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Novel Water-Soluble 7-(Acylhydrazono)-Formyl Camptothecins As Potent Inhibitors of DNA Topoisomerase I[1]

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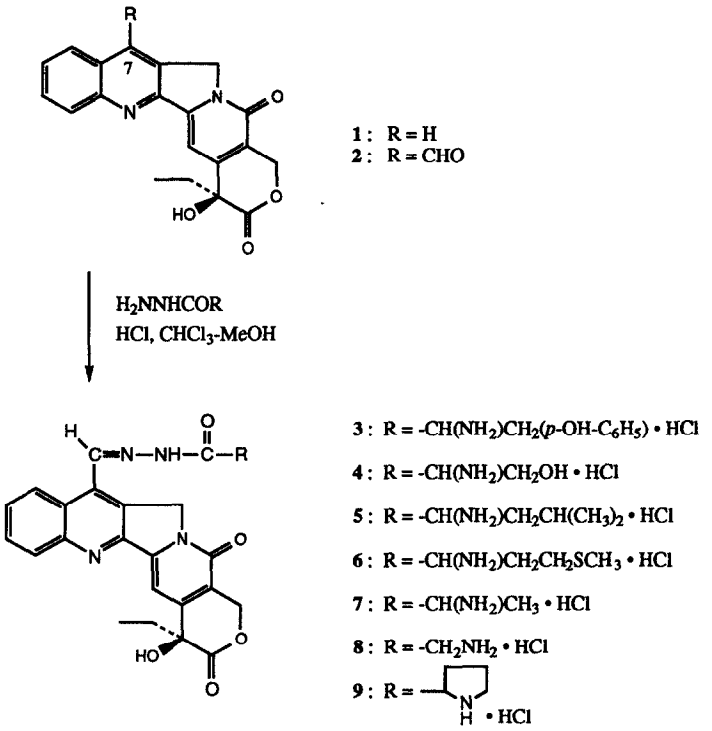
Abstract : Seven water-soluble 7-(acylhydrazono)-formyl camptothecins were synthesized and evaluated for their ability to cause protein-linked DNA breaks and to inhibit topoisomerase I activity. Compared with camptothecin, compound **3** was more potent in these two assays but was less toxic in several cancer cell lines.

Camptothecin (**1**)[2] and some of its derivatives are potent inhibitors of DNA topoisomerase I.[3,4] Among the numerous camptothecin derivatives, hycamptamine,[5] 9-aminocamptothecin,[6] and CPT-11[7] are being tested clinically as anticancer drugs[8] against colon and other cancers in Europe, the U.S.A., and Japan. The development of camptothecin derivatives as potential anticancer drug candidates frequently suffers from inadequate water solubility. In our endeavors to synthesize water-soluble camptothecins, 7-formyl camptothecin was chosen as the starting material for preparation of hydrophilic group-bearing derivatives. This compound has shown improved solubility in organic solvents and an excellent ability to form imines or hydrazones. We have synthesized a series of novel water-soluble 7-(acylhydrazono)-formyl camptothecins and have evaluated their inhibitory effect on DNA topoisomerase I.

The 7-(acylhydrazono)-formyl camptothecins (**3-9**), were synthesized (Scheme **1**) from 7-formyl camptothecin (**2**), which can be prepared from **1** by a literature method[9]. Briefly, compound **2**, was dissolved in $CHCl_3$-MeOH or CH_2Cl_2-MeOH. The acyl hydrazine in HCl solution was then added to the above solution. After the mixture wasallowed to stand for 2 hr at room temperature or was heated to 60 °C for 10 minutes, it was evaporated *in vacuo* and purified by Sephadex column chromatography to yield



Scheme 1

Table 1. Protein-linked DNA Breaks and Relaxation Activity Inhibition of Topoisomerase I of 7-(Acylhydrazono)-Formyl Camptothecin Derivatives[11]

Compound No.	Protein-linked DNA breaks in cells (25 μg/mL), %	Relaxation activity inhibition of Topoisomerase I (25 μg/mL), %
1	100.0	100.0
3	181.0	163.5
4	79.8	145.0
5	111.7	136.4
6	111.0	197.1
7	93.7	98.0
8	110.3	115.9
9	154.6	100.0

Table 2. Cytotoxicity of 7-(Acylhydrazono)-Formyl Camptothecin Derivatives (IC_{50}, $\mu g/mL$)[11]

Compound No.	Cell lines KB	KB VP-16^R/7μM	KB VCR^R/20μM	HepG2-T14	MCF-7	SW480
1	0.01	0.04	0.03	0.09	0.02	0.01
3	0.10	5.00	1.00	1.00	5.00	0.40
4	0.12	5.00	3.00	3.50	1.00	1.00
5	0.10	1.00	0.40	0.40	0.15	0.04
6	0.10	0.50	0.20	2.00	1.00	0.80
7	0.10	5.00	3.00	2.50	1.00	0.60
8	0.10	3.00	1.00	0.60	0.40	0.10
9	0.04	0.60	5.00	1.25	0.80	0.70

the target compounds (**3**-**9**). Because the reaction is performed in a hydrochloric acid-containing solution and an amino group is present in the acylhydrazine, the corresponding water-soluble HCl salt will be formed.

Tables 1 and 2 show the bioassay results for these new camptothecin derivatives (**3**-**9**). Generally, the relaxation activity inhibition of topoisomerase I and the protein-linked DNA breaks correlated with the cytotoxicity. One of the derivatives, compound **3**[10], caused more protein-linked DNA breaks and inhibition of topoisomerase I activity and was less toxic to the cells, when compared with **1**. The cell death caused by **3** may result from a mechanism other than DNA single-strand breaks. Further development of **3**-related compounds as potential anticancer drug candidates is in progress.

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Reference and Notes

1. Antitumor Agents 149. For part 148, see Zhang, Y. L.; Guo, X.; Cheng, Y. C.; Lee, K. H.; *J. Med. Chem.* Submitted.
2. Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888.
3. Hsiang, Y. H.; Hertzberg, R.; Hecht, S.; Liu, L.F. *J. Biol. Chem.* **1985**, *260*, 14873-14878.
4. Hsiang, Y. H.; Liu, L. F.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Kirschenbaum, S.; Silber, R.; Potmesil, M. *Cancer Res.* **1989**, *49*, 4385-4389.

5. Johnson, P. K.; McCabe, F. L.; Faucette, L. F.; Hertzberg, R. P.; Kingsbury, W. D.; Boehm, J. C.; Caranfa, M. J.; Holden, K. G. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 623
6. Wani, M. C.; Nicholas, A. W.; Wall, M. E. *J. Med. Chem.* **1986**, *29*, 2358-2363.
7. Fukuoka, M.; Negoro, S.; Niitani, H.; Taguchi, T. *Proc. Am. Soc. Clin. Oncol.* **1990**, *9*, 874-884.
8. *Washington Insight*, **1989**, 2, 6, and **1990**, 3, 6.
9. Miyasaka, T; Sawada, S.; Nokata, K. *Heterocycles*, **1981**, *16*, 1719.
10. 7-(L-Tyrosylhydrazono)-formyl-camptothecin • hydrochloride **(3)**: Yield 95%; yellow powder; mp 250 °C (decomp.); IR (KBr) 3400 (OH), 3300-2000 (very broad, NH_3^+), 1740 (γ-lactone), 1690 (carbonyl of amino acid residue), 1650 (carbonyl of amide), 1635 (C=N); ^1H NMR (CD_3OD) δ 0.86 (t, J = 7 Hz, 3H,18-H), 1.78 (q, J = 7 Hz, 19-H), 3.09 (dd, J = 26, 7 Hz , 2H, CH_2 of tyrosine residue), 4.28 (m, AB type, 2H, 5-H), 4.87 (t, J = 7Hz, 1H, NH_2-C*H*- of tyrosine residue), 5.18, 5.33 (d, J = 16 Hz, 1H each, 17-H), 6.47, 6.78, 6.98, and 7.10 (d, J = 8 Hz, 1H each, aromatic protons of tyrosine residue), 6.91 (s, 1H, 14-H), 7.26 and 7.35 (t, J = 7.5 Hz, 1H each, 10-H, 11-H), 7.50 and 7.61 (d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.15 (s, 1H, HC=N-N). *Anal.* Calcd. for $C_{30}H_{28}N_5O_6Cl$ • 1.5 H_2O: C 58.44, H 5.06, N 11.35; Found C 58.84, H 4.79, N 11.08.
11. Bioassay: Assays for inhibition of DNA topoisomerase I and for production of cellular protein-linked DNA breaks, as well as for cytotoxicity in cancer cells, were carried out according to the procedures described previously.[3,12,13]
12. Lee, K. H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L. S.; Dai, H. J.; Chen, C. H.; Liu, S. Y.; Cheng, Y. C. *J. Nat. Prod.* **1989**, *52*, 606.
13. Carmichael, J.; Gegraff, W. G.; Gezdar, A. F.; Minna J. D.; Mitchael J. B. *Cancer Res.* **1987**, *47*, 936-942.

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