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Analogs of Camptothecin

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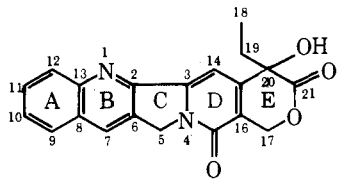
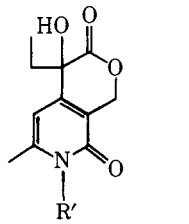
Several compounds having portions of the camptothecin ring system were prepared. These compounds were screened against L1210 lymphoid leukemia with negative results. Two of the analogs which contained the pyridine and hydroxylactone D and E rings were also screened for inhibition of DNA and RNA syntheses in HeLa cells. Each of these analogs had decreased activity as compared with camptothecin and there was no degradation of DNA in the HeLa cells. This suggests that the D and E rings are not a sufficient requirement for camptothecin-like activity.

The early animal tests showed the alkaloid camptothecin (1) to be a potent antineoplastic agent;[1] however, the toxic effects and lack of activity against gastrointestinal cancer showed 1 to be ineffective as a chemotherapeutic agent.[2] The effectiveness of camptothecin against leukemia in experimental animals and the unusual effects it has on macromolecular synthesis[3] have caused an interest in determining the structural features of camptothecin associated with this activity.

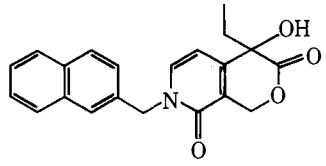


camptothecin (1)

Previous studies have indicated that the functional groups of the hydroxylactone ring E must be present to maintain the activity of camptothecin. Thus substitution of the hydroxyl group at C-20 by halogen,[4] acetoxy,[4] ethyl,[5] or hydroxymethyl[6] gives compounds showing reduced or lack of cytotoxicity in animals. Replacement of the hydroxyl group by hydrogen gave a desoxy compound inactive against tumors[4] but showing inhibition of DNA–RNA synthesis.[3] Reduction of the C-21 carbonyl group also caused a loss of activity.[4] The activity of camptothecin is maintained if the lactone ring is opened to the alkali metal salt or amide.[4] These data suggest that the D and E rings of camptothecin are necessary for activity. Only limited information about the biological activity of simple analogs of camptothecin is available; however, the report that **2** has only 0.01 the activity of camptothecin[7] and the weak inhibition of RNA synthesis of **3**[6] suggested that the D and E rings were a necessary but not a sufficient requirement for activity.



2, R = R′ = H
3, R = CH_3; R′ = n-C_4H_9



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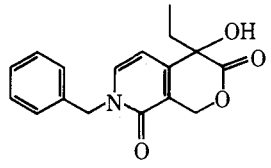

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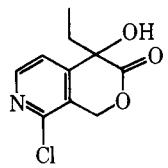


6

Results and Discussion

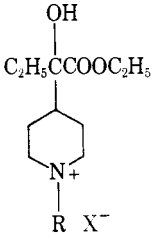
In an attempt to determine the structural requirements required for activity, several series of analogs were prepared, three of which (**4**, **5**, and **6**) contain the D and E ring systems comparable to camptothecin (1). The syntheses were described previously.[8] The analog **4** contains the same number of cyclic atoms as camptothecin; however, the nitrogen at position 1 is replaced by carbon and the C-2–3 bond is not closed. This very close analog was inactive in the L1210 leukemia screen (Table I) and was less active than camptothecin as an inhibitor of nucleic acid synthesis in HeLa cells.[9] Replacement of the β-naphthylmethyl group on nitrogen by a benzyl group (**5**) gave a marked decrease in inhibition of macromolecular synthesis. Neither of these analogs caused degradation of DNA, a property possibly related to the suppression of tumor growth,[3] and thus the inactivity in the L1210 screen was expected.

Table I lists additional compounds related in structure to camptothecin (1) whose syntheses have been reported.[10] **7** contains the atoms of D and E rings and **8** has all the atoms of camptothecin without closure of the lactone ring. **6** has the D and E ring with the carbonyl group of the pyridone replaced by chlorine.[8] These compounds, as well as the analogs with the B and D rings (type I), the A, B, and D rings (type II), and the B, C, and D rings (type III),[10] were inactive in the L1210 leukemia screen.

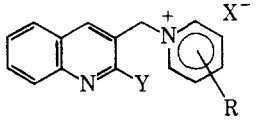
Table I. Screening Data

NSC compd	Tumor[a]	Single dose, mg/kg	Act.,[b] T/C (%)	Multiple dose, mg/kg, injected	No./injected	Act.,[b] T/C (%)	Status[e]
1 camptothecin[f]	LE	0.5	163				
(100880)	KB		ED_{50} 3×10^{-2} μg[c]				
7 (160846)	LE			400	3	100	F
	KB		ED_{50} 1:100[c]		3		2
8 (160847)	LE	200	Toxic				
		50	115				F
9 (160848)	LE	100	96				F
10 (160849)	LE	100	Toxic				
		50	95				F
	KB		ED_{50} 1:31[c]				2
11 (160853)	KB		ED_{50} 1:18[c]				2
13 (160854)	LE	50	Toxic	25	3	89	F
				12.5	9	97	F
14 (160855)	LE			100	3	Toxic	
				50	3	97	F
			ED_{50} 1:17[c]				2
12 (160856)	LE	100	Toxic				
		50	105				F
15 (160857)	LE	50	Toxic	12.5	3	102	F
				25	9	113[d]	P
				18	9	105	F
16 (160858)	LE	50	Toxic				
		25	96				F
6 (166611)	LE	200	94	200	3	103	F
4 (177363)	LE	200	94				F

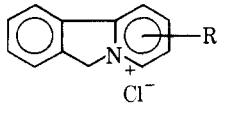
[a] LE = L1210 lymphoid leukemia, KB = cell culture of human epidermoid carcinoma of the nasopharynx. [b] The minimum toxic dose or the activity of the maximum nontoxic dose. [c] The tissue culture data (KB) are reported in the number of dilutions required to kill 50% of the cells. [d] One cure was noted. [e] F, failed criterion for activity; P, passed criterion for activity at that phase of the screen; 2, failed to show activity in the tissue culture screen. [f] See ref 4.



7, R = H; X = Cl
8, R = 3-(2-chloroquinolyl)methyl; X = Br

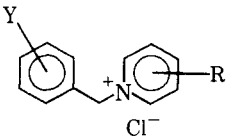


type I



type II

9, R = 4-CH_3; Y = I; X = Br
10, R = 4-CH_3; Y = O═; X = Br
11, R = 1,3-$(CH_3)_2$
12, R = 4-CH_3



type III

13, R = 4-CH_3; Y = H
14, R = 3-CH_3; Y = H
15, R = 2,4-$(CH_3)_2$; Y = 2-Cl
16, R = 3,5-$(CH_3)_2$; Y = 2-Cl

Conclusion

These results support the hypothesis that the D and E rings are required for activity; however, an additional, planar, aromatic system seems to be required. **4**, the most active of the analogs, has a completely mobile naphthalene ring, for free rotation is possible around either of the methylene bonds of the nitrogen substituent. It is probable that the 2–3 bond is required for activity, for the orbital overlap of the two π systems would increase the barrier to rotation about the 2–3 bond to more than 5.0 kcal/mol even in the absence of a 4–5 or 5–6 bond. This would give an effective planar system to natural camptothecin. This is in agreement with the postulated structural requirements proposed from molecular orbital calculations.[11]

Acknowledgment. This research was supported in part by a grant from the National Cancer Institute of the NIH (CA-12149) for which the authors express appreciation. The preliminary screening data for anticancer activity were determined by the National Service Center of the Drug Development Branch of the National Cancer Institute. The authors express appreciation to Dr. Harry Wood for assistance in arranging for obtaining these data.

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Book Reviews†

Synthetic Methods of Organic Chemistry. Volume 28. By W. Theilheimer. Yearbook 1974. S. Karger, Basel and New York. xx + 652 pp. 16 × 23 cm.

There can hardly be anyone with modern graduate training in synthetic organic chemistry who has not browsed at one time or another through the library set of "Synthetic Methods of Organic Chemistry," the continuing series masterfully edited by William Theilheimer.

This 28th volume lives up to the scholarly tradition of its predecessors. The method of classification of chemical transformations is explained thoroughly in the preface and need not be discussed here. There is the usual cumulative subject index, in this instance for Volumes 26–28. Several specialized indexes based on the now famous Theilheimer notation system are included, and there is also a useful short review entitled "Trends in Synthetic Organic Chemistry–1974" which deserves to be singled out.

In all honesty this Reviewer is troubled by a disquieting impression that many chemists, especially perhaps medicinal chemists, still view this reference source with a mixture of awe and puzzlement. A typical remark might be "I know it's there, but I don't use it much." The reasons are probably several: the multilingual format, a ponderous system of organization and symbology, and occasional annoying lapses in coverage ("I couldn't find my beautiful paper cited!"); all these things may cause some to glance through the book once and, shrugging their shoulders, return it to the shelf forevermore.

Needless to say, the initiated Theilheimer *afficionado* knows better. He knows, for example, that he may find the specific reagent or chemical reaction he is looking for, or again that he may not—*it almost doesn't matter!* What he can be certain to find is an astonishing harvest of chemical knowledge more varied than he could ever imagine. And if even a tiny fraction of this knowledge finds a lasting place in his personal bag of synthetic tricks, the effort will have been amply justified.

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Reagents for Organic Synthesis. Volume 4. By Mary Fieser and Louis F. Fieser. Wiley-Interscience, New York, N.Y. 1974. 660 pp. 15.5 × 22.5 cm. $24.95.

This fourth volume in the series covers the 1970–1972 synthetic organic literature. The format used previously is repeated with the reagents indexed not only as to subject but also as to reactions and compound type. Volume references are also given for those reagents appearing in Volumes 1–3. Almost 300 new reagents are reviewed for the first time, which indicates the rapid rate at which new reagents are being introduced. A significant deletion in this volume is that this is the first of the many books published by the Fiesers that does not include a picture of a cat. This series continues to be a most valuable reference source for synthetic organic chemists.

†Unsigned book reviews are by the editorial staff.

Biosynthesis and Enzymic Hydrolysis of Penicillins and Cephalosporins. By E. P. Abraham. University of Tokyo Press, Tokyo, Japan. 1974. 86 pp. $7.00.

The two essays included in this volume summarize Professor Abraham's presentations of the E. R. Squibb lectures at the Waksman Institute of Microbiology (Rutgers University) in 1973. He focuses on the following in his review of the biosynthesis: amino acid precursors; intracellular peptides; roles of peptides P1, P2, and P3; biosynthetic studies with *Cephalosporium* and *Penicillium* protoplasts; mechanisms of ring closure; roles of 6-aminopenicillanic acid and isopenicillin I; and control mechanisms. The following aspects of β-lactamases are mentioned: significance of enzymic hydrolysis in chemotherapy; evolution and induction of β-lactamases; properties relating to active sites; and approaches to clinical problems arising from the production of β-lactamases. Although few experimental details are included in the essays, 88 and 138 references are cited for the first and second topics, respectively, and some relate to work published in 1974.

The major value of these essays lies in Professor Abraham's clear presentation of the problems under study (frequently with high priority in his own laboratory), the strategy involved, and the value of the results obtained. His 35 years of experience in penicillin and related research programs provides a unique perspective not usually available in most research programs. The uninitiated will find these essays the best available summary of the current status of these two problems; the expert will be stimulated to reevaluate his current programs studying one or more aspects of penicillins and cephalosporins.

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Receptors for Reproductive Hormones. Edited by B. W. O'Malley and A. R. Means. Plenum Press, New York, N.Y. xii + 458 pp. 17 × 25 cm. $25.00.

This book is volume 36 in a series entitled "Advances in Experimental Medicine and Biology," and it consists of the proceedings of the Conference on Receptors for Reproductive Hormones held on July 10–11, 1972, at Vanderbilt University, Nashville, Tenn. The conference was supported by the Center for Population Research, National Institutes for Child Health and Human Development. Twenty papers were presented at the conference by many of the leading researchers in the rapidly developing area of sex steroid and gonadotropin receptors.

The 20 papers can be divided into five main parts. The first seven chapters deal with estrogen receptors in general, with four of them dealing particularly with the nuclear events relating to the mechanism of action. The current concept of the estrogen–receptor complex is covered in the first chapter, discussing the model of one estrogen binding site per uterine receptor, a physical change of the cytosol complex to its nuclear state, and the features of the model still to be proven or modified. The second chapter is concerned with the influence of endogenous estrogen on the translocation of the estrogen–receptor complex to its nuclear locus and a proposed two-phase uterotrophic action of estrogen. Estrogen–receptor transformation is dealt with in the third paper, supporting the hypothesis that the important step is receptor transforma-