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Synthesis, cytotoxicity and DNA cross-linking activity of symmetrical dimers based upon the epoxide domain of the azinomycins

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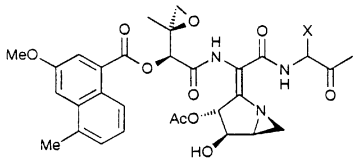
A series of dimeric compounds 1a–c designed around the epoxide domain of the azinomycins have been synthesised and demonstrated to be highly efficient DNA interstrand cross-linking agents.

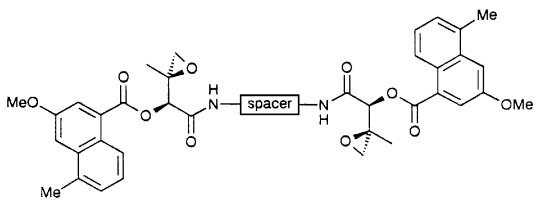
Chemical agents capable of inducing DNA interstrand cross-links (ISC's) comprise an extremely important class of clinical cancer chemotherapeutic agent.[1] Indeed, several important drugs used for the treatment of this disease (*e.g.* cisplatin, chlorambucil and melphalan) are known to induce ISC formation. In 1986, two new cytotoxic agents named azinomycin A and B were isolated from the culture broths of *Streptomyces griseofuscus* S42227 which were shown to possess significant *in vivo* anti-tumour activity (Fig. 1).[2] Using synthetic oligonucleotide duplexes, Armstrong *et al.* demonstrated that azinomycin B causes interstrand cross-links in the major groove of DNA by alkylation at N-7 of guanine (G) and reaction with another purine residue (A or G) two bases along on the complementary DNA strand.[3] More recently, Fujiwara *et al.* provided the first direct evidence for the involvement of the electrophilic epoxide and aziridine moieties in the DNA cross-linking event using self-complementary oligodeoxynucleotide $[d(TAGCTA)_2]$.[4] Whilst reactions with the nucleophilic purine residues of DNA is clearly essential for ISC formation, the poor chemical stability of these agents especially the 1-azabicyclo-[3.1.0]hexane ring system is undesirable from the point of view of possible drug development. Indeed, the high reactivity of this heterocyclic system has severely hampered efforts to complete the total synthesis of these natural products.[5] Since several studies have determined that simplified derivatives based upon the epoxide domain of the azinomycins are highly cytotoxic,[6] we reasoned that dimeric structures based upon this motif joined by a suitable linker might serve as effective DNA cross-linking agents (Fig. 1). Such compounds might be expected to display enhanced chemical stability with respect to the natural products. Furthermore, by fine tuning the nature of the aromatic residues and the linker, agents capable of targeting specific base sequences can be imagined.

To test our hypothesis, we chose to prepare three bisepoxides **1a–c** containing flexible hydrocarbon linker units of varying lengths between the two epoxide subunits. The synthesis of these bisepoxides was accomplished in a straightforward fashion from enantiomerically pure epoxy ester (2*S*,3*S*)-**2**.[7] Selective cleavage of the benzyl ester by hydrogenation and coupling of the resultant acid with 0.5 molar eq. of ethylenediamine furnished bisepoxide **1a** in 69% yield (Scheme 1).† Similarly, (2*S*,3*S*)-**2** was transformed into bisepoxides **1b** and **1c** using 0.5 molar eq. of 1,4-diaminobutane and 1,6-diaminohexane, respectively. All three bisepoxides were produced as crystalline solids which displayed good thermal and hydrolytic stability.‡

With these bisepoxides in hand, we turned our attention to studying their interstrand cross-linking activities using an agarose gel assay.[8] Monoepoxide **3**[7] was used as a control in these experiments. In contrast to epoxide **3** which displays no ISC activity at concentrations up to 50 μM, bisepoxide **1a** induces cross-links at concentrations as low as 0.1 μM and effects 100% cross-linking at 10 μM (Fig. 2). Bisepoxides **1b** and **1c** bearing progressively longer linkers between the two epoxide centres also induce ISC in double stranded DNA. Bisepoxide **1b** induces some ISC's at 0.1 μM with 100% ISC's being observed at just 1 μM; **1c** induces appreciable levels of cross-linking at the somewhat higher concentration of 10 μM. It is interesting to observe that bisepoxides **1a** and **1b** are more effective ISC agents in this assay than the clinically important anti-cancer drugs cisplatin, melphalan and chlorambucil (data not shown). To gain preliminary information concerning the

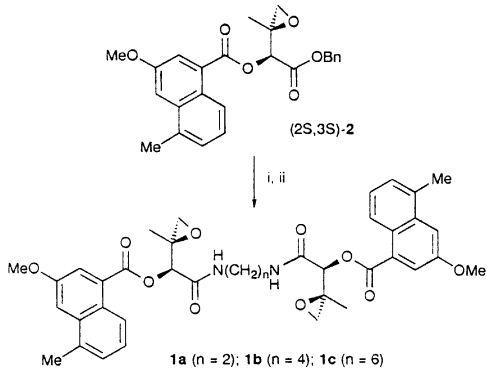


Azinomycin A (X = H); Azinomycin B (X = CHO)



dimeric epoxides: chemically stable DNA interstrand cross-linking agents?

Fig. 1



1a (n = 2); **1b** (n = 4); **1c** (n = 6)

Scheme 1 *Reagents and conditions*: (i) 10% Pd/C, H_2, MeOH, 1 h; (ii) diamine (see text), PyBOP®, Et_3N, HOBt, DMF, 69% (**1a**), 46% (**1b**), 80% (**1c**). PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate.

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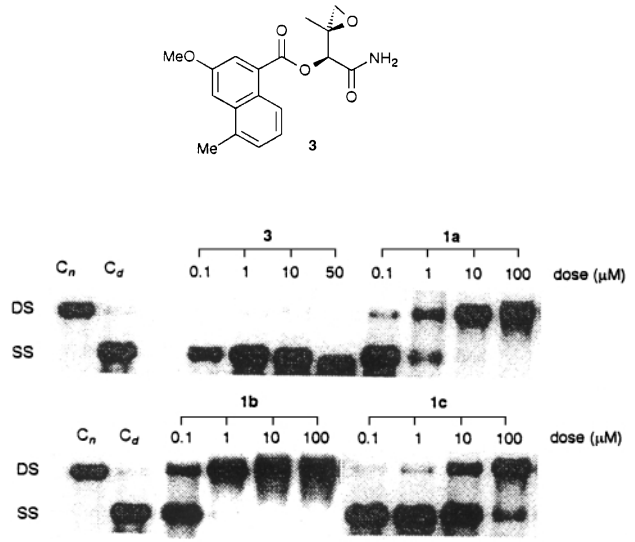

Fig. 2 Agarose cross-linking gel for bisepoxides **1a–c** and epoxide **3**. Plasmid DNA was treated with the agents at the concentrations shown for 2.5 h prior to denaturation and gel electrophoresis. C_n and C_d are control non-denaturated and denatured samples, respectively. DS and SS indicate the positions of double and single stranded DNA, respectively.

Table 1 Cytotoxicity data for **1a–c**

Human tumour cell lines	IC_{50} (μM)[a]		
	1a	**1b**	**1c**
A2780	< 0.05	0.065	0.11
CH1	< 0.05	< 0.05	0.08
CH1cisR[b]	< 0.05	< 0.05	0.08
SKOV-3	1.4	0.82	1.2
HT29	0.42	0.55	0.50
K562	0.067	0.027	0.062

[a] Dose of drug inhibiting growth by 50% following a 96 h exposure (1 h in the case of K562) as determined by the SRB assay[10] (MTT assay[11] in the case of K562). [b] Cell line with acquired resistance to cisplatin.

sequence selectivity of bisepoxides **1a–c**, we have evaluated them in a Taq DNA polymerase stop assay.[9] All three bisepoxides and epoxide **3** induce Taq stops preferentially at G residues indicating alkylation at these bases. Interestingly, the bisepoxides block polymerase at fewer bases than the corresponding monoepoxide **3**, indicating enhanced sequence specificity.

The cytotoxicity of bisepoxides **1a–c** was determined against a small panel of human tumour cell lines [A2780, CH1, SKOV-3 (all ovarian), HT-29 (colon), K-562 (leukemia)] (Table 1). All the compounds display potent cytotoxicity in a range of cell lines including one with acquired resistance to the drug cisplatin (CH1cisR) where no cross resistance was observed. In some cell lines, epoxide **3** shows comparable potency to these bisepoxides (*e.g.* CH1, IC_{50} 0.056 μM), however in other lines it is markedly less potent (*e.g.* K562, IC_{50} 0.493 μM).

In conclusion, we have demonstrated that the chemically stable bisepoxides **1a–c** are easy to prepare and are highly effective ISC agents. Our findings suggest that a spacer consisting of a chain of approximately four carbon atoms is optimal for cross-linking ability. Furthermore, we have determined that these bisepoxides are highly cytotoxic. In future studies, we plan to determine how the nature of the aromatic residue and the conformational flexibility of the linker influence ISC activity and how the sequence specificity of these agents can be enhanced.

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Notes and references

† *Selected physical and spectroscopic data*: **1a**: mp 66–68 °C; $[\alpha]_D^{20}$ = +76.6 (*c* 0.57, $CHCl_3$); ν_{max} (Nujol®)/cm^{-1} 3369 (NH), 1726 (ester C=O), 1685 (amide C=O); δ_H (400 MHz; $CDCl_3$) 8.62 (2H, m, ArH), 7.98 (2H, d, *J* = 2.6 Hz, ArH), 7.37 (2H, d, *J* = 2.6 Hz, ArH), 7.32–7.27 (4H, m, ArH), 6.96 (2H, br s, NH), 5.31 (2H, s, H-2), 3.88 (6H, s, OCH_3), 3.56–3.37 (4H, m, CONHCH_2-), 2.96 (2H, d, *J* = 4.7 Hz, H-4), 2.66 (2H, d, *J* = 4.7 Hz, H-4′), 2.62 (6H, s, $ArCH_3$), 1.49 (6H, s, CH_3); δ_C (100.6 MHz; $CDCl_3$) 167.6 (s, C=O), 165.5 (s, C=O), 155.8 (s, ArC), 134.3 (s, ArC), 133.1 (s, ArC), 127.71 (s, ArC), 127.68 (d, ArCH), 127.0 (s, ArC), 125.1 (d, ArCH), 123.8 (d, ArCH), 122.5 (d, ArCH), 108.2 (d, ArCH), 76.0 (d, C-2), 56.0 (s, C-3), 55.4 (q, OCH_3), 52.6 (t, C-4), 39.0 (t, CONHCH_2), 20.0 (q, Ar-CH_3), 18.0 (q, CH_3); Observed (MH^+): 685.2760; $C_{38}H_{41}N_2O_{10}$ requires 685.2760. **1b**: mp 95 °C; $[\alpha]_D^{20}$ = +32 (*c* 0.31, $CHCl_3$); Observed (M^+): 712.2993; $C_{40}H_{44}N_2O_{10}$ requires 712.2996. **1c**: mp 105 °C; $[\alpha]_D^{20}$ = +50 (*c* 0.43, $CHCl_3$); Observed (M^+): 740.3310; $C_{42}H_{48}N_2O_{10}$ requires 740.3309; found: C, 68.15; H, 6.63; N, 3.49. $C_{42}H_{48}N_2O_{10}$ requires C, 68.09; H, 6.53; N, 3.78%.

‡ No degradation was observed after stirring **1a** in DMSO and pH 7.4 buffer (1 : 1) for 7 days.

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