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In Vitro Cytotoxicity of 5-Aminosubstituted 20(*S*)-Camptothecins. Part 1[†]

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Abstract—A number of 5-aminosubstituted 20(*S*)-camptothecin analogues were prepared via semi-synthesis starting from 20(*S*)-camptothecin and 9-methoxy 20(*S*)-camptothecin. In vitro anti-cancer activity of these analogues was determined using 60 human tumor cell line assay. Although water solubility of most of these compounds was improved compared to 20(*S*)-camptothecin, their anti-cancer activity was considerably diminished. However, only smaller substituents such as methylamine or hydroxylamine as present in **8s** and **8t**, respectively, showed good activity with improved water solubility. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

Camptothecin is an alkaloid isolated from the Chinese[1] plant *Camptotheca acuminata* and the plant *Nothapodytes foetida* of Indian[2] origin. The molecule has a pentacyclic ring structure embodying pyrrolo[3,4-*b*]-quinoline moiety (rings A, B and C), conjugated pyridone moiety (ring D) and a chairal centre within a six membered α-hydroxy lactone (ring E). It has exhibited significant anti-cancer activity in L1210 mice leukemia and Walker 256 sarcoma in rats. However, in the 1970's the human clinical studies[3,4] on 20(*S*)-camptothecin **1** indicated severe side effects due to its high toxicity and water insolubility which curtailed its further clinical progress. Later in 1985, following the advent of Hsiang and co-workers'[5] discovery that topoisomerase I[6–9] is the specific target of 20(*S*)-camptothecin, the research interests on camptothecin have become manifold and led to the generation of several hundreds of synthetic (for some of the recent synthetic analogues, see refs 10–14) and semi-synthetic (for some of the recent semi-synthetic analogues, see refs 15–20) analogues to date. Although in the process several highly potent analogues of **1** have been made, poor water solubility and unacceptable toxic side effects of the drug during its clinical studies have disrupted the entry of many of these analogues into further development. The only analogues of 20(*S*)-camptothecin **1** which have successfully crossed these hurdles and entered into the market recently are Topotecan **2**[21,22] and Irnotecan **3**.[23] Topotecan **2** has recently been approved for the treatment of breast cancer and second line treatment of ovarian cancers in many countries.[24] Irnotecan **3** has been the drug of choice for colorectal cancer in US, Japan and Europe.[25,26] However, irnotecan has shown some toxic side effects and severe diarrhea in Japan due to its pro-drug form and the release of varied amounts of toxic active metabolite, 10-hydroxy-7-ethylcamptothecin(SN-38), in the body.[27] Several other analogues are in various stages of their development.[28–30] Most of these analogues represent only A and B ring modified derivatives of camptothecin **1**. A very limited number of C, D or E ring modified analogues of **1** were reported in the literature as these analogues have produced either total loss of activity or less potent molecules in comparison to camptothecin **1** as per the literature reports (Chart 1) (for C-ring modified analogues, see refs 31–33; for D-ring analogues, see ref 34; for E-ring analogues, see refs 35–37. Recently an E-ring modified analogue BN-80245 claimed to be a potent inhibitor of cell growth and topoisomerase I[16]).

For example, Sawada reported[31] that 5-hydroxy, alkoxy and acetoxy 20(*S*)-camptothecins did not show any anti-tumor activity in L1210 in mice. Recently, Wang et al.,[32] prepared 5α- and 5β-hydroxymethyl-20(*S*)-camptothecins and found them to be inactive in DNA-Topo I

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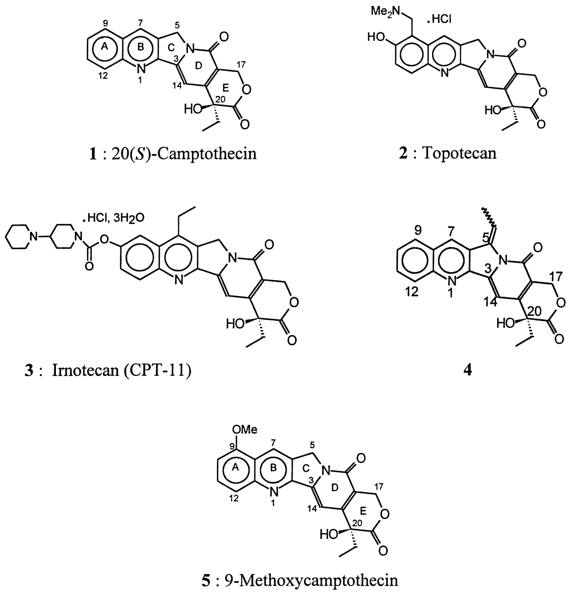


1 : 20(*S*)-Camptothecin

2 : Topotecan

3 : Irnotecan (CPT-11)

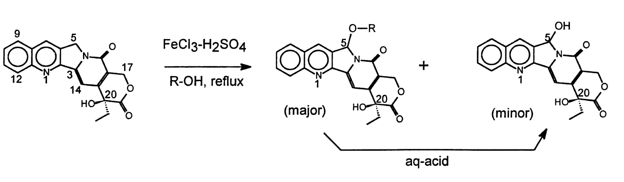
4

5 : 9-Methoxycamptothecin

Chart 1.

inhibition assay as well as human cancer cell line cytotoxicity assay. The reason for this inactivity was attributed to the requirement of planarity of the camptothecin molecule to exhibit its cytotoxicity. They claimed that the substituents at C-5 position are being projected out of the plane, which may interfere with the enzyme and cause ineffective binding to the DNA-Topo I cleavable complex, thereby reducing its anti-tumor activity. However, Sugimori et al.,[39] made 20(*S*)-5-ethylidenecamptothecin **4** which exhibited the same order of potency as 20(*S*)-camptothecin **1** in in vitro P388 cell line assay suggesting that a certain degree of bulk tolerance is allowed at C-5 position of 20(*S*)-camptothecin **1**.

In continuation of our research interests in the design of semi-synthetic analogues of plant based pharmacophores (for our other areas of research, see refs 40–42), we have made several C-5 substituted 20(*S*)-camptothecin analogues in our laboratory in order to study the effect of a wide range of substituents at C-5 position of **1** on its cytotoxic activity. With this endeavor we have developed a novel synthetic transformation in which the naturally occurring 20(*S*)-camptothecin can be transformed to 5-alkoxy and 5-hydroxy camptothecins using $FeCl_3$–H_2SO_4 recipe in an alcoholic medium (eq (1)).



(1)

Aqueous acid hydrolysis of 5-alkoxy camptothecins produced the corresponding 5-hydroxy derivative exclusively. Utilizing the 'masked aldehyde' functionality embedded in 5-hydroxy camptothecin, we have synthesized several camptothecin analogues having a number of substituents at C-5 carbon via oxygen (-O-), nitrogen (-NH-) and carbon ($-CH_2-$) linkages (Chart 2). Contrary to the literature reports,[31–33] our research findings have shown excellent in vitro cytotoxic activity for most of these analogues[43–45] when tested against 60 human tumor cell line assay at National Cancer Institute (NCI), Bethesda, Washington, DC. The synthesis of C-5-NH-substituted camptothecin analogues **8** which are hitherto unknown and the in vitro cytotoxicity obtained against 60 human tumor cell line assay will be discussed here. Also, water solubility of some of these analogues was determined as we anticipated that the presence of additional nitrogen atom into the camptothecin molecule might impart more water solubility.

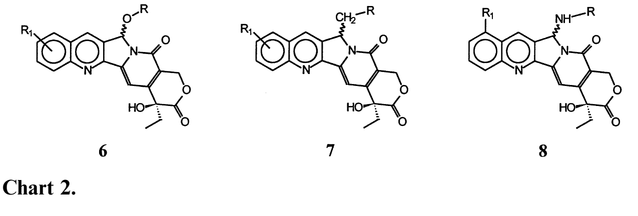
Chemistry

The C-5-NH-substituted 20(*S*)-camptothecin analogues described here were prepared by a three-step semi-synthetic methodology starting from 20(*S*)-camptothecin **1** and 9-methoxy-20-(*S*)-camptothecin **5** which are isolated from the Indian plant *Nothapodytes foetida* following literature method.[2] In the first step, camptothecin **1** and 9-methoxycamptothecin **5** were treated with $FeCl_3$ in the presence of 95% sulfuric acid and ethanol under reflux conditions to produce the corresponding 5-ethoxy derivatives **9a** and **9b**, respectively. Aqueous acid hydrolysis of **9a** and **9b** gave 5-hydroxycamptothecins **10** and **11**, respectively (Scheme 1).

Exploiting the '*masked aldehyde*' functionality embedded in the form of *hemi-aminal* group, the 5-hydroxycamptothecins **10** and **11** were treated with various types of primary amines represented by **12**,

$R\text{-}(CH_2)_n\text{-}NH_2$--------------------**12**

in the presence of a base to obtain 5-NH-substituted 20(*S*)-camptothecin derivatives **8** via the in-situ cyclisation of the intermediate imine compound. The length of the alkylchain in **12** was varied from $n=0$ to 4 and the R group represents a variety of substituents such as halogen, hydroxy, alkoxy, amine, substituted amine, cyclic amine, aryl and cyclohexyl groups. All the 5-NH-substituted camptothecin analogues represented by **8** were isolated as a mixture of 20(*S*),5(*R*) and 20(*S*),5(*S*) diastereomers whose ratio was determined by HPLC as approximately 1:1. These diastereomers were separable by high pressure liquid chromatography (HPLC) technique. Only some selected compounds were isolated as

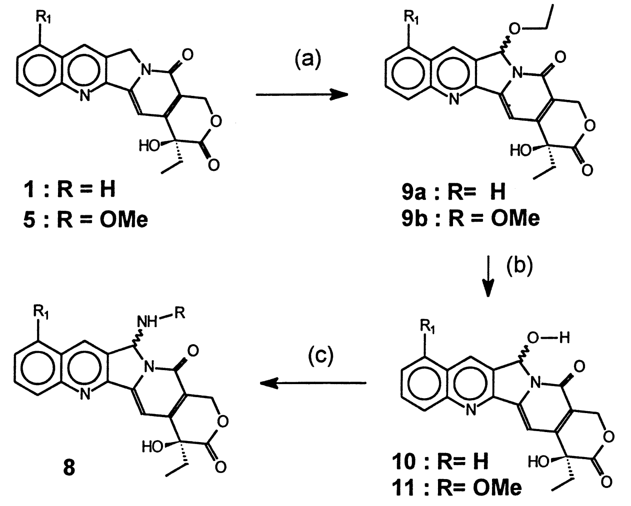


6

7

8

Chart 2.



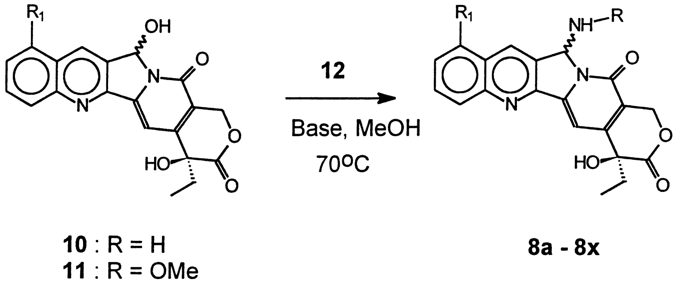
Scheme 1. Reagents: (a) $FeCl_3$, EtOH, H_2SO_4, 85°C 16 h, 70%; (b) 50% HCl, EtOH, 100°C, 16 h, 70%; (c) pyridine or NEt_3, **12**, MeOH, 70°C, 60–70%.

independent diastereomers using flash column chromatography. However, for the purpose of determining their broad in vitro anti-cancer activity against human tumor cell line assay, these compounds were used as a mixture of two diastereomers (approximately 1:1 ratio by HPLC) only.

In general, all the 5-NH-substituted camptothecin analogues represented by **8** were prepared by the treatment of 5-hydroxy camptothecins **10** or **11** with primary amine **12** dissolved in dry methanol in the presence of a base such as pyridine or triethylamine and heating the reaction mixture to reflux temperatures until the starting material is consumed (vide Experimental) (Scheme 1). Four different catagories of primary amines were selected to prepare a variety of 5-NH-substituted camptothecin analogues **8**. *Category 1*: Hydroxy substituted amines such as hydroxylamine hydrochloride, 2-ethanolamine, serinol (2-amino-1,3-propanediol) and 4-hydroxy butylamine. *Category 2*: Diamino alkanes such as ethylene diamine, *N*,*N*-dimethylethylenediamine, *N*,*N*,-diethylethyl-enediamine, *N*,*N*,-diethyl-1,3-propanediamine,1-(2-aminoethyl)pyrrolidine,1-(2-aminoethyl)piperidine,1-(2-aminoethyl) morpholine and 1-(2-aminoethyl)piperazine. *Category 3*: Aryl substituted amines such as benzylamine, furfuryl amine. *Category 4*: Other amines such as methyl amine, 2-chloroethyl amine, 2-(1-cyclohexenyl)ethyl amine and 5-aminovaleric acid. The products obtained were purified by column chromatography and characterised with the support of their analytical data. Broadly, all the compounds of **8** have shown a singlet around 6.5 ppm, diagnostic of $C_5\underline{H}$-NH- proton in their ^1H NMR spectrum and a peak around 79 ppm, diagnostic of $\underline{C_5}$H-NH- carbon in their ^{13}C NMR spectrum.

Biological Activity

All the compounds were tested to determine their in vitro cytotoxicity against 60 human tumor cell line assay[46,47] at National Cancer Institute (NCI), Bethesda, Washington, DC. Table 1 presents the IC_{50} value (the average concentration of the test compound required to produce 50% growth inhibition (GI_{50}) in all the 56 cell lines tested) for all the 5-NH-substituted camptothecin analogues.



In general, the C-5-NH substituted camptothecin analogues have shown much diminished anti-cancer activity when compared to the 20(*S*)-camptothecin **1**. Among the compounds tested, 9-methoxy analogues have showed more tolerance for C-5-NH-substitution than the corresponding camptothecin analogues (see Table 1: IC_{50} of **8b** versus **8c**, **8d** versus **8e**, **8f** versus **8g**, **8r** versus **8q**). Bulky substitutents at C-5 position of both the camptothecins **1** and **5** were not tolerated and produced inactive compounds (**8h**–**8p**) except the compounds **8v** and **8w** which showed good activity. Finally, small substituents at C-5 position as present in the compounds **8s** and **8t** showed better activity out of all the compounds tested. The in vitro anti-cancer activity of the compounds **8s**, **8t**, **8v** and **8w** against 20 human tumor cell line assay representing at least two or three cell lines per each cancer subpanel was presented in Table 2.

Water solubility

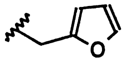
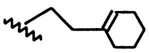
Solubility of some of the C-5-NH-substituted camptothecin analogues was determined in water as well as in pH 5 buffer solution following the reported procedure.[48] Table 3 presents the data obtained for some of the selected compounds. As expected the solubility of these compounds was ranging from 0.08 to 21 mg per mL of water. Compared to the parent camptothecin **1** whose water solubility was 0.002 mg/mL, these compounds showed significant increase in their solubility.

Thus, for the first time 5-aminosubstituted 20(*S*)-camptothecin derivatives were prepared via semi-synthesis. Although most of these derivatives showed high water solubility, they are all found to be inactive in human tumor cell line assay. Only small substituents at the 5-amino group as in **8s** or **8t** exhibited good in vitro anticancer activity.

Experimental

In general all the reactions were carried out under nitrogen atmosphere. The solvents used in the reactions were dried prior to use. FT-IR spectra were recorded on a Perkin–Elmer 1650 FT-IR spectrophotometer. Reagent bases such as pyridine and triethylamine were

Table 1. In vitro cytotoxicity data of 5-aminosubstituted 20(*S*)-camptothecin analogues (**8**)

S No.	$R_1 =$	Primary amine **12**	R	IC_{50} µM
a	H	Ethanolamine	CH_2CH_2OH	52.5
b	H	4-Hydroxy butylamine	$CH_2CH_2CH_2CH_2OH$	34.6
c	OMe	4-Hydroxy butylamine	$CH_2CH_2CH_2CH_2OH$	7.58
d	H	Serinol	$CH(CH_2OH)_2$	50.0
e	OMe	Serinol	$CH(CH_2OH)_2$	6.60
f	H	*N*,*N*-Dimethylethylene diamine	$CH_2CH_2NMe_2$	30.0
g	OMe	*N*,*N*-Dimethylethylene diamine	$CH_2CH_2NMe_2$	9.50
h	OMe	*N*,*N*-Diethylethylene diamine	$CH_2CH_2{\cdot}NEt_2$	19.9
i	OMe	*N*,*N*-Diethylpropylene diamine	$CH_2CH_2CH_2NEt_2$	10.0
j	OMe	Ethylene diamine	$CH_2CH_2NH_2$	13.5
k	H	1-(2-Aminoethyl)pyrrolidine	$CH_2CH_2N(CH_2)_4$	47.8
l	OMe	1-(2-Aminoethyl)pyrrolidine	$CH_2CH_2N(CH_2)_4$	31.6
m	H	1-(2-Aminoethyl)piperidine	$CH_2CH_2N(CH_2)_5$	23.4
n	OMe	1-(2-Aminoethyl)piperidine	$CH_2CH_2N(CH_2)_5$	28.0
o	OMe	1-(2-Aminoethyl)morpholine	$CH_2CH_2N(CH_2CH_2OCH_2CH_2)$	33.8
p	H	1-(2-Aminoethyl)piperazine	$CH_2CH_2N(CH_2CH_2NHCH_2CH_2)$	35.5
q	H	Benzylamine	CH_2Ph	46.7
r	OMe	Benzylamine	CH_2Ph	13.6
s	H	Methyl amine	Me	4.67
t	H	Hydroxylamine hydrochloride	OH	2.39
u	H	2-Chloroethyl amine	CH_2CH_2Cl	19.9
v	OMe	Furfuryl amine		5.37
w	OMe	1-(Cyclohexenyl)ethyl amine		3.16
x	OMe	5-Amino caproicacid	$(CH_2)_5COOH$	16.9
20(*S*)-Camptothecin				0.04
Topotecan				0.16

dried prior to use in reactions. ^1H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively, on a Varian Gemini 200 MHz FT NMR spectrometer. Mass spectra were recorded on Hewlett Packard GC–MS model no. 5989.

5-Hydroxycamptothecin 10. To a mixture of 20(*S*)-camptothecin **1** (2 g) and ferric chloride (2 g) dissolved in 80 mL of ethanol, 10 mL of 95% sulfuric acid was carefully added dropwise and continued heating at 70°C for 24 h. Excess acid and ethanol were removed under vacuum and the residue was extracted with ethylacetate. Organic layer was washed with water, brine and dried over anhyd sodium sulfate. Concentration of the solvent afforded 1.8 g of yellowish powder containing 5-ethoxycamptothecin **9a** and 5-hydroxycamptothecin **10** in the ratio of 8:1. The above mixture was dissolved in 25 mL of ethanol and 50 mL of 50% aq HCl and the contents were heated to reflux for 30 h. The excess water and ethanol were removed as an azeotropic mixture to obtain a residue which was poured into ice water. The resulting precipitate was filtered and dried over P_2O_5 under vacuum to obtain yellow powder. Silica gel column purification of the solid powder using a solvent mixture of ethyl acetate and chloroform afforded 1.4 g of 5-hydroxycamptothecin **10**. IR: 3367, 1749, 1658, 1591 cm^{-1}; ^1H NMR ($CDCl_3 + DMSO$-d_6): δ 8.50 (s, 1H), 8.20 (d, $J = 8$ Hz, 1H), 7.94 (d, $J = 8$ Hz, 1H), 7.85 (t, $J = 6.8$ Hz, 1H), 7.64 (t, $J = 6.8$ Hz, 1H), 7.58 (s, 0.5H), 7.56 (s, 0.5H), 7.06 (s, 0.5H), 7.01 (s, 0.5H), 6.95 (br d, 1H, D_2O exchangeable), 5.67 (d, $J = 16.5$ Hz, 1H), 5.25 (d, $J = 16.5$ Hz, 1H), 5.05 (br d, 1H, D_2O exchangeable), 2.05–1.86 (m, 2H), 1.06 (t, $J = 7$ Hz, 3H); Mass (*m*/*z*): 364 (M + 1), 348, 320, 277, 236, 91, 57.

5-Hydroxy-9-methoxycamptothecin 11. To a mixture of 9-methoxycamptothecin **5** (1 g) and ferric chloride (1 g), dissolved in 50 mL of ethanol, 5 mL of sulfuric acid was added dropwise and continued heating at 85°C for 22 h. Excess acid and ethanol were removed under vacuum and the residue was extracted with ethylacetate. Organic layer was washed with water, brine and dried over anhyd sodium sulfate. Concentration of the solvent afforded dark brownish powder containing 9-methoxy-5-ethoxycamptothecin **9b** and 9-methoxy-5-hydroxycamptothecin **11** in the ratio of 2:1. To the above residue, dissolved in 15 mL of ethanol, 25 mL of 80% aq HCl was added and heated to reflux for 16 h. At the end, excess water and ethanol were removed as an azeotropic mixture and the residue was poured into ice water. The resulting precipitate was filtered and dried over P_2O_5 under vacuum. Column purification of the residual powder afforded 720 mg of 9-methoxy 5-hydroxycamptothecin **11**; IR: 3398, 1749, 1656, 1616, 1577, 1465, 1383, 1154 cm^{-1}; ^1H NMR ($CDCl_3 +$ DMSO-d_6): δ 8.81 (s, 1H), 7.81–7.61 (m, 2H), 7.50 (d, $J = 5.5$ Hz, 1H), 7.12–6.71 (m, 2H), 5.70 (d, $J = 16$ Hz, 1H), 5.30 (d, $J = 16$ Hz, 1H), 4.06 (s, 3H), 1.98–1.75 (m, 2H), 1.10–0.98 (m, 3H); Mass (*m*/*z*): 394 (M + 1), 377, 348, 266, 149, 88, 57.

General experimental procedure for the synthesis of 5-aminosubstituted camptothecins 8. 5-Hydroxycamptothecin **10** or 9-methoxy-5-hydroxy camptothecin **11** and

Table 2. In vitro cytotoxicity data of selected 5-aminosubstituted CPT analogues[a]

Cell panel/ cell line	Compound **8s**	Compound **8t**	Compound **8v**	Compound **8w**
Leukemia				
HL-60	0.22	0.37	—	—
MOLT-4	0.15	0.18	0.30	0.26
Non small cell lung cancer				
HOP 62	1.73	0.39	1.23	1.63
H 460	—	0.49	4.96	14.10
H 522	2.91	0.21	2.87	2.17
Colon cancer				
COLO 205	16.6	3.90	22.9	10.2
HCT 116	7.00	3.10	2.88	4.17
SW 620	5.53	1.92	6.78	4.16
CNS cancer				
U 251	3.79	1.16	2.47	2.08
SF-295	1.13	0.25	0.63	0.53
Melanoma				
M-14	2.20	0.37	3.22	1.42
UACC-62	—	0.37	5.19	0.97
Ovarian cancer				
OVCAR-8	3.73	1.61	2.05	3.14
SK-OV-3	5.25	—	3.20	9.09
Renal cancer				
ACHN	0.98	0.41	1.16	1.17
A 498	16.2	4.32	3.40	6.72
Breast cancer				
MCF-7	1.25	0.34	1.21	1.26
MCF7/ADR	1.95	2.19	1.24	1.21
T 47D	4.64	0.20	2.61	7.74

[a] All the above values are given in μM concentration and refer to GI_{50} values. The term GI_{50} stands for the concentration of the drug required for 50% growth inhibition of the human tumor cells under study.

an amine **12** (1.5 equiv) were suspended in dry methanol and heated to reflux in the presence of pyridine or teiethylamine (10 equiv) until the starting material is consumed. Reaction mixture was concentrated to dryness and the residue was extracted with 5% methanol–ethylacetate. Organic layer was washed with brine and dried over anhyd sodium sulfate. Evaporation of the solvent and purification of the residue over 100–200 mesh silica gel column using ethylacetate–chloroform as an eluent provided the 5-amino substituted camptothecin analogue.

5-(2′-Hydroxyethylamino)camptothecin 8a. A mixture of 5-hydroxycamptothecin **10** (200 mg) and 2-aminoethanol (60 mg) were dissolved in methanol and heated in the presence of pyridine for 16 h to get 140 mg of 5-(2′-hydroxyethylamino) camptothecin as a white solid; IR: 3331, 1745, 1656, 1590, 1404, 1158, 1103, 1044 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.41 (s, 1H), 8.26 (d, $J=11.3$ Hz, 1H), 7.96 (d, $J=11.3$ Hz, 1H), 7.82 (t, $J=6.8$ Hz, 1H), 7.68 (t, $J=6.8$ Hz, 1H), 7.62 (s, 1H), 6.76 (s, 1H), 5.63 (d, $J=16$ Hz, 1H), 5.24 (d, $J=16$ Hz, 1H), 3.85 (br s, 1H, D_2O exchangeable), 3.51 (br t, 2H), 2.51–2.38 (m, 1H), 2.28–2.15 (m, 1H), 2.02–1.69 (m, 2H), 1.06 (t, $J=7.5$ Hz, 3H); ^{13}C NMR (DMSO-d_6): δ 172.26, 157.64, 151.52, 150.36, 148.78, 144.10, 132.63, 130.68 (2C), 128.97, 128.77, 128.15, 127.59, 120.46, 96.44, 79.09, 76.60, 72.30, 65.20, 43.31, 30.29, 7.75; Mass (m/z): 409 (M + 1), 389, 361,347, 319, 91, 57.

5-(4′-Hydroxybutyl amino)-9-methoxycamptothecin 8c. A mixture of 5-hydroxy-9-methoxycamptothecin **11** (100 mg) and 4-aminobutanol (42 mg) were suspended in 20 mL of methanol and heated to reflux in the presence of pyridine (0.4 mL) for 15 h to get 48 mg of 5-(4′-hydroxybutylamino)-9-methoxycamptothecin as a solid; IR: 3357, 1748, 1653, 1599, 1462, 1365, 1267, 1187, 815 cm^{-1}; Partial ^1H NMR ($CDCl_3$): δ 6.67 (s, 0.5H), 6.61 (s, 0.5H), 5.70 (d, $J=16$ Hz, 1H), 5.30 (d, $J=16$ Hz, 1H), 4.06 (s, 3H), 3.81 (s, 1H, D_2O exchangeable), 3.50 (m, 2H), 3.42 (br s, 1H, D_2O exchangeable), 2.40–1.85 (m, 4H), 1.55–1.35 (m, 4H), 1.04 (m, 3H); Mass (m/z): 465 (M + 1), 378, 334, 319, 249, 205, 169, 97, 91.

5-(1′,3′-Dihydroxypropyl-2′-amino)camptothecin 8d. A mixture of 5-hydroxy-camptothecin **10** (100 mg) and serinol (60 mg) were suspended in 10 mL of methanol and heated to reflux in the presence of triethylamine (0.5 mL) for 16 h to get 75 mg of 5-(1′,3′-dihydroxypropyl-2′-amino)camptothecin as a solid; IR: 3361, 1751, 1658, 1595, 1225, 1048 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 8.41 (s, 1H), 8.26 (d, $J=11.3$ Hz, 1H), 7.96 (d, $J=11.3$ Hz, 1H), 7.82 (t, $J=6.8$ Hz, 1H), 7.68 (t, $J=6.8$ Hz, 1H), 7.62 (s, 1H), 6.75 (s, 0.5H), 6.65 (s, 0.5H), 6.02 (s, 1H, D_2O exchangeable), 5.62 (d, $J=16$ Hz, 1H), 5.23 (d, $J=16$ Hz, 1H), 4.25–4.05 (m, 2H, D_2O exchangeable), 3.49–3.22 (m, 4H), 2.78–2.58 (m, 2H), 2.05–1.85 (m, 2H), 1.01 (t, $J=7.5$ Hz, 3H); Mass (m/z): 419 (M-H_2O), 406, 375, 362, 347, 90.

5-(*N*,*N*-Dimethylethylenediamino)camptothecin 8f. A mixture of 5-hydroxy-camptothecin **10** (200 mg) and *N*,*N*-dimethylethylenediamine (100 mg) were suspended in 20 mL of methanol and heated to reflux in the presence of triethylamine (1 mL) for 8 h to get 145 mg of 5-(*N*,*N*-dimethylethylenediamino) camptothecin as a solid; IR: 3416, 1747, 1657, 1595, 1383, 1154, 1105, 1046 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6): δ 8.40 (s, 1H), 8.27 (d, $J=11.3$ Hz, 1H), 7.95 (d, $J=11.3$ Hz, 1H), 7.80 (t, $J=6.8$ Hz, 1H), 7.68 (t, $J=6.8$ Hz, 1H), 7.62 (s, 1H), 6.72 (s, 0.5H), 6.65 (s, 0.5H), 5.66 (d, $J=16.5$ Hz, 0.5H), 5.64 (d, $J=16.5$ Hz, 0.5H), 5.28 (d, $J=16.5$ Hz, 0.5H), 5.26 (d, $J=16.5$ Hz, 0.5H), 5.49 (br s, 1H, D_2O

Table 3. Solubility of 5-aminosubstituted camptothecins[a]

Compound	8a	8d	8k	8p	8s	8t	8u	8v	8w	1
Water	0.6	0.1	16	21	0.15	0.04	0.07	0.09	0.007	0.002
pH 5 Buffer	0.5	0.08	16	16	0.15	0.04	0.05	—	—	0.003

[a] The above values refer to mg/mL of the compound under study.

exchangeable), 2.58–2.05 (m, 4H), 2.25 (s, 6H), 2.05–1.84 (m,2H), 1.05 (t, *J* = 7.5 Hz, 3H); Mass (*m*/*z*): 436 (M + 1), 391, 363, 347, 303, 120, 85.

5-(*N*,*N*-Dimethylethylenediamino)-9-methoxycamptothecin 8g. A mixture of 5-hydroxy-9-methoxycamptothecin **11** (100 mg) and *N*,*N*-dimethylethylenediamine (46 mg) were suspended in 25 mL of methanol and heated to reflux in the presence of triethylamine (1 mL) for 6 h. It furnished 30 mg of 5-(*N*,*N*-dimethylethylenediamino)-9-methoxy-camptothecin as a solid; IR: 3412, 1743, 1665, 1583, 1260, 1167 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 8.87 (s, 1H), 7.73 (m,3H), 7.56 (s, 1H), 7.01 (d, *J* = 7.8 Hz, 0.5H), 6.66 (d, *J* = 12 Hz, 0.5H), 5.80 (d, *J* = 11 Hz, 0.5H), 5.70 (d, *J* = 11 Hz, 0.5H), 5.40 (d, *J* = 11 Hz, 1H), 4.07 (s, 3H), 3.32 (br s, D_2O exchangeable), 2.56–1.82 (m, 6H), 2.25 (s, 6H), 2.30 (1H, D_2O exchangeable), 1.04 (m, 3H); Mass (*m*/*z*); 465 (M + 1), 439, 421, 392, 377, 333, 149, 125, 97.

5-Benzylaminocamptothecin 8q. A mixture of 5-hydroxy-camptothecin **10** (100 mg) and benzylamine (40 mg) were suspended in 6 mL of methanol and heated to 75°C in the presence of triethylamine (0.3 mL) for 10 h to get 60 mg of 5-benzylaminocamptothecin as a solid; IR: 3429, 1751, 1655, 1584, 1156, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.40 (s, 1H), 8.27 (d, *J* = 11.3 Hz, 1H), 7.95 (d, *J* = 11.3 Hz, 1H), 7.82 (t, *J* = 6.8 Hz, 1H), 7.68 (t, *J* = 6.8 Hz, 1H), 7.62 (s, 1H), 7.12–6.95 (m, 5H), 6.75 (s, 0.5H), 6.68 (s, 0.5H), 5.64 (d, *J* = 16.5 Hz, 0.5H), 5.54 (d, *J* = 16.5 Hz, 0.5H), 5.24 (d, *J* = 16.5 Hz, 0.5H), 5.14 (d, *J* = 16.5 Hz, 0.5H), 4.05 (br s, 1H, D_2O exchangeable), 3.75 (s, 1H, D_2O exchangeable), 3.48–3.21 (m, 2H), 2.00–1.79 (m, 2H), 1.19–0.95 (m, 3H); Mass (*m*/*z*): 454 (M + 1), 378, 348, 106, 57.

5-Methylaminocamptothecin 8s. A mixture of 5-hydroxy-camptothecin **10** (100 mg) and 40% aq methylamine (0.2 mL) were dissolved in 10 mL of methanol and heated to 65°C for 16 h to get 75 mg of 5-methylamino-camptothecin as a solid. Purification of the solid over flash column chromatography using 200–400 mesh silica gel and ethylacetate–chloroform as eluent provided the two independent diastereomers (yield: 10 mg each and 40 mg mixture of two isomers); mp: 157°C: IR: 3360, 2925, 1746, 1656, 1594, 1460, 1153, 1042, 762 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.42 (s, 1H), 8.28 (d, *J* = 11.3 Hz, 1H), 7.96 (d, *J* = 11.3 Hz, 1H), 7.82 (t, *J* = 6.8 Hz, 1H), 7.68 (t, *J* = 6.8 Hz, 1H), 7.62 (s, 1H), 6.71 (s, 1H), 5.72 (d, *J* = 16 Hz, 1H), 5.28 (d, *J* = 16 Hz, 1H), 3.95 (br s, 1H, D_2O exchangeable), 2.82 (br s, 1H, D_2O exchangeable), 1.98 (s, 3H), 1.94 (m, 2H), 1.05 (t, *J* = 7 Hz, 3H); Mass (*m*/*z*): 377 (M + 1), 362, 348, 333, 304, 247, 218, 169, 95. Anal. calcd for $C_{21}H_{19}N_3O_4$; C, 66.83; H, 5.07; N, 11.13. Found: C, 66.95; H, 5.12; N, 11.15.

5-Hydroxylaminocamptothecin 8t. A mixture of 5-hydroxycamptothecin **10** (500 mg) and hydroxyl-ammoniumchloride (190 mg) were suspended in methanol and heated in the presence of pyridine for 20 h to get 5-hydroxylamino camptothecin **8t** (350 mg) as solid material. Purification of the solid over flash column chromatography using 200–400 mesh silica gel and ethyl acetate–chloroform as eluent provided the two independent diastereomers (yield: 40 mg each); mp: 190°C; IR: 3745, 3400, 1744, 1654, 1602, 1156, 1046 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 8.41 (s, 1H), 8.27 (d, *J* = 11.3 Hz, 1H), 7.96 (d, *J* = 11.3 Hz, 1H), 7.82 (t, *J* = 6.8 Hz, 1H), 7.68 (t, *J* = 6.8 Hz, 1H), 7.62 (s, 1H), 7.05 (br s, 1H, D_2O exchangeable), 6.58 (s, 1H), 5.72 (d, *J* = 16.5 Hz, 1H), 5.25 (d, *J* = 16.5 Hz, 1H), 5.0 (br s, 1H, D_2O exchangeable), 2.00–1.80 (m, 2H), 1.15–0.95 (m, 3H); Mass (*m*/*z*): 361 (M-18), 348, 317, 218, 57. Anal. calcd for $C_{20}H_{17}N_3O_5$: C, 63.32; H, 4.51; N, 11.07. Found: C, 63.45; H, 4.58; N, 11.12.

5-(2′-Chloroethylamino)camptothecin 8u. A mixture of 5-hydroxycamptothecin **10** (50 mg) and 2-chloroethylamine (20 mg) were suspended in 6 mL of methanol and heated to reflux in the presence of triethylamine (1 mL) for 24 h to get 30 mg of 5-(2′-chloroethylamino)-camptothecin as a solid.; IR: 3422, 1746, 1657, 1600, 1226, 1155, 1046, 762 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.42 (s, 1H), 8.28 (d, *J* = 11.3 Hz, 0.5H), 8.26 (d, *J* = 11.3 Hz, 0.5H), 7.96 (d, *J* = 11.3 Hz, 1H), 7.82 (t, *J* = 6.8 Hz, 1H), 7.68 (t, *J* = 6.8 Hz, 1H), 7.62 (s, 1H), 6.71 (s, 0.5H), 6.65 (s, 0.5H), 5.63 (d, *J* = 16 Hz, 1H), 5.24 (d, *J* = 16 Hz, 1H), 3.85 (br s, 1H, D_2O exchangeable), 3.70 (s, 1H, D_2O exchangeable), 3.45 (br t, 2H), 2.75–2.52 (m, 1H), 2.45–2.22 (m, 1H), 2.05–1.79 (m, 2H), 1.18–0.99 (m, 3H); Mass (*m*/*z*): 426 (M + 1), 390, 376, 347, 305, 79.

5-Furfurylamino 9-methoxycamptothecin 8v. A mixture of 5-hydroxy-9-methoxy-camptothecin **11** (500 mg) and freshly distilled furfuryl amine (0.5 mL) were suspended in 50 mL of methanol and heated to reflux in the presence of pyridine (1.5 mL) and 4-dimethyl-amino-pyridine (40 mg) for 16 h. It furnished 100 mg of 5-furfurylamino-9-methoxy-camptothecin **8v** as yellow solid after column purification. IR: 3372, 1746, 1667, 1594, 1228, 1045, 727 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.88 (s, 1H), 7.83–7.70 (m,2H), 7.55 (s,1H), 7.15 (d, *J* = 8.6 Hz, 1H), 6.97 (d, *J* = 7 Hz, 1H), 6.69 (s, 0.5H), 6.62 (s, 0.5H), 6.00 (d, *J* = 10 Hz, 1H), 5.76–5.55 (m, 2H), 5.28–5.14 (m, 2H), 4.07 (s, 3H), 3.90–3.40 (m, 2H), 1.98–1.77 (m, 2H), 1.08–1.00 (m, 3H); Mass (*m*/*z*): 474 (M +), 392, 378, 334, 191, 121, 96. Anal. calcd for $C_{26}H_{23}N_3O_6$: C, 65.95; H, 4.89; N, 8.87. Found: C, 66.12; H, 4.76; N, 8.79.

5-[2-(1-Cyclohexenyl)ethyl]amino-9-methoxycamptothecin 8w. A mixture of 5-hydroxy-9-methoxy-camptothecin **11** (200 mg) and freshly distilled 2-(1-cyclohexenyl)ethyl amine (0.2 mL) were suspended in 10 mL of methanol and heated to reflux in the presence of pyridine (0.2 mL) for 12 h and provided 60 mg of **8w** as solid after column purification; IR: 1740, 1667, 1617, 1463, 1268, 1154, 831 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.86 (s, 1H), 7.97–7.72 (m, 2H), 7.59 (s, 0.5H), 7.57 (s, 0.5H), 6.95 (d, *J* = 7 Hz, 1H), 6.68 (s, 0.5H), 6.63 (s, 0.5H), 5.72–5.62 (m, 1H), 5.36–5.22 (m, 2H), 4.05 (s, 3H), 2.17–1.82 (m, 8H), 1.80–1.53 (m, 6H), 1.01 (t, *J* = 7.4 Hz, 3H); Mass (*m*/*z*): 530 (M +), 502, 377, 333, 248, 124, 86. Anal. calcd for $C_{29}H_{31}N_3O_5$: C, 69.44; H, 6.23; N, 8.37. Found: C, 69.34; H, 6.35; N, 8.21.

Solubility

Solubility of camptothecin analogues in water as well as in pH 5.0 buffer was determined by HPLC method which separated the lactone and carboxylate forms. HPLC system equipped with Waters 510 pump, 996 photodiode array detector, 77251 rheodyne injector, C-18 column (250×4.6 mm, 5 µm particle size) was used for the analyses. The solubilities were determined in water as well as pH 5.0 in 0.1 M sodium acetate buffer by equilibrating an excess of compound in 0.3 mL each and subjected to rotation on a laboratory rotator for 24 h at 25°C. The samples were filtered through 0.22 µm Millipore (Millex GV4) filter and injected on the column. Calibration curves were obtained by plotting drug concentration versus peak areas of standard sample.

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