Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1075–1077

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A 3,4-Epoxypiperidine Structure as a Novel and Simple DNA-Cleavage Unit

Kazuyuki Miyashita, Myunji Park, Sayako Adachi, Sayori Seki, Satoshi Obika and Takeshi Imanishi*

Graduate School of Pharmaceutical Sciences Osaka University, 1-6 Yamadaoka, Suita, Osaka 565–0871, Japan

Received 26 December 2001; accepted 26 January 2002

Abstract—Based on the 4-hydroxy-1-azabicyclo[3.1.0]hexane structure of azinomycin, a 3,4-epoxypiperidine structure was designed as a novel and simple alkylating molecular unit, and some 3,4-epoxypiperidine derivatives were found to show DNA-cleavage activity, the structural requirements for which were revealed. © 2002 Elsevier Science Ltd. All rights reserved.

An alkylating agent is an attractive group of biologically active compounds such as an anticancer agent or as an enzyme inhibitor. Although a number of molecules causing alkylation of nucleophiles in a biological system have been evaluated, it is still required to create a molecular unit having a more potent alkylating ability.[1]

In order to design a novel molecule having DNA-alkylating ability, we focused on azinomycins (Fig. 1) which are an antitumor antibiotic isolated from *Streptomyces* sp.[2] and are known to work as a DNA cross-linking agent. As is easily expected from the structure, 1-azabicyclo[3.1.0]hexane and oxirane ring units are responsible for the activity and were found to alkylate a guanosine and/or an adenosine residue of a genomic DNA duplex.[3] Because of its important biological activity and its characteristic structure, a number of synthetic studies on azinomycin and its analogues have been conducted,[4] and the first total synthesis of azinomycin has been achieved recently by Coleman's group.[5] Through these studies, it has become known that the 4-hydroxy-1-azabicyclo[3.1.0]hexane ring system is highly unstable: the hydroxyl protected compounds are stable enough to isolate, but deprotection of the hydroxyl group greatly lowers the stability.[6] However, no information regarding the decomposition mechanism and the decomposition product has ever been reported.

As some examples of ring contraction from a piperidine ring system having a leaving group at C-3 to a 1-azabicyclo[3.1.0]hexane ring system via an intramolecular substitution reaction have been reported,[7] it occurred to us that the neighboring hydroxyl group of the aziridine ring of **1** might promote the ring opening to transform into an epoxypiperidine as shown in Figure 2. We assumed, if such promotion occurs, the 3,4-epoxypiperidine could also work as an alkylating agent. Based on this hypothesis, we synthesized 3,4-epoxypiperidine derivatives **2** and studied their DNA-cleavage activity.

The epoxides *cis*- and *trans*-**2** were synthesized as shown in Scheme 1. Unsaturated alcohol **3**[8] was epoxidized with *m*-CPBA to give *cis*-epoxide exclusively, sub-

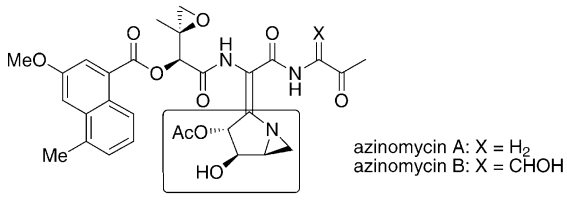


Figure 1. Structures of azinomycins A and B.

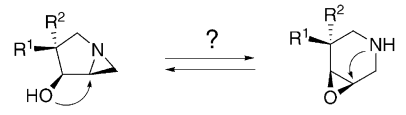


Figure 2. Design concept for 3,4-epoxypiperidine.

*Corresponding author. Tel.: +81-6-6879-8200; fax: +81-6-6879-8204; e-mail: imanishi@phs.osaka-u.ac.jp

PII: S0960-894X(02)00085-9

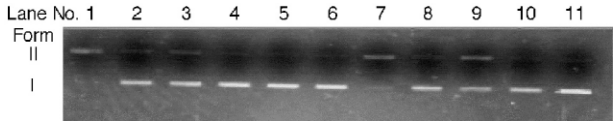


Figure 3. DNA cleavage activity for *trans*-**2**, *cis*-**2**, **6**, (*S*)-*trans*-**2**, and (*R*)-*trans*-**2**.[11] Form I: supercoiled pBR 322 DNA; Form II: relaxed DNA, lanes 1 and 2: *trans*-**2**; lanes 3 and 4: *cis*-**2**; lanes 5 and 6: **6**; lanes 7 and 8: (*S*)-*trans*-**2**; lanes 9 and 10: (*R*)-*trans*-**2**; lane 11: control. Concentrations of the compounds are 100 and 10 μM, respectively.

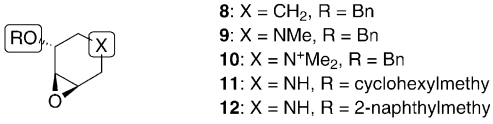
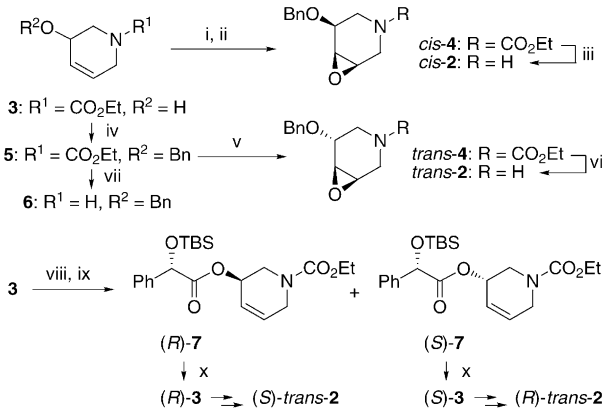


Figure 4. Structures of racemic *trans*-derivatives **8**–**12**.



Scheme 1. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2 (88%); (ii) BnBr, NaH, THF (91%); (iii) MeLi, THF then H_2O (quant); (iv) BnBr, NaH, THF (95%); (v) *m*-CPBA, CH_2Cl_2 (*trans*/*cis* = ca. 4:1, *trans*-**4**, 78%); (vi) MeLi, THF then H_2O (quant); (vii) MeLi, THF then H_2O (quant); (viii) *O*-*tert*-butyldimethylsilyl-(*S*)-mandelic acid, EDC·HCl, DMAP, THF (81%); (ix) silica gel chromatography; (x) 0.2 M NaOH–EtOH (90%).

sequent benzylation of which afforded *cis*-**4**. On the other hand, **3** was benzylated, then oxidized with *m*-CPBA to give an isomeric epoxide *trans*-**4** as a main product. The ethoxycarbonyl group of *cis*- and *trans*-**4** was removed by treatment with methyllithium to afford *cis*- and *trans*-**2**,[9] respectively, in almost quantitative yield.[10] In order to investigate the role of the epoxide structure, olefinic compound **6** was also prepared similarly.

At first, the activity of *cis*-**2**, *trans*-**2** and **6** with respect to DNA was examined by a relaxation assay of supercoiled plasmid DNA.[11] As shown in Figure 3, relaxation was observed with *trans*-**2** at the concentration of 100 μM (lane 1), while weak activity was observed on treatment with *cis*-**2** at the same concentration (lane 3). In contrast, the olefinic compound **6** was inactive (lanes 5 and 6). These results clearly show that the 3,4-epoxypiperidine structure has DNA-cleavage activity as expected, and also indicate the important role of the epoxide structure and the stereochemistry.

The optically active compounds, (*S*)-*trans*-**2** and (*R*)-*trans*-**2**, were also obtained by optical resolution of the corresponding mandelate ester **7** as shown in Scheme 1. The resulting (*R*)- and (*S*)-**3**[12] were transformed to (*S*)- and (*R*)-*trans*-**2**, respectively. The (*S*)-isomer was found to be more active than the (*R*)-isomer (lanes 7 and 8 versus lanes 9 and 10) (Fig. 4).

Focusing on the other two functional groups, the amino group and the benzyloxy group, in the molecule of *trans*-**2**, we further examined the DNA-cleavage activity of other racemic *trans*-derivatives, *trans*-**4** and **8**–**12**,[13] to investigate the structure–activity relationship (Fig. 5).

The amino group was found to be essentially important, because ethoxycarbonyl derivative *trans*-**4** and cyclohexane derivative **8** did not show the activity at all (lanes 3–6). In contrast, the activity of *N*-methyl derivative **9** was lower than that of *trans*-**2**, but remained (lanes 7 and 8). *N,N*-Dimethylammonium derivative **10** also showed faint activity (lanes 9 and 10), suggesting that, on DNA-cleavage, the amino group could be transformed to the corresponding ammonium form to interact with a phosphate linkage. Taking account of the fact that the ammonium derivative **10** has the activity, it is obvious that the alkylation of the DNA takes place via a nucleophilic cleavage of the epoxide form,[14] but the possibility still remains that the alkylation involves two pathways, via an epoxide form and an aziridine form, as the activity of **10** was much weaker than that of *trans*-**2**.

Compound **11** in which the benzyl group of *trans*-**2** was replaced with a cyclohexylmethyl group completely lost the activity (lanes 11 and 12), while **12** having a 2-naphthylmethyl group showed greatly increased activity (lanes 13 and 14). These results strongly suggest that the aromatic ring works as an intercalator.

In conclusion, although details of the mechanism for the DNA cleavage of the 3,4-epoxypiperidines are still unclear, the present results showed that 3,4-epoxypiperidines can work as a novel and potent DNA-cleavage unit, and also revealed the structural requirements for the activity. As is obvious from the results, the free amino group and the aromatic group play an important role in the DNA-cleavage. As the urethane derivative *trans*-**4** was inactive, a trigger function, activating the

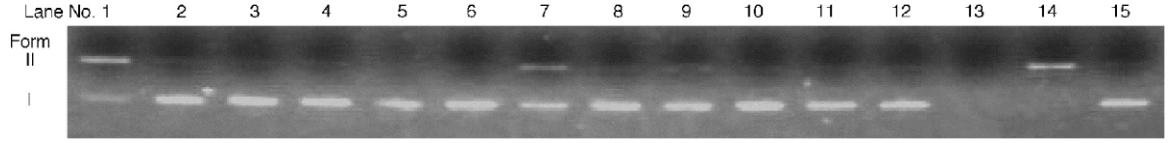
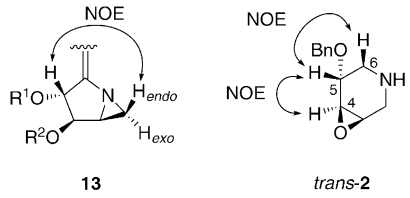


Figure 5. DNA cleavage activity for *trans*-**2**, **4** and **8**–**12**.[11] Form I: supercoiled pBR 322 DNA; Form II: relaxed DNA, lanes 1 and 2: *trans*-**2**; lanes 3 and 4: **4**; lanes 5 and 6: **8**; lanes 7 and 8: **9**; lanes 9 and 10: **10**; lanes 11 and 12: **11**; lanes 13 and 14: **12**; lane 15: control. Concentrations of the compounds are 100 and 10 μM, respectively.

molecule under particular conditions, would be easily introduced by choosing a proper *N*-protecting group. Although the DNA cleavage activity of the present compounds are not high compared with that of clinically employed DNA cleavage agents, it is also anticipated to increase the activity by combination with a proper target recognition site because of the simple structure. Further studies on the mechanism and on its application are in progress in our laboratory.

References and Notes

1. For example, Kauffman, J. E.; Foye, W. O. In *Principles of Medicinal Chemistry*, 2nd ed.; Foye, W. O., Ed.; Lea & Febiger: Philadelphia, 1981: p 837. Rajski, S. R.; Williams, R. M. *Chem. Rev*. **1998**, *98*, 2723.
2. (a) Yokoi, K.; Nagaoka, K.; Nakashima, T. *Chem. Pharm. Bull*. **1986**, *34*, 4554. (b) Armstrong, R. W.; Moran, E. J. *Tetrahedron Lett*. **1991**, *57*, 2208.
3. Armstrong, R. W.; Salvati, M. E.; Nguyen, M. *J. Am. Chem. Soc*. **1992**, *114*, 3144. Alcaro, S.; Coleman, R. S. *J. Org. Chem*. **1998**, *63*, 4620. Fujiwara, T.; Sato, I. *Tetrahedron Lett*. **1999**, *40*, 315. Hartley, J. A.; Hazrati, A.; Kelland, L. R.; Khanim, R.; Shipman, M.; Suzenet, F.; Walker, L. F. *Angew. Chem. Int. Ed*. **2000**, *39*, 3467. Alcaro, S.; Coleman, R. S. *J. Med. Chem*. **2000**, *43*, 2783.
4. For review: Hodgkinson, T. J.; Shipman, M. *Tetrahedron* **2001**, *57*, 4467.
5. Coleman, R. S.; Li, J.; Navarro, A. *Angew. Chem. Int. Ed*. **2001**, *40*, 1736.
6. Coleman, R. S.; Kong, J.-S. *J. Am. Chem. Soc*. **1998**, *120*, 3538. Coleman, R. S.; Richardson, T. E.; Carpenter, A. J. *J. Org. Chem*. **1998**, *63*, 5738. Hashimoto, M.; Terashima, S. *Heterocycles* **1998**, *47*, 59.
7. Banks, R. E.; Besheesh, M. K.; Lawrence, N. J.; Pritchard, R. G.; Tovell, D. J. *Chem. Commun*. **1999**, 47. Tehrani, K. A.; Syngel, K. V.; Boelens, M. J.; Contreras, J.; Kimpe, N. D.; Knight, D. W. *Tetrahedron Lett*. **2000**, *41*, 2507.
8. Imanishi, T.; Shin, H.; Hanaoka, M.; Momose, T.; Imanishi, I. *Chem. Pharm. Bull*. **1982**, *30*, 3617.
9. It has been reported that, in the 1-azabicyclo[3.1.0]hexane structure **13**, NOE correlation is observed between the hydrogens shown below.[2a,15] ^{1}H NMR study of *trans*-**2** taken in $CDCl_3$ revealed that the hydrogen at C-5 has NOE correlation only with the hydrogens at C-4 and C-6, showing that *trans*-**2** has an epoxide structure at least in $CDCl_3$ (Fig. 6). ^{1}H NMR data for *cis*-**2** and *trans*-**2** are as follows. *cis*-**2** ($CDCl_3$) δ: 2.66, 2.84 (2H, AB in ABX, $J_{AX}=5$ Hz, $J_{BX}=5$ Hz, $J_{AB}=14$ Hz, C6-H), 3.01, 3.18 (2H, AB in ABX, $J_{AX}=3$ Hz, $J_{BX}=$ca. 0 Hz, $J_{AB}=15$ Hz, C2-H), 3.25 (1H, dd, $J=3$, 4 Hz, C3-H), 3.41 (1H, t-lile, $J=4$ Hz, C4-H), 3.73 (1H, ddd, $J=4$, 5, 5 Hz, C5-H), 4.70 (2H, AB, $J=12$ Hz, PhC<u>H$_2$</u>O–), 7.35–7.40 (5H, m, aromatic H). *trans*-**2** ($CDCl_3$) δ: 2.47 (1H, dd, $J=5$, 14 Hz, C6-H), 3.01 (1H, dd, $J=7$, 14 Hz, C6-H), 3.10, 3.19 (2H, AB in ABX, $J_{AX}=2$, $J_{BX}=$ca. 0 Hz, $J_{AB}=16$ Hz, C2-H), 3.13 (1H, d, $J=2$ Hz, C3-H), 3.27 (1H, d, $J=5$ Hz, C4-H), 3.62 (1H, dd, $J=5$, 7 Hz, C5-H), 4.66 (2H, AB, $J=12$ Hz, PhC<u>H$_2$</u>O–), 7.31–7.36 (5H, m, aromatic H).



13 *trans*-**2**

Figure 6. NOE correlation observed in **13** and *trans*-**2**.

10. The epoxides *cis*- and *trans*-**2** were stable in a solution of usual organic solvents, but sometimes underwent decomposition after concentration, suggesting that polymerization took place. Therefore, *cis*- and *trans*-**2** were prepared from *cis*- and *trans*-**4** before use and were employed immediately.
11. To a solution of supercoiled pBR322 DNA (17 μg) in pH 7.4 TE buffer (9 μL) was added a DMSO solution of the compounds (1 μL, 1 mM and 100 μM), and the whole was incubated for 24 h at 37 °C to complete strand cleavage.[16] The resulting DNAs were analyzed by electrophoresis on 0.7% native agarose gel at 7.4 v/cm for 20 min. Piperidine-treatment for 30 min at 90 °C in place of the incubation for 24 h at 37 °C was also examined to complete strand cleavage, but showed the same result.
12. The absolute configurations of **3** were determined as shown by Mosher's method.
13. Cyclohexane derivative **8** was prepared from 2-cyclohexen-1-ol similarly to *trans*-**2**. *N*-Methyl derivatives, **9** and **10**, were prepared by methylation of *trans*-**2**. Alkoxy derivatives, **11** and **12**, were prepared by the same method as that for *trans*-**2**.
14. Some typical exmples of epoxide derivatives which alkylate DNA: Benasutti, M.; Ejadi, S.; Whitlow, M. D.; Loechler, E. L. *Biochemistry* **1988**, *27*, 472. Hara, M.; Yoshida, M.; Nakano, H. *Biochemistry* **1990**, *29*, 10449. Chan, K. L.; Sugiyama, H.; Saito, I.; Hara, M. *Tetrahedron Lett*. **1991**, *32*, 7719.
15. Armstrong, R. W.; Moran, E. J. *J. Am. Chem. Soc*. **1992**, *114*, 371.
16. Nakatani, K.; Okamoto, A.; Saito, I. *Angew. Chem., Int. Ed. Engl*. **1997**, *36*, 2794.