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# Novel C-ring Analogues of 20(S)-Camptothecin-Part-2 : Synthesis and *in Vitro* Cytotoxicity of 5-C-Substituted 20(S)-Camptothecin Analogues

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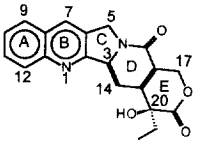
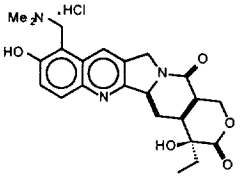
*Abstract : A series of 5-C-substituted 20(S)-camptothecin analogues were synthesised and evaluated their in vitro anti-cancer activity. Several of these analogues have showed excellent activity against human tumor cell lines.* © 1999 Elsevier Science Ltd. All rights reserved.

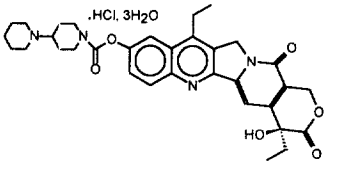
For the past three decades, the research studies on a medicinally important plant alkaloid '20(S)-Camptothecin' **1**[1] have engaged the special attention of medicinal chemists and pharmacologists due to its excellent anti-cancer activity. Since 20(S)-camptothecin **1** itself could not make much clinical progress due to its high toxicity, low water solubility and transformation of active 'lactone form' into inactive 'open acid form' observed in human clinical studies[2], several hundreds of camptothecin analogues were prepared to date[3] and their clinical efficacies were evaluated in various laboratories. The analogues that have successfully crossed these hurdles so far and entered into the market recently are topotecan[4] and irnotecan[5] and several other anlogues[6] are in various stages of their development.



20(S)-Camptothecin **1**



Topotecan



CPT-11 (Irnotecan)

The structure activity relationship[7] emerged out of the pharmacological study of several 20(S)-camptothecin analogues indicated that any modification of C-, D- or E-ring of **1** has resulted into diminished activity or total loss of anti-cancer activity. In particular, the 5-C-substituted camptothecin derivatives were reported to be incative by Lee[7b] and Fukuda[7c]. In continuation of our research programme aimed at the design of novel analogues of plant based pharmacophores[8], we have synthesised several 5-substituted 20(S)-camptothecin analogues[9] having a variety of A- and B-ring substituents and evaluated their *in vitro* anti-cancer activity against 60 human tumor cell line assay at NCI, Bethesda, Washington, USA. Interestingly, most of these analogues exhibited excellent *in vitro* anti-

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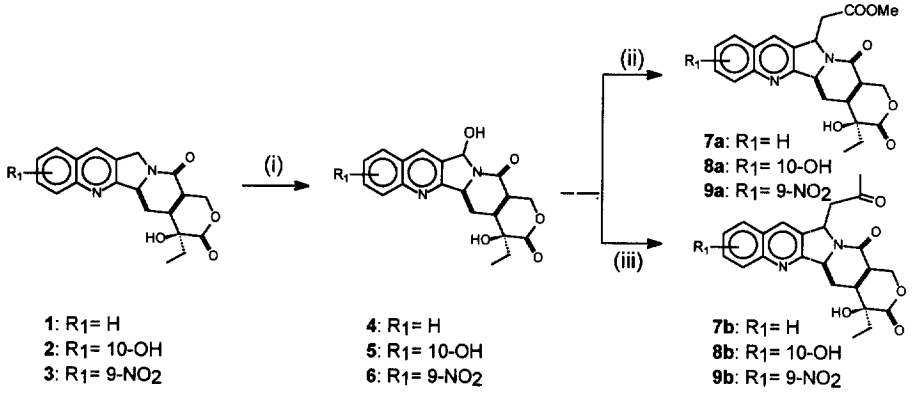
cancer activity against various cancer cell lines. The synthesis and the biological activity of the 5-C-substituted analogues of 20(S)-camptothecin are reported here in this communication

Accordingly, 5-hydroxy camptothecins **4-6** were prepared as shown in Scheme 1 from the corresponding 20(S)-camptothecins **1-3** by a new synthetic transformation developed in our laboratory[9]. Utilising the '*masked aldehyde*' functionality embeded in 5-hydroxycamptothecins, treatment of **4-6** with Wittig reagent such as methyl (triphenyl-phosphoranylidene)acetate in the presence of pyridine in toluene solution afforded the corresponding esters **7a-9a** respectively.Under the similar reaction conditions, reaction of 1-triphenylphosphoranylidene-2-propanone with **4-6** produced the corresponding acetone derivatives **7b-9b**[10] respectively, Scheme1.

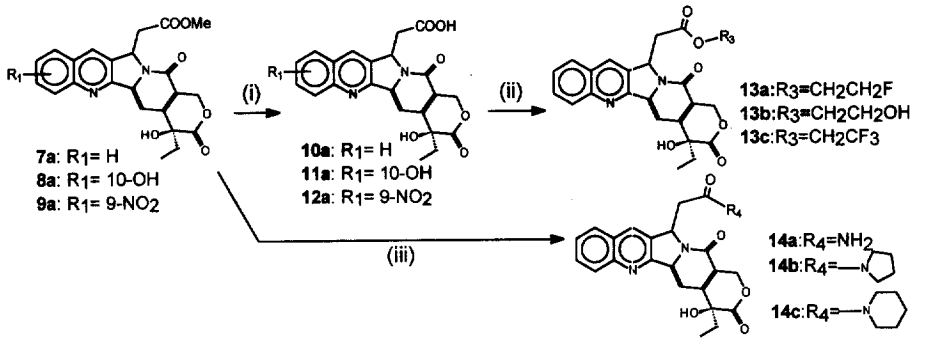
**SCHEME 1**




**Reagents and Conditions**:(i)$FeCl_3$,$H_2SO_4$,EtOH,80°C; 50%aq.HCl,EtOH,100°C; (ii) $Ph_3P$=CH COOMe, toluene, Py, 110°C, 24h,70-80%; (iii) $Ph_3P$=CHCOC$H_3$, toluene, Py or $NEt_3$,110°C, 24-48h, 75-85%;

Alkaline hydrolysis of methyl esters **7a-9a** provided the corresponding 5-carboxymethyl analogues **10a-12a** respectively, Scheme 2. Derivatisation of the acid **10a** with 2-fluoroethanol, ethylene glycol or 2,2,2-trifluoroethanol under acidic reaction conditions furnished novel ester derivatives **13a-13c** respectively[10]. Similarly reaction of ester **7a** with ammonia, pyrrolidine or piperidine under reflux conditions provided the corresponding amides **14a-14c** respectively.

**SCHEME 2**




**Reagents and Conditions** : (i) 1N KOH,EtOH, rt, 3-4h, 75%; (ii) 2-Fluoroethanol for **13a**; Ethylene glycol for **13b**; Trifluoroethanol for **13c**; 1,2-Dichloroethane, $H_2SO_4$, 80°C,12-16h, 70-75%; (iii) ammonia for **14a**; Pyrrolidine for **14b**; Piperidine for **14c**; reflux, 10-12h, 60-70%;

**Biological Activity** : The *in vitro* anti-cancer activity of most of these compounds[11] were determined by testing against 60 human tumor cell line assay at NCI, Bethesda, Washington, USA. Some of these compounds were also tested at our in-house screening facility

**Table 1**: *In vitro* cytotoxicity (GI50) of 5-C-substituted 20(S)-camptothecin analogues :

| | HUMAN TUMOR CELL LINES | | | | | | |
|---|---|---|---|---|---|---|---|
| Compd. | SF 268 | OVCAR 8 | MCF7 /ADR | DU-145 | ACHN | HOP 62 | UACC 62 |
| 7a | 18.6 | 16.3 | 7.2 | 15.5 | 5.29 | --- | 6.44 |
| 8a | 2.32 | 1.72 | --- | 0.6 | 0.44 | 2.09 | 1.72 |
| 9a | 26.2 | 12.6 | ---- | 8.51 | 4.61 | 5.25 | 4.04 |
| 7b | 7.15 | 7.39 | 3.88 | 3.92 | 3.03 | --- | 3.54 |
| 8b | 4.00 | 3.50 | 5.00 | 2.00 | 1.00 | 4.00 | 7.50 |
| **9b** | **0.14** | **1.12** | **0.50** | **0.28** | **0.17** | **0.13** | **0.30** |
| **13a** | **0.05** | **0.04** | **6.00** | **0.10** | **0.08** | **>30** | **0.20** |
| 13b | 1.00 | 0.25 | 6.00 | 1.00 | 10 | >30 | 0.20 |
| 13c | 1.00 | 0.10 | 4.00 | 0.10 | 0.80 | 20 | 0.07 |
| 14a | 29 | 20 | --- | 12 | 16 | >30 | 16 |
| **14b** | **---** | **0.76** | **0.79** | **0.45** | **0.21** | **0.29** | **0.41** |
| **14c** | **0.65** | **3.57** | **3.03** | **0.32** | **0.34** | **0.53** | **0.33** |
| Topotecan | 0.08 | 0.20 | 0.07 | 0.04 | 0.04 | 0.05 | 0.03 |

All the above values were given in μM conc. The term GI50 stands for the conc. of the drug required to produce 50% growth inhibition of the cells under study. Representative cell lines are SF 268(CNS), OVCAR 8(Ovarian), MCF7/ADR( Breast), DU-145(Prostate), ACHN (Renal), HOP 62(Lung), UACC 62(Melanoma).

against a set of 7 selected human tumor cell lines representing one cell line per each cancer subpanel following the NCI protocol[12]. As per the *in vitro* cytotoxicity data presented in table 1, the 5-C-substituted camptothecin analogues have showed good anti-cancer activity. In the case of carbomethoxymethyl analogues, 10-hydroxy derivative **8a** showed better activity than **7a** and **9a**. But, in the case of acetone derivatives, 9-nitro analogue **9b** is found to be more potent than **7b** or **8b**. Among the ester derivatives prepared, substituted alkyl ester analogues **13a** and **13c** are found to be more active than the simple alkyl ester derivatives **7a, 8a** and **9a**. Similarly, compared to unsubstituted amide derivative **14a**, cyclic amide analogues **14b** and **14c** exhibited excellent activity against all the cell lines. However, none of the acid derivatives **10a, 11a** or **12a** showed any significant anti-tumor activity even at 100 μM concentration. Overall, compounds **9b**,**13a** and **14b** were considered to be the most active analogues for which further studies such as MTD(maximum tolerated dose), *in vivo* efficacy and lactone stability in mice and human plasma are warranted.

Thus the biological avtivity data of the above 5-C-substituted 20(S)-camptothecin analogues clearly demonstrated that not all the C-ring analogues of **1** produce inactive compounds as reported in the literature[7]. Certainly the type of substituent at the C-5 carbon of **1** has a marked role to play in altering the anti-cancer activity of the parent molecule.

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10)The two diastereomers[20(S),5(S) and 20(S),5(R)] of each of the C-5-C substituted camptothecins are well separated on tlc and the independent diastereomers are isolated by flash silica gel column chromatography using a combination of acetone-chloroform

solvent system as eluent. Spectral data of one of the diastereomers of selected compounds are presented here : **Compound 7a** : mp: 248°C; IR: 3447,1750,1724,1660, 1612,1154 $cm^{-1}$ ;$^1$H NMR (200 MHz, $CDCl_3$) : δ 8.41( s, 1H), 8.22(d,J=8.2Hz,1H), 7.92(d, J=8.2Hz,1H), 7.82(t,J=8Hz,1H), 7.68(t, J=8Hz,1H),7.64(s,1H),6.05(dd, J=8.60, 2.8Hz, 1H), 5.70( d, J=16Hz, 1H), 5.27(d, J=16Hz, 1H), 3.94 (dd,J=17Hz, 3.4Hz, 1H), 3.79(s, 3H), 2.92 (dd,J=17Hz, 8.6Hz, 1H), 1.90(q, 2H),1.04(t, J=8Hz, 3H) ; Mass(m/z) : 420($M^+$) 402, 376, 361, 317, 277, 218, 91; **Compound 13a**: mp : 175°C; IR : 3444, 1742, 1660, 1599, 1158 $cm^{-1}$; $^1$H NMR (200MHz,$CDCl_3$) : δ 8.41 (s,1H), 8.22(d,J=8.2Hz,1H), 7.91(d,J=8.2Hz,1H), 7.83 (t, J=7.4Hz,1H),7.65(t,J=8Hz,1H),7.64(s,1H), 6.06(dd,J=8.2Hz, 3.4Hz, 1H), 5.68 (d,J= 16Hz, 1H), 5.27 (d,J=16Hz, 1H), 4.66(t,J=4Hz,1H), 4.42(t,J= 4Hz,2H), 4.28(t,J=4Hz,1H), 3.93(dd, J=14Hz,3.2Hz, 1H), 3.76 (s,$D_2O$ exchangeble,1H), 3.06(dd,J=16Hz, 8.0Hz, 1H), 1.92(q,2H),1.04(t,J=8Hz, 3H); Mass(m/z) : 452, 434, 408, 389, 317, 218; **Compound 14b**: mp : 257°C; IR : 3382, 1758, 1659, 1631,1589,1157, 621 $cm^{-1}$; $^1$H NMR(200MHz,$CDCl_3$): δ8.72(s,1H),8.20(d,J=8.2 Hz,1H),7.94 (d, J=8.2 Hz,1H),7.80(t,J=8.0Hz,1H),7.64(s,1H), 7.62(t,J=8Hz,1H), 6.04(dd,J=8.6Hz, 2.8Hz, 1H), 5.70(d,J=16Hz,1H), 5.28(d,J=16Hz,1H), 4.15(dd,J=14.8Hz,2.8Hz,1H), 3.76(s, $D_2O$ exchangeble,1H), 3.65-3.18(m,4H),2.38(dd, J=16Hz,10Hz,1H),2.13-1.81(m,6H), 1.04 (t,J=8Hz,3H); Mass (m/z) : 460($M^+$), 416, 388, 360,316, 191,98.

**11)** All the C-5-C-substituted compounds submitted for *in vitro* anti-cancer activity represent a mixture of two diastereomers 20(S),5(S) and 20(S),5(R) app. in the ratio of 1:1(HPLC).

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