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SYNTHESIS AND CYTOTOXIC ACTIVITY OF ALKYLIDENE- AND ALKYL-SUBSTITUTED CAMPTOTHECINS.

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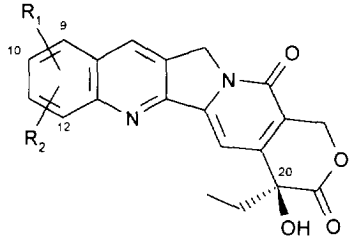
Abstract: A new family of camptothecin derivatives is described. Their synthesis, *in vitro* cytotoxicity, and topoisomerase I inhibition is reported. © 1997 Elsevier Science Ltd.

Camptothecin (**1**), firstly isolated by Wani and Wall from *Camptotheca Acuminata*,[2] is a potent inhibitor of DNA topoisomerase I.[3] Some of its derivatives[4] are on the market as anticancer drugs in Europe, USA and Japan. Among them 9-amino camptothecin[5] (9-AC, **2**) has emerged as one of the most promising clinical candidates for cancer treatment. During the course of our work aimed to the synthesis of 9-AC (**2**), we developed a Pd(0) catalysed deoxygenation on **3**.[6] Accordingly, we decided to extend the Pd(0) catalysis to C-C bond formation, by the use of the Heck reaction.[7] Now we wish to report the synthesis, and the preliminary *in vitro* cytotoxicity, of a new series of camptothecin analogs (**6**), derived from the application of the Heck reaction to the camptothecin nucleus. Compounds (**6**) are endowed with good *in vitro* cytotoxicity.



1 R_1 = H, R_2 = H
2 R_1 = 9-NH_2, R_2 = H
3 R_1 = 10-OH, R_2 = H
4 R_1 = 9-Br, R_2 = H
5 R_1 = 10-OTf, R_2 = H
5a R_1 = 10-OTs, R_2 = 9-NO_2

The Heck reaction on a CPT nucleus lacking an activating group (such as nitro group in **5a**) required use of a leaving group better than tosylate and the choice of the proper reaction conditions and phosphine ligands. Compounds of formula **6a-g** were synthesised from halogeno- or trifluoromethansulfonyl- camptothecins of formula **4** and **5** (scheme 1).[8] Reaction of 9-Br-CPT (**4**) in DMF at 100°C overnight with methyl acrylate and acrylamide in presence of $Pd(OAc)_2$, and 1,1'-bis-(diphenylphosphino)ferrocene (DPPF) as ligand, afforded **6a**,[9] and **6b** in 70%, and 53% yield respectively. The reaction of **4** with methyl 2-acetamidoacrylate in the same conditions was faster (4 hours) and afforded **6c** in 78%y. Only the Z form was present, while a slow (2 weeks) equilibration E/Z took place when **6c** was dissolved in DMSO. Trimethyl vinyl silane reacted in one hour to

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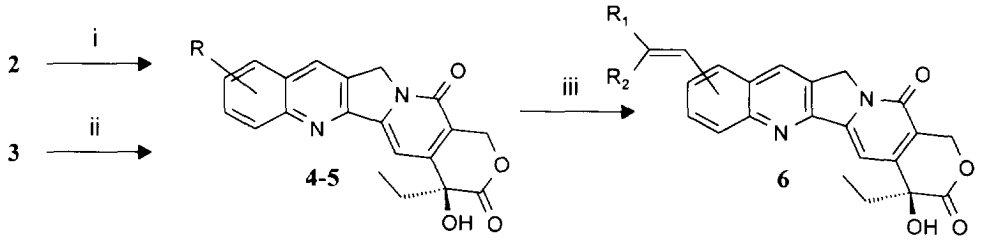
give a mixture of **6e** (50%y.) and **6f** (25%y.), that could be easily separated. **6f** was obtained also from a brief treatment of **6e** with trifluoroacetic acid.

Scheme 1.



9-derivatives

6a R_1 = COOMe, R_2 = H; **6b** R_1 = $CONH_2$, R_2 = H; **6c** R_1 = COOMe, R_2 = $NHCOCH_3$;

6d R_1 = Ph, R_2 = H; **6e** R_1 = $Si(Me)_3$, R_2 = H; **6f** R_1 = H, R_2 = H;

10-derivatives

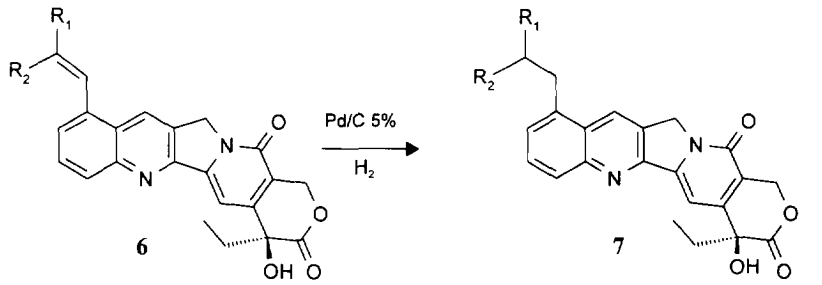
6g R_1 = COOMe, R_2 = H;

i: 16% HBr, $NaNO_2$, rt; CuBr, 16% HBr, 70°C, 2hs; ii: Et_3N, CH_2Cl_2, Tf_2O, 0°C; iii: DMF, 5% mol. $Pd(OAc)_2$, 5.5% mol. Dppf, 100 °C, alkene 5 mol. equiv., 53-78% y.

The reaction with styrene was complete in 5 hours to give **6d** (63%y.). Reaction of **5** in DMF at 80°C with methylacrylate and $Pd(OAc)_2$ /DPPF as catalytic system gave, after 24 hrs, **6g** (57%y.). Addition of one equivalent of LiCl or NaOAc did improve the reactivity,[10] being the reaction complete respectively in 1 and 3 hours, but led to the formation of inseparable by-products. In all cases in compounds **6a-g**, when R_2 = H, the E form was formed exclusively.

Eventually some selected compounds (**6a**, **6c**, and **6d**) were reduced under classical conditions (Pd/C, room temperature) to afford the corresponding alkyl derivatives **7a-c**,[9] according to scheme 2.

Scheme 2.



7a R_1 = COOMe, R_2 = H; **7b** R_1 = COOMe, R_2 = $NHCOCH_3$; **7c** R_1 = Ph, R_2 = H;

Table 1 presents the *in vitro* cytotoxic activity on L1210 murine leukemia cells[11] and the topoisomerase I inhibition[12] of some selected derivatives **6**.

The introduction in 9 position of the camptothecin nucleus of an unsubstituted alkylidene moiety, as in **6f**, provides a compound endowed with a cytotoxic activity comparable to 9-AC. The 2-substitution on the alkenyl residue also influenced the overall cytotoxicity. The presence of a polar amide group (**6b-c**) led to a decrease in cytotoxic activity in respect to the parent compound and to less polar or non polar substituents (**6a** and **6d**). Substitution in 10 position also led to a decrease in cytotoxic activity (**6a** *vs.* **6g**).

Table 1. *In vitro* activity against L1210 murine leukemia cells and relaxation activity inhibition of topoisomerase I of selected camptothecin derivatives **6**.

Compound	Cytotoxicity on L1210 IC_{50} [a] (nM ± S.E.)	Topo I inhibition IC_{50} [b] (μM ± S.E.)
6a	7.6 ± 4.1	6.7 ± 3.1
6b	121.1 ± 13.2	3.2 ± 0.9
6c	1184.9 ± 286.8	31.9 ± 7.3
6d	15.8 ± 1.3	> 100
6f	7.5 ± 0.3	4.2 ± 1.7
6g	62.3 ± 11.8	> 100
9-AC (2)	12.7 ± 0.7	1.8 ± 0.8

a) Concentration inhibiting 50% of cell growth after 48h continuous treatment. b) Concentration inhibiting by 50% the relaxation of 250 ng of SV40 DNA obtained with 0.5 U topoisomerase I at 37°C for 30 min.

Table 2 presents the *in vitro* cytotoxic activity on L1210 murine leukemia cells[11] and the topoisomerase I inhibition[12] of derivatives **7a-c**. The reduction of the alkylidene double bonds of **6a** and **6d** to afford the more flexible, non conjugated alkyl substituted derivatives **7a** and **7c** did not lead to relevant differences in cytotoxicity in respect to the parent compounds. A five fold increase in cytotoxicity was instead observed in **7b** compared to the parent **6c**. In both series there was no correlation between cytotoxicity and topoisomerase I inhibiting activity. In this respect other factors, like the ability of the compounds to accumulate into the cells and to maintain sustained intracellular levels, could play an important role.

Table 2. *In vitro* activity against L1210 murine leukemia cells and relaxation activity inhibition of topoisomerase I of camptothecins **7a-c**.

Compound	Cytotoxicity on L1210 IC_{50} [a] (nM ± S.E.)	Topo I inhibition IC_{50} [b] (μM ± S.E.)
7a	6.2 ± 1.1	41.8 ± 3.8
7b	183.1 ± 32.6	11.3 ± 2.3
7c	16.3 ± 1.3	> 100
9-AC (2)	12.7 ± 0.7	1.8 ± 0.8

a) Concentration inhibiting 50% of cell growth after 48h continuous treatment. b) Concentration inhibiting by 50% the relaxation of 250 ng of SV40 DNA obtained with 0.5 U topoisomerase I at 37°C for 30 min.

Further work is in progress to gain a more precise picture of the structure-activity relationships and of the mechanism of action in this class of compounds and to ascertain their antitumor activity on selected tumor models.

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9. Spectral data of representative compounds. **6a**: ^{1}H NMR (DMSO-d_6) δ, ppm: 9.07 (1H, s); 8.45 (1H, d, J = 15.5 Hz); 8.23 (1H, d, J = 8.5 Hz); 8.14 (1H, d, J = 7.1 Hz); 7.88 (1H, dd, J = 7.6 and 8.2 Hz); 7.34 (1H, s); 6.8 (1H, d, J = 15.7 Hz); 6.53 (1H, s); 5.42 (2H, s); 5.27 (2H, s); 3.79 (3H, s); 1.86 (2H, m); 0.87 (3H, t, J = 7.32 Hz). **7c**: ^{1}H NMR (DMSO-d_6) δ, ppm: 8.89 (1H, s); 8.03 (1H, d, J = 8.49 Hz); 7.79-7.73 (1H, m); 7.55 (1H, d, J = 7.03 Hz); 7.33 (1H, s); 6.51 (1H, s); 5.42 (2H, s); 5.28 (2H, s); 3.59 (3H, s); 3.42-3.36 (2H, m); 2.88-2.77 (2H, m); 1.91-1.80 (2H, m); 0.87 (3H, t, J = 7.33 Hz).
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