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# SYNTHESIS AND ANTITUMOR ACTIVITY OF A NEW CLASS OF WATER SOLUBLE CAMPTOTHECIN DERIVATIVES.

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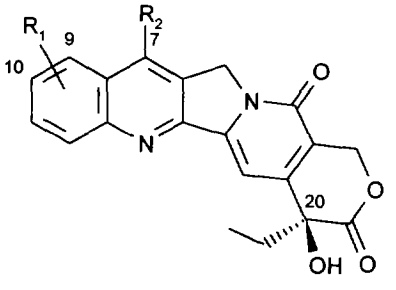
**Abstract:** A new family of water soluble camptothecin derivatives is described. Their synthesis, *in vitro* cytotoxicity, and *in vivo* antitumor activity is reported. Compounds **5a** and **5c** displayed excellent *in vivo* antitumor activity both ip and iv.

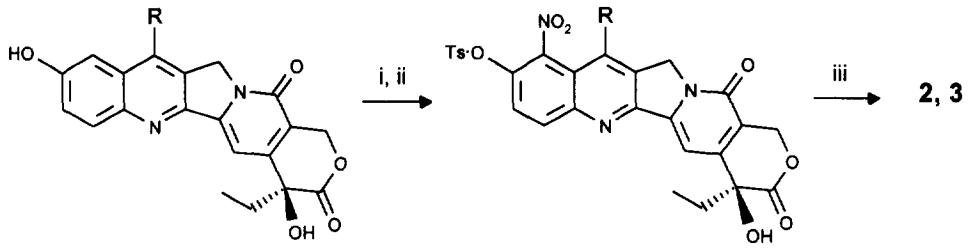
Camptothecin (**1**), firstly isolated by Wani and Wall from *Camptotheca Acuminata*,[2] is a potent inhibitor of DNA topoisomerase I.[3] Some of its derivatives[4] are being tested clinically as anticancer drugs in Europe, USA and Japan. Among them 9-amino camptothecin[5] (9-AC, **2**) has emerged as one of the most promising clinical candidates for cancer treatment. Nevertheless its water insolubility and the difficulties associated with the synthesis of multigram quantities have retarded its clinical development until recently.[5] Now we wish to report our preliminary results on the synthesis and antitumor activity of a new series of water-soluble amino camptothecin derivatives, amidino camptothecins (**5**), endowed with high *in vitro* cytotoxicity and good *in vivo* activity.



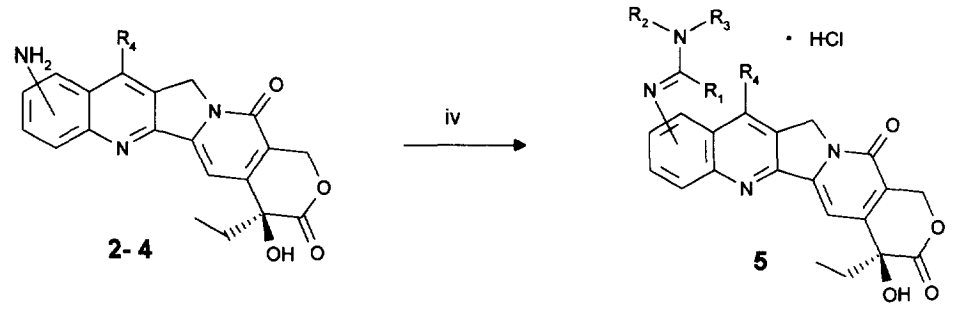

**1** $R_1$ = H, $R_2$ = H
**2** $R_1$ = 9-$NH_2$, $R_2$ = H
**3** $R_1$ = 9-$NH_2$, $R_2$ = Et
**4** $R_1$ = 10-$NH_2$, $R_2$ = H

The amidino derivatives of formula **5a-h** were readily prepared from the appropriate amino camptothecins of formula **2-4**. 9-AC (**2**) and its 7-ethyl analogue (**3**) were prepared in good overall yield according to a new efficient procedure we have recently developed[6] (scheme 1). 10-amino camptothecin (**4**) was prepared following a literature procedure.[7]

**Scheme 1.**




R = H, Et R = H, Et




$R_4$ = H, Et $R_4$ = H, Et

**9-substituted**

**5a** $R_1$ = H, $R_2$ = $R_3$ = Me, $R_4$ = H;

**5b** $R_1$ = $R_2$ = $R_3$ = $R_4$ = H;

**5c** $R_1$ = $R_2$ = $R_4$ = H, $R_3$ = Me;

**5d** $R_1$ = H, $R_2$ = Me, $R_3$ = Ph, $R_4$ = H;

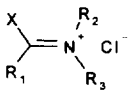
**5e** $R_1$ = $R_4$ = H , $R_2$-$R_3$ = -$(CH_2)_2$-O-$(CH_2)_2$-;

**5f** $R_1$ = $R_2$ = $R_3$ = Me, $R_4$ = H;

**5g** $R_1$ = H, $R_2$ = $R_3$ = Me, $R_4$ = Et;

**10-substituted**

**5h** $R_1$ = H, $R_2$ = $R_3$ = Me, $R_4$ = H.

i: $HNO_3/H_2SO_4$, rt, 83%; ii: *p*-$CH_3$-$PhSO_2Cl$, $CH_2Cl_2$, $Et_3N$, DMAP, rt, 80-87%; iii: $Pd(OAc)_2$, Dppf, HCOOH, $Et_3N$, 90°C, 75-89%; iv:  , DMF, rt, overnight, X = OMe, Cl, 34-79%.

Reaction with freshly prepared formimidates or imidoyl chlorides[8] afforded after the appropriate work-up the corresponding carbon and nitrogen substituted amidines **5a-h**[9] in low to moderate yield, according to scheme 1.

All compounds were isolated as hydrochloride salts, and have shown a high increase in water solubility when compared to the starting amines. Typical values range from 1 to 10 mg/mL (pH = 5.5, rt). Table 1 presents the *in vitro* cytotoxic activity on L1210 murine leukemia cells[10] and the topoisomerase I inhibition[11] of derivatives **5a-h**. The presence of diverse substituents in the amidino moiety (**5c** and **5d**) affords compounds with good cytotoxic activity ($IC_{50}$ 28.1 - 52.0 nM), only slightly reduced in comparison with 9-AC (**2**, $IC_{50}$ 12.7 nM). The presence of identical substituents on the amidine nitrogen (**5a**, **5b**, **5e**, and **5h**) leads to a decrease in cytotoxic activity, although it remains relevant ($IC_{50}$ range 92.5 - 124.9 nM). On the other hand the

introduction of an alkyl substituent on the amidine carbon is associated with a marked reduction in cytotoxicity (**5f**). The cytotoxicity data are not strictly correlated with the inhibiting activity in the enzymatic assay on topoisomerase I, in particular for **5f**. 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 1. *In vitro* activity against L1210 murine leukemia cells and relaxation activity inhibition of topoisomerase I of camptothecins **5a-g**.

| Compound | Cytotoxicity on L1210 $IC_{50}$ [a] (nM ± S.E.) | Topo I inhibition $IC_{50}$ [b] (μM ± S.E.) |
|---|---|---|
| **5a** | 107.7 ± 12.4 | 19.2 ± 7.1 |
| **5b** | 92.5 ± 12.2 | 20.4 ± 2.9 |
| **5c** | 52.0 ± 2.6 | 6.6 ± 1.5 |
| **5d** | 28.1 ± 1.9 | 2.2 ± 0.9 |
| **5e** | 124.9 ± 5.1 | 69.0 ± 2.5 |
| **5f** | 688.8 ± 127.9 | 3.5 ± 0.9 |
| **5g** | 52.4 ± 3.9 | 3.6 ± 0.1 |
| **5h** | 94.5 ± 3.4 | 42.0 ± 12.0 |
| **9-AC (2)** | 12.7 ± 0.7 | 4.2 ± 0.7 |

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.

The *in vivo* antileukemic activity of compounds **5a**, **5b** and **5c** is presented in Table 2. All compounds are effective in increasing the survival time of leukemic mice both after ip and iv treatments. In particular **5a** and **5c** maintained the same efficacy in both models, at variance with 9-AC.

Table 2. *In vivo* activity against L1210 ascitic and disseminated leukemia by ip and iv single treatment of selected amidino camptothecins.

| Compound | L1210 ip[a] T/C%[b] (OD,[c] mg/kg) | L1210 iv [d] T/C%[b] (OD,[c] mg/kg) |
|---|---|---|
| **5a** | 175 (15) | 200 (20)[e] |
| **5b** | 219 (15) | 143 (15) |
| **5c** | 169 (10) | 167 (15)[e] |
| **9-AC (2)** | 163 (1.5) | 138 (2.5) |

a) Ascitic leukemia; treatment ip + 1. b) T/C% median survival time of treated mice/median survival time of untreated control x 100. c) Optimal dose. d) Disseminated leukemia; treatment iv + 1. e) Highest dose tested.

Further work is in progress to gain a more precise picture of the mechanism of action and structure-activity relationships in this class of products. Nevertheless the observed high water solubility and excellent *in vivo* ip and iv antitumor activity on L1210 of compounds **5a** and **5c** warrant further studies.

**References and notes.**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8. For a general preparation of amidines and imidates see for instance *The Chemistry of Amidines and Imidates*, Vol. 20, S. Patai ed., 1975; *Amidines and Imidates*, Vol. 21, S. Patai ed., 1991.
9. 9-(dimethylamino-methyleneamino)-20(S)-camptothecin (**5a**). An excess of freshly prepared dimethyl chloroformimidate chloride was added to a solution of 150 mg of **2** in 20 mL of DMF at room temperature. The mixture was stirred overnight, evaporated *in vacuo*, and the residue was taken-up with water. Reverse phase column chromatography and lyophilization afforded 150mg (79%) of **5a**, as hydrochloride salt. Free base $^1$H NMR (DMSO-$d_6$) δ (ppm) 0.87 (3H, t, J = 7.3 Hz); 1.85 (2H, m); 3.10 (6H, s); 5.27 (2H, s); 5.41 (2H,s); 6.50 (1H, s); 7.08 (1H, m); 7.31 (1H, s); 7.68 (2H, m); 7.94 (1H, s); 9.04 (1H, s). The geometry of the carbon nitrogen double bond was investigated in compound **5f**, isolated as free base. NOESY experiments showed that the double bond assumes the E configuration at room temperature. However, treatment with HCl (0.1M, 1.1 eq, rt) leads to equilibration.
10. The assay for cytotoxic activity was carried out according to a procedure previously described: Geroni, C.; Pesenti, E.; Tagliabue, G.; Ballinari D.; Mongelli, N.; Broggini, M.; Erba, E.; D'Incalci, M.; Spreafico, F.; Grandi, M. *Int. J. Cancer* **1993**, *53*, 308.
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