Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 9, pp. 1097-1100, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00191-1

TOPOISOMERASE II INHIBITORS. SYNTHETIC HYBRIDIZATION OF 4-QUINOLONES AND ANTHRACYCLINES

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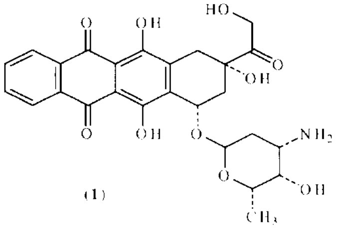
Abstract. A series of hybrid molecules (**14-16**) combining certain structural features of both 4-quinolone antimicrobial inhibitors of DNA gyrase and anthracycline inhibitors of mammalian topoisomerase II were prepared synthetically in six chemical steps from digiferrol dimethyl ether (**3**). The resulting hybrids were, unfortunately, inactive against both bacteria and mammalian cells as well as purified topoisomerases at doses where ciprofloxacin and doxorubicin are highly active. © 1997 Elsevier Science Ltd.

Introduction

Topoisomerases II are enzymes essential for altering the conformation of DNA by making transitory double-strand breaks followed by strand passage and resealing.[1] Certain clinically significant drugs are believed to work at least in significant part by forming stable ternary complexes (DNA-enzyme-drug) resulting in failure of this cycle leading to defective DNA and cell death. Anthracyclines (4-demethoxydoxorubicin (**11**) for example), m-AMSA, DHAQ and etoposide are antitumor agents attacking mammalian topoisomerase II.[2] The 4-quinolones (ciprofloxacin (**2**) for example) are antimicrobial agents attacking bacterial and fungal DNA topoisomerase II and likely topoisomerase IV as well.[3] Only at comparatively high doses is this selectivity difference bridged in particular quinolones.[4] The intimate molecular details underlying these phenomena are presently speculative.



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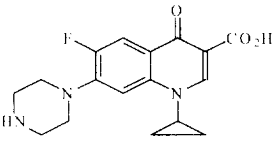
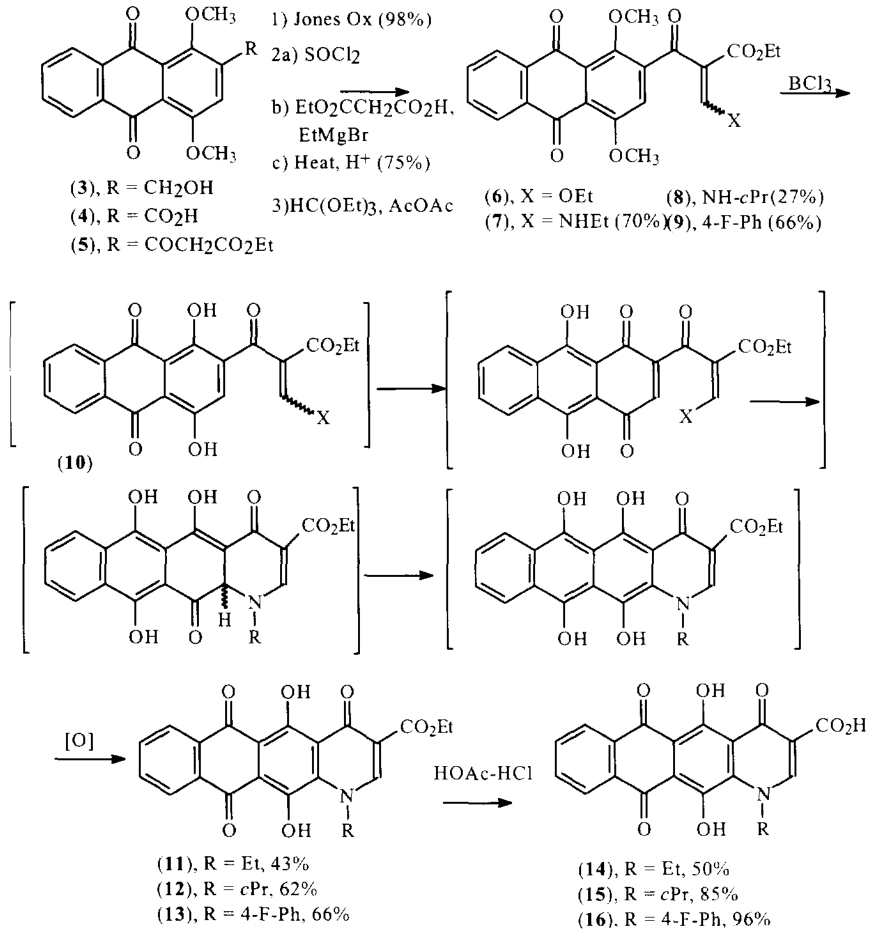


(2)

We felt it to be worthwhile to blend the salient molecular features of anthracyclines and quinolones into hybrid single molecules to see if the comparative effect on these enzymes would be informative and, perhaps, lead to therapeutically useful substances. Indications that some fluoroquinolones are active against both bacterial and mammalian topoisomerases was encouraging.[4]

After several abortive attempts, the following synthetic route proved efficient. Digiferol dimethyl ether (**3**)[5] (prepared from leucoquinizarine by the Marschalk reaction,[6] methylation, and isolation by chromatography on silica gel) was oxidized to the corresponding acid (**4**) in 98% yield with the Jones reagent. The next several steps parallel the Grohe synthesis of quinolone antiinfectives.[7] Formation of the β-keto ester (**5**; a mixture of keto and enol forms) was achieved by conversion of the acid to the acid chloride by refluxing with excess thionyl chloride followed by reaction with ethyl hydrogen malonate in the presence of ethyl magnesium bromide and heating in aqueous acid to decarboxylate. This three step sequence went in 75% overall yield without purification of intermediates. Treatment of **5** with triethyl orthoformate and acetic anhydride gave the geometric mixture of enol ethers (**6**). This was unstable so was used without delay to prepare the desired intermediate enamines as *cis-trans* mixtures by addition-elimination with the appropriate amines (**7**; R=NHEt, 70%; **8**; R=NH-*c*-Pr, 27%; **9**; R=NH-4-F-Ph, 66% yield). After a number of failed trials with other reagents, the deblocking and cyclization was found to proceed



(**3**), R = CH_2OH
(**4**), R = CO_2H
(**5**), R = $COCH_2CO_2Et$

(**6**), X = OEt (**8**), NH-*c*Pr(27%)
(**7**), X = NHEt (70%)(**9**), 4-F-Ph (66%)

(**10**)

(**11**), R = Et, 43%
(**12**), R = *c*Pr, 62%
(**13**), R = 4-F-Ph, 66%

(**14**), R = Et, 50%
(**15**), R = *c*Pr, 85%
(**16**), R = 4-F-Ph, 96%

without requiring isolation of intermediates by ether cleavage with BF_3 to the presumed anthraquinones (**10**) as individual mixtures of tautomeric and *cis-trans* forms, one of which presumably undergoes an intramolecular Michael-type cyclization (as illustrated). An apparent spontaneous air oxidation during work up generated the individual targeted amines as their esters (**11**; R=Et, 43%; **12**; R=*c*-Pr, 62%; **13**; R=4-F-Ph, 66%). Hydrolysis of these cyclized products with methanolic-HCl (the usual reagent) was unsatisfactory but with HOAc-HCl high yields of the target compounds (**14**, R=Et, 50%; **15**, R=*c*-Pr, 85%; **16**, R=4-F-Ph, 96%) were obtained in a high state of purity, aided by their poor solubility in most solvents.

Target compounds **14-16** were assayed for their capacity to induce enzyme-dependent strand breakage into supercoiled plasmid DNA, a phenomenon which correlates well with microbial cell death. No measurable activity was observed against *E. coli*-derived DNA gyrase II at concentrations two orders of magnitude higher than the concentration where ciprofloxacin is highly active.[8] They were also inactive *in vitro* in an agar dilution-streak assay against a battery of Gram-positive and Gram-negative bacteria at 100 μg/ml in comparison with ciprofloxacin which is active at least at 1 μg/ml in these tests. No activity was seen against a panel of fungal pathogens at 100 μg/ml in contrast to amphotericin B which served as a positive control. Furthermore, these analogs were inactive against calf thymus-derived topoisomerase II at 100 μg/ml, a concentration where etoposide is highly active. Likewise, analogs **14-16** were inactive against A549, HT-29, B16F10 and P-388 tumor cell lines in culture at concentrations where doxorubicin is highly active (IC_{50} values = 0.0031-0.012 μg/ml).[9]

These biological findings are disappointing and comparatively uninformative. Many speculative rationalizations could be advanced to account for them. Whatever the ultimate reason, putative molecular mode of action hypotheses for inhibition of either topoisomerase must accommodate these findings.

Acknowledgment: This work was supported in part by grants AI-13155 and AI-36650 from the National Institutes of Health, Allergy and Infectious Diseases Institute.

References and Notes

1. (a) Wang, J. C. In *DNA Topoisomerases: Biochemistry and Molecular Biology*; Liu, L. F., Ed.; Academic Press: New York, 1994; pp 1-19. (b) Fostel, J. M.; Shen, L. L. In *Design of Enzyme Inhibitors as Drugs*; Sandler, M.; Smith, H. J., E's.; Oxford University: Oxford, 1994; pp 564-624.
2. (a) Liu, L. F. In *DNA Topology and Its Biological Effects*. Cozzarelli, N. R.; Wang, J. C., Eds.; Cold Spring Harbor Lab Press: New York, 1990, pp 371-389. (b) Osheroff, N.; Corbett, A. H.; Johnson, M. J. In *DNA Topoisomerases: Biochemistry and Molecular Biology*: Liu, L. F., Ed.; Academic Press: New York, 1994; pp 105-126.
3. (a) Mitscher, L. A.; Shen, L. L. In *Nucleic Acid Targeted Drug Design*; Propst, C. L.; Perun, T. J., Eds.; M. Dekker: New York, 1992; pp 423-474. (b) Kato, J.-I.; Ikeda, H. In *Molecular Biology of DNA Topoisomerases and its Application to Chemotherapy*; Ando, T.; Ikeda, H.; Ogura, M., Eds.; C.R.C.; Boca Raton, 1993; pp 32-37. (c) Shen, L. L.; Baranowski, J.; Fostel, J.; Montgomery, D. A.; Lartey, P. A. *Antimicrob. Agents Chemother.* **1992**, *36*, 2778-2784.

4. (a) Gootz, T. D.; Osheroff, N. In *Quinolone Antimicrobial Agents, 2nd Ed*; Hooper, D. C.; Wolfson, J. S., Eds.; American Society for Microbiology: New York, 1993, pp 139-160. (b) Sato, K.; Hoshino, K.; Mitsuhashi, S. *Prog. Chem. Research.* **1992**, *38*, 121-132.
5. Hahn, D. V.; Sequin, E.; Tillequin, F.; Monneret, C.; Koch, M. *Chem Pharm. Bull.* **1989**, *37*, 3294-3300.
6. (a) Marschalk, C.; Koenig, F.; Ouroussoff, N. *Bull. Soc. chim. France*, **1936**, *3*, 1545-1555. (b) Marschalk, C. *Bull. Soc. chim. France.* **1939**, *6*, 655-665.
7. (a) Grohe, K.; Heitzer, H. *Liebig's Ann. Chem.*, **1987**, 29-37. (b) *ibid.*, 871-879.
8. Shen, L. L.; Mitscher, L. A.; Sharma, P.; O'Donnell, T. J.; Chu, D. W. T.; Cooper, C. S.; Rosen, T.; Pernet, A. G. *Biochemistry*, **1989**, *28*, 3886-3894.
9. Chu, D. T. W.; Hallas, R.; Clement, J. J.; Alder, J.; McDonald, E.; Plattner, J. J. *Drugs Exptl. Clin. Res.* **1992,** *18*, 275-282.

(*Received in USA* 30 *October* 1995; *accepted* 20 *March* 1997)