Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 239–241

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Chemical Synthesis and Cytotoxicity of some Azinomycin Analogues Devoid of the 1-Azabicyclo[3.1.0]hexane Subunit

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Received 7 October 1999; accepted 22 November 1999

Abstract—A series of compounds related to the left-hand domain of the azinomycins have been made and evaluated for cytotoxic activity against a small panel of human tumour cell lines. The epoxide ring is shown to be essential for biological activity. Cytotoxicity is also shown to be sensitive to changes in the substitution pattern on the aromatic ring and the amide group.

In 1986, azinomycins A **1** and B **2** were isolated from the culture broths of *Streptomyces griseofuscus* S42227 and were found to exhibit potent in vitro cytotoxic activity and significant in vivo anti-tumour activity (Fig. 1).[1–4] Armstrong et al.[5] have shown that azinomycin B causes interstrand cross-links in the major groove of duplex DNA by initial alkylation at guanine and subsequent reaction at a second purine residue two bases along on the complimentary strand of the DNA duplex. Further studies by Fujiwara et al.[6] have revealed more details concerning the chemical events which lead to these interstrand cross-links. Along with the azinomycins, an additional metabolite **3** devoid of the 1-azabicyclo[3.1.0]hexane ring system was isolated from the culture broths of *S. griseofuscus* S42227.[1–3] It was originally concluded that this material lacked anti-bacterial or anti-tumour activity,[3] however further studies by Terashima[7] have indicated that this compound possesses strong cytotoxic activity against P388 murine leukemia [IC_{50} (μg/mL) 0.0036].[7] In other work, azinomycin–lexitropsin hybrid molecules (e.g. **4**) containing the left hand domain of the azinomycins have been shown to possess DNA-cleaving activity.[8] From these and other studies, it appears that the epoxide domains of compounds **1**–**4** play a key role in their biological activity. In this Letter, we describe the synthesis and cytotoxic activity of a range of compounds related to epoxide **1** in which systematic modifications have been made to this structural motif. These studies provide some new insights into the nature of the interactions between the epoxide domains of agents **1**–**4** and DNA.

A series of primary amides **7a**–**e** possessing different aromatic chromophores were made from homochiral epoxide (2*S*,3*S*)-**5** to evaluate how the nature of the aromatic group influences cytotoxicity. These compounds were made by acylation of the secondary hydroxyl group of **5**[9,10] and subsequent conversion of the

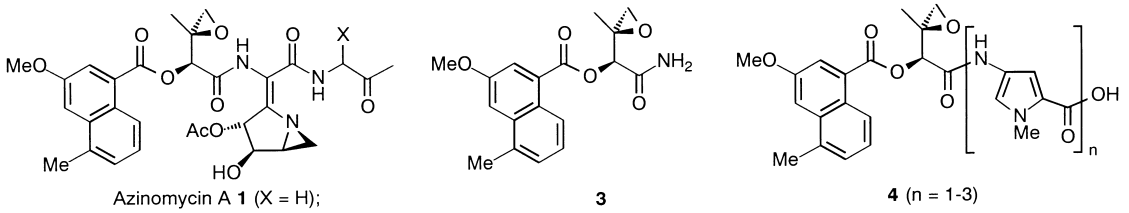

Figure 1.

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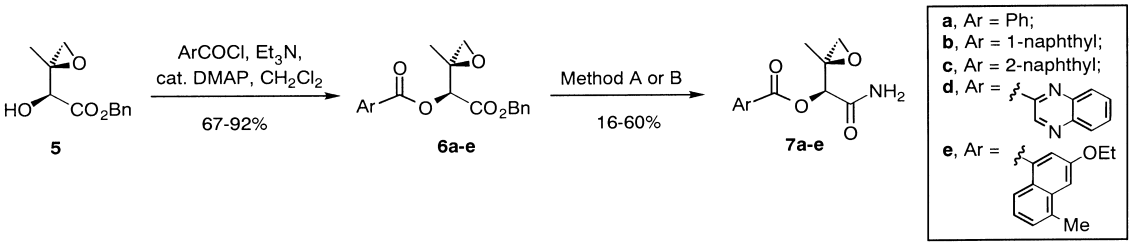
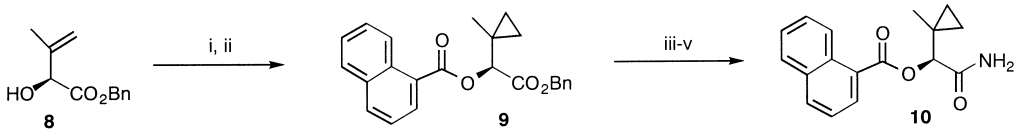


Scheme 1. Method A: (i) H_2, Pd/C, MeOH; (ii) NH_4OH, Et_3N, HOBt, PyBOP, DMF. Method B: (i) H_2, Pd/C, MeOH; (ii) 4-methoxybenzlamine, Et_3N, HOBt, PyBOP, DMF; (iii) CAN, $MeCN/H_2O$.



Scheme 2. Reagents and conditions: (i) Et_2Zn, CH_2I_2, CH_2Cl_2, 87%; (ii) 1-naphthoyl chloride, Et_3N, DMAP, CH_2Cl_2, 91%; (iii) H_2, Pd/C, MeOH; (iv) 4-methoxybenzylamine, Et_3N, HOBt, PyBOP, DMF, 93% from **9**; (v) CAN, $MeCN/H_2O$, 73%.

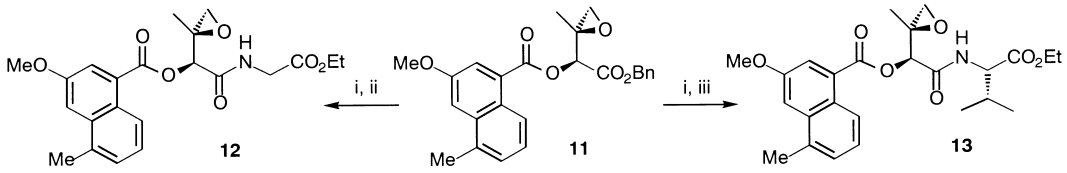
benzyl ester into the primary amide (Scheme 1). Two different methods were used for this later conversion, although we have found that the more direct approach is generally higher yielding (i.e. Method A). In addition, (2*R*,3*R*)-**3** was made in an identical fashion to our published route to (2*S*,3*S*)-**3** by using AD-mix-β in the asymmetry inducing dihydroxylation step.[9,10]

In accordance with the postulated mechanism of action of the azinomycins,[5,6] we imagined that the cytotoxic activity of metabolite **3** might arise from monoalkylation of DNA by the reactive epoxide moiety. To test this hypothesis, (2*S*)-**10** was made in which the epoxide ring was replaced by the isosteric but quite inert cyclopropane ring. This was achieved by cyclopropanation[11] of homochiral allylic alcohol (2*S*)-**8**[9,10] followed by conversion to (2*S*)-**10** along similar lines to that described above (Scheme 2). From the chemical standpoint, it is notable that no concomitant cleavage of the cyclopropane ring was observed during the hydrogenation step.

To study the role of the amide substitution pattern, secondary amides **12** and **13** were made from (2*S*,3*S*)-**11**[10] by selective cleavage of the benzyl ester followed by coupling with glycine ethyl ester and (*S*)-valine ethyl ester respectively (Scheme 3).

With these compounds in hand, our attention turned to assessing their biological activity. All the compounds were tested for in vitro cytotoxic activity against a small panel of human tumour cell lines {A2780, A2780cisR,[12] CH1, SKOV-3 (all ovarian) and HT29 (colon)} (Table 1).[13] Consistent with Terashima's observations,[7] we have determined that epoxide (2*S*,3*S*)-**3** is highly cytotoxic and has potency comparable to the established anti-tumour agents mitomycin C and cisplatin (entry 1, cf. entries 11 and 12). The enantiomer of this compound, (2*R*,3*R*)-**2**, was found to be slightly less potent indicating some level of chiral recognition (entry 1, cf. entry 2). The epoxide ring is clearly important for cytotoxic activity as cyclopropane **10** was essentially inactive (entry 4, cf. entry 8). The potency of these compounds is highly dependent on the nature of the aromatic residue (entry 1, cf. entries 3–7). Even a very subtle change from the methyl to ethyl ether at C-3 of the naphthalene residue results in a substantial loss of potency (entry 1, cf. entry 7). It is also notable that epoxide **7d** containing the 2-quinoxaloyl group, an established DNA intercalating group,[14] was completely inactive.[15] Additionally, it is interesting that we observed a loss of potency when the amide functionality was further substituted (entry 1 cf. entries 9 and 10).

In the case of four representative compounds ((2*S*,3*S*)-**3** (2*R*,3*R*)-**3**, (2*S*,3*S*)-**7b** and (2*S*)-**10**), we have obtained more direct evidence that these compounds induce cytotoxicity by alkylating DNA. This was accomplished by undertaking DNA binding-sequence specificity studies using the taq polymerase stop assay.[16] In these experiments, good correlation was observed between DNA binding and cell growth inhibition potency. Cyclopropyl amide **10** did not produce any taq stop sites over background DMSO control levels at up to 50 μM. The other three compounds produced binding affinities which paralled their potency in the cytotoxicity tests. Further studies directed towards gaining greater



Scheme 3. Reagents and conditions: (i) H_2, Pd/C, MeOH; (ii) $EtO_2CCH_2NH_2.HCl$, Et_3N, HOBt, PyBOP, DMF; 89% from **11**; (iii) (*S*)-$EtO_2CCH(^iPr)NH_2.HCl$, Et_3N, HOBt, PyBOP, DMF, 85% from **11**.

Table 1.

Entry	Compound	Cytotoxic activity[a] against:				
		A2780	A2780cisR	CH1	SKOV-3	HT29
1	(2*S*,3*S*)-**3**	< 0.05	0.076	< 0.05	1.25	0.33
2	(2*R*,3*R*)-**3**	0.058	0.155	0.062	2.3	0.61
3	(2*S*,3*S*)-**7a**	20.5	>25	>25	>25	>25
4	(2*S*,3*S*)-**7b**	0.44	1.1	0.55	5.1	2.15
5	(2*S*,3*S*)-**7c**	0.39	1.35	0.17	13.0	2.6
6	(2*S*,3*S*)-**7d**	>25	>25	>25	>25	>25
7	(2*S*,3*S*)-**7e**	1.8	3.5	1.4	12.0	4.3
8	(2*S*)-**10**	>25	>25	>25	>25	>25
9	(2*S*,3*S*)-**12**	0.34	0.83	0.26	7.3	2.25
10	(2*S*,3*S*)-**13**	0.35	0.56	0.315	2.95	2.25
11	Mitomycin C	0.05	0.06	0.04	0.2	0.04
12	Cisplatin	0.3	3.0	0.1	4.4	1.7

[a]96-h incubation, IC_{50} (μM) values.

insight into the molecular mechanism of action of these and other azinomycin analogues are ongoing and these results will be disclosed in due course.

Acknowledgements

We gratefully acknowledge the financial support provided by the Cancer Research Campaign (to T. J. H. and F. S.). We are indebted to the EPSRC National Mass Spectrometry Centre for performing some of the mass spectral measurements and the EPSRC Chemical Database Service at Daresbury.[17]

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12. A2780cisR is a subline of A2780 possessing acquired resistance (ca. 10-fold) to the commonly used DNA cross-linking reagent cisplatin.
13. Cytotoxicity assay: Cells were added to 96-well microtitre plates at 5×10^3/well and allowed to attach overnight. The compounds were dissolved immediately before use in DMSO (at 20 mM) and diluted in tissue culture growth medium prior to adding to the cell lines at a range of final concentrations (from 100 μM down to 2.5 nM). Drugs remained in contact with the cells throughout a 96-h incubation period before assessing for growth inhibitory effects using the Sulforhodamine B (SRB) assay. For a description of this SRB assay, see Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.; Bokesch, H.; Kennedy, S.; Boyd, M. R. *J. Nat. Cancer Inst*. **1990**, *82*, 1107.
14. For example, see Wang, A. H.-J.; Ughetto, G.; Quigley, G. J.; Hakoshima, T.; van der Marel, G. A.; van Boom J. H.; Rich, A. *Science* **1984**, *225*, 1115.
15. This lack of biological activity may be attributed to poor transport through the cell membrane. While we have not directly measured the lipophilicity of this compound, we have noted that it displays poor solubility in organic solvents compared with the other analogues.
16. The technique involved incubation of these compounds with plasmid DNA of known sequence (2 h at 37 °C). Cisplatin was run as a control in these experiments. For a description of this assay, see Ponti, M.; Forrow, S. M.; Souhami, R. L.; D'Incalci, M.; Hartley, J. A. *Nucl. Acid Res*. **1991**, *19*, 2929.
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