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Novel C-Ring Analogues of 20(*S*)-camptothecin. Part 3: Synthesis and Their In Vitro Cytotoxicity of A-, B- and C-ring Analogues

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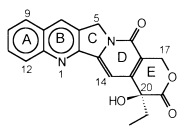
Abstract—Several 5-substituted alkoxy 20(*S*)-camptothecin analogues having A- and B-ring substituents were prepared via semi-synthesis. Most of these compounds were found to exhibit potent anti-cancer activity based on their in vitro cytotoxicity data obtained against human tumor cell lines.

Recently[1,2] we have reported a new synthetic methodology to transform 20(*S*)-camptothecin (CPT) **1** to 5-alkoxysubstituted 20(*S*)-camptothecin **2** using $FeCl_3$–H_2SO_4 recipe. In order to establish the generality of this method, we have carried out this transformation on several A- and B-ring substituted 20(*S*)-camptothecins for the synthesis of the corresponding 5-alkoxysubstituted camptothecin analogues. Also, this will help us in understanding the structure–activity relationship (SAR) of 5-substituted CPT analogues on their anti-cancer activity. The synthesis of these analogues and their in vitro anti-cancer activity against 60 human tumor cell line assay performed at NCI, Bethesda, Washington, DC, USA are reported here.



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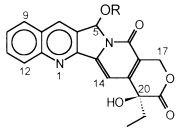

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Accordingly, reaction of A- and B-ring substituted 20(*S*)-camptothecins **3**–**9** (these compounds were prepared according to the methods described in refs 3 and 4) with ethanol and concd sulfuric acid in the presence of $FeCl_3$ produced the corresponding 5-ethoxycamptothecins **3a**–**9a** along with 5-hydroxycamptothecins **3b**–**9b** as minor products. Hydrolysis of **3a**–**9a** using 50% aqueous HCl provided the 5-hydroxy derivatives **3b**–**9b** respectively (Scheme 1).

Among the various C-5 substituted alkoxy-20(*S*)-camptothecin analogues prepared in our laboratory, we have found that the groups such as 2-hydroxyethoxy, 2-fluoroethoxy, 2,2,2-trifluoroethoxy and 2-methoxyethoxy at C-5 position have shown better activity in comparison to 5-alkoxy camptothecin analogues. Therefore, we intended to synthesize 5-substituted camptothecins having selected groups on A- and B-rings at strategic carbons such that the resulting A-, B- and C-ring substituted CPT analogues may exhibit pronounced anti-cancer activity. Keeping this in mind, compounds **3b**, **5b**, **7b** and **9b** were reacted with ethylene glycol, 2-fluoroethanol, 2-methoxyethanol and 2,2,2-trifluoroethanol in the presence of *p*-toluenesulfonic acid using Dean–Stork apparatus to obtain the corresponding 5-substituted derivatives **3c**–**3f, 5c**–**5f, 7c**–**7f** and **9c**–**9f** respectively,[5] in good yield (Scheme 2). In general, virtually every camptothecin known in the literature can be transformed to produce 5-alkoxysubstituted camptothecin analogue by following this method.

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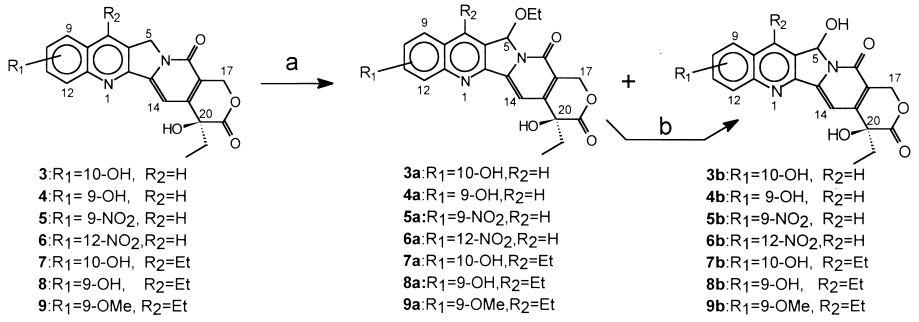
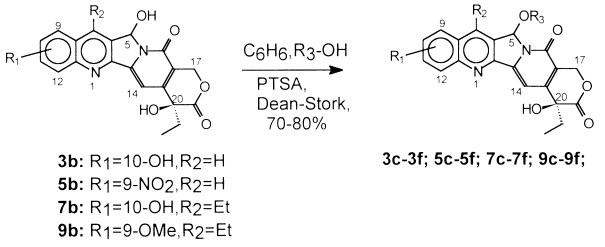


Scheme 1. Reagents: (a) $FeCl_3$, EtOH, 95% H_2SO_4, 85 °C, 20 h, 70–80%; (b) 50% HCl, EtOH, 100 °C, 20 h, 65–75%.

Biological Activity

The in vitro anti-cancer activity of most of these compounds was determined at National Cancer Institute (NCI), Bethesda, Washington, DC, USA using 60 human tumor cell line assay. However, some of the compounds were tested at our cell biology facility against seven human tumor cell lines representing one cell line per each cancer cell panel following NCI protocol.[6,7] The activity data presented here corresponds to approx. 1:1 mixture of both the diastereomers having 20(*S*),5(*S*) and 20(*S*),5(*R*) configuration. Among the 5-substituted camptothecin analogues, 12-nitro derivatives were found to be totally inactive even at 100 μM concn. Although 9-hydroxy 20(*S*) camptothecin is known to be inactive as per the literature reports,[8] 9-hydroxy-5-ethoxycamptothecin **4a** was found to be the most potent compound among the derivatives **3a**–**9a** with an IC_{50} (average concn of the drug required to produce 50% cell growth inhibition in 56 cell lines) value of 0.2 μM (Table 1). The in vitro cytotoxicity data presented in Table 2 correspond to the compounds **3c**–**3f, 5c**–**5f, 7c**–**7f** and **9c**–**9f** against certain selected human tumor cell lines. As expected, most of these compounds



Scheme 2.

Table 1. In vitro cytotoxicity of A-, B-ring substituted 5-ethoxy 20(*S*)-camptothecins

Compd	R_1	R_2	IC_{50} (μM)
3a	10-OH	H	2.88
4a	9-OH	H	0.20
5a	9-NO_2	H	27.5
6a	12-NO_2	H	>30
7a	10-OH	Et	5.0
8a	9-OH	Et	4.67
9a	9-OMe	Et	5.37
20(*S*)-Camptothecin			0.04
Topotecan			0.16

Table 2. In vitro cytotoxicity (GI50) data of A-, B- and C-ring substituted camptothecin analogues[a]

Compd	R_1	R_2	R_3	SF 268	OVC AR 8	MCF7/ADR	DU-145	ACHN	HOP62	UACC62
3c	10-OH	H	CH_2CH_2OH	2.1	2.52	—	3.26	3.40	2.44	2.12
3d	10-OH	H	CH_2CH_2F	20	4.5	4.0	0.25	0.5	10	10
3e	10-OH	H	CH_2CH_2OMe	5.0	4.5	< 0.01	0.68	1.2	4.0	0.52
3f	10-OH	H	CH_2CF_3	0.03	0.26	0.20	0.10	0.10	0.06	0.10
5c	9-NO_2	H	CH_2CH_2OH	4.66	7.14	—	4.13	3.36	2.45	2.46
5d	9-NO_2	H	CH_2CH_2F	0.31	0.74	0.20	0.63	0.25	0.36	0.40
5e	9-NO_2	H	CH_2CH_2OMe	1.0	0.97	—	0.44	0.43	0.40	0.47
5f	9-NO_2	H	CH_2CF_3	0.09	0.13	0.07	0.06	0.02	0.5	0.16
7c	10-OH	Et	CH_2CH_2OH	nd[b]	nd	nd	nd	nd	nd	nd
7d	10-OH	Et	CH_2CH_2F	>30	0.5	0.1	1.0	8.0	25	1.0
7e	10-OH	Et	CH_2CH_2OMe	>30	0.1	0.2	0.55	0.6	20	0.6
7f	10-OH	Et	CH_2CF_3	2.0	0.5	0.4	1.0	0.4	2.0	0.07
9c	9-OMe	Et	CH_2CH_2OH	1.10	8.40	5.53	5.02	3.82	6.47	—
9d	9-OMe	Et	CH_2CH_2F	1.89	1.92	1.99	0.79	1.06	1.22	2.02
9e	9-OMe	Et	CH_2CH_2OMe	0.59	0.85	0.51	1.14	0.52	0.66	2.54
9f	9-OMe	Et	CH_2CF_3	2.13	2.63	1.84	3.90	2.83	1.58	4.68
Topotecan				0.08	0.2	0.07	0.04	0.04	0.05	0.03

[a]All the above values refer to GI50 in μM concn. The term GI50 stands for the concn of the drug required to produce 50% growth inhibition of the cells under study. Representative human tumor cell lines are SF 268 (CNS), OVCAR 8 (ovarian), MCF7/ADR (breast), DU-145 (prostate), ACHN (renal), HOP 62 (lung), UACC 62 (melanoma).
[b]nd, not determined.

Table 3. In vivo anti-tumor activity of compounds **4a** and **3f**[a]

Compd	Dose (mg/kg)	Growth delay (% T-C/C)		
		NCI-H23 (lung)	DU-145 (prostate)	A-498 (renal)
4a	200	12	33	6
	134	15	27	8
	90	8	9	23
3f	200	58[b]	21	27[b]
	134	58[b]	4A	46
	90	12	8	46
Topotecan	10	60	339	47
	5	6	35	67
	2.5	10	83	11

[a]Dose schedule, Q4D×3; host, athymic nude mice; route, iv.
[b]Toxic dose.

showed excellent activity against all the cancer cell lines tested. In particular, compounds **3f, 5d, 5f**, **7f** and **9e** have exhibited GI50 at <1.0 μM concn in all the cell lines. Compounds **3f** and **7f** having trifluoroethoxy group at C-5 position appears to be the most potent out of all (Table 2). In general, B-ring substitution of 5-substituted camptothecin analogues produced less potent compounds, whereas A-ring substitution (except 12-position) provided relatively more potent compounds. The in vivo study of the compounds **3f** and **4a** (as a mixture of both the diastereomers app. in 1:1 ratio) were carried out at NCI against lung, prostate and renal xenograft models. To our surprise, compound **4a** which showed excellent in vitro activity is found to be inactive in all the three xenograft models. However, **3f** exhibited tumor growth delay in prostate and renal xenografts (Table 3). Further evaluation of in vivo efficacy of other interesting compounds in this series is in progress.

In summary, a new and fairly general methodology was developed for the synthesis of 5-substituted camptothecin derivatives possessing a wide number of substituents on A- and B-rings of 20(*S*)-camptothecin **1**. Also, the biological activity results clearly established that the substituent at C-5 position in **1** do produce a large number of active analogues contrary to the literature reports.[9,10] Further study is progressing in the design of a therapeutically more efficacious camptothecin analogue having a suitable substituent at C-5 position of **1**.

References and Notes

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5. The two diastereomers 20(*S*),5(*S*) and 20(*S*),5(*R*) of each of these analogues are well separated on TLC and the independent diastereomers are isolated by flash silica gel column chromatography using acetone–chloroform or methanol–chloroform solvent mixtures as eluent. The spectral data of one of the diastereomers of the selected compounds are given here : Compound **3f**: IR: 3420, 1748, 1664, 1605, 1159, 1001 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.48 (s, D_2O exchangeable, 1H), 8.45 (s, 1H), 8.04 (d, $J=9$ Hz, 1H), 7.47 (d, $J=9$ Hz, 1H), 7.40 (s, 1H), 7.19 (s, 1H), 7.01 (s, 1H), 6.58 (s, D_2O exchangeable, 1H), 5.41 (s, 2H), 5.00–4.60 (m, 2H), 1.87 (m, 2H), 0.89 (brs, 3H); ^{13}C NMR (DMSO-d_6): δ 172.43, 157.76, 157.12, 151.22, 147.50, 144.34, 143.75, 131.04, 130.80, 129.90, 129.16, 124.23, 124.00, 120.63, 109.68, 96.66, 89.73, 72.37, 68.80, 65.19, 30.42, 7.85; Mass (m/e): 462 (M + 1), 418, 364, 320, 263; Compound **4a**: IR: 3368, 1745, 1662, 1616, 1086, 817 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.91 (s, D_2O exchangeble, 1H), 8.75 (s, 1H), 7.80–7.45 (m, 2H), 7.22 (s, 1H), 7.05 (d, $J=9$ Hz, 1H), 6.91 (s, 1H), 6.55 (s, D_2O exchangeable, 1H), 5.41 (s, 2H), 4.18–3.90 (m, 2H), 1.87 (m, 2H), 1.21 (m, 3H), 0.89 (br s, 3H); Mass (m/e): 408 (M + 1), 390, 364, 335, 320.
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