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Novel Cytotoxic 7-Iminomethyl and 7-Aminomethyl Derivatives of Camptothecin

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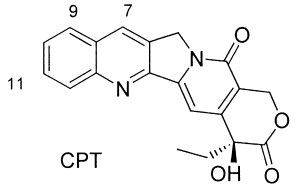
Abstract—A series of new 7-iminomethyl derivatives of camptothecin were obtained from camptothecin-7-aldehyde and aromatic, alicyclic and aliphatic amines. Their hydrogenation led to the corresponding amines. All the imines and the less polar amines showed a marked increase of the cytotoxic activity against H460 non-small lung carcinoma cell line, with respect to topotecan. The lipophilicity of the substituent in position 7 of camptothecin seems to play an important role for cytotoxic potency. The 7-phenyl-iminomethyl derivative showed efficacy comparable to topotecan in vivo against NSCLC H460 xenografted in athymic nude mice.

Camptothecin (CPT), an alkaloid isolated from *Camptotheca acuminata* by Wall and Wani in 1966,[1] showed potent cytotoxic activity. Interest for its application as an antitumour agent declined due to toxic secondary effects, until it was discovered that the cellular target of the drug is topoisomerase I,[2] an enzyme essential for religation of DNA during a number of critical cellular processes, including replication, transcription and repair.[3] Camptothecin, topoisomerase I and DNA form a so-called 'cleavable complex'[4] that induces topoisomerase I-mediated DNA breaks by preventing DNA religation.



These results prompted the synthesis of many derivatives and analogues.[5] Two of them, topotecan (Hycamtin®) and irinotecan (Camptosar®) are now used in clinical practice, whereas some other ones are in various stages of clinical development.[6] Much effort, including that leading to the two above cited drugs, has been spent towards increasing the water solubility of camptothecin, in order to obtain compounds with improved pharmacological profile and enhanced efficacy against human tumors.

From structure–activity relationship studies[7] it appears that the ring-E lactone and the natural 20*S*-configuration are essential for antitumor activity. Whereas activity of compounds with substitutions in rings C and D is critically dependent on the size and type of substituents, most structural modifications have concerned rings A and B, where wide possibilities of variation exist, especially in positions 7, 9, 10 and 11. Recently, the structures of the topoisomerase I covalent and non-covalent complexes with a 22-base pair DNA duplex have been solved by X-ray analysis. On this basis and on structure–activity relationships, a binding mode for camptothecin has been proposed.[8] In this and in an analogous model,[9] there is wide space for substitutions in position 7 of camptothecin without steric clash.

Recent results,[10] also of our own work,[11] have indicated the importance of lipophilic groups in position 7 of camptothecin for potent cytotoxic activity. The scarce solubility in water of these compounds does not represent

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a disadvantage, due to the possibility of successful administration per os of camptothecin derivatives.[11,12] We envisaged, however, that such groups could be easily introduced in position 7 of camptothecin, at the same time maintaining a substantial solubility in water, via imination of the easily available camptothecin-7-aldehyde (**1**).[13]

We report here on the synthesis and in vitro antitumor evaluation of a new series of 20*S*-7-iminomethylsubstituted camptothecins that can also be easily converted to the corresponding amines. Whereas no such imines were known in the literature, some 7-aminomethylcamptothecins deriving from cyclic amines have been obtained by total synthesis.[14] The amine **3a** was reported[15] to be available via a Minisci free-radical aminomethylation of camptothecin, but in our hands this method gave no trace of the compound, although repeated in various experimental conditions.

The 7-iminoderivatives were obtained in good yield from 20*S*-camptothecin-7-aldehyde (**1**), in its turn prepared according to the procedure described by Sawada,[13] by using the $Yb(OTf)_3$-catalyzed condensation reported by Kobayashi et al.[16] (Scheme 1). The reaction works well with aromatic, alicyclic and aliphatic amines. The imines are stable compounds, also in mild acid solutions, except for the photosensitivity of **2d**. The hydrogenation to amines with Pd/C occurred in fair yields (Scheme 1). Compound **3a** was obtained by hydrogenation of the corresponding oxime.[13]

Synthesis of Imines[17]

General procedure

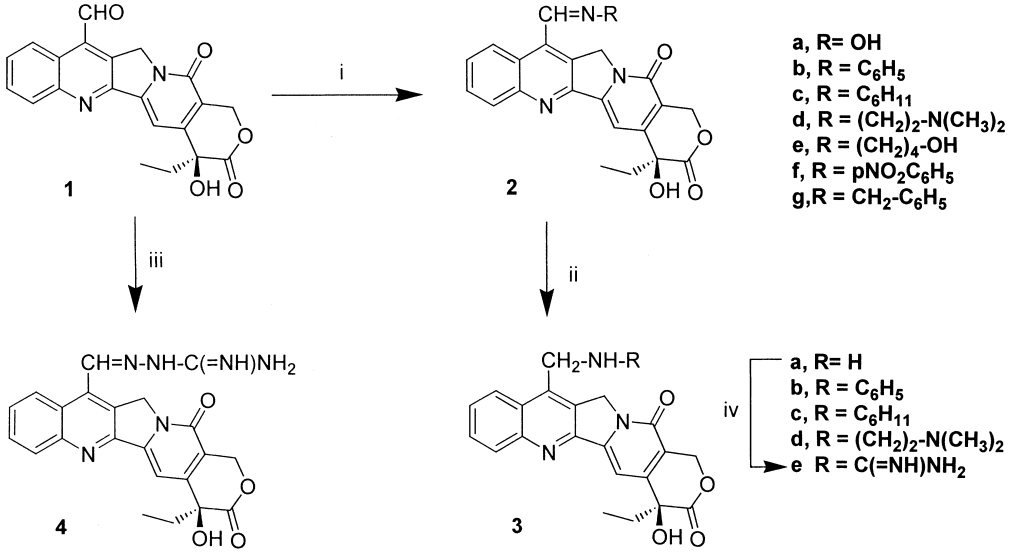
To a suspension of $Yb(OTf)_3$ (16 mg, 0.03 mmol) in 5 mL of anhydrous CH_2Cl_2 containing 4 Å MS, a solution of 7-formylcamptothecin (100 mg, 0.26 mmol) in 20 mL of CH_2Cl_2 is added, followed by a solution of the amine (0.26 mmol) in 0.5 mL of CH_2Cl_2. The resulting mixture is stirred at room temperature until the reaction is complete. After filtering the sieves, 20 mL of water are added and the two phases are separated. The aqueous layer is rapidly extracted three times with dichloromethane. The combined organic phases are dried and evaporated, and the product purified by flash chromatography on silica gel.

2b. The solution is stirred 4 h. Flash chromatography (eluent: CH_2Cl_2/MeOH 98:2). Orange powder. Yield 51%, mp 255–258 °C dec., ^{1}H NMR (DMSO-d_6) δ: 0.83 (t, $J=7$ Hz, H_3-18), 1.7–1.9 (m, H_2-19), 5.37 (s, H_2-17), 5.5 (s, H_2-5), 6.45 (s, -OH), 7.25–7.35 (m, H-14; H arom), 7.4–7.5 (m, 4H arom), 7.7 (m, H-11), 7.85 (m, H-10), 8.16 (dd, H-12), 8.9 (dd, H-9), 9.55 (s, CH=N).

2c. The solution is stirred 2 days. Flash chromatography (eluent: CH_2Cl_2/MeOH 99.1:0.9). Yellow powder. Yield 68%. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.3–1.9 (m, H_2-19; 5-CH_2-), 3.6–3.7 (m, CH–N), 5.4 (s, H_2-17), 5.45 (s, H_2-5), 6.55 (s, -OH), 7.35 (s, H-14), 7.8 (m, H-11), 7.9 (m, H-10), 8.25 (dd, H-12), 8.8 (dd, H-9), 9.5 (s, CH=N).

2d. The solution is stirred overnight. Flash chromatography (eluent: CH_2Cl_2/MeOH 96:4). Light brown powder (to be stored at 4 °C). Yield 84%. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.75–1.95 (m, H_2-19), 2.84 (s, -$N(CH_3)_2$), 3.4–3.55 (m, CH_2–N-), 4.15–4.27 (m, -CH_2–N=), 5.35–5.45 (m, H_2-17; H_2-5), 6.55 (s, -OH), 7.35 (s, H-14), 7.85 (m, H-11), 7.9 (m, H-10), 8.3 (dd, H-12), 8.8 (dd, H-9), 9.62 (s, CH=N).

2e. The solution is stirred overnight. Flash chromatography (eluent: hexane/ethyl acetate 2:8, then MeOH). Yellow powder. Yield 35%. ^{1}H NMR (DMSO-d_6) δ 0.87 (t, $J=7$ Hz, H_3-18), 1.5–1.7 (m, -CH_2-), 1.75–1.95 (m, H_2-19; -CH_2-), 3.45–3.55 (m, CH_2–O), 3.85–3.95 (t,



Scheme 1. (i) RNH_2, $Yb(TfO)_3$, CH_2Cl_2; (ii) H_2/Pd/C; (iii) $NH_2NHC(=NH)NH_3^+$ HCO_3^-, py, EtOH; (iv) 1*H*-pyrazolecarboxamidine HCl, DIEA, DMF.

CH_2–N), 4.45 (t, -OH), 5.42 (s, H_2-17), 5.45 (s, H_2-5), 6.53 (s, -OH), 7.4 (s, H-14), 7.86 (m, H-11), 7.96 (m, H-10), 8.3 (dd, H-12), 8.85 (dd, H-9), 9.5 (s, CH=N).

2f. The solution is stirred 2 days. Flash chromatography (eluent: CH_2Cl_2/MeOH 98:2). Yellow powder. Yield 35%. Mp 260–265 °C dec. ^{1}H NMR (DMSO-d_6) δ: 0.85 (t, $J=7$ Hz, H_3-18), 1.7–1.9 (m, H_2-19), 5.35 (s, H_2-17), 5.48 (s, H_2-5), 6.45 (s, -OH), 7.3 (s, H-14), 7.6–7.7 (m, 2 Ar), 7.8 (m, H-11), 7.9 (m, H-10), 8.25 (dd, H-12), 8.35–8.40 (m, 2 Ar), 8.9 (dd, H-9), 9.67 (s, CH=N).

2g. The solution is stirred 2 days. Flash chromatography (eluent: hexane/ethyl acetate 3:7). Yellow powder. Yield 55%. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.7–1.9 (m, H_2-19), 5.15 (s, -CH_2-), 5.3–5.5 (m, H_2-17; H_2-5), 6.55 (s, -OH), 7.25–7.55 (m, 5 Ar; H-14), 7.85 (m, H-11), 7.95 (m, H-10), 8.26 (dd, H-12), 8.85 (dd, H-9), 9.7 (s, CH=N).

Synthesis of Amines[17]

General procedure

The imine (0.05 mmol) is dissolved in 10 mL of methanol. 20 mg of 10% Pd/C are then added and the resulting suspension is stirred under hydrogen atmosphere until the imine disappears in TLC. The catalyst is filtered and washed with dichloromethane and methanol. The solvent is evaporated and the resulting amine is purified by flash chromatography.

3a. 7-hydroxyiminomethylcamptothecin[14] was hydrogenated for 2 h. Flash chromatography (eluent: CH_2Cl_2/MeOH from 97:3 to 85:15). Yellow powder. Yield 52%. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.7–1.9 (m, H_2-19), 4.37 (s, CH_2–N), 5.43 (s, H_2-17), 5.5 (s, H_2-5), 6.55 (s, -OH), 7.35 (s, H-14), 7.72 (m, H-11), 7.86 (m, H-10), 8.2 (dd, H-12), 8.35 (dd, H-9). MS m/z: 377 (M^+ 17), 360 (48), 316 (100), 288 (23).

3b. Hydrogenation: 1 h. Flash chromatography (eluent: CH_2Cl_2/MeOH 98:2, and rechromatography with ethyl acetate). Yellow powder. Yield 50%. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.7–1.9 (m, H_2-19), 5 (d, $J=7$ Hz, CH_2–N), 5.32 (s, H_2-17), 5.41 (s, H_2-5), 6.42 (t, $J=7$ Hz, -NH-), 6.5 (s, -OH), 6.55–6.65 (m, 3 Ar), 7.05–7.15 (2 Ar), 7.32 (s, H-14), 7.75 (m, H-11), 7.9 (m, H-10), 8.25 (dd, H-12), 8.48 (dd, H-9). MS m/z 453 (M^+ 0.5), 360 (100), 316 (50), 231 (20), 93 (45).

3c. Hydrogenation: 2 h. Flash chromatography (eluent: CH_2Cl_2/MeOH 98:2). Yellow powder. Yield 10%.^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.05–2 (m, H_2-19 and cyclohex.), 4.35 (s, CH_2–N), 5.45 (s, H_2-17; H_2-5), 6.55 (s, -OH), 7.32 (s, H-14), 7.75 (m, H-11), 7.85 (m, H-10), 8.18 (dd, H-12), 8.4 (dd, H-9).

3d. Hydrogenation: 4 h. Flash chromatography (eluent: from CH_2Cl_2/MeOH 9:2 to MeOH). Yellow-brown powder. Yield 20%. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.75–1.85 (m, H_2-19), 2.45 (s, -N(CH_3)$_2$), 2.7–2.9 (m, -CH_2–CH_2-), 4.38 (s, CH_2–N), 5.45 (s, H_2-17; H_2-5), 6.55 (s,-OH), 7.35 (s, H-14), 7.75 (m, H-11), 7.9 (m, H-10), 8.2 (dd, H-12), 8.45 (dd, H-9).

Compound 3e. A solution of 30 mg (0.08 mmol) of **3a**, 11.7 mg (0.08 mmol) of 1*H*-pyrazole-1-carboxamidine hydrochloride and 10.3 mg (0.08 mmol) of diisopropylethylamine in 0.5 mL of DMF was stirred 2 days at room temperature. Ether was then added to precipitate the crude product which was collected, washed with ether and dried. Purification by flash chromatography (eluent: CH_2Cl_2/MeOH from 9:1 to 1:1) afforded 7 mg of the desired product as a yellow solid. Yield 19%. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.75–1.85 (m, H_2-19), 4.20 (t, $J=6$Hz, -*NH*–CH_2), 5.15 (d, $J=6$Hz, -*CH_2*–NH-), 5.38 (s, H_2-17), 5.48 (s, H_2-5), 6.55 (s, -OH), 7.36 (s, H-14), 7.50 (brs NH_3^+), 7.80 (m, H-11), 7.95 (m, H-10), 8.15–8.25 (m, H-12; H-9).

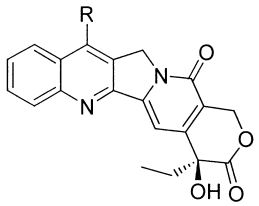
Compound 4. A solution of aminoguanidine bicarbonate (53 mg, 0.4 mmol), 7-formyl-CPT (**1**) (50 mg, 0.13 mmol) and pyridine (1.4 mL) in 10 mL of ethanol was refluxed 3 h. The solvent was evaporated and a solution of water/ethanol 1:1 was added to the residue. The precipitate was filtered and dried to afford 50 mg of **5** as a yellow solid. Yield 89%.^{1}H NMR (DMSO-d_6) δ: 0.87 (t, $J=7$ Hz, H_3-18), 1.75–1.85 (m, H_2-19), 5.45 (s, H_2-17), 5.51 (s, H_2-5), 6.27 (br.s, 4H guan.), 6.55 (s, -OH), 7.39 (s, H-14), 7.75 (m, H-11), 7.90 (m, H-10), 8.21 (dd, H-12), 8.63 (dd, H-9), 9.0 (s, CH=N).

The compounds prepared were evaluated for their cytotoxicity against a human non-small lung carcinoma cell line, H460, using topotecan as a reference compound. This cell model was chosen for its sensitivity to topoisomerase I inhibitors, likely related to overexpression of the target enzyme.[18] H460 is also a useful cell model for in vivo studies of antitumor efficacy for its reproducible growth in athymic mice. Cells were cultured in RPMI-1640 containing 10% fetal calf serum. Cytotoxicity was assessed by growth inhibition assay after 1 h drug exposure. Cells in the logarithmic phase of growth were harvested and seeded in duplicates into six-well plates. Twenty-four hours after seeding, cells were exposed to the drug and harvested 72 h after exposure and counted with a Coulter counter. IC_{50} is defined as the inhibitory drug concentration causing a 50% decrease of cell growth over that of untreated control.

The results of the cytotoxicity studies are summarized in Table 1.

From the data it appears that all the imines prepared, independently from the group attached to the nitrogen atom, show a marked increase of the cytotoxic activity, with respect to topotecan in the same conditions. Hydrogenation of the imino double bond does not appreciably affect the activity, except for **3a** and **3e** These two are much more polar compounds than the other ones (see Table 1, where logP values (however approximate, because of the calculation method) are reported), thus suggesting that as in other cases of 7-

Table 1. In vitro cytotoxic activity of CPT derivatives on H460 cell line



Compound	R	IC_{50} (μM) H460	LogP (calc.)[19]
Topotecan		1.38	
2b	CH=N–C_6H_5	0.13	3.19
2c	CH=N–C_6H_{11}	0.37	3.08
2d	CH=N–CH_2–CH_2–N$(CH_3)_2$	0.12	1.53
2e	CH=N–$(CH_2)_4$–OH	0.36	1.57
2f	CH=NC_6H_4–p–NO_2	0.28	2.34
2g	CH=N–CH_2–C_6H_5	0.28	3.26
3a	CH_2–NH_2	2.37	0.37
3b	CH_2–NH–C_6H_5	0.31	2.56
3c	CH_2–NH–C_6H_{11}	0.35	2.44
3d	CH_2–NH–$(CH_2)_2$–N$(CH_3)_2$	0.45	0.89
3e	CH_2–NH–C(=NH)NH_3^+ Cl^-	12.65	−0.79
4	CH=N–NH–C(=NH)NH_2	39.3	0.67

Table 2. Antitumor activity of **2b** and topotecan (TPT) in the treatment of NSCLC H460 xenografted sc in athymic nude mice (po, q4d×4)[a]

Compound	Dose (mg/kg)	TVI (%)[b]	BWL (%)[c]	Lethal toxicity[d]
2b	2	33	2	0/5
	6	51	7	0/4
	18	89	1	0/5
TPT	15	95	12	0/4

[a]For methods see ref 20.
[b]Tumor volume inhibition % in treated versus control mice assessed 3 days after last treatment.
[c]Body weight loss % 3 days after last treatment.
[d]Dead/treated mice.

substituted camptothecins[10,11] the lipophilicity of the substituent contributes positively to the cytotoxic activity. Also, the distance of the lipophilic group from the camptothecin nucleus might influence the activity. This hypothesis is consistent with the low activity of compound **4**, and of the hydrazone of **1** reported by Sawada.[13]

Compound **2b** was tested for its in vivo activity against non-small cell lung carcinoma H460 xenografted sc in athymic nude mice. The data reported in Table 2 indicate that the compound has an efficacy comparable to topotecan at non-toxic doses, that is it has a better tolerability at effective doses.

In conclusion, this work provides a simple synthetic methodology for the preparation of a wide range of 7-iminomethyl and 7-aminomethyl substituted camptothecins, endowed with potent cytotoxic activity.

Acknowledgements

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