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The Synthesis of 5-Substituted Camptothecins As Potential Inhibitors of DNA Topoisomerase I[1]

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Abstract: Four new 5-substituted camptothecins (**4-7**) have been synthesized and evaluated for DNA topoisomerase I inhibition. The results suggested that the pyridone moiety in the D ring of camptothecin plays a crucial role in determining its activity and that the 5 position of the C ring should be unsubstituted for retention of activity.

The early structure-activity relationship studies[2, 3] of camptothecin (**1**) showed that the structural requirements for antitumor activity include the presence of the planar ABCD ring system and the β-hydroxy lactone moiety of ring E. Substitution of ring A with small groups, such as 10-hydroxy, is also permissible, with many of these analogs being considerably more active than **1**. In the search for clinically useful camptothecin analogs, a number of derivatives have been synthesized based on the above knowledge. In the design of these analogs, much effort has been directed towards introducing a water-soluble moiety into the 9 or 10 position of the A ring of the camptothecin. The most successful examples are the 7,10-substituted analog CPT-11 and the 9-substituted analogs Topotecan and 9-aminocamptothecin, which are being tested clinically as anticancer drugs against colon and other cancers in Japan,[4] Europe,[5] and the U.S.A.,[6, 7] respectively. In our previous report, a series of novel water-soluble 7-(aminoacylhydrazono)-formyl camptothecins with

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increased topoisomerase I inhibitory activities were synthesized. Our results suggested that the 7 position of the B ring is a suitable location for introducing a polar moiety into camptothecin to produce analogs with enhanced topoisomerase I inhibitory activity.[8, 9]

As an extension to this study, an investigation of the 5-substituted camptothecins was undertaken. A few 5-substituted camptothecins have been reported; however, no biological data are available.[10] The generation of α,β-epimers has also made this position more difficult to manipulate. On the other hand, the significance of the pyridone moiety of the D ring on camptothecin's activity is still unclear. To explore the practicality of active 5-substituted camptothecins and to clarify the significance of the pyridone moiety, we have synthesized four new 5-substituted camptothecins and have evaluated their inhibitory effects on DNA topoisomerase I.

5β- and 5α-Hydroxymethyl camptothecin (**4** and **5**) together with the 5,5-dihydroxymethyl derivative (**6**) were synthesized by refluxing **1** and formaldehyde in DMF for 1 hour in the presence of 4-piperidinopiperidine.[11] Prolonged reaction time (more than 5 hours) will totally convert the 5-α,β-hydroxymethyl derivatives into the 5,5-dihydroxymethyl derivative. The mixture of **4** and **5** isomers was separated by repeated silica gel chromatography and the structures of these compounds were confirmed by spectral data.[12] The stereochemistries of the two epimers were established by the X-ray crystal analysis of **4** (Fig. 1) as its trihydrate.[13]

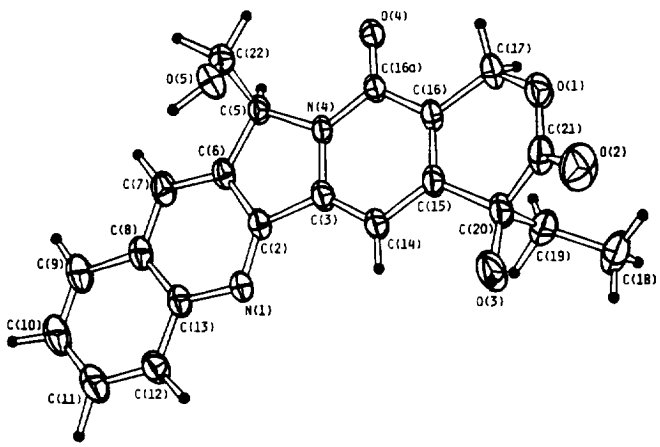
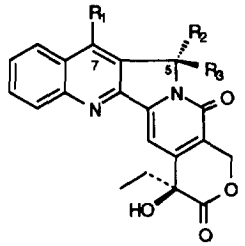


Figure 1. ORTEP diagram (40% probability ellipsoids) showing the solid-state conformation of 4 in crystals of the trihydrate; small filled circles represent hydrogen atoms.

Table 1. Biological Evaluation of Compounds 1-7



Compound No	R_1	R_2	R_3	Protein Linked DNA Breaks in KB Cells (%, 25 μg/ml X 1hr)[14]
1	H	H	H	100
2	CHO	H	H	106.8
3	CH_2OH	H	H	97.6
4	H	H	CH_2OH	20.4*
5	H	CH_2OH	H	8.7
6	H	CH_2OH	CH_2OH	0
7	H	H, $COC_6H_2(OCH_3)_3$		0

* Compounds 4-7 are all inactive against BT549, PLC, SW480, and A549 cell lines (ID_{50} 500-5000 or > 5000 nM).[14]

5-(3', 4', 5'-Trimethoxybenzoyl) camptothecin (7) was synthesized by reaction of 20-acetyl camptothecin with NaH and 3, 4, 5-trimethoxybenzoyl chloride, followed by deacetylation. However, the separation of the resulting isomers was unsuccessful.

For comparison with the activity of the 5-substituted analogs, 7-formyl- (2) and 7-hydroxymethyl (3) camptothecin were synthesized by a literature method.[10]

Table 1 shows the bioassay results for the 5- and 7-substituted camptothecin derivatives (2-7). Generally, the 7-substituted analogs (2 and 3) retained the potency of the parent camptothecin. This result was consistent with that observed with our previously synthesized 7-(aminoacylhydrazono)-formyl camptothecins. In contrast, the 5-substituted camptothecin derivatives (4-7) showed a dramatic loss of activity. Although 4 and 5 possess the same substitution (CH_2OH) found in 3, their activities drop to 20.4% and 8.7% respectively. The decrease in activity is somewhat related to the steric demands of the substituents. The α,β-disubstituted compound 6 and the bulkier substituted compound 7

lost all activity. Since the 5 position is adjacent to the pyridone moiety of the D ring, the above results suggest that, along with other essential moieties, the pyridone moiety may play a critical role in determining DNA topoisomerase I inhibitory activities. Hydrogen bonding between the 5-hydroxymethyl and the pyridone moiety or adjacent steric hindrance may make docking of the pyridone ring to the active site of the receptor more difficult. Hence, we conclude that to maintain the function of the pyridone moiety, the 5 position of the C ring should be free of any steric hindrance from both the α and β side and, thus, introducing substituents into this position will lead to loss of activity.

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11. Procedure for synthesis of compounds **4-6**: Formaldehyde (37 %, 0.2 ml) and 4-piperidinopiperidine (100 mg) were added to a suspension of camptothecin (**1**, 100 mg) in dimethylformamide (10 ml). The mixture was refluxed for 1 hr and the solvent was evaporated under reduced pressure. The residue was purified on a silica gel column, eluting with chloroform/methanol (50:1), to give a mixture of **4** and **5**, together with **6** (52 mg, 50 %). The mixture of **4** and **5** was separated by repeated silica gel column chromatography, eluting with chloroform/methanol (100:1), to give **4** (14 mg, 14 %) and **5** (15 mg, 15 %) respectively.
12. 5β-Hydroxymethyl camptothecin (**4**): colorless plates; mp 269-270°C (decomp.); IR (KBr) 3400 (OH), 1740 (γ-lactone), 1645 (amide carbonyl) ; ^{1}H NMR ($CDCl_3$+CD_3OD, 1:1) δ: 0.82 (t, *J* = 7.0 Hz, 3H, 18-H), 1.74 (q, *J* = 7.0 Hz, 2H, 19-H), 3.16 (s, 1H, OH), 4.09, 4.20 (both dd, *J* = 3.7, 11.5 Hz, 1H each, C*H*$_2$OH), 5.08, 5.42 (both d, *J* = 16.4 Hz, 1H each, 17-H), 5.59 (t, *J* = 3.7 Hz, 1H, 5-H), 7.47, 7.63 (both t, *J* = 8.0 Hz, 1H each, 10-H, 11-H), 7.49 (s, 1H, 14-H), 7.79 (d, *J* = 8.0 Hz, 1H, 9-H), 7.98 (d, *J* = 8.0 Hz, 1H, 12-H), 8.31 (s, 1H, 7-H). *Anal.* Calcd. for $C_{21}H_{18}N_2O_5 \bullet H_2O$: C 63.62, H 5.09, N 7.07; Found C 63.81, H 5.19, N 6.97. 5α-Hydroxymethyl camptothecin (**5**): colorless plates; mp 254-255°C (decomp.); IR (KBr) 3400 (OH), 1745 (γ-lactone), 1650 (amide carbonyl) ; ^{1}H NMR (d_5-pyridine) δ: 0.59 (t, *J* = 7.0 Hz, 3H, 18-H), 1.59 (q, *J* = 7.0 Hz, 2H, 19-H), 3.19 (s, 1H, OH), 4.31, 4.75 (both dd, *J* = 3.8, 11.3 Hz, 1H each, C*H*$_2$OH), 4.96, 5.45 (both d, *J* = 16.4 Hz, 1H each, 17-H), 5.60 (t-like, 1H, 5-H), 7.19, 7.38 (both t, *J* = 8.0 Hz, 1H each, 10-H, 11-H), 7.52 (d, *J* = 8.0 Hz, 1H, 9-H), 7.68 (s, 1H, 14-H), 8.01 (d, *J* = 8.0 Hz, 1H, 12-H), 8.20 (s, 1H, 7-H). 5-Dihydroxymethyl camptothecin (**6**): colorless plates; mp 247-248°C (decomp.); IR (KBr) 3400 (OH), 1742 (γ-lactone), 1650 (amide carbonyl) ; ^{1}H NMR ($CDCl_3$) δ: 1.07 (t, *J* = 7.0 Hz, 3H, 18-H), 1.91 (q, *J* = 7.0 Hz, 2H, 19-H), 3.85 (s, 2H, OH X 2), 4.18, 4.22 (both d, *J* = 10.5 Hz, 1H each, C*H*$_2$OH), 4.48, 4.60 (both d, *J* = 10.5 Hz, 1H each, C*H*$_2$OH), 5.28, 5.69 (both d, *J* = 16.4 Hz, 1H each, 17-H),

7.68, 7.84 (both t, J = 8.0 Hz, 1H each, 10-H, 11-H), 7.74 (s, 1H, 14-H), 7.97 (d, J = 8.0 Hz, 1H, 9-H), 8.22 (d, J = 8.0 Hz, 1H, 12-H), 8.44 (s, 1H, 7-H).

13. *Crystal data*: $C_{21}H_{18}N_2O_5.3H_2O$; MW = 432.43, triclinic, space group $P1(C_1^1)$, a = 8.075(1) Å, b = 10.370(2) Å, c = 6.497(1) Å, α = 100.32(1)°, β = 95.37(1)°, γ = 109.84(1)°, V = 496.4(3) Å^3, Z = 1, $D_{calcd.}$ = 1.446 g cm^{-3}, μ(CuKα radiation, λ = 1.5418 Å) = 9.0 cm^{-1}. Intensity data ($\pm h$, $\pm k$, $+l$; 2025 non-equivalent reflections) were recorded on an Enraf-Nonius CAD-4 diffractometer [CuKα radiation, graphite monochromator; ω-2θ scans, θ_{max} = 75°] from a crystal of dimensions 0.07 x 0.17 x 0.40 mm. The crystal structure was solved by direct methods (MULTAN11/82). Full-matrix least-squares refinement (Enraf-Nonius SDP) of atomic positional and thermal parameters (anisotropic C, N, O; isotropic H) converged (max. shift:esd = 0.03) at R = 0.054 (R_w = 0.075) over 1775 reflections with $I > 3.0\sigma(I)$. Atomic coordinates, bond lengths, bond angles, and torsion angles have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.
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