SYNTHESIS AND ANTILEUKEMIC ACTIVITY OF (±)-20-DEOXYAMINOCAMPTOTHECIN ANALOGUES[1)]

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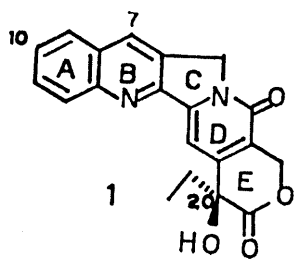
The camptothecin analogues (±)-7-ethyl-10-methoxy-20-deoxyaminocamptothecin (2) and (±)-7-ethyl-10-hydroxy-20-deoxyaminocamptothecin·HCl (3) were synthesized from indolizine compound 4 via Friedländer condensation to construct a pentacyclic ring system, and were tested in a P388 mouse antileukemia assay. Compounds 2 and 3 were more active and less toxic than (+)-camptothecin (1), and therefore had higher therapeutic ratios.

KEYWORDS camptothecin; aminocamptothecin; Friedländer condensation; alkaloid; antileukemic activity

(+)-Camptothecin (1), a heterocyclic alkaloid originally isolated from Camptotheca acuminata (Nyssaceae) by Wall et al. in 1966, has potent antileukemic and antitumor activities,[2)] but a highly toxic effect on both animals and humans.[3)] Thus, structural modification of 1 for a chemotherapeutic agent is necessary, and a number of analogues have been prepared in the course of studies to define structure-activity relationships (SAR).[4,5,6)] The SAR studies revealed that the hydroxy group at the C-20 position in ring E is an absolute requirement for its in vivo antitumor activity since either 20-deoxycamptothecin or 20-chlorocamptothecin is essentially inactive.[5,6)]

To continue our total-synthesis study of optically active (+)-camptothecin,[1,7)] we changed the hydroxy group at the C-20 position into an amino group, and so found that (±)-7-ethyl-10-methoxy-20-deoxyaminocamptothecin (2) and (±)-7-ethyl-10-hydroxy-20-deoxyaminocamptothecin·HCl (3) were more active than the parent compound 1 in a P388 mouse antileukemia assay. Here we describe the synthesis and antileukemic activities of compounds 2 and 3.

To start the synthetic route to amino analogues 2 and 3, we used indolizine compound 4, obtained in the process of the total synthesis of 1.[1,7)] Mesylation of 4 with methanesulfonyl chloride in pyridine afforded 5 in 39% yield [mp 148–152°C (dec.); MS m/z 385 (M^+); IR (KBr): 1746 (lactone), 1671 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 3.30 (3H, s, Ms), 6.55 (1H, s, arom-H)]. Compound 5 was treated with NaN_3 in DMF at 60°C to give azide 6 as an oil in quantitative yield [IR (film): 2120 (N_3) 1746 (lactone) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 6.42 (1H, s, arom-H)]. Azide 6 was deketalized with 80% aqueous trifluoroacetic acid (TFA) at 0°C to afford tricyclic ketone 7 as an oil in 95% yield [IR (film): 2100 (N_3) cm^{-1}].



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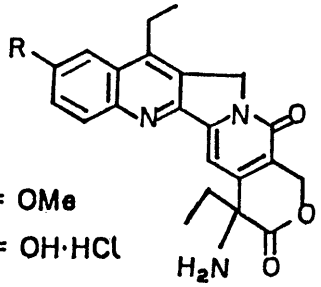
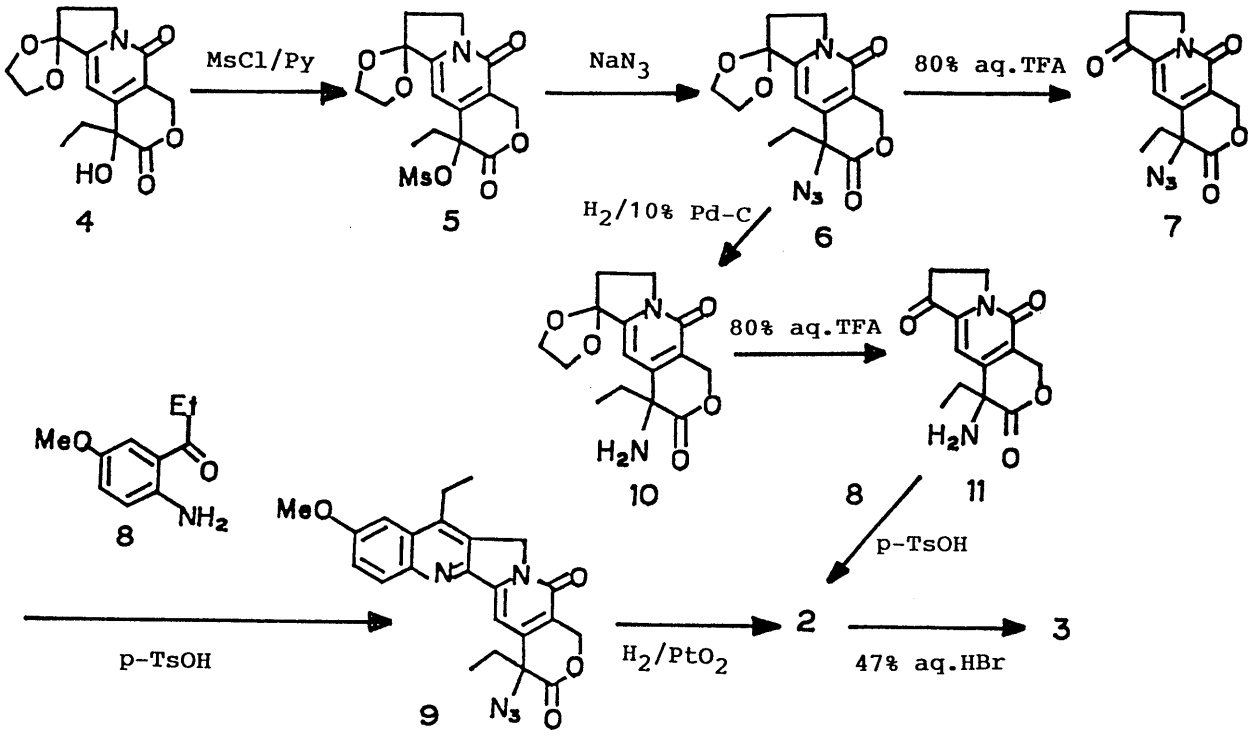


2 R = OMe

3 R = OH·HCl



Friedländer condensation was used to construct a pentacyclic ring system, using toluene in the presence of an acid-catalyst. Thus, compound **7** and 2'-amino-5'-methoxypropiophenone (**8**)[8] in toluene were heated under reflux in the presence of p-TsOH using a Dean-Stark trap to produce **9** as a colorless solid in 63% yield [mp 230-245°C (dec.); MS m/z 431 (M^+); IR (KBr): 2122 (N_3), 1752 (lactone) cm^{-1}; ^{1}H-NMR ($CDCl_3$+ CD_3OD) δ: 4.02 (3H, s, OMe), 7.52 (1H, s, arom-H)]. The azido group of **9** was hydrogenated over PtO_2 to give **2**[9] as a crystalline powder in 47% yield. On the other hand, compound **6** was hydrogenated over 10% Pd-C to give **10** in 53% yield [mp 165-175°C; IR (KBr): 1737 (lactone) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 6.70 (1H, s, arom-H)]. Compound **10** was deketalized by 80% aqueous TFA to afford **11** quantitatively [mp 160-170°C (dec.); MS m/z 262 (M^+); IR (KBr): 1752 (lactone) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 7.17 (1H, s, arom-H)]. Similarly, Friedländer condensation of **11** with **8** gave **2** in 45% yield. The TLC data and ^{1}H-NMR spectrum of a sample of **2** were identical with those of the one obtained from **9**. Compound **2** was demethylated in refluxing 47% aqueous HBr, and a crude product was purified by reversed-phase HPLC [developer: MeOH-H_2O (2:3) adjusted to pH 3 with dilute aqueous HCl] to afford **3**[10] in 45% yield.

Compounds **2** and **3** were tested in a P388 mouse leukemia assay and the results are summarized in Table I. Synthetic (+)-camptothecin (**1**)[7] was used as a control. Compounds **2** and **3** were more active than **1**. Compound **2**, in particular, exhibited higher activity than **1** or **3**. One out of 6 mice treated with 120 mg of **2** per kg and 3 out of 6 with 240 mg/kg survived from death by leukemia for more than 40 days. The methoxy group at the C-10 position seems to be more important for the activity than the corresponding hydroxy group. The maximum tolerated dose (MTD) of **2** was higher than that of **1**. It is interesting that **3** was nontoxic up to 480 mg/kg, and prolonged the survival time to 186% of the control. Therefore, the therapeutic ratios (TR) of compounds **2** and **3** were 14.1 and more than 22.6, respectively, against 4.0 in compound **1**.

The hydroxy group at the C-20 position has been reported to be essential for antileukemia activity.[5,6] However, the results of this *in vivo* assay suggested that the introduction of the amino group at the C-20 position may lead to a new development of camptothecin analogue studies. A further study of structural modification of ring E is in progress in our laboratory and will be reported elsewhere.

Table I. Antitumor Activity of Camptothecin Analogues against P388 Leukemia in Mice[a)]

Compound	Dose (mg/kg)	T/C[b)] (%)	Survivors for over 40 days	MTD[c)] (mg)	TR value[d)]
1[e)]	15	128	0/6	120	4.0
	30	130	0/6		
	60	142	0/6		
	120	176	0/6		
	240	144	0/6		
2[f)]	30	166	0/6	240	14.1
	60	210	0/6		
	120	270	1/6		
	240	>402	3/6		
	480	115	0/6		
3[f)]	60	151	1/6	>480	>22.6
	120	153	0/6		
	240	179	0/6		
	480	186	0/6		

a) P388 cells (10^6) were transplanted intraperitoneally (ip) into CDF_1 mice on day 0 and the compounds were administered ip on day 1. b) T/C = (median survival time of treated/control mice) x 100. c) MTD = maximum tolerated dose. d) TR value = MTD/ILS_{30} (ILS_{30}: amount required to give a T/C of 130). e) Injected as an aqueous solution of the sodium salt. f) Injected as a suspension in H_2O containing 0.9% NaCl, 0.9% benzyl alcohol, 0.4% Tween 80 and 0.5% CMC.

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9) Compound 2: mp 285-300°C (dec.); MS m/z 405 (M^+); IR (KBr): 1730 (lactone), 1662 (C=O), 1602 cm^{-1}; ^{1}H-NMR ($CDCl_3$+ CD_3OD) δ: 1.00 (3H, t, J = 7 Hz), 1.44 (3H, t, J = 8 Hz), 1.8-2.2 (2H, m), 3.0-3.6 (2H, m), 4.03 (3H, s), 5.28 (2H, s), 5.37, 5.67 (2H, AB q, J = 18 Hz), 7.3-7.7 (2H, m), 7.74 (1H, s), 8.12 (1H, d, J = 9 Hz).

10) Compound 3: mp 200-220°C (dec.); MS m/z 391 (M^+); IR (KBr): 1735 (lactone), 1655 (C=O), 1600; ^{1}H-NMR (D_2O) δ: 1.16 (6H, t, J = 7 Hz), 2.1-3.0 (4H, m), 4.4-4.9 (2H, br s), 5.4-6.0 (2H, br s), 6.78 (1H, d, J = 3 Hz), 7.14 (1H, s), 7.16 (1H, dd, J = 3 Hz, 9 Hz), 7.71 (1H, d, J = 9 Hz).

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