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Synthesis of Novel Water-Soluble 7-(Aminoacylhydrazono)-formyl Camptothecins With Potent Inhibition of DNA Topoisomerase I†

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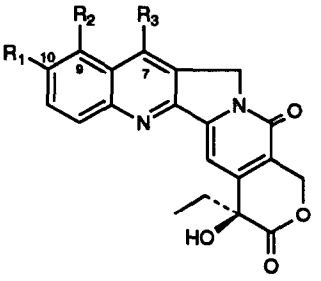
Abstract—Eighteen new water-soluble 7-(aminoacylhydrazono)-formyl camptothecins were synthesized and evaluated for their ability to cause protein-linked DNA breaks and to inhibit topoisomerase I activity. Compared with camptothecin, five of the compounds were as potent or more potent in these two assays but were less toxic in several cancer cell lines. The results suggest that the 7 position in the B ring is a suitable location for introducing a polar moiety into camptothecin producing analogues with enhanced topoisomerase I inhibiting activity.

Introduction

Camptothecin (**1**) (Fig. 1) is an anti-tumor alkaloid, which was first isolated from the Chinese tree *Camptotheca acuminata* (Nyssaceae) by Wall and co-workers in 1966.[2] Camptothecin (**1**) has drawn great attention worldwide as a potent inhibitor of DNA topoisomerase I.[3,4] Among the numerous camptothecin derivatives, CPT-11,[5] Topotecan (SK&F 104864),[6,7] and 9-aminocamptothecin[8] are being tested clinically as anti-cancer drugs against colon and other cancers in Japan, Europe, and the U.S.A., respectively. The development and use of camptothecin derivatives as potential anti-cancer drug candidates suffer frequently 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 was first prepared from camptothecin in 1981 by Miyasaka and co-workers[9] and has shown improved solubility in organic solvents and an excellent reactivity. The amino acid hydrazides have been selected as hydrophilic moieties because they contain polar amino groups and can be easily prepared from corresponding amino acid esters and hydrazine. We have synthesized a series of novel water-soluble 7-(aminoacylhydrazono)-formyl camptothecins and have evaluated their inhibitory effect on DNA topoisomerase I. Some results have been reported in the previous communication,[10] and we describe herein the full details concerning these novel DNA topoisomerase I inhibitors.



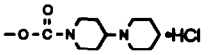
	R_1	R_2	R_3
Camptothecin (1):	H	H	H
CPT-11 (2):		H	C_2H_5
Topotecan (3):	OH	$-CH_2N(CH_3)_2$	H
7-Formyl Camptothecin (4):	H	H	CHO

Figure 1. The structures of camptothecin and its derivatives.

†Antitumor Agents 153. For paper 152 in this series, see Ref. 1.

Chemistry

Commercially available acylhydrazines and camptothecin were obtained from Aldrich Chemical Co. or other commercial sources. Commercially unavailable acylhydrazines were prepared according to a literature method,[11] and 7-formyl camptothecin was synthesized from camptothecin by Miyasaka's method.[9] The compounds can be formed, according to one general method, by the reaction method illustrated in Figure 2. Briefly, 7-formyl camptothecin (**4**) was dissolved in $CHCl_3$-EtOH or $CHCl_3$-MeOH. The amino acyl hydrazine in HCl solution was added to the above solution, then the mixture was allowed to stand for 2 h at room temperature or was warmed to 60 °C for 10 min. After the reaction, the solvent was removed under vacuum and the residue was purified via Sephadex column chromatography to give the desired compounds (e.g. compounds **5–22** in Fig. 3).

Because the reaction is performed in hydrochloric acid-containing solution and a suitable amino group exists in the acylhydrazide, the corresponding water-soluble HCl salt is formed.

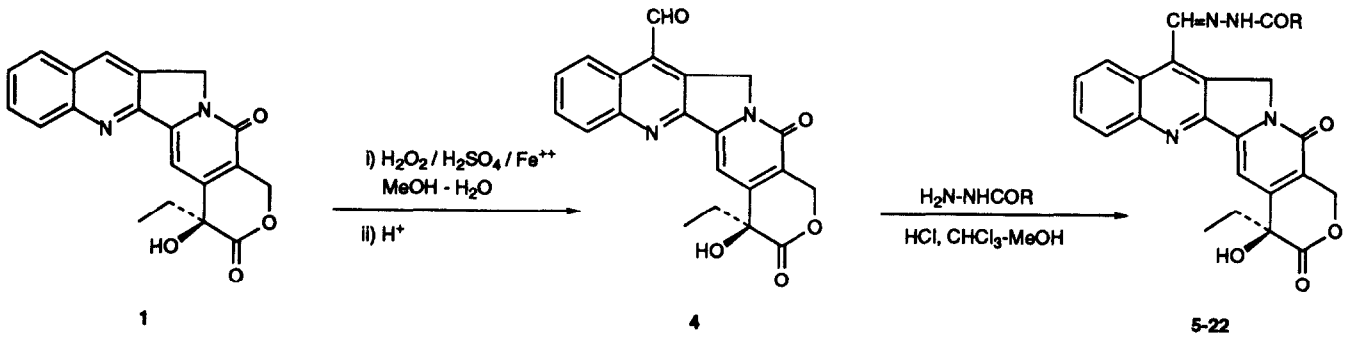


Figure 2. Synthesis of 7-(aminoacylhydrazono)-formyl camptothecin analogues.

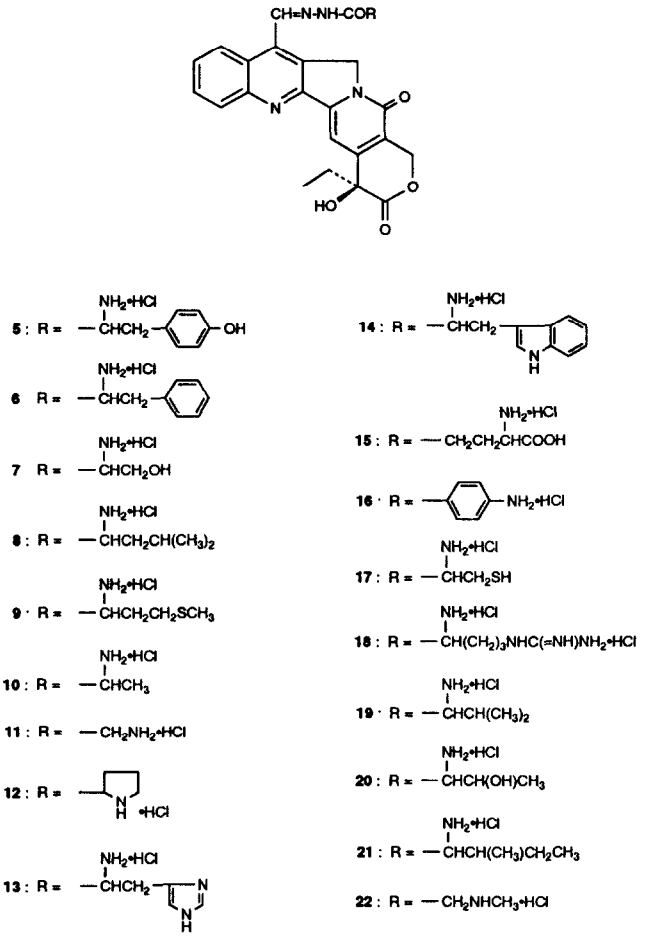


Figure 3. The structures of 7-(aminoacylhydrazono)-formyl camptothecin analogues.

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]

Table 1 shows the bioassay results for several of the new camptothecin analogs. Generally, the relaxation activity inhibition of topoisomerase I and the protein-linked DNA breaks correlated with the cytotoxicity. Five of the derivatives (**5, 6, 8, 9,** and **12**) were as potent or more potent in causing protein-linked DNA breaks and inhibiting topoisomerase I activity, though they were less toxic to the cells when compared with camptothecin. Among them, aromatic amino acid residue-bearing compounds **5** and **6** were the most potent in both assays. The cell death caused by these five compounds may result from mechanisms other than DNA single-strand breaks. Two of the derivatives (**7** and **11**) caused fewer protein-linked DNA breaks but more or equal inhibition of topoisomerase I activity; the reverse was true for compound **22**. Compounds **10** and **13–20** were less active in both assays.

In the cases of CPT-11 and topotecan, the hydrophilic groups were introduced into either the 10 or 9 position in the A ring. The above results suggest that the 7 position in the B ring is also a suitable location for introducing a polar moiety into camptothecin.

Experimental

Melting points were determined on a Fisher–John melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlantic, GA. ^{1}H NMR spectra were measured at 300 MHz on a Bruker 300 spectrometer and chemical shifts are reported in δ (ppm) units relative to the internal reference Me_4Si. Infrared (IR) spectra were recorded on a Perkin Elmer IR 400 spectrometer as KBr pellets. Mass spectra (MS) data were obtained on a TRIO 1000 mass spectrometer. Gel filtration chromatography was performed on Sephadex G-15 gel (Pharmacia Fine Chemicals, mesh 40–120 μ).

General procedure for synthesis of 7-(aminoacylhydrazono)-formyl camptothecin compounds

Synthesis of compounds **5–22** followed the procedure below. To a solution of 7-formyl camptothecin (**4**) (50 mg, 0.13 mmol) in 10 mL $CHCl_3$ and 10 mL MeOH, an appropriate acyl hydrazine (0.65 mmol) in 0.5 mL 10 % hydrochloric acid was added. The mixture was allowed to stand for 2 h at room temperature or was warmed for 10 min at 60 °C to complete the reaction. The solvent was then removed under vacuum. The residue was purified by Sephadex G-15 gel filtration chromatography

Table 1. Biological activities of 7-(aminoacylhydrazono)-formyl camptothecin analogs

Compound No.	Inhibition of Cell Proliferation (μg/mL) KB	KB VP-16^R/7μM	KB VP-16^R/10μM	KB VCR^R/20μM	Protein linked DNA Breaks in cells (25μg/mL), %	Inhibition of Topo I (25μg/mL), %
1	0.01	0.04	0.03	0.03	100.0	100.0
5	0.10	5.00	1.00	1.00	129.3	163.5
6	0.04	3.00	0.08	0.20	144.0	118.0
7	0.12	5.00	1.00	3.00	69.4	145.0
8	0.10	1.00	0.10	0.40	111.7	120.0
9	0.10	0.50	0.10	0.20	111.0	136.0
10	0.10	5.00	0.20	3.00	93.7	98.0
11	0.10	3.00	0.50	1.00	84.5	104.0
12	0.04	0.60	1.00	5.00	117.5	100.0
13	1.00	5.00	3.00	5.00	56.9	33.0
14	5.00	5.00	4.00	1.00	65.2	67.0
15	1.00	0.50	0.60	< 0.30	69.6	89.0
16	0.30	0.30	0.60	1.00	69.0	13.0
17	3.00	1.00	0.60	0.30	71.8	5.0
18	3.00	1.00	< 0.30	< 0.30	81.8	62.0
19	< 0.30	2.00	0.60	< 0.30	65.2	55.0
20	1.00	5.00	5.00	1.00	40.8	55.0
21	< 0.30	0.50	1.00	< 0.30	89.4	93.0
22	>10.00	1.00	2.00	0.30	114.7	89.0

(eluting with $CHCl_3$:MeOH:H_2O, 6:4:1) to give the desired 7-(aminoacylhydrazono)-formyl camptothecin.

Compounds **5–22** formed by the above synthetic method have the following characteristics.

7-(L-Tyrosylhydrazono)-formyl-camptothecin.hydrochloride (5)

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) cm^{-1}; ^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 coupling, 2H, 5-H), 4.87 (t, J = 7 Hz, 1H, NH_2-C*H*- of tyrosine residue), 5.18, 5.33 (both d, J = 16 Hz, 1H each, 17-H), 6.47, 6.78, 6.98, 7.10 (all d, J = 8 Hz, 1H each, aromatic protons of tyrosine residue), 6.91 (s, 1H, 14-H), 7.26, 7.35 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 7.50, 7.61 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.15 (s, 1H, *H*C=N-N); Anal. ($C_{30}H_{28}N_5O_6Cl·1.5H_2O$) C, H, N.

7-(L-Phenylalanylhydrazono)-formyl-camptothecin·hydrochloride (6)

Yield 93 %; yellow powder, mp 252 °C (decomp.); IR (KBr) 3430 (OH), 3300–2000 (very broad, NH_3^+), 1740 (γ-lactone), 1695 (carbonyl of amino acid residue), 1655 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 1.04 (t, J = 7 Hz, 3H, 8-H), 1.99 (q, J = 7 Hz, 2H, 19-H), 3.37 (d, J = 7 Hz, 2H, CH_2 of phenylalanine residue), 5.25 (t, J = 7 Hz, 1H, NH_2-C*H*- of phenylalanine residue), 5.42 (m, AB type coupling, 2H, 5-H), 5.45, 5.67 (both d, J = 16 Hz, 1H each, 17-H), 7.06 (t, J = 7.5 Hz, 1H, 4'-H of phenylalanine residue), 7.18 (t, J = 7.5 Hz, 2H, 3'-H and 5'-H of phenylalanine residue), 7.36 (d, J = 7.5 Hz, 2H, 2'-H and 6'-H of phenylalanine residue), 7.73 (s, 1H, 14-H), 7.81, 7.93 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.24, 8.30 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.36 (s, 1H, *H*C=N-N); Anal. ($C_{30}H_{28}N_5O_5Cl·2H_2O$) C, H, N.

7-(L-Serinylhydrazono)-formyl-camptothecin·hydrochloride (7)

Yield 85 %; yellow powder, mp 273 °C (decomp.); IR

(KBr) 3430 (OH), 3300–2000 (very broad, NH_3^+), 1745 (γ-lactone), 1700 (carbonyl of amino acid residue), 1655 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (D_2O) : δ 1.03 (t, J = 7 Hz, 3H, 18-H), 1.99 (q, J = 7 Hz, 2H, 19-H), 4.10 (dd, J = 6.5, 12 Hz, 1H of C$\underline{H}_2$OH), 4.35 (dd, J = 3.5, 12 Hz, 1H of C$\underline{H}_2$OH), 5.04 (t, J = 3.5 Hz, 1H, NH_2-C$\underline{H}$-), 5.43, 5.62 (both d, J = 16 Hz, 1H each, 17-H), 5.53 (d, J = 10 Hz, 2H, 5-H), 7.71 (s, 1H, 14-H), 7.81, 7.93 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.26, 8.39 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 9.08 (s, 1H, $\underline{H}$C=N-N); Anal. ($C_{24}H_{24}N_5O_6Cl \cdot 2\ H_2O$) C, H, N.

*7-(L-Leucinylhydrazono)-formyl-camptothecin·hydrochloride (**8**)*

Yield 83 %; yellow powder, mp 236 °C (decomp.); IR (KBr) 3430 (OH), 3300–2000 (very broad, NH_3^+), 1745 (γ-lactone), 1700 (carbonyl of amino acid residue), 1655 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 1.01 (d, J = 6.5 Hz, 6H, -CH(C$\underline{H}_3$)$_2$), 1.09 (m, 1H, -C$\underline{H}$-(CH$_3$)$_2$), 1.33 (t, J = 7 Hz, 3H, 18-H), 1.80 (m, 2H, C$\underline{H}_2$-CH(CH$_3$)$_2$), 1.97 (q, J = 7 Hz, 2H, 19-H), 4.31 (dd, J = 6.5, 14 Hz, 1H, NH_2-C$\underline{H}$-CH$_2$-), 5.40 (m, AB type coupling, 2H, 5-H), 5.42, 5.61 (both d, J = 16 Hz, 1H each, 17-H), 7.70 (s, 1H, 14-H), 7.81, 7.93 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.26, 8.44 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 9.07 (s, 1H, $\underline{H}$C=N-N); Anal. ($C_{27}H_{30}N_5O_5Cl \cdot H_2O$) C, H, N.

*7-(L-Methionylhydrazono)-formyl-camptothecin·hydrochloride (**9**)*

Yield 93 %; yellow powder, mp 234 °C (decomp.); IR (KBr) 3430 (OH), 3300–2000 (very broad, NH_3^+), 1740 (γ-lactone), 1692 (carbonyl of amino acid residue), 1652 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 0.89 (t, J = 7 Hz, 3H, 18-H), 1.92 (q, J = 7 Hz, 2H, 19-H), 2.19, 2.31, 2.61, 2.81 (all *m*, 1H each, 4H of -C$\underline{H}_2$-C$\underline{H}_2$-SCH$_3$), 4.98 (dd, J = 4, 8 Hz, 1H, -C$\underline{H}$-NH$_2$), 5.40 (m, AB type coupling, 2H, 5-H), 5.28, 5.48 (both d, J = 16 Hz, 1H each, 17-H), 5.43 (br d, J = 3 Hz, 2H, 5-H), 7.56 (s, 1H, 14-H), 7.67, 7.79 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.12, 8.26 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.96 (s, 1H, $\underline{H}$C=N-N); Anal. ($C_{26}H_{28}N_5O_5ClS \cdot 2H_2O$) C, H, N.

*7-(L-Alanylhydrazono)-formyl-camptothecin·hydrochloride (**10**)*

Yield 92 %; yellow powder, mp 245 °C (decomp.); IR (KBr) 3420 (OH), 3300–2000 (very broad, NH_3^+), 1745 (γ-lactone), 1690 (carbonyl of amino acid residue), 1652 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (D_2O): δ 1.03 (t, J = 7 Hz, 3H, 18-H), 1.79 (d, J = 7 Hz, 3H, CH_3 of alanine residue), 1.99 (q, J = 7 Hz, 2H, 19-H), 5.43, 5.62 (both d, J = 16 Hz, 1H each, 17-H), 5.53 (br d, J = 7.5 Hz, 2H, 5-H), 7.71 (s, 1H, 14-H), 7.81, 7.93 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.26, 8.39 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 9.09 (s, 1H, $\underline{H}$C=N-N); Anal.($C_{24}H_{24}N_5O_5Cl \cdot 2H_2O$) C, H, N.

Elemental analyses for componds 5–22

No.	Formula	Calculated	Found
5	$C_{30}H_{28}N_5O_6Cl \cdot 1.5H_2O$	C 58 44 H 5.06 N 11.35	C 58.84 H 4.79 N 11.08
6	$C_{30}H_{28}N_5O_5Cl \cdot 2H_2O$	C 59.09 H 5.29 N 11.49	C 58.72 H 5.23 N 11.05
7	$C_{24}H_{24}N_5O_6Cl \cdot 2H_2O$	C 51.60 H 5 23 N 12.54	C 51.81 H 5.30 N 12.93
8	$C_{27}H_{30}N_5O_5Cl \cdot H_2O$	C 58.11 H 5.78 N 12.55	C 58.15 H 5.58 N 12.58
9	$C_{26}H_{28}N_5O_5ClS \cdot 2H_2O$	C 52.57 H 5.43 N 11.79	C 52. 43 H 5.55 N 12.01
10	$C_{24}H_{24}N_5O_5Cl \cdot 2H_2O$	C 53.99 H 5.29 N 13.12	C 54.03 H 5.47 N 13.03
11	$C_{23}H_{22}N_5O_5Cl \cdot 2H_2O$	C 53.13 H 5.04 N 13.47	C 53.41 H 5.24 N 13.57
12	$C_{26}H_{26}N_5O_5Cl \cdot 2H_2O$	C 55.80 H 5.41 N 12.52	C 55.99 H 5.70 N 12.48
13	$C_{27}H_{26}N_7O_5Cl \cdot 2H_2O$	C 54.05 H 5.04 N 16.34	C 54.31 H 4.95 N 16.27
14	$C_{32}H_{29}N_6O_5Cl \cdot 3H_2O$	C 57.64 H 5.29 N 12.61	C 57.68 H 5.34 N 12.41
15	$C_{26}H_{26}N_5O_7Cl \cdot 2H_2O$	C 52.75 H 5.11 N 11.83	C 52.64 H 4.93 N 12.02
16	$C_{28}H_{24}N_5O_5Cl \cdot 2H_2O$	C 57.78 H 4.85 N 12.03	C 58.02 H 5.07 N 11.96
17	$C_{24}H_{24}N_5O_5ClS \cdot H_2O$	C 52.60 H 4.78 N 12.78	C 52.78 H 5.15 N 13.01
18	$C_{27}H_{32}N_8O_5Cl_2 \cdot 3H_2O$	C 48.15 H 5.69 N 16.64	C 48.51 H 5 86 N 16.43
19	$C_{26}H_{28}N_5O_5Cl \cdot H_2O$	C 57.40 H 5.56 N 12.87	C 57.23 H 5.78 N 13.03
20	$C_{25}H_{26}N_5O_5Cl \cdot 2H_2O$	C 54.80 H 5.52 N 12.78	C 54.67 H 5.35 N 12.99
21	$C_{27}H_{30}N_5O_5Cl \cdot 2.5H_2O$	C 55 48 H 6.04 N 11.99	C 55.44 H 6.34 N 12.27
22	$C_{24}H_{24}N_5O_5Cl \cdot 1.5H_2O$	C 54.97 H 5.19 N 13.36	C 55.06 H 5.22 N 12.99

*7-(L-Glycylhydrazono)-formyl-camptothecin·hydrochloride (**11**)*

Yield 94 %; dark yellow powder, mp 241 °C (decomp.); IR (KBr) 3400 (OH), 3300–2000 (very broad, NH_3^+), 1740 (γ-lactone), 1700 (carbonyl of amino acid residue), 1650 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (D_2O) : δ 1.03 (t, J = 7 Hz, 3H, 18-H), 1.98 (q, J = 7

Hz, 2H, 19-H), 4.49 (s, 2H, CH_2 of glycine residue), 5.43, 5.62 (both d, J = 16 Hz, 1H each, 17-H), 5.42 (br d, J = 6 Hz, 2H, 5-H), 7.66 (s, 1H, 14-H), 7.77, 7.88 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.18, 8.34 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 9.03 (s, 1H, HC=N-N); Anal. ($C_{23}H_{22}N_5O_5Cl\cdot 2H_2O$) C, H, N.

7-(L-Prolylhydrazono)-formyl-camptothecin·hydrochloride (12)

Yield 92 %; yellow powder, mp 251 °C (decomp.); IR (KBr) 3420 (OH), 3300-2000 (very broad, NH_3^+), 1740 (γ-lactone), 1690 (carbonyl of amino acid residue), 1652 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 1.02 (t, J = 7 Hz, 3H, 18-H), 1.98 (q, J = 7 Hz, 2H, 19-H), 2.18, 2.33, 2.95 (all m, total 4H, $-CH_2-CH_2-$ of proline residue), 3.56 (m, 2H, $-CH_2-N-$), 5.23 (t, J = 7 Hz, 1H, $-CH_2-CH-N-$), 5.41, 5.58 (both d, J = 16 Hz, 1H each, 17-H), 5.47 (br d, J = 12 Hz, 2H, 5-H), 7.65 (s, 1H, 14-H), 7.78, 7.90 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.21, 8.32 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 9.02 (s, 1H, HC=N-N); Anal. ($C_{26}H_{26}N_5O_5Cl\cdot 2H_2O$) C, H, N.

7-(L-Histidylhydrazono)-formyl-camptothecin·hydrochloride (13)

Yield 96 %; yellow powder, mp 239 °C (decomp.); IR (KBr) 3530 (NH of pyrizo ring), 3460 (OH), 3300–2000 (very broad, NH_3^+), 1760 (γ-lactone), 1692 (carbonyl of amino acid residue), 1652 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (D_2O) : δ 1.00 (t, J = 7 Hz, 3H, 18-H), 1.98 (q, J = 7 Hz, 2 H, 19-H), 3.55 (t, J = 7 Hz, 2H, $NH_2-CH-CH_2$), 4.55 (t, J = 7 Hz, $NH_2-CH-CH_2$-), 5.31 (m, AB type coupling, 2H, 5-H), 5.37, 5.56 (both d, J = 16 Hz, 1H each, 17-H), 7.13, 7.48 (both s, 1H each, H of imidazole ring), 7.56 (s, 1H, 14-H), 7.65, 7.84 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 7.87, 7.99 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.71 (s, 1H, HC=N-N); Anal. ($C_{27}H_{26}N_7O_5Cl\cdot 2H_2O$) C, H, N.

7-(L-Tryptophylhydrazono)-formyl-camptothecin·hydrochloride (14)

Yield 96 %; yellow powder, mp 245 °C (decomp.); IR (KBr) 3400 (OH), 3300–2000 (very broad, NH_3^+), 1745 (γ-lactone), 1692 (carbonyl of amino acid residue), 1648 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 1.02 (t, J = 7 Hz, 3H, 18-H), 1.98 (q, J = 7 Hz, 2H, 19-H), 3.31, 3.5 (both dd, J = 4.5, 14 Hz, 1H each, CH_2 of tryptophan residue), 4.98 (m, AB type coupling, 2H, 5-H), 5.17 (dd, J = 4.5, 10 Hz, 1H, $-CH-NH_2$ of tryptophan residue), 5.43, 5.66 (both d, J = 16 Hz, 1H each, 17-H), 6.40 (m, 3H, aromatic protons of indole ring), 7.06 (s, 1H, 14-H), 7.43 (m, 1H, aromatic protons of indole ring), 7.69 (s, 1H, a-H of indole ring), 7.78, 7.92 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.19, 8.24 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.58 (s, 1H, HC=N-N); Anal. ($C_{32}H_{29}N_6O_5Cl\cdot 3H_2O$) C, H, N.

7-(L-Glutamylhydrazono)-formyl-camptothecin·hydrochloride (15)

Yield 90 %; yellow powder, mp 240 °C (decomp.); IR (KBr) 3430 (OH), 3300–2000 (very broad, NH_3^+), 1735 (γ-lactone), 1715 (carbonyl of amino acid residue), 1645 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (D_2O) : δ 1.03 (t, J = 7 Hz, 3H, 18-H), 1.30 (m, 2H, $-CH-CH_2-CH_2-CO-$), 1.98 (q, J = 7 Hz, 2 H, 19-H), 2.40 (m, 2H, $-CH-CH_2-CH_2-CO-$), 4.18 (t, J = 7 Hz, $NH_2-CH-CH_2-$), 5.41 (br s, 2H, 5-H), 5.42, 5.62 (both d, J = 16 Hz, 1H each, 17-H), 7.55 (s, 1H, 14-H), 7.73, 7.84 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.14, 8.33 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.95 (s, 1H, HC=N-N); Anal. ($C_{26}H_{26}N_5O_7Cl\cdot 2H_2O$) C, H, N.

7-(4-Aminobenzoylhydrazono)-formyl-camptothecin·hydrochloride (16)

Yield 95 %; yellow powder, mp 270 °C (decomp.); IR (KBr) 3550 (NH of benzene ring), 3340 (OH), 3300–2000 (very broad, NH_3^+), 3230 (NH of 4-amino benzoyl group), 1737 (γ-lactone), 1650 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) : δ 0.90 (t, J = 7 Hz, 3H, 18-H), 1.90 (q, J = 7 Hz, 2 H, 19-H), 5.46, 5.48 (both br s, 2H each, 17-H, 5-H), 6.67, 7.78 (both d, J = 8.5 Hz, 2H each, 4H of aminobenzoyl moiety), 7.37 (s, 1H, 14-H), 7.85, 7.94 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.26, 8.53 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.32(s, 1H, HC=N-N); Anal. ($C_{28}H_{24}N_5O_5Cl\cdot 2H_2O$) C, H, N.

7-(L-Cysteinylhydrazono)-formyl-camptothecin·hydrochloride (17)

Yield 94 %; dark yellow powder, mp 235 °C (decomp.); IR (KBr) 3420 (OH), 3300–2000 (very broad, NH_3^+), 1740 (γ-lactone), 1708 (carbonyl of amino acid residue), 1655 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6) : δ 1.02 (t, J = 7 Hz, 3H, 18-H), 1.99 (q, J = 7 Hz, 2 H, 19-H), 3.32 (d, J = 14 Hz, 2H, $-CH_2-SH$), 4.50 (br s, 1H, $-(NH_2)-CH-CH_2-SH$), 5.43, 5.62 (both d, J = 16 Hz, 1H each, 17-H), 5.44 (d, J = 4.5, 2H, 5-H), 7.67 (s, 1H, 14-H), 7.77, 7.89 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.19, 8.35 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 9.04 (s, 1H, HC=N-N); Anal. ($C_{24}H_{24}N_5O_5ClS\cdot H_2O$) C, H, N.

7-(L-Arginylhydrazono)-formyl-camptothecin·hydrochloride (18)

Yield 75 %; dark orange powder, mp 246 °C (decomp.); IR (KBr) 3400 (OH), 3300–2000 (very broad, NH_3^+), 1735 (γ-lactone), 1695 (carbonyl of amino acid residue), 1650 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (D_2O) : δ 0.89 (t, J = 7 Hz, 3H, 18-H), 1.5–2.2 (m, 6H, $CH_2 \times 3$), 3.13 (t, J = 7 Hz, 2H, $-NH-CH_2$), 3.85 (t, J = 7 Hz, $NH_2-CH-CH_2$-), 5.25, 5.34 (both d, J = 16 Hz, 1H each, 17-H), 5.37 (br s, 2H, 5-H), 7.56 (s, 1H, 14-H), 7.74, 7.84 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 7.83, 7.93 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.68 (s, 1H, HC=N-N); Anal. ($C_{27}H_{32}N_8O_5Cl_2\cdot 3H_2O$) C, H, N.

*7-(L-Valylhydrazono)-formyl-camptothecin·hydrochloride (**19**)*

Yield 81 %; yellow powder, mp 232 °C (decomp.); IR (KBr) 3370 (OH), 3300–2000 (very broad, NH_3^+), 1750 (γ-lactone), 1690 (carbonyl of amino acid residue), 1650 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 1.00 (t, J = 7 Hz, 3H, 18-H), 1.13 (d, J = 6 Hz, 6H, -CH-(C$\underline{H}_3$)2), 1.36 (m, 1H, -C$\underline{H}$-(CH_3)$_2$), 1.93 (q, J = 7 Hz, 2 H, 19-H), 3.98 (d, J = 6 Hz, 1H, NH_2-C$\underline{H}$-CH-), 5.22 (m, AB type coupling, 2H, 5-H), 5.34, 5.48 (both d, J = 16 Hz, 1H each, 17-H), 7.46 (s, 1H, 14-H), 7.66, 7.77 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.00, 8.17 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.92 (s, 1H, $\underline{H}$C=N-N); Anal. ($C_{26}H_{28}N_5O_5Cl$•H_2O) C, H, N.

*7-(L-Threonylhydrazono)-formyl-camptothecin·hydrochloride (**20**)*

Yield 75 %; yellow powder, mp 221 °C (decomp.); IR (KBr) 3400 (OH), 3300–2000 (very broad, NH_3^+), 1740 (γ-lactone), 1695 (carbonyl of amino acid residue), 1650 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 0.89 (t, J = 7 Hz, 3H, 18-H), 1.18 (d, J = 6.5 Hz, 3H, CH_3 of threonine residue), 1.84 (q, J = 7 Hz, 2H, 19-H), 3.31 (d, J = 5.2 Hz, 1H, -CO-CH(NH_2)-), 4.02 (qd, J = 5.2, 6.5 Hz, OH-C$\underline{H}$-CH_3), 5.28, 5.49 (both d, J = 16 Hz, 1H each, 17-H), 5.41 (m, AB type coupling, 2H, 5-H), 7.58 (s, 1H, 14-H), 7.68, 7.80 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.14, 8.29 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.99 (s, 1H, $\underline{H}$C=N-N); Anal. ($C_{25}H_{26}N_5O_5Cl$·$2H_2O$) C, H, N.

*7-(L-Isoleucylhydrazono)-formyl-camptothecin·hydrochloride (**21**)*

Yield 89 %; yellow powder, mp 245 °C (decomp.); IR (KBr) 3420 (OH), 3300–2000 (very broad, NH_3^+), 1735 (γ-lactone), 1685 (carbonyl of amino acid residue), 1655 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 0.88, 0.89 (both t, J = 7 Hz, 3H, -CH_2-C$\underline{H}_3$ × 2), 1.20 (d, J = 7 Hz, 3H, -CH-CH_3), 1.56 (m, 2H, -CH-C$\underline{H}_2$-CH_3), 1.85 (q, J = 7 Hz, 2H, 19-H), 3.20 (d, J = 4.5 Hz, 1H, -C$\underline{H}$(NH_2)-), 5.28, 5.47 (both d, J = 16 Hz, 1H each, 17-H), 5.37 (m, AB type coupling, 2H, 5-H), 7.55 (s, 1H, 14-H), 7.67, 7.79 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 8.12, 8.25 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.95 (s, 1H, $\underline{H}$C=N-N); Anal. ($C_{27}H_{30}N_5O_5Cl$·$2.5H_2O$) C, H, N.

*7-(Sarcosylhydrazono)-formyl-camptothecin·hydrochloride (**22**)*

Yield 81 %; yellow powder, mp 270 °C (decomp.); IR (KBr) 3410 (OH), 3300–2000 (very broad, NH_3^+), 1730 (γ-lactone), 1685 (carbonyl of amino acid residue), 1650 (carbonyl of amide), 1635 (C=N) cm^{-1}; ^1H NMR (CD_3OD) : δ 0.88 (t, J = 7 Hz, 3H, 18-H), 1.85 (q, J = 7 Hz, 2H, 19-H), 2.94 (s, 3H, N-CH_3), 5.30 (m, AB type coupling, 2H, 5-H), 4.38 (br s, 2H, -CO-C$\underline{H}_2$-N-), 5.23, 5.34 (both d, J = 16 Hz, 1H each, 17-H), 6.95 (s, 1H, 14-H), 7.41, 7.53 (both t, J = 7.5 Hz, 1H each, 10-H, 11-H), 7.58, 7.68 (both d, J = 7.5 Hz, 1H each, 9-H, 12-H), 8.27 (s, 1H, $\underline{H}$C=N-N); Anal. ($C_{24}H_{24}N_5O_5Cl$·$1.5H_2O$) C, H, N.

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