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Bioorganic & Medicinal Chemistry Letters 15 (2005) 653–656
Bioorganic & Medicinal Chemistry Letters

Truncated azinomycin analogues intercalate into DNA

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Received 24 September 2004; revised 11 November 2004; accepted 15 November 2004
Available online 8 December 2004

Abstract—The design and synthesis of a potentially more therapeutically-viable azinomycin analogue **4** based upon **3** has been completed. It involved coupling of a piperidine mustard to the acid chloride of the azinomycin chromophore. Both the designed azinomycin analogue **4** and the natural product **3** bind to DNA and cause unwinding, supporting an intercalative mode of binding.
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The azinomycins (**1**, **2**) are potent antitumour antibiotics, isolated from *Streptomycetes griseofucus* (Fig. 1).[1] Their densely functionalized structures[1] have made them interesting targets for synthetic organic chemists,[2] while their mode of action has been investigated in a smaller number of investigations.[2,3] These latter studies have addressed the ability of the compounds to alkylate and crosslink DNA,[4] their sequence selectivity[5,6] and the ability of the compounds to intercalate into the double helix.[6,7] The therapeutic potential of the azinomycins has been less well studied, mainly due to the unstable nature of the antibiotics, which makes their utility very limited. Some analogues, either designed[8] or as partial structures in synthetic routes to the agents,[9] have been disclosed. Here, we describe our own initial investigations of the synthesis of azinomycin analogues, which led to the observation that these compounds intercalate into the DNA duplex. Contrary to previous reports, we discovered that the natural product partial structure **3** also has similar characteristics, which suggest that it intercalates into the duplex.

Compound **3** represents the 'left-half' structure of the azinomycins and was also isolated as a natural product (Fig. 2).[10] Although initial studies suggested that it was

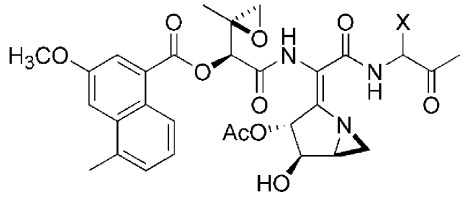

Figure 1. Azinomycin A **1** X = H, azinomycin B **2** X = CHO.

inactive in tumour cell lines, further investigations with **3** have demonstrated that it possesses potent antitumour activity in its own right,[11] that it alkylates DNA non-sequence selectively at all guanines[6] and that partial structures that cannot alkylate have a non-covalent DNA binding affinity, assessed by equilibrium dialysis methods, of around 10^{-3} M.[6] While one group has suggested DNA intercalation of the naphthalene through investigations of viscometric and unwinding effects,[6] Coleman et al. used noncovalently binding analogues of **3** (although not **3**) and, with similar assays, found that intercalation did not occur.[7] They also used fluorescence contact transfer[12] with **3** to support the lack of intercalation of the chromophore.

Although **3** has more therapeutic potential than **1** and **2**, we feel that the presence of the epoxide and ester bonds limit its utility. However, it makes a good lead compound to begin investigations of analogues that may have properties useful in the design of prodrugs. Due to our interest in potential bioreductives,[13] we designed

Keywords: Azinomycin; Intercalation; DNA unwinding; Synthesis; Analogues.
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doi:10.1016/j.bmcl.2004.11.037

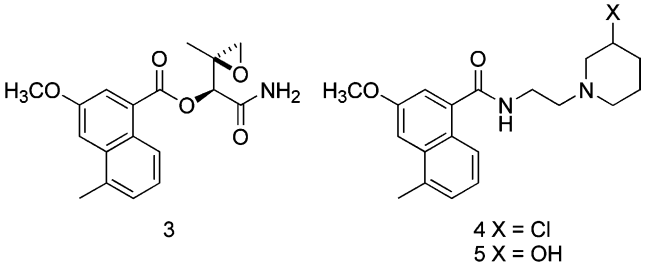


Figure 2. Compounds **3**–**5** used in this study.

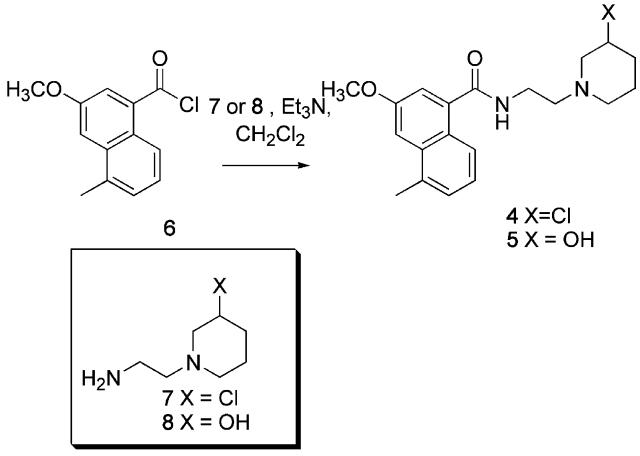
4, a nitrogen mustard similar to **3** in that it could alkylate DNA and place the chromophore in close proximity to the duplex, but contains a more robust amide bond between the chromophore and the alkylating group (Fig. 2).

Compound **4** was synthesized from the reaction of naphthoate acid chloride **6**[14] and the piperidine analogue **7**.[15] The acid chloride was generated by treatment of the acid with thionyl chloride under reflux (92% yield) and was used without further purification. The piperidine derivative **7** was generated from the Boc-protected amine via treatment with HCl in EtOAc (2 M). Direct addition of **7** was found to be more efficient than addition of the hydroxyl compound **8** followed by halogenation and gave the product **4** in 55% yield after purification.[16] Compound **5**, the control nonalkylating version of **4**, was synthesized in a similar fashion from the reaction of the piperidine alcohol **8** with **6** (Scheme 1).[17]

With **4** in hand, it was possible to begin investigations of its biological properties. Unwinding of supercoiled ϕX174 DNA, whilst not a definitive assay, has been used extensively to show DNA binding by intercalation for a number of bisintercalators.[18] Similar unwinding assays have been used for other intercalator–alkylator complexes.[19] The supercoiled DNA is relaxed by binding of the intercalator, then begins to form negative supercoils at higher concentrations. Compound **4** clearly unwinds supercoiled DNA in a similar fashion to known intercalators (Fig. 3a). Complete unwinding is observed at a 1:1 ratio of drug to base pairs. Compound **5**, the hydroxyl-analogue of **4** that cannot alkylate DNA, does not unwind the duplex. Although, as noted, DNA unwinding is not a definitive assay for intercalation, it is striking that such a simple compound possesses characteristics very similar to those of known intercalators.

Having found that, upon alkylation, the naphthoate group seemed to intercalate into the helix, we then studied the interaction of the natural product **3** with ϕX174 DNA. Compound **3** was synthesized by the method of Shipman and co-workers[20] although, as we required all four diastereoisomers of **3**, the method was adapted to allow formation of both the (2*S*, 3*S*)- and (2*S*, 3*R*)-compounds.[21] Unwinding of supercoiled DNA by **3** was similar to that of **4**, suggesting that in accordance with the viscometric results of Zang and Gates,[6] the natural product epoxide is certainly unwinding supercoiled DNA, probably by intercalative binding.

One further possibility for these compounds was that DNA nicking, rather than unwinding, was occurring with the supercoiled ϕX174 DNA. Clinically used antitumour agents such as melphalan are able to cleave supercoiled DNA to a relaxed (Form I) and linear (Form II) form even under the mild conditions of the experiment for unwinding (Fig. 3c). When the unwinding experiment is carried out with a classical intercalator such as echinomycin using agarose gels containing ethidium bromide (EtBr), the DNA reverts to a supercoiled form, presumably as EtBr competes with the intercalator (data not shown). With a nicked DNA duplex, the reaction is irreversible as nicking occurs prior to electrophoresis. Repetition of the DNA unwinding experiment with **3** and **4** with agarose gels containing EtBr showed no unwinding of the duplex (data not shown), while the melphalan gel is unaffected (Fig. 3c). This suggests that the unwinding/relaxation of supercoiled DNA is a pro-



Scheme 1. Synthesis of **4** and **5**.

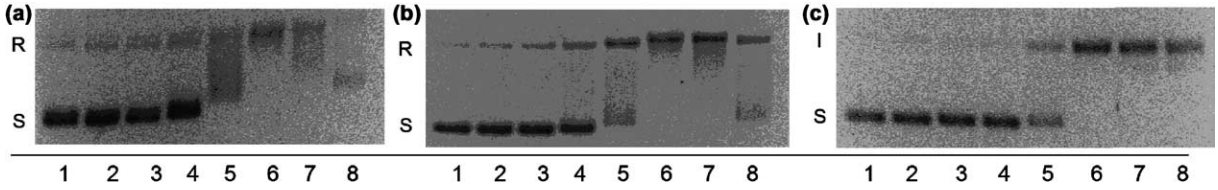


Figure 3. (a) and (b) DNA unwinding by (a) **4**, (b) **3**. Supercoiled ϕX174 DNA. Lane (1) DNA only; Lanes (2–8) 0.001, 0.01, 0.1, 1.0, 2.0, 3.0 drug/bp ratio. Concentration DNA 0.34 mM bp; agarose gel electrophoresis was carried out and the gels were then stained with ethidium bromide. A similar experiment with EtBr in the gel showed no unwinding, (c) DNA cleavage by melphalan. Agarose gel electrophoresis was carried out with EtBr present in the gel. A gel with post-electrophoresis staining with EtBr looked exactly similar. Concentrations are as for the unwinding gels. S = supercoiled, R = relaxed, I = form I DNA.

cess that does not involve DNA cleavage.† This latter result is also suggestive of a reversible interaction between the azinomycins and DNA, which may allow the natural product to equilibrate to a favoured alkylation site. More experiments are underway to ascertain the veracity of this observation.

The cytotoxicity of compounds **3** (IC_{50} 50 nM), **4** and **5** (both inactive) against the prostate cancer cell line DU145 was determined using the MTT assay. The lack of activity of **4** was somewhat surprising and may reflect a change in the uptake of the intercalator–alkylator conjugate on going from the uncharged epoxide to the piperidine-mustard, which would carry a positive charge at the pH of the experiment. However, other intercalator–piperidine-mustard conjugates give good cell-culture-based antitumour activities.[15] Compound **3** maintains the potent activity seen in previous studies with this compound.[11] The lack of activity of **4** was disappointing in view of its ability to alkylate and unwind DNA in a similar fashion and we are currently generating analogues of **4** with different alkylating functionalities in order to understand this loss of activity.

Taken together, we believe these results, along with those already published, suggest a mechanism of action for the azinomycins and, perhaps, a different biological effect (leading to the same outcome—cell death) for the crosslinking and noncrosslinking agents. The low affinity of the naphthoate moiety is enough to locate these compounds close to the DNA helix. This poor binding, probably represented by rapid association and dissociation rates at nonsequence selective intercalation sites on the duplex, is enough to locate the alkylating moieties close to DNA nucleophiles. Alkylation by the natural products can presumably occur either at the epoxide or aziridine moiety when a G is suitably positioned either adjacent to, or three bases from the intercalation site.[22] When a monoalkylation event occurs, the chromophore may intercalate into the DNA helix. However, when a G is suitably positioned for crosslinking, the intercalation of the chromophore becomes energetically unfavourable[6] and a different drug–DNA adduct is formed. In the truncated analogues, the naphthalene is able to intercalate and, due to the covalent nature of the adduct, maintains a high affinity for DNA. Intercalation necessarily involves duplex distortion, whereas the crosslinking events with the natural product are likely to distort the DNA in a different fashion. Interestingly, Hartley et al.[23] found that a noncrosslinking analogue of azinomycin A had similar antitumour activity to the crosslinking compound, suggesting either that the two adducts have similar potent activity or that the activity of the monoadduct predominates. Our own studies of crosslinking agents suggest that monoalkylation is sufficient for biological activity and that crosslinking may even be detrimental.[24] Only high resolution structural studies and biological studies of the signals transduced as a consequence of binding of these agents will ultimately answer remaining questions as to the mechanism of action of the azinomycins. In the meantime, studies will continue to find therapeutically useful analogues of these intriguing natural products.

† It should be noted that incubation times >2 h with **3** and **4** led to observation of time-dependant DNA cleavage, confirming that alkylation is occurring.

Acknowledgements

We thank ULSOP for a Millennium studentship (MCH) and the Engineering and Physical Science Research Council for funding (Grant No. GR/R47646/01), the School of Pharmacy Mass Spectrometry service for analyses.

References and notes

1. (a) Nagaoka, K.; Matsumoto, M.; Oono, J.; Yokoi, K.; Ishizeki, S.; Nakashima, T. *J. Antibiot.* **1986**, *39*, 1527; (b) Yokoi, K.; Nagaoka, K.; Nakashima, T. *Chem. Pharm. Bull.* **1986**, *34*, 4554.
2. Hodgkinson, T. J.; Shipman, M. *Tetrahedron* **2001**, *57*, 4467.
3. Casely-Hayford, M.; Searcey, M. The azinomycins. Discovery, Synthesis and DNA-Binding Studies. In *DNA and RNA Binders From Small Molecules to Drugs*; Demeunynck, M., Bailly, C., Wilson, W. D., Eds.; Wiley-VCH: Weinheim, 2002; pp 676–696.
4. Armstrong, R. W.; Salvatti, M. E.; Nguyen, M. *J. Am. Chem. Soc.* **1992**, *114*, 3144.
5. Coleman, R. S.; Perez, R. J.; Burk, C. H.; Navarro, A. *J. Am. Chem. Soc.* **2002**, *124*, 13008.
6. Zang, H.; Gates, K. S. *Biochemistry* **2000**, *39*, 14968.
7. Coleman, R. S.; Burk, C. H.; Navarro, A.; Brueggemeier, R. W.; Diaz-Cruz, E. S. *Org. Lett.* **2002**, *40*, 3545.
8. Hartley, J. A.; Hazrati, A.; Hodgkinson, T. J.; Kelland, L. R.; Khanim, R.; Shipman, M.; Suzenet, F. *Chem. Commun.* **2000**, 2325–2326.
9. Hashimoto, M.; Matsumoto, M.; Yamada, K.; Terashima, S. *Tetrahedron* **2003**, *59*, 3089.
10. Ishizeki, S.; Ohtsuka, M.; Irinoda, K.; Kukita, K.-I.; Nagaoka, K.; Nakashima, T. *J. Antibiot.* **1987**, *40*, 60.
11. Hashimoto, M.; Matsumoto, M.; Yamada, K.; Terashima, S. *Tetrahedron Lett.* **1994**, *35*, 2207.
12. Suh, D.; Chaires, J. B. *Bioorg. Med. Chem.* **1995**, *3*, 723.
13. Searcey, M.; Patterson, L. H. *Curr. Med. Chem.—Anti-Cancer Agents* **2004**, *4*, 457.
14. Shibuya, M. *Tetrahedron Lett.* **1983**, *24*, 1175.
15. Pors, K.; Schnyder, S.; Bibby, M. C.; Teesdale-Spittle, P. H.; Hartley, J. A.; Searcey, M.; Patterson, L. H., submitted for publication.
16. **4** As a colourless oil (50 mg, 58%). ^1H NMR δ_H ppm ($CDCl_3$, 400 MHz) 8.07 (1H, d, J = 8 Hz, ArH), 7.33–7.27 (4H, m, ArH), 6.56 (1H, br s, NH), 3.94 (3H, s, OCH_3), 3.62–3.60 (2H, m, CH_2), 2.65 (3H, s, $ArCH_3$), 2.62 (2H, m, CH_2), 2.39 (1H, m, CH), 1.83 (2H, m, CH_2), 1.56 (2H, m, CH_2), 1.31 (2H, m, CH_2). δ_C ppm (100 MHz; $CDCl_3$) 169.21 (C=O), 156.37 (ArC), 136.89 (ArC), 134.24 (ArC), 133.15 (ArC), 127.63 (ArC), 125.62 (ArC), 124.21 (ArC), 123.53 (ArC), 117.04 (ArC), 105.05 (ArC), 61.01 (CH_3), 56.18 (CH_2), 55.83 (CH_2), 52.73 (CH_2), 50.80 (CH_2), 36.60 (CH), 34.43 (CH_2), 24.25 (CH_2), 19.93 (CH_3). FABMS, m/z $(M+H)^+$ 361.

17. **5** Was a reddish brown oil (60 mg, 58%). ^{1}H NMR δ_H ppm ($CDCl_3$, 400 MHz) 8.04 (1H, d, J = 8 Hz, ArH), 7.32–7.28 (4H, m, ArH), 6.51 (1H, br s, NH), 3.94 (3H, s, OCH_3), 3.76 (1H, br s, OH), 3.62–3.58 (2H, q, CH_2), 2.64 (3H, s, $ArCH_3$), 2.61–2.58 (2H, t, CH_2), 2.41 (1H, m, CH), 1.76 (2H, m, CH_2), 1.66 (2H, m, CH_2), 1.48 (2H, m, CH_2). δ_C ppm (100 MHz; $CDCl_3$) 169.32 (C=O), 156.36 (ArC), 136.91 (ArC), 134.23 (ArC), 133.17 (ArC), 127.63 (ArC), 125.58 (ArC), 124.23 (ArC), 123.44 (ArC), 117.02 (ArC), 104.99 (ArC), 66.35 (CH), 60.32 (CH_2), 56.91 (CH_3), 55.46 (CH_2), 53.50 (CH_2), 50.77 (CH_2), 36.74 (CH_2), 31.94 (CH_2), 21.92 (CH_2), 19.92 (CH_3). FABMS, m/z $(M+H)^+$ 343.
18. Boger, D. L.; Ledeboer, M.; Kume, M.; Searcey, M.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 11375.
19. Gourdie, T. A.; Prakash, A. S.; Wakelin, L. P.; Woodgate, P. D.; Denny, W. A. *J. Med. Chem.* **1991**, *34*, 240.
20. 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.
21. Casely-Hayford, M. A.; David, M.-H.; James, C. H.; Patterson, L. H.; Bailly, C.; Searcey, M., submitted for publication.
22. Although no sequence selectivity data on long sequences of DNA has been published for the original compounds, Armstrong (Ref. 4) noted that alkylation at G appeared to occur nonsequence selectively for carzinophilin (azinomycin B).
23. Hartley, J. A.; Hazrati, A.; Kelland, L. R.; Khanim, R.; Shipman, M.; Suzenet, F.; Walker, L. F. *Angew. Chem., Int. Ed.* **2000**, *39*, 3467.
24. Casely-Hayford, M.; Pors, K.; Patterson, L. H.; Hartley, J. A.; Searcey, M., submitted for publication.