Tetrahedron Letters 50 (2009) 3648–3650

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Azinomycin bisepoxides containing rigid aromatic linkers: synthesis, cytotoxicity and DNA interstrand cross-linking activity

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ARTICLE INFO

Article history:
Received 15 January 2009
Revised 9 March 2009
Accepted 16 March 2009
Available online 21 March 2009

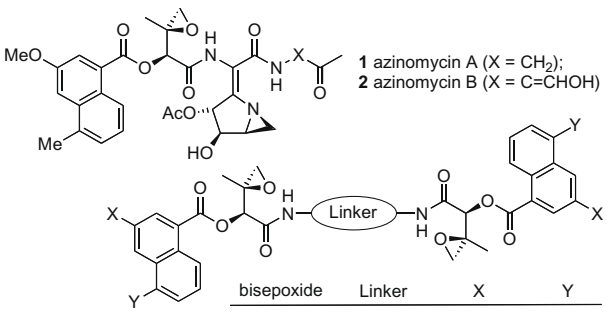
ABSTRACT

The synthesis of a series of azinomycin bisepoxides containing rigid linkers is achieved through two different strategies. Double copper-mediated C–N bond formation under Buchwald-type conditions can be realised but yields are poor with fully intact epoxide substrates. The bisepoxides are made in improved yields through the simultaneous formation of two amide bonds. Bioassays reveal that **5**, containing a 1,3-diaminobenzene linker, is a potent in vitro DNA interstrand cross-linking agent with significant cytotoxicity in the NCI 60-human cancer cell panel (GI_{50} = 0.15 μM).

DNA interstrand cross-linking (ISC) agents such as cisplatin, chlorambucil, and melphalan represent an extremely important class of clinical cancer chemotherapeutics.[1] Currently considerable effort is focused on the identification of new medicines that operate by this mode of action with improved therapeutic profiles.[2] Azinomycins A (**1**) and B (**2**) are structurally unique natural products that possess significant in vivo antitumour activity and appear to act by disruption of cellular DNA replication by ISC formation (Fig. 1).[3,4] Unfortunately, the 1-azabicyclo[3.1.0]hexane core of these compounds is unstable, making them unsuitable for development as potential anti-cancer therapeutics.

Previously, we have demonstrated that dimeric structures based upon the epoxide domain of the azinomycins are highly potent ISC agents, that display much improved chemical stability.[5,6] For example, bisepoxide **3** induces appreciable amounts of in vitro DNA ISC in the low nM range, and displays good cytotoxicity in the NCI 60 human cancer cell panel (GI_{50} = 31 nM) (Fig. 1).[5b] Thus far, all the bisepoxides studied have incorporated flexible linkers between the epoxide subunits. We reasoned that by reducing the number of rotatable bonds within the linker, we might reduce the entropic cost of binding to the DNA backbone, and hence increase potency. To test this hypothesis, we targeted the synthesis of bisepoxides **4**–**6** based upon a simple disubstituted aromatic nucleus. The precise distance and spatial disposition of the reacting epoxide centres being fine tuned by careful choice of the substitution pattern around the aromatic ring. In this Letter, we describe two different synthetic approaches to this new compound class, and preliminary biological findings with these more rigid derivatives.

In considering routes to bisepoxides **4–6**, we were attracted to the idea of effecting two concomitant Cu-mediated C–N bond-forming reactions between epoxyamide subunits and 1,2- 1,3- or 1,4-dihalobenzenes.[7,8] At the outset of this work, we were concerned about the compatibility of the electrophilic epoxide ring and with the conditions commonly employed for amide N-arylation.[8] Nevertheless, we felt this approach merited investigation as if successful, the wide availability of aromatic and heteroaromatic dihalides would allow large numbers of bisepoxides to be generated rapidly and screened in a highly divergent manner. To explore this strategy, (2*S*,3*S*)-**7** was prepared according to published methods,[9] and reacted with 0.5 equiv of 1,4-diiodobenzene



bisepoxide	Linker	X	Y
3		OMe	Me
4a		H	H
4b		OMe	Me
5		OMe	Me
6		OMe	Me

Figure 1.

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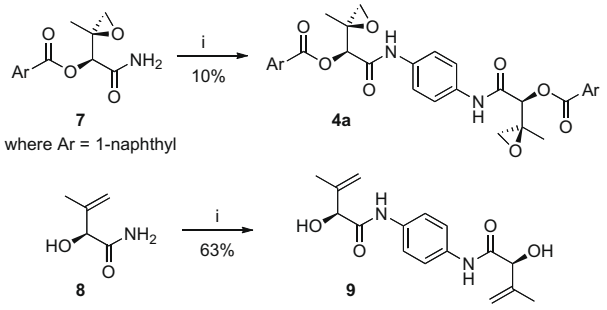
doi:10.1016/j.tetlet.2009.03.123

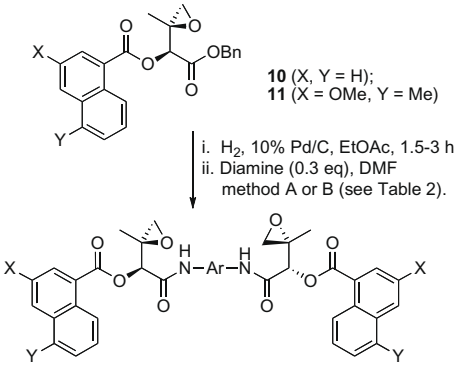


Scheme 1. Reagents and conditions: 1,4-diiodobenzene (0.5 equiv), CuI (1 equiv), DMEDA (2 equiv), K_2CO_3 (2 equiv), K_3PO_4 (2 equiv), dioxane, 50 °C, 22 h.

in the presence of stoichiometric amounts of CuI and *N*,*N*′-dimethyl-ethylenediamine (DMEDA) (Scheme 1). Bisepoxide **4a** was isolated in 10% yield as a single diastereomer from this reaction. However, the efficiency of this coupling was very poor with no starting amide or monoamidated products recovered, suggesting that **7** is incompatible with the reaction conditions. Evidence in support of this supposition was obtained by reacting amide (2*S*)-**8**,[10] devoid of the epoxide ring, under identical conditions. In this instance, bisarylation proceeded smoothly providing **9** in a much improved yield (Scheme 1). Although bisamide **9** might be converted into **4a** by stereocontrolled epoxidation and acylation, the need for additional synthetic steps discouraged us from further pursuing N-arylation as a route to these compounds.

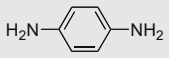
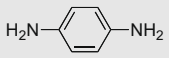
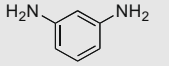
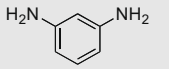
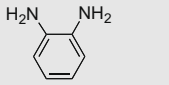
As an alternative strategy, we explored coupling of *o*-, *m*- or *p*-diaminobenzene with the appropriately substituted epoxy acid. Benzyl esters **10** and **11** were prepared as their (2*S*,3*S*)-enantiomers using published methods.[9] Hydrogenolysis and coupling with 0.5 equiv of the appropriate diaminobenzene using either EDCI/HOBt or HATU as activating agent effected amide bond formation (Scheme 2 and Table 1). Using this approach, bisepoxide **4a** could be produced in much improved yield (50% cf 10% in Scheme 1). Other rigid bisepoxides, namely **4b** and **5**, were prepared using the same approach.[11] One limitation was noted, in that sterically congested **6** could not be formed from 1,2-diaminobenzene with only monoacylation being observed.

The new bisepoxides 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 appreciable cytotoxicity. Mean values for GI_{50}, LC_{50} and TGI for bisepoxides **4a**, **b** and **5** are given in Table 2, together with those obtained previously for **3**.[5b] The relative order of anticancer activities of these dimers is **3** > **5** > **4b** > **4a** as deduced from the mean values of 50% growth inhibition (GI_{50}) taken across all 60 cell lines. This order of potency



Scheme 2.

Table 1
Synthesis of bisepoxides **4–6** by amide bond formation

Entry	Diamine	Epoxide	Method[a]	Product	Yield (%)
1		**10**	A	**4a**	50[b]
2		**11**	A	**4b**	59
3		**11**	A	**5**	34
4		**11**	B	**5**	46
5		**11**	A	**6**	0[c]

[a] Method A: EDCI, HOBt, DMF, 0 °C→rt, 20 h; method B: HATU, DIEA, DMF, 24 h, rt.
[b] 0.5 equiv of diamine used.
[c] Only formation of monoepoxide detected by ES–MS.

is similar when the data from mean cytotoxicities (LC_{50}) (**3** > **5** ≈ **4b** > **4a**) or total growth inhibitions (TGI) (**3** > **5** > **4b** > **4a**) are compared.

The DNA interstrand cross-linking activities of these bisepoxides were determined with an agarose gel assay using the pBR322 linearised plasmid.[12] The ^{32}P 5′-end labelled duplex was incubated with **3** (0.01–1000 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 phosphor-image analysis. The efficiency of ISC formation follows the order **3** > **5** > **4b** > **4a** (Table 2). This trend directly correlates with the order of in vitro cytotoxicity suggesting that the biological activity of these agents is related to their ability to form DNA ISC. In all cases, the extent of DNA ISC formation was shown to be dependent on bisepoxide concentration with increasing ISC at higher drug concentrations (data not shown).

Several features of the biological data merit further comment. It is clear that use of these aromatic linkers reduces potency in comparison to related systems based on flexible hydrocarbon linkers (**5** vs **3**). This suggests that the most accessible conformations of **4** and **5** are not optimal for reaction with the nucleophilic N-7 guanine atoms on the complementary DNA strands which most likely produce the ISC.[5] The observation that **5** is more active than **4b** is consistent with earlier observations that a three carbon linker between the epoxide units is optimal for biological activity.[5b] Interestingly, bisepoxide **4b** displayed significantly higher cytotoxicity and ISC activity than its counterpart **4a** lacking the MeO and Me substituents on the naphthalene ring.[13]

In conclusion, we have explored the synthesis of a series of azinomycin-like bisepoxides containing aromatic linkers using two

Table 2
Cytotoxicity and DNA ISC activity data

Bisepoxide	GI_{50} (μM)	Cytotoxicity[a] LC_{50} (μM)	TGI (μM)	DNA ISC activity[b] (%)
4a	4.4	74	25	1.7
4b	0.39	62	8.7	28
5	0.15	62	3.4	56
3[c]	0.031	3.2	0.21	100

[a] Measured against the NCI panel of 60 cancer cell lines.
[b] At 100 nM. See text for details.
[c] Data taken from Ref. 5b.

alternative strategies. The best yields were obtained by coupling 1,3- or 1,4-diaminobenzene with the requisite epoxy acids using EDCI/HOBt or HATU as activators. The new bisepoxides displayed good ISC efficiencies and appreciable in vitro cytotoxicities although they were less potent than structurally related compounds containing flexible hydrocarbon linkers. Future work will focus on identifying the origin of these differences in potency, and on designing new anti-cancer agents based upon this motif.

Acknowledgements

This work was supported financially by the University of Warwick and CRUK (C2259/A3083). We thank the EPSRC National Mass Spectrometry Service Centre for mass measurements, EPSRC (EP/C007999/1) for the provision of additional mass spectrometers and the NCI anti-cancer screening programme for the in vitro cytotoxicity data.

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10. Amide (2*S*)-**8** was prepared from (2*S*)-benzyl 2-hydroxy-3-methylbut-3-enoate (Ref. 9) by heating in a sealed tube with 7 M ammonia in methanol (40 °C, 16 h, 79%).
11. *Selected spectroscopic data for* **4a**: mp = 144–144.5 °C. $[\alpha]_D$ +3.8 (*c* 1.1, $CHCl_3$); IR (film) 3338, 2974, 1693, 1611, 1543, 1127 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) 8.94 (2H, d, *J* 8.5 Hz, ArH), 8.36 (2H, dd, *J* 7.3, 1.2 Hz, ArH), 8.08 (2H, d, *J* 8.0 Hz, ArH), 7.99 (2H, s, NH), 7.91 (2H, d, *J* 8.0 Hz, ArH), 7.64 (2H, m, ArH), 7.58–7.52 (8H, m, ArH), 5.33 (2H, s, H-2), 3.10 (2H, d, *J* 4.5 Hz, H-4), 2.87 (2H, d, *J* 4.5 Hz, H-4), 1.60 (6H, s, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$) 165.9 (C), 164.6 (C), 134.2 (CH), 133.8 (C), 133.7 (C), 131.4 (C), 130.8 (CH), 128.6 (CH), 128.2 (CH), 126.5 (CH), 125.68 (C), 125.58 (CH), 124.5 (CH), 120.7 (CH), 76.2 (C-2), 56.2 (C-3), 53.7 (C-4), 17.6 (CH_3). HRMS (ES^+) calculated for $C_{38}H_{36}N_3O_8$ requires 662.2497 $[M+NH_4^+]$; found 662.2508; **4b**: mp = 157.5–160.5 °C. $[\alpha]_D$ = +33.8 (*c* 1.1, $CHCl_3$); IR (film) 3337, 2976, 1694, 1611, 1543, 1191 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) 8.61 (2H, m, ArCH), 8.00 (2H, s, NH), 7.96 (2H, d, *J* 2.8 Hz, ArCH), 7.49–7.48 (6H, m, ArCH), 7.36–7.34 (4H, m, ArCH), 5.32 (2H, s, H-2), 3.98 (6H, s, $ArOCH_3$), 3.08 (2H, d, *J* 4.4 Hz, H-4), 2.85 (2H, d, *J* 4.4 Hz, H-4), 2.67 (6H, s, $ArCH_3$), 1.59 (6H, s, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$) 165.7 (C), 164.5 (C), 155.8 (C), 134.3 (C), 133.7 (C), 133.2 (C), 128.0 (C), 127.8 (CH), 126.8 (C), 125.2 (CH), 123.7 (CH), 122.1 (CH), 120.6 (CH), 108.4 (CH), 76.3 (C-2), 56.2 (C-3), 55.5 (CH_3), 53.5 (C-4), 20.1 (CH_3), 17.7 (CH_3). HRMS (ES^+) calculated for $C_{42}H_{44}N_3O_{10}$ requires 750.3021 $[M+NH_4^+]$; found 750.3019; **5**: mp = 131–133 °C. $[\alpha]_D$ = +56.4 (*c* 1.2, $CHCl_3$); IR (film) 3349, 2934, 1684, 1614, 1540 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) 8.60 (2H, m, ArH), 8.13 (2H, s, NH), 7.94 (2H, d, *J* 2.5 Hz, ArCH), 7.80 (1H, s, ArCH), 7.44 (2H, d, *J* 2.5 Hz, ArCH), 7.38–7.33 (6H, m, 6 × ArCH), 7.23–7.19 (1H, m, ArCH), 5.30 (2H, s, H-2), 3.95 (6H, s, $ArOCH_3$), 3.09 (2H, d, *J* 4.4 Hz, H-4), 2.84 (2H, d, *J* 4.4 Hz, H-4), 2.66 (6H, s, $ArCH_3$), 1.58 (6H, s, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$) 165.7 (C), 164.7 (C), 155.7 (C), 137.6 (C), 134.3 (C), 133.1 (C), 129.5 (CH), 127.9 (C), 127.7 (CH), 126.8 (C), 125.2 (CH), 123.7 (CH), 122.1 (CH), 116.2 (CH), 111.5 (CH), 108.5 (CH), 76.3 (C-2), 56.2 (C-3), 55.5 (CH_3), 53.4 (C-4), 20.0 (CH_3), 17.7 (CH_3). HRMS (ES^+) calculated for $C_{42}H_{44}N_3O_{10}$ requires 750.3021 $[M+NH_4^+]$; found 750.3025.
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