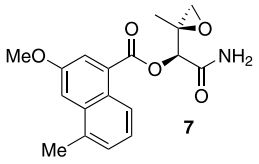
[a] Experiments conducted using the DNA duplex produced by annealing: 5′-d(TATTATGCCATTATT)-3′ (**5**)/3′-d(ATAATACGGTAATAA)-5′ (**6**). The 5′-end of either **5** or **6** was ^{32}P labelled with T4 polynucleotide kinase and [^{32}P]ATP prior to annealing with the unlabelled complementary strand. *Reaction conditions:* 400 μM drug, 9 μM duplex DNA, 45 mM sodium cacodylate (pH 7), v/v 10% DMSO, 8 °C, 20 h. Experiments were performed in triplicate (mean values reported).

further apart than in azinomycin B), high levels of cross-links are produced. Finally, it should be noted that we cannot fully account for differences in the reactivity seen with the less potent analogues. For example, it is unclear why **3a** produces large amounts of monoalkylation with labelled **6** but **3b–d** do not.

Bisepoxides **3a–d** and **3f** were evaluated in vitro against the NCI 60 human tumour cell lines derived from nine cancer types (leukaemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney). All of them exhibited significant cytotoxicity. The relative order of anticancer activities of the five dimers is **3b** ≈ **3f** > **3c** > **3a** > **3d** as deduced from the mean values of 50% growth inhibition (GI_{50}) taken across all 60 cell lines (Table 2). This order of potency is similar when the data from mean cytotoxicities (LC_{50}) (**3b** > **3f** ≈ **3c** > **3d** ≈ **3a**) or total growth inhibitions (TGI) (**3b** > **3f** > **3c** > **3a** > **3d**) are compared. The introduction of the aminomethyl group clearly results in ca. 3–10-fold increase in cytotoxicity (Table 1, **3d** cf. **3f**). These data correlate well with those obtained in the DNA ISC assays. Monoepoxide **7**[6c] (Fig. 5), unable to generate cross-links but known to alkylate DNA,[6e] is less potent than the dimers against the 60-cell line panel as judged by 50% growth inhibition.

Table 2. Summary of cytotoxicity data obtained from NCI panel of 60 cancer cell lines

Bisepoxide	Cytotoxicity		
	GI_{50}, nM	LC_{50}, μM	TGI, μM
3a	72	62	2.4
3b	31	3.2	0.21
3c	53	14.8	0.55
3d	178	50	3.4
3f	28	14.4	0.35
7	214	33	2.0



7

Figure 5. Structure of monoepoxide **7**.

In conclusion, we have prepared a series of azinomycin-like bisepoxides **3**, which display both high ISC efficiencies and appreciable in vitro cytotoxicities. In the 'all-carbon' series, a three methylene spacer length proved optimal as judged by comparative DNA cross-linking assays and tumour cytotoxicity data. A further 2–4-fold increase in ISC activity can be achieved by the introduction of a methylamino group into the linker.

Acknowledgments

We are indebted to BBSRC (B15997) for financial support of this work, Dr. Kevin Moffat of the University of Warwick for his help and assistance, and the NCI anti-cancer screening programme for the in vitro cytotoxicity data.

References and notes

1. Rajski, S. R.; Williams, R. M. *Chem. Rev.* **1998**, *98*, 2723–2795.
2. For recent illustrative examples, see: Bando, T.; Narita, A.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **2003**, *125*, 3471–3485; Richter, S. N.; Maggi, S.; Mels, S. C.; Palumbo, M.; Freccero, M. *J. Am. Chem. Soc.* **2004**, *126*, 13973–13979; Hartley, J. A.; Spanswick, V. J.; Brooks, N.; Clingen, P. H.; McHugh, P. J.; Hochhauser, D.; Pedley, R. B.; Kelland, L. R.; Alley, M. C.; Schultz, R.; Hollingshead, M. G.; Schweikart, K. M.; Tomaszewski, J. E.; Sausville, E. A.; Gregson, S. J.; Howard, P. W.; Thurston, D. E. *Cancer Res.* **2004**, *64*, 6693–6699; Paz, M. M.; Kumar, G. S.; Glover, M.; Waring, M. J.; Tomasz, M. *J. Med. Chem.* **2004**, *47*, 3308–3319; He, H.; Tian, T.; Wang, P.; Wu, L.; Xu, J.; Zhou, X.; Zhang, X.; Cao, X.; Wu, X. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3013–3016.
3. Nagaoka, K.; Matsumoto, M.; Oono, J.; Yokoi, K.; Ishizeki, S.; Nakashima, T. *J. Antibiot.* **1986**, *39*, 1527–1532.
4. For reviews, see: Hodgkinson, T. J.; Shipman, M. *Tetrahedron* **2001**, *57*, 4467–4488; Casely–Hayford, M.; Searcey, M. In *Small Molecule DNA and RNA Binders*; Demeunynck, M., Bailly, C., Wilson, W. D., Eds.; Wiley–VCH: Weinheim, 2003; Vol. 1, pp 676–696.

5. (a) Lown, J. W.; Majumdar, K. C. *Can. J. Biochem.* **1977**, *55*, 630–635; (b) Armstrong, R. W.; Salvati, M. E.; Nguyen, M. *J. Am. Chem. Soc.* **1992**, *114*, 3144–3145; (c) Fujiwara, T.; Saito, I.; Sugiyama, H. *Tetrahedron Lett.* **1999**, *40*, 315–318; (d) Hartley, J. A.; Hazrati, A.; Kelland, L. R.; Khanim, R.; Shipman, M.; Suzenet, F.; Walker, L. F. *Angew. Chem., Int. Ed.* **2000**, *39*, 3467–3470; (e) Coleman, R. S.; Perez, R. J.; Burk, C. H.; Navarro, A. *J. Am. Chem. Soc.* **2002**, *124*, 13008–13017; (f) Landreau, C. A. S.; LePla, R. C.; Shipman, M.; Slawin, A. M. Z.; Hartley, J. A. *Org. Lett.* **2004**, *6*, 3505–3507; (g) LePla, R. C.; Landreau, C. A. S.; Shipman, M.; Jones, G. D. D. *Org. Biomol. Chem.* **2005**, *3*, 1174–1175.
6. (a) Hashimoto, M.; Matsumoto, M.; Yamada, K.; Terashima, S. *Tetrahedron Lett.* **1994**, *35*, 2207–2210; (b) Shishido, K.; Haruna, S.; Iitsuka, H.; Shibuya, M. *Heterocycles* **1998**, *49*, 109–112; (c) Hodgkinson, T. J.; Kelland, L. R.; Shipman, M.; Suzenet, F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 239–241; (d) Zang, H.; Gates, K. S. *Biochemistry* **2000**, *39*, 14968–14975; (e) Coleman, R. S.; Burk, C. H.; Navarro, A.; Brueggemeier, R. W.; Diaz-Cruz, E. S. *Org. Lett.* **2002**, *4*, 3545–3548; (f) Casely-Hayford, M. A.; Pors, K.; Patterson, L. H.; Gerner, C.; Neidle, S.; Searcey, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 653–656.
7. Hartley, J. A.; Hazrati, A.; Hodgkinson, T. J.; Kelland, L. R.; Khanim, R.; Shipman, M.; Suzenet, F. *Chem. Commun.* **2000**, 2325–2326.
8. Bryant, H. J.; Dardonville, C. Y.; Hodgkinson, T. J.; Hursthouse, M. B.; Malik, K. M. A.; Shipman, M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1249–1255.
9. *Selected physical and spectroscopic data of **3f***: mp 68–69 °C; $[\alpha]_D^{26}$ +26.0 (*c* 0.57, $CHCl_3$); IR (film) 3377, 2937, 1724, 1674 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.60–8.55 (2H, m, 2 × H-8′), 7.87 (2H, d, J = 2.5 Hz, 2 × H-2′), 7.38 (2H, d, J = 2.5 Hz, 2 × H-4′), 7.33–7.27 (4H, m, 2 × H-7′, 2 × H-6′), 7.02 (2H, br s, 2 × NH), 5.08 (2H, s, 2 × H-2), 3.93 (6H, s, 2 × OCH_3), 3.53–3.45 (2H, m, 2 × C*H*HN), 3.30–3.22 (2H, m, 2 × CH*H*N), 2.90 (2H, d, J = 4.8 Hz, 2 × epoxide CH*H*), 2.78 (2H, d, J = 4.8 Hz, 2 × epoxide C*H*H), 2.62 (6H, s, 2 × 5′-CH_3), 2.55–2.48 (4H, m, 2 × CH_2NCH_3), 2.21 (3H, br s, CH_2NCH_3), 1.53 (6H, s, 2 × 3-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7 (s, CO), 166.0 (s, CO), 155.8 (s, C-3′), 134.3 (s), 133.2 (s), 128.4 (s), 127.7 (d), 126.8 (s), 125.1 (d), 123.8 (d, C-8′), 122.2 (d, C-2′), 108.1 (d, C-4′), 76.6 (d, C-2), 56.6 (t, CH_2NCH_3), 56.3 (s, C-3), 55.6 (q, OMe), 53.4 (t, C-4), 41.6 (q, NCH_3), 36.9 (t, CH_2NH), 20.2 (q, C-5′-Me), 17.9 (q, C-3-Me); HRMS (LSIMS) calcd for $C_{41}H_{48}N_3O_{10}$: 742.3340; found 742.3325.
10. Hartley, J. A.; Berardini, M. D.; Souhami, R. L. *Anal. Biochem.* **1991**, *193*, 131–134.
11. Three additional 15-mer oligonucleotide duplexes were studied under the same conditions reported in Table 1. In each case, the duplex was constructed from the same unreactive A–T sequence with modifications being made to the central triplet sequence. Specifically, the sequences studied were: 5′-d(GTC)-3′/3′-d(CAG)-5′ (**8**); 5′-d(GCT)-3′/3′-d(CGA)-5′ (**9**); 5′-d(GTT)-3′/3′-d(CAA)-5′ (**10**). **3a**, **3b** and **3f** produced measurable amounts of ISC formation (10%, 14% and 70%, respectively) with duplex **8**. However, only **3f** produced measurable amounts of ISC (30% and 21%, respectively) with duplexes **9** and **10**.