Antitumor Agents. 7.[1] Synthesis and Antitumor Activity of Novel Hexacyclic Camptothecin Analogues

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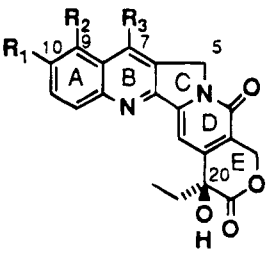
Eleven novel hexacyclic and three 7,9-disubstituted pentacyclic derivatives of camptothecin were synthesized and evaluated for in vitro cytotoxic activity against P388, HOC-21, and QG-56 and in vivo antileukemic activity against P388 in mice. Hexacyclic compounds which have an additional 5-, 6-, or 7-membered ring cyclized at positions 7 and 9 of camptothecin were prepared by intramolecular cyclization of pentacyclic camptothecin derivatives or Friedländer condensation of the appropriate bicyclic amino ketone and tricyclic ketone. All of the hexacyclic compounds exhibited compatible or superior activity of 7-ethyl-10-hydroxycamptothecin (SN-38) in in vitro assays without regard to the size or type of the additional ring, and three of six compounds showed more than 300% *T/C* on in vivo assay. These results suggest that the potency of the hexacyclic ring system is higher than that of the original pentacyclic ring system of camptothecin and that the conformational rigidity of substituents at positions 7 and 9 is favorable for antitumor activity.

Camptothecin (**1a**), an alkaloid with strong antitumor activity in experimental tumors, was isolated from *Camptotheca acuminata* by Wall and co-workers in 1966.[2] Although the development of **1a** was stopped because of a variety of unacceptable side effects on humans,[3] the unique mechanism of action of camptothecin as an inhibitor of topoisomerase I, which was reported by Liu and co-workers in 1985,[4] revived interest in the drug. Among the many camptothecin analogues which have been investigated over more than 25 years, CPT-11 (**2**),[5] topotecan (**3**),[6] 9-aminocamptothecin (**4**)[7] are currently undergoing extensive clinical trials. Previously, we reported the synthesis and biological activity of various ring A-, B-, C-, and E-modified camptothecin analogues.[1,8] These studies showed that the intact ring system of camptothecin is essential for antitumor activity and that the northern part of the camptothecin molecule might be a suitable site for functionalization to obtain more potent analogues of **1a**. In this paper, we describe the modifications of the northern part of the camptothecin molecule, in short at positions 7, 9, and 10, and the novel findings that the unique hexacyclic ring system (Figure 2) exhibits a superior antitumor activity to the original pentacyclic ring system of **1a**.



1a $R_1=R_2=R_3=H$

2 $R_1=$ OCON–N · HCl

$R_2=H$, $R_3=Et$

3 $R_1=OH$, $R_2=CH_2NMe_2$ · HCl

$R_3=H$

4 $R_1=H$, $R_2=NH_2$, $R_3=H$

5 $R_1=OH$, $R_2=H$, $R_3=Et$

Figure 1.

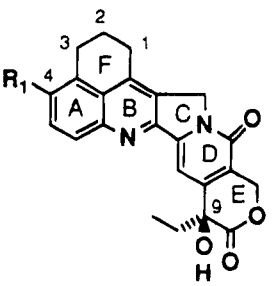


Figure 2.

Chemistry

Fourteen analogues were prepared by total synthesis (Scheme 1). Amino ketones **7**,[9] **8**, and **9**, which were derived from **6**[9] in several steps, were subjected to condensation with **10a**[10] or **10b**[11] to give 7,9-disubstituted camptothecins **11**, **12a,b**, and **13**. Compound **12b** gave novel hexacyclic compound **15** upon methanolysis and acidification. Hydrolysis of **13** gave water-soluble hexacyclic compound **16** as a hydrochloride salt, together with (20*S*)-7-methyl-9-(aminomethyl)camptothecin (**14**). To prepare various hexacyclic compounds, **28a,b** and **29**–**33**, we employed the Friedländer condensation of appropriate bicyclic amino ketones, **21**, **22**, **24**,[12] **25**,[13] **26**,[14] and **27**,[15] with **10a** or **10b**. Compounds **21** and **22** were prepared from **17** and **18**,[16] respectively, in three steps involving reaction with β-propiolactone, hydrogenation or deprotection, and cyclization. In the case of condensation of amino ketone **21** with **10a**, compound **32** was not obtained directly but enamine **34**

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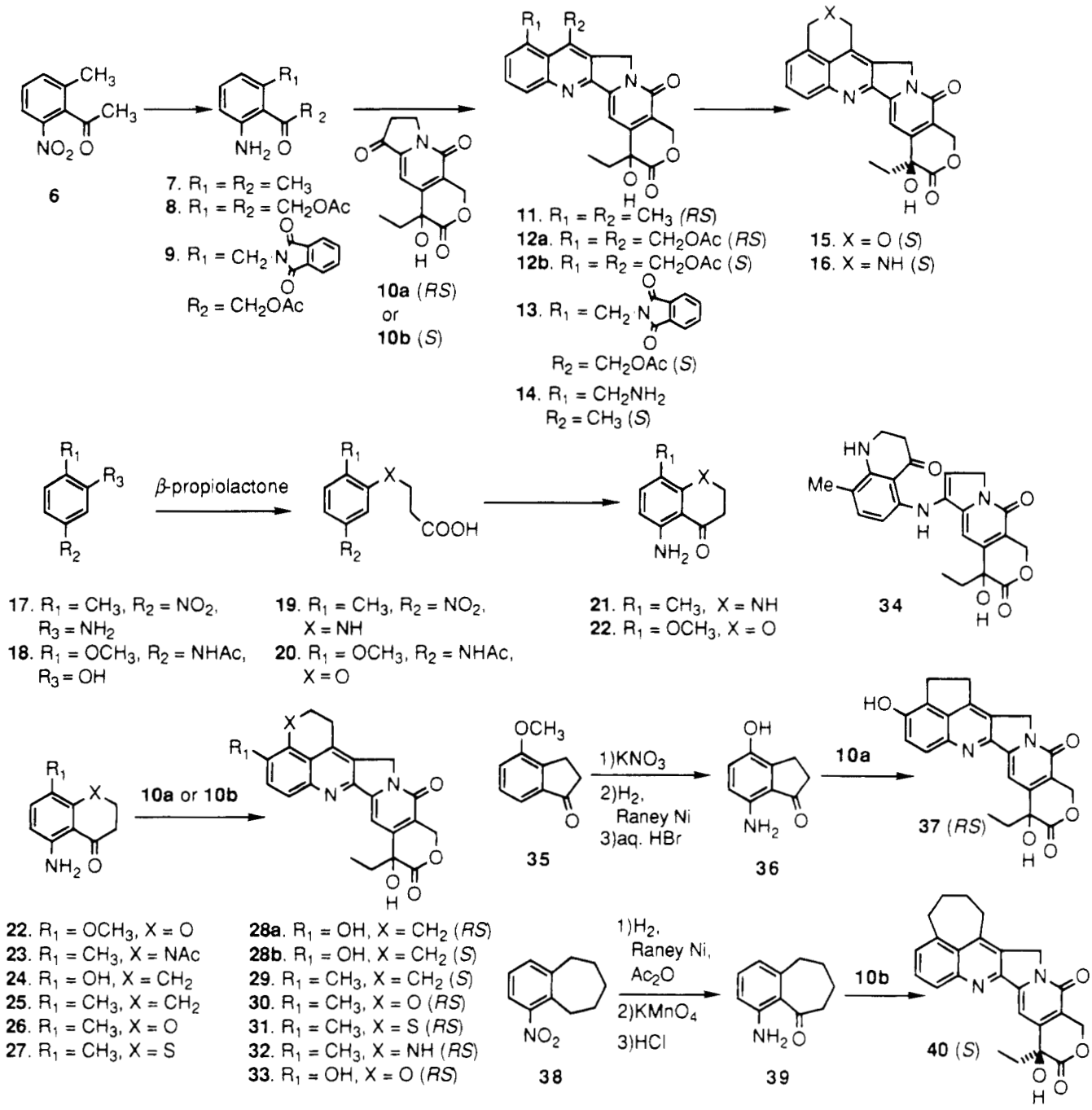
Scheme 1



was. As it was suspected that the basicity of the nitrogen atom in the dihydroquinoline ring of **34** stopped further intramolecular cyclization, the nitrogen atom of **21** was protected by an acetyl group to give **23**, and this was followed by condensation with **10a** and deprotection to provide **32**. Nitration of 4-methoxyindanone (**35**)[17] followed by catalytic reduction and hydrolysis afforded amino ketone **36**, which gave **37** upon condensation with **10a**. 9-Aminobenzosuberone (**39**) was prepared from **38**[18] in three steps: hydrogenation, oxidation, and hydrolysis. Compound **39** was condensed with **10b** to give **40**.

Results and Discussion

The biological test results for 14 of the new camptothecin analogues are presented in Table 1. In order to obtain more meaningful comparisons of relative activities, SN-38 (**5**),[5] which is an active metabolite of CPT-11, was tested at the same time.

We have previously reported that the northern part of the camptothecin molecule involving 5-, 7-, 9-, and 10-positions might be a suitable site for functionalization.[1] Also, it has been reported that substitution at either position 7 or 9 with an alkyl group such as a methyl, ethyl, propyl, or benzyl retained or increased the antitumor activity,[6,19] while reports of 7,9-disubstituted camptothecin analogues are rare. We supposed that the cause of the lower activity of **12a** is not the bulkiness but the flexibility of the acetoxymethyl groups and that conformational restriction of substituents at positions 7 and 9 is probably required for antitumor activity. These considerations led us to design the novel hexacyclic analogues of camptothecin because cyclization at positions 7 and 9 was expected to make the

Table 1. Comparison of Antiproliferative Activities and Antitumor Potencies of Camptothecin Analogues in Three Cell Line Cultures[a] and P388 Mouse Leukemia Assays[b]

	IC_{50} (ng/mL)			% *T/C*[c]				
	cell line			dose (mg/kg)				
compd[d] no.	P388	HOC-21	QG-56	480	240	120	60	30
1a* [g]	4.74 (2.44)[f]	14.7 (1.09)	4.63 (1.80)		144	176	142	130
1b[g,i]					163	148	128	128
5* [g]	1.95–6.93 (1)	24.6–123.1 (1)	0.94–3.17 (1)		67.0	258 (1)[e]	191	146
11	3.76 (1.02)	>500 (>4.06)	0.60 (0.55)					
12a	40.5 (5.84)	>500 (>4.94)	7.41 (7.72)					
14*	5.02 (2.21)	44.76 (1.28)	6.36 (2.01)					
15*	0.95 (0.42)	12.28 (0.35)	1.14 (0.60)					
16*	3.39 (1.62)	37.75 (1.19)	2.98 (1.39)					
28a[g]	3.80 (0.51)	26.4 (0.46)	0.50 (0.19)	88.5 (1)	>392 (5)	354 (1)	201	182
28b*	1.60 (0.22)	12.6 (0.22)	0.26 (0.10)					
29*	0.78 (0.30)	5.88 (0.18)	0.68 (0.22)					
30[h]	4.50 (1.13)	41.9 (0.92)	1.38 (0.85)	>376 (4)	376 (2)	273 (1)	260 (1)	
31[h]	3.77 (0.94)	35.8 (0.78)	3.36 (2.07)	>400 (5)	290	293 (1)	224 (1)	205
32[h]	4.46 (0.87)	41.8 (1.01)	1.68 (1.04)	180 (1, 7.5)[j]	174 (1, 3.8)	207 (1, 1.9)		
33[g]	4.05 (0.80)	79.0 (0.78)	1.10 (0.57)	19	20	233	221 (1)	147
37[h]	2.22 (1.14)	15.9 (0.84)	1.25 (0.57)	78.2	90.9	110 (1)	206 (1)	167
40*	1.22 (0.62)	15.6 (0.51)	1.73 (0.71)					

[a] In vitro antiproliferative activities of the drugs against three cell lines (P388, mouse leukemia; HOC-21, human ovarian cancer; QG-56, human lung squamous cell carcinoma) were measured by MTT assay after 3 days of incubation and expressed as the doses required to inhibit the growth of 50% of the cells cultivated (IC_{50}, ng/mL). [b] P388 cells (1×10^6) were transplanted intraperitoneally (ip) into CDF1 mice on day 0; compounds were administered ip on day 1.[24] [c] % *T/C* = (median survival time of treated/control animals) × 100. [d] Compounds with * have the *S* configuration; the others are racemic. [e] Figures in parentheses indicate number of mice surviving for 40 days, out of the six mice tested in each case. [f] Figures in parentheses indicate IC_{50} of tested compounds/IC_{50} of SN-38. [g] Injected as an aqueous solution of the sodium salt. [h] Injected as a suspension in water containing 0.9% NaCl, 0.09% benzyl alcohol, 0.4% Tween 80, and 0.5% carboxymethyl cellulose. [i] **1b** is racemic camptothecin.[20] [j] The in vivo activity of compound **32** was measured at the low dose. Figures in parentheses indicate the number of cured mice and the dose.

substituents of camptothecin more rigid. As expected, compound **28a**, which has a new 6-membered ring, named ring F, was twice as active as SN-38 (**5**) in an in vitro assay. Compound **28b** which has an *S* configuration at position 9 was approximately twice as active as racemic **28a** on in vitro assay, as natural (20*S*)-camptothecin (**1a**) is about twice as potent as racemic camptothecin (**1b**).[20] Compound **29**, which has a methyl group at position 4, also demonstrated strong antitumor activity. For investigation of the effect of a heteroatom at position 3 in ring F on antitumor activity, the 3-oxa-, 3-thia-, and 3-aza hexacyclic analogues **30**–**32** were synthesized as racemates. As these three compounds showed the same order of activity as SN-38 (**5**), the corresponding 9*S* compounds were expected to be more active than **5**. To measure the effect of a heteroatom at position 2, the 2-oxa- and 2-aza hexacyclic compounds **15** and **16** were investigated. Compound **15** was twice as active as **5**, while compound **16** was approximately as active as **5**. It is interesting to note that the hexacyclic compound **16** exhibited higher activity than the pentacyclic compound **14**, which had an open form of ring F of compound **16**. This result also demonstrates that the potency of a hexacyclic ring system is higher than that of the original pentacyclic ring system of camptothecin. On the whole, the novel hexacyclic compounds are more potent essentially than SN-38 in in vitro assays, without regard to the type or position of the heteroatom. Thus, such results suggest that the electronic factor at positions 2 and 3 cannot play an important role in the antitumor activity. To investigate the function of the size of the additional ring F, we synthesized and evaluated compounds **37** and **40**, which had a 5- and a 7-membered ring F, respectively. Since racemic compound **37** showed the same order of activity as SN-38 (**5**), compound **37** with the *S* configuration should be twice as active as **5**. Compound **40**, which had no substituent in ring A, was also twice as active as **5**. There was little difference in antitumor activity among the compounds with a 5-, 6-, or 7-membered ring F.

Six compounds were evaluated for antitumor activity in mice bearing P388 leukemia. All compounds displayed strong antitumor potency. Compound **28a** showed a % *T/C* of over 392 and effected cures in five mice out of six at a dose of 240 mg/kg. Though 3-oxa compound **30** and 3-thia compound **31** were as active as **28a**, 3-aza compound **32** was highly toxic at higher doses, but at the lower dose of 1.9 mg/kg, it was found to be active with a % *T/C* of 207 and one mouse out of six was cured.

It is apparent from our current study that novel hexacyclic analogues of camptothecin exhibit a high potency. It is presumed that the hexacyclic ring system, which is rigid or has less conformational variation than 7- or 9-substituted compounds, can easily form a ternary complex with DNA and topoisomerase I because the rigidity of a ligand contributes to diminishing the decrease of entropy in docking.[21] Besides our hexacyclic compounds possessing ring F, it is known that cyclization of side chains enhanced biological activity of camptothecin derivatives. Hexacyclic compound 10,11-(methylenedioxy)camptothecin which has an additional ring next to ring A is reported to be much more active than 10,11-dimethoxycamptothecin in topoisomerase I inhibition and in vivo antitumor assay.[22] Though the reason of the difference of activity has been explained in terms of planarity and encroachment of substituents,[22] the entropic effect would be also concerned. Since hexacyclic compounds **28a**, **37**, and **40** were almost equally active regardless of the size of ring F, we presume that there is more bulk tolerance for various modifications of ring F. We plan to synthesize hexacyclic compounds which are substituted at ring A or F to increase the activity or the water-solubility.

Experimental Section

Melting points were found using a Yanaco MP-S3 or MP-500D apparatus and are uncorrected. IR spectra were obtained on a Hitachi 270-30 infrared spectrophotometer. Mass spectra were recorded on a JEOL JMS-D300, a JMS-HX110, or a JMS-AX505W spectrometer. ^{1}H NMR spectra were obtained on a JEOL JNM-FX90Q or a JNM-EX400 spectrometer; all values are reported in ppm (δ) downfield from $(CH_3)_4$-Si. Elemental analyses were obtained on a Heraeus CHN-O-Rapid or a Perkin-Elmer 2400CHN instrument. Optical rotations were measured with a Horiba SEPA-200 polarimeter. Column chromatography was performed using silica gel 60 F_{254} (70-230 mesh) (Merck).

2′-(Acetoxymethyl)-6′-aminophenacyl Acetate (8). A solution of 2′-methyl-6′-nitroacetophenone (**6**) (10.6 g, 59.2 mmol), *N*-bromosuccinimide (15.8 g, 88.8 mmol), and benzoyl peroxide (100 mg, 0.41 mmol) in benzene (250 mL) was heated to reflux under N_2 for 12 h. The reaction mixture was washed successively with 1 N NaOH, water, and brine and dried (Na_2SO_4). The solvent was removed to give a yellow solid, 2′-(bromomethyl)-6′-nitroacetophenone (15.5 g, 100%), which was used in the next step without further purification: MS (FD) *m/z* 257, 259 (M^+, $M^+ + 2$); ^{1}H NMR ($CDCl_3$) δ 2.64 (s, 3H), 4.47 (s, 2 H), 7.58 (t, 1H, J = 8 Hz), 7.80 (dd, 1H, J = 1, 8 Hz), 8.17 (dd, 1H, J = 1, 8 Hz). 2′-(Bromomethyl)-6′-nitroacetophenone (15.5 g, 60.1 mmol) and potassium acetate (40 g, 408 mmol) in AcOH (267 mL) were heated at 100 °C for 4 h. The solvent was evaporated to produce a residue that was dissolved in AcOEt and washed with water and dried (Na_2SO_4). Evaporation of the solvent left a brown oil, which was chromatographed (20% AcOEt/*n*-hexane) to give 2′-(acetoxymethyl)-6′-nitroactophenone as a solid (8.64 g, 62%): mp 88–89 °C; IR (KBr) 1738, 1704 cm^{-1}; MS (FAB) *m/z* 238 (MH^+), 178 (M^+ − Ac); ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3H), 2.60 (s, 3H), 5.10 (s, 2H), 7.58 (t, 1H, J = 8 Hz), 7.77 (dd, 1H, J = 2, 8 Hz), 8.18 (dd, 1H, J = 2, 8 Hz). 2′-(Acetoxymethyl)-6′-nitroacetophenone (4.0 g, 16.9 mmol) and bromine (2.7 g, 16.9 mmol) in AcOH (90 mL) were warmed to 70 °C for 1.3 h. The reaction mixture was concentrated, and the residue was dissolved in AcOEt. The mixture was washed with water and dried (Na_2SO_4). After removal of the solvent, the solid residue and fine powdered sodium acetate (3.2 g, 39.0 mmol) in DMSO (30 mL) were heated at 80 °C for 2.5 h. The reaction mixture was partitioned between water and AcOEt, and the organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (20% AcOEt/*n*-hexane) to give 2′-(acetoxymethyl)-6′-nitrophenacyl acetate (2.21 g, 44%): mp 69 °C; IR (KBr) 1740 cm^{-1}; MS (FAB) *m/z* 296 (MH^+), 236 (M^+ − AcO); ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H), 2.10 (s, 3H), 5.06 (s, 2H), 5.15 (s, 2H), 7.64 (t, 1H, J = 8 Hz), 7.79 (dd, 1H, J = 2, 8 Hz), 8.20 (dd, 1H, J = 2, 8 Hz). A suspension of this compound (68 mg, 0.23 mmol) and PtO_2 (4 mg) in MeOH (2 mL) was hydrogenated at atmospheric pressure until the uptake of hydrogen was complete. After the catalyst was filtered and washed with MeOH, the solvent was evaporated to give **8** (49 mg, 80%): MS (EI) *m/z* 265 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H), 2.20 (s, 3H), 5.02 (s, 2H), 5.06 (s, 2H), 6.67 (d, 1H, J = 8 Hz), 6.73 (d, 1H, J = 8 Hz), 7.18 (t, 1H, J = 8 Hz).

6′-Amino-2′-(phthalimidomethyl)phenacyl Acetate (9). To a solution of 2′-(bromomethyl)-6′-nitroacetophenone (2 g, 7.7 mmol) in DMF (30 mL) was added potassium phthalimide (2.9 g, 15.5 mmol), and the solution was heated at 90 °C for 9 h. After removal of the solvent at reduced pressure, the residue was suspended in water and the precipitate was collected. The solid obtained was washed successively with 10% aqueous NaOH, water, and THF to give 2′-(phthalimidomethyl)-6′-nitroacetophenone (1.06 g, 42%): ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H), 4.83 (s, 2H), 7.54 (d, 1H, J = 8 Hz), 7.60–7.95 (m, 5H), 8.12 (dd, 1H, J = 3, 8 Hz). Bromine (246 mg, 1.54 mmol) was added to a suspension of 2′-(phthalimidomethyl)-6′-nitroacetophenone (500 mg, 1.54 mmol) in AcOH (200 mL), and the mixture was heated at 90 °C for 1 h. The reaction mixture was poured into water, and the resulting precipitate was collected, washed with water, and dried to give 6′-nitro-2′-(phthalimidomethyl)-2-bromoacetophenone (555 mg, 89%): ^{1}H NMR (DMSO-d_6) δ 4.76 (d, 2H, J = 0.5 Hz), 4.89 (s, 2H), 7.77 (d, 1H, J = 8 Hz), 7.90 (s, 4H), 8.28 (distorted d, 1H, J = 8 Hz). A mixture of the above bromide (100 mg, 0.25 mmol) and AcOCs (240 mg, 1.25 mmol) in DMF (3 mL) was stirred at room temperature for 1 h. The reaction mixture was partitioned between water and $CHCl_3$, and the organic phase was dried (Na_2SO_4) and concentrated to give 6′-nitro-2′-(phthalimidomethyl)phenacyl acetate (66 mg, 69%): ^{1}H NMR ($CDCl_3$) δ 2.09 (s, 3H), 4.94 (s, 2H), 5.32 (s, 2H), 7.55–8.00 (m, 6H), 8.12 (distorted d, 1H, J = 8 Hz). A suspension of the above nitro compound (118 mg, 0.31 mmol) and PtO_2 (30 mg) in dioxane (5 mL) and EtOH (5 mL) was hydrogenated at atmospheric pressure for 1 h. The catalyst was filtered, and the solvent was evaporated in vacuo to give **9** (105 mg, 97%): ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H), 4.71 (s, 2H), 5.35 (s, 2H), 6.58 (d, 1H, J = 8 Hz), 6.69 (d, 1H, J = 8 Hz), 7.10 (t, 1H, J = 8 Hz), 7.60–7.90 (m, 4H).

(20*RS*)-7,9-Dimethylcamptothecin (11). 2′-Amino-6′-methylacetophenone (**7**) (246 mg, 1.65 mmol), 7,8-dihydro-4-ethyl-4-hydroxy-1*H*-pyrano[3,4-*f*]indolizine-3,6,10(4*H*)-trione (**10a**) (362 mg, 1.38 mmol), and *p*-TsOH·H_2O (10 mg, 0.05 mmol) were brought to reflux for 6.5 h in toluene (15 mL). The precipitate obtained after cooling was collected by filtration and washed successively with AcOEt and $CHCl_3$. Recrystallization (AcOH) gave pure **11** as a pale yellow solid (432 mg, 83%): mp 290 °C dec; IR (KBr) 3448, 1758, 1659, 1602 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.89 (t, 3H, J = 7 Hz), 1.88 (q, 2H, J = 7 Hz), 2.95 (s, 6H), 5.24 (s, 2H), 5.42 (s, 2H), 7.30 (s, 1H), 7.44 (dd, 1H, J = 1, 8 Hz), 7.66 (t, 1H, J = 8 Hz), 7.97 (dd, 1H, J = 1, 8 Hz). Anal. ($C_{22}H_{20}N_2O_4 \cdot {}^3/_4H_2O$) C, H, N.

(20*RS*)-7,9-Bis(acetoxymethyl)camptothecin (12a). A mixture of compound **8** (47 mg, 0.18 mmol), **10a** (47 mg, 0.18 mmol), and *p*-TsOH·H_2O (2 mg, 0.01 mmol) in toluene (9 mL) was heated to reflux for 5 h. Evaporation of the solvent left a dark brown tar, which was chromatographed (1% MeOH/$CHCl_3$). The product obtained was recrystallized succesively from $CHCl_3$/*n*-hexane and AcOEt/MeOH to give **12a** (40 mg, 46%): mp 245 °C dec; IR (KBr) 1746, 1659, 1602 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3H, J = 7 Hz), 1.90 (q, 2H, J = 7 Hz), 2.11 (s, 3H), 2.12 (s, 3H), 5.31, 5.74 (ABq, 2H, J = 17 Hz), 5.51 (s, 2H), 5.70 (s, 2H), 5.77 (s, 2H), 7.64 (s, 1H), 7.79 (d, 1H, J = 7 Hz), 7.81 (d, 1H, J = 4 Hz), 8.29 (dd, 1H, J = 4, 7 Hz). Anal. ($C_{26}H_{24}N_2O_8 \cdot {}^1/_4H_2O$) C, H, N.

(20*S*)-7,9-Bis(acetoxymethyl)camptothecin (12b). A mixture of compound **8** (575 mg, 2.17 mmol), **10b** (567 mg, 2.17 mmol), and pyridinium *p*-toluenesulfonate (100 mg, 0.40 mmol) in toluene (40 mL) was heated to reflux for 2 h. The reaction mixture was partitioned between water and AcOEt, and the organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by preparative TLC (5% MeOH/$CHCl_3$) to give **12b** (496 mg, 46%). The ^{1}H NMR spectrum data of **12b** were identical with those of **12a**.

(20*S*)-7-(Acetoxymethyl)-9-(phthalimidomethyl)camptothecin (13). A mixture of compound **9** (100 mg, 0.28 mmol) and **10b** (75 mg, 0.28 mmol) in toluene (20 mL) was heated to reflux for 10 min. The resultant suspension was treated with *p*-TsOH·H_2O (5 mg) and heated to reflux for an additional 2 h. The mixture was concentrated in vacuo, and the residue was subjected to chromatography (1% MeOH/$CHCl_3$) to afford **13** (82 mg, 50%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3H, J = 7 Hz), 1.70–2.10 (m, 2H), 2.17 (s, 3H), 5.31, 5.76 (ABq, 2H, J = 16 Hz), 5.55 (s, 2H), 5.62 (s, 2H), 5.87 (s, 2H), 7.64 (s, 1H), 7.85–8.04 (m, 5H), 8.20 (d, 1H, J = 8 Hz).

(20*S*)-9-(Aminomethyl)-7-methylcamptothecin Hydrochloride (14) and (9*S*)-9-Ethyl-1,2-dihydro-9-hydroxy-3*H*,12*H*-pyrano[3′,4′:6,7]indolizino[1,2-*c*]benzo[*ij*][2,6]-naphthyridine-10,12(9*H*,15*H*)-dione Hydrochloride (16). A suspension of compound **13** (200 mg, 0.35 mmol) in 47% aqueous HBr (7 mL) was heated at 100 °C in a nitrogen atmosphere for 5 h. The reaction mixture was concentrated in vacuo. After the residue was dissolved in EtOH and concentrated to dryness twice to remove excess HBr, the residue was washed with ether and dissolved in water. The solution was subjected to HPLC (Capcell Pak C_{18}; acetonitrile/water/1 N HCl = 10/50/0.3) to afford **14** (25 mg, 17%) and **16** (6.5 mg, 4%). **14**: mp 170 °C dec; MS (EI) *m/z* 391 (M^-); HRMS

m/z 391.1532 (M^+); ^1H NMR (DMSO-d_6) δ 0.91 (t, 3H, J = 8 Hz), 1.7–2.1 (m, 2H), 2.97 (s, 3H), 4.6–5.0 (m, 2H), 5.33 (s, 2H), 5.41, 5.46 (ABq, 2H, J = 16 Hz), 7.36 (s, 1H), 7.77 (d, 1H, J = 7 Hz), 7.87 (t, 1H, J = 7 Hz), 8.21 (d, 1H, J = 7 Hz), 8.59 (bs, 3H). **16**: mp >130 °C dec; MS (EI) *m/z* 389 (M^+); HRMS *m/z* 389.1368 (M^+) ($C_{22}H_{19}N_3O_4$ = 389.1376); ^1H NMR (DMSO-d_6) δ 0.90 (t, 3H, J = 7 Hz), 1.84–1.95 (m, 2H), 4.78 (s, 2H), 4.90 (s, 2H), 5.38 (s, 2H), 5.42, 5.46 (ABq, 2H, J = 18 Hz), 6.50 (bs, 1H), 7.41 (s, 1H), 7.70 (d, 1H, J = 7 Hz), 7.93 (dd, 1H, J = 7, 9 Hz), 8.18 (d, 1H, J = 9 Hz), 9.99 (bs, 2H). Anal. ($C_{22}H_{19}N_3O_4 \cdot HCl \cdot {}^5/_2H_2O$) C, H, N.

(9*S*)-9-Ethyl-9-hydroxy-3,9,12,15-tetrahydropyrano[3,4,5-*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,12(1*H*)-dione (15). To a solution of **12b** (92 mg, 0.19 mmol) in a mixture of $CHCl_3$ (3 mL) and MeOH (3 mL) was added MeONa (40 mg, 0.74 mmol). After 30 min, the solvents were removed in vacuo. The residue was dissolved in 47% aqueous HBr (3 mL) and heated at 80 °C for 1 h. After the reaction mixture was neutralized with $NaHCO_3$, the resulting precipitate was collected by filtration and washed successively with water and MeOH. The solid was purified by chromatography (0.5% MeOH/$CHCl_3$) and recrystallization ($CHCl_3$/MeOH) to afford **15** (20 mg, 27%): mp 270–285 °C dec; IR (KBr) 1746, 1659, 1611 cm^{-1}; MS (FD) *m/z* 390 (M); ^1H NMR (DMSO-d_6) δ 0.89 (t, 3H, J = 7 Hz), 1.88 (q, 2H, J = 7 Hz), 5.09 (s, 2H), 5.23 (s, 2H), 5.42 (s, 2H), 7.35 (s, 1H), 7.45 (dd, 1H, J = 2, 8 Hz), 7.80 (t, 1H, J = 8 Hz), 8.01 (dd, 1H, J = 2, 8 Hz). Anal. ($C_{22}H_{18}N_2O_5 \cdot {}^1/_2H_2O$) C, H, N.

3-(2-Methyl-5-nitroanilino)propionic Acid (19). A solution of 2-methyl-5-nitroaniline (**17**) (60 g, 0.39 mol) in acetonitrile (300 mL) was heated to reflux and treated with β-propiolactone (25 mL, 0.38 mol) over 30 min. The mixture was heated to reflux for an additional 3.5 h. After concentration of the solution, the residue was dissolved in 10% aqueous NaOH (1 L) and washed with ether. Acidification to pH 2 using concentrated HCl and filtration gave **19** (36.4 g, 43%): ^1H NMR (DMSO-d_6) δ 2.17 (s, 3H), 2.59 (t, 2H, J = 7 Hz), 3.40 (bt, 2H, J = 7 Hz), 5.4–5.7 (m, 1H), 7.20 (d, 1H, J = 9 Hz), 7.26 (d, 1H, J = 2 Hz), 7.40 (dd, 1H, J = 2, 9 Hz).

3-[5-(Acetylamino)-2-methoxyphenoxy]propionic Acid (20). To a solution of KOH (3.26 g, 49.7 mmol), (3-hydroxy-4-methoxyphenyl)acetamide (**18**) (9 g, 49.7 mmol), 18-crown-6 (100 mg, 0.38 mmol) in water (50 mL), and 1,4-dioxane (50 mL) was added β-propiolactone (4 g, 49.7 mmol), and the solution was stirred at room temperature for 16 h. The reaction mixture was diluted with water (100 mL) and washed with AcOEt. The resultant aqueous solution was acidified with 10% aqueous HCl and extracted with AcOEt. The combined organic solution was washed with water and concentrated to dryness. The residue was recrystallized (EtOH) to give **20** (2.8 g, 22%): ^1H NMR (DMSO-d_6) δ 1.99 (s, 3H), 2.69 (t, 2H, J = 7 Hz), 3.70 (s, 3H), 4.10 (t, 2H, J = 7 Hz), 6.85 (d, 1H, J = 9 Hz), 7.08 (dd, 1H, J = 3, 9 Hz), 7.29 (d, 1H, J = 3 Hz).

5-Amino-8-methyl-2,3-dihydroquinolin-4(1*H*)-one (21). A mixture of **19** (5.2 g, 23.2 mmol) and PtO_2 (100 mg) in EtOH (70 mL) and 1,4-dioxane (70 mL) was stirred in an atmosphere of H_2. The catalyst was removed, and the solution was concentrated in vacuo. The residue was added in portions to stirred polyphosphoric acid (80 g) at 100–110 °C. After stirring was continued for 3 h, the mixture was poured into water and the pH was adjusted to 11 using 10% aqueous NaOH. The mixture was extracted with $CHCl_3$, and the combined extracts were concentrated and subjected to flash chromatography (40% AcOEt/*n*-hexane) to afford **21** (2.24 g, 55%): ^1H NMR ($CDCl_3$) δ 1.92 (s, 3H), 2.59 (t, 2H, J = 7 Hz), 3.48 (t, 2H, J = 7 Hz), 5.80 (d, 1H, J = 9 Hz), 6.83 (d, 1H, J = 9 Hz).

5-Amino-8-methoxy-4-chromanone (22). A solution of **20** (1 g, 3.95 mmol) in 6 N aqueous HCl (20 mL) was refluxed for 1 h, and the solvent was removed to give 3-(5-amino-2-methoxyphenoxy)propionic acid hydrochloride (960 mg, 98%): ^1H NMR (DMSO-d_6) δ 2.72 (t, 2H, J = 7 Hz), 3.77 (s, 3H), 4.16 (t, 2H, J = 7 Hz), 6.98 (br, 3H). A solution of this acid (960 mg, 3.88 mmol) in concentrated H_2SO_4 (10 mL) was warmed to 50 °C for 1 h. The mixture was poured into ice/water and neutralized with Na_2CO_3. The aqueous solution was extracted with AcOEt, and the organic phase was washed with water, dried, and concentrated to give **22** (190 mg, 25%) as a yellow solid: IR (KBr) 3466, 3352, 1647, 1566, 1485 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.79 (t, 2H, J = 7 Hz), 3.80 (s, 3H), 4.52 (t, 2H, J = 7 Hz), 6.16 (d, 1H, J = 9 Hz), 6.96 (d, 1H, J = 9 Hz).

1-Acetyl-5-amino-8-methyl-2,3-dihydroquinolin-4(1*H*)-one (23). To a solution of **21** (266 mg, 1.51 mmol) and pyridine (0.13 mL, 1.61 mmol) in benzene (10 mL) was added benzyl chloroformate (0.22 mL, 1.54 mmol). After being stirred at room temperature for 1 h, the mixture was washed successively with water and brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed (66% AcOEt/*n*-hexane) to afford 5-[(benzyloxycarbonyl)amino]-8-methyl-2,3-dihydroquinolin-4(1*H*)-one (402 mg, 86%): ^1H NMR ($CDCl_3$) δ 2.08 (s, 3H), 2.78 (t, 2H, J = 8 Hz), 3.58 (t, 2H, J = 8 Hz), 5.19 (s, 2H), 7.14 (d, 1H, J = 9 Hz), 7.1–7.6 (m, 5H), 7.65 (d, 1H). To a solution of this compound (400 mg, 1.29 mmol) in pyridine (0.34 mL, 4.20 mmol) and benzene (20 mL) was added acetyl chloride (0.28 mL, 3.94 mmol), and the mixture was heated to reflux for 1.5 h. The mixture was partitioned between AcOEt and water, and the organic phase was dried (Na_2SO_4). Evaporation of the solvent afforded 1-acetyl-5-[(benzyloxycarbonyl)amino]-8-methyl-2,3-dihyroquinolin-4(1*H*)-one (446 mg, 98%) as a colorless solid: ^1H NMR ($CDCl_3$) δ 1.57 (s, 3H), 1.95 (bs, 3H), 2.1–2.4 (m, 2H), 2.5–3.6 (m, 2H), 5.21 (s, 2H), 7.25–7.5 (m, 6H), 8.35 (d, 1H, J = 9 Hz). This amide (446 mg, 1.27 mmol), 10% Pd/C (60 mg), AcOH (0.06 mL), water (2 mL), MeOH (20 mL), and 1,4-dioxane (10 mL) were combined, and the mixture was stirred in an atmosphee of H_2 for 8 h. The reaction mixture was filtered and concentrated. The residue was chromatographed (50% AcOEt/*n*-hexane) to give 1-acetyl-5-amino-8-methyl-2,3-dihydroquinolin-4(1*H*)-one (**23**) (272 mg, 99%) as a yellowish-green solid: ^1H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.14 (s, 3H), 2.5–3.0 (m, 2H), 3.0–3.5 (m, 1H), 4.8–5.2 (m, 1H), 6.0–6.5 (m, 2H), 6.53 (d, 1H, J = 9 Hz), 7.14 (d, 1H, J = 9 Hz).

(9*RS*)-9-Ethyl-2,3-dihydro-4,9-dihydroxy-1*H*,12*H*-benzo[*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,13-(9*H*,15*H*)-dione (28a). A solution of 8-amino-3,4-dihydro-5-hydroxy-1(2*H*)-naphthalenone hydrochloride (**24**) (1.44 g, 6.74 mmol) and **10a** (1.94 g, 7.37 mmol) in AcOH (120 mL) was heated at 100 °C for 11 h. The precipitate obtained after cooling was collected by filtration and washed successively with AcOEt and MeOH. The material was dissolved in 0.6 N NaOH (240 mL) and washed successively with CH_2Cl_2 and AcOEt. The precipitate obtained after acidifying the mixture with concentrated HCl was collected by filtration and washed successively with water and MeOH to give **28a** (1.35 g, 45%): mp 280 °C dec; IR (KBr) 3424, 1758, 1653, 1587 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, 3H, J = 7 Hz), 1.6–2.3 (m, 4H), 2.7–3.3 (m, 4H), 5.20 (s, 1H), 5.41 (s, 2H), 7.27 (s, 1H), 7.48 (d, 1H, J = 9 Hz), 7.85 (d, 1H, J = 9 Hz). Anal. ($C_{23}H_{20}N_2O \cdot {}^2/_3H_2O$) C, H, N.

(9*S*)-9-Ethyl-2,3-dihydro-4,9-dihydroxy-1*H*,12*H*-benzo-[*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,13(9*H*,-15*H*)-dione (28b). A mixture of compound **24** (1.79 g, 8.38 mmol) and **10b** (2.00 g, 7.60 mmol) in AcOH (120 mL) was heated at 110 °C for 18 h. The reaction mixture was concentrated, and the residue was washed successively with AcOEt, saturated aqueous $NaHCO_3$, $CHCl_3$, and MeOH. Recrystallization (AcOH) gave **28b** (1.57 g, 51%) as a pale yellow solid: mp 221–226 °C dec; the IR and ^1H NMR spectral data of **28b** were identical with those of **28a**; MS (FAB) *m/z* 405 (MH^+); $[\alpha]_D = -23°$ (c = 0.24, DMSO). Anal. ($C_{23}H_{20}N_2O \cdot {}^1/_2H_2O$) C, H, N.

(9*S*)-9-Ethyl-2,3-dihydro-9-hydroxy-4-methyl-1*H*,12*H*-benzo[*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,-13(9*H*,15*H*)-dione (29). A mixture of 8-amino-3,4-dihydro-5-methyl-1(2*H*)-naphthalenone (**25**) (36 mg, 0.20 mmol) and **10b** (54 mg, 0.20 mmol) in AcOH (5 mL) was heated to reflux for 3 h. After the reaction mixture was concentrated, the residue obtained was purified with preparative TLC (2% MeOH/$CHCl_3$) to give **29** (22 mg, 27%) as a pale yellow solid: mp 265 °C dec; IR (KBr) 3416, 2944, 1748, 1660, 1602, 1512, 1456, 1366 cm^{-1}; MS (FD) *m/z* 402 (M^+); HRMS *m/z* 402.1580;

^{1}H NMR (DMSO-d_6) δ 0.89 (t, 3H, J = 7 Hz), 1.82−1.93 (m, 2H), 2.06 (t, 2H, J = 6 Hz), 2.41 (s, 3H), 2.99 (bs, 2H), 3.10 (t, 2H, J = 6 Hz), 5.15 (d, 2H, J = 2 Hz), 5.42 (s, 2H), 6.49 (s, 1H), 7.28 (s, 1H), 7.62 (d, 1H, J = 9 Hz), 7.86 (d, 1H, J = 9 Hz).

(9*RS*)-9-Ethyl-1,2-dihydro-9-hydroxy-4-methyl-12*H*-pyrano[4,3,2-*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,13(9*H*,15*H*)-dione (30). A mixture of 5-amino-8-methyl-4-chromanone (**26**) (644 mg, 3.63 mmol), **10a** (857 mg, 3.25 mmol), and *p*-TsOH·H_2O (10 mg, 0.05 mmol) in AcOH (150 mL) was heated to 100 °C for 11.5 h. After the mixture was concentrated to 50 mL and the suspension was diluted with $CHCl_3$ (50 mL) and AcOEt (50 mL), the precipitate was collected by filtration and washed successively with $CHCl_3$, 1 N NaOH, 10% aqueous HCl, water, and MeOH to give **30** (322 mg, 24%): mp 300 °C dec; IR (KBr) 3406, 1743, 1659, 1614 cm^{-1}; MS (FD) m/z 404 (M^+); ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7 Hz), 1.89 (q, 2H, J = 7 Hz), 2.36 (s, 3H), 2.54 (t, 2H, J = 5 Hz), 4.53 (t, 2H, J = 5 Hz), 5.25 (s, 2H), 5.44 (s, 2H), 6.51 (s, 1H), 7.31 (s, 1H), 7.66 (s, 2H). Anal. ($C_{23}H_{20}N_2O_5$) C, H, N.

(9*RS*)-9-Ethyl-1,2-dihydro-9-hydroxy-4-methyl-12*H*-thiino[4,3,2-*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,13(9*H*,15*H*)-dione (31). A mixture of 5-amino-8-methyl-4-thiochromanone (**27**) (1.82 g, 9.42 mmol) and **10a** (2.22 g, 9.42 mmol) in toluene (100 mL) was brought to reflux. After being heated for 30 min, the suspension was treated with *p*-TsOH·H_2O (700 mg, 3.68 mmol) and heated to reflux for an additional 7.5 h using a Dean−Stark trap. The precipitate obtained after cooling was collected by filtration and washed with AcOEt. A suspension of the crude material in a mixture of EtOH (100 mL) and 1 N HCl (20 mL) was heated to reflux for 2 h. After the mixture was cooled, the precipitate was collected by filtration and washed successively with water, EtOH, AcOEt, and ether to give **31** (2.0 g, 51%): mp >300 °C; IR (KBr) 1746, 1653, 1599, 1557 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7 Hz), 1.89 (q, 2H, J = 7 Hz), 2.43 (s, 3H), 3.2−3.6 (m, 4H), 5.24 (s, 2H), 5.47 (s, 2H), 7.33 (s, 1H), 7.67 (d, 1H, J = 9 Hz), 7.86 (d, 1H, J = 9 Hz). Anal. ($C_{23}H_{20}N_2O_4S$) C, H, N.

(9*RS*)-9-Ethyl-1,2-dihydro-9-hydroxy-4-methyl-3*H*,12*H*-pyrano[3′,4′:6,7]indolizino[1,2-*c*]benzo[*ij*][2,7]naphthyridine-10,13(9*H*,15*H*)-dione (32). A mixture of **23** (123 mg, 0.56 mmol) and **10a** (134 mg, 0.56 mmol) in toluene (20 mL) was brought to reflux. After 30 min, the suspension was treated with *p*-TsOH·H_2O (50 mg, 0.263 mmol) and heated to reflux for an additional 7 h using a Dean−Stark trap. The precipitate obtained after cooling was collected by filtration and washed successively with AcOEt, EtOH, water, EtOH, and ether to give (9*RS*)-3-acetyl-9-ethyl-1,2-dihydro-9-hydroxy-4-methyl-3*H*,12*H*-pyrano[3′,4′:6,7]indolizino[1,2-*c*]benzo[*i*,*j*][2,7]-naphthyridine-10,13(9*H*,15*H*)-dione (170 mg, 65%). A suspension of this camptothecin analogue (100 mg, 0.22 mmol) in concentrated HCl (10 mL) was heated at 100 °C for 1.5 h. After the reaction mixture was neutralized with $NaHCO_3$, the precipitate obtained was filtered and washed successively with water, EtOH, AcOEt, and ether to give **32** (30 mg, 33%): mp >300 °C; IR (KBr) 3400, 1740, 1658, 1612 cm^{-1}; MS (FAB) m/z 404 (MH^+); ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7 Hz), 1.89 (q, 2H, J = 7 Hz), 2.25 (s, 3H), 3.0−3.6 (m, 4H), 5.17 (s, 2H), 5.41 (s, 1H), 6.00 (bs, 1H), 6.43 (s, 1H), 7.26 (d, 1H, J = 9 Hz), 7.29 (s, 1H), 7.45 (d, 1H, J = 9 Hz). Anal. ($C_{23}H_{21}N_3O_4 \cdot {}^3/_4H_2O$) C, H, N.

(*RS*)-6-[(2,3-Dihydro-8-methyl-4-oxoquinol-5-yl)amino]-4-ethyl-4-hydroxy-1*H*-pyrano[3,4-*f*]indolizine-3,10(4*H*,8*H*)-dione (34). A mixture of **21** (344 mg, 1.95 mmol) and **10a** (461 mg, 1.95 mmol) in toluene (20 mL) was brought to reflux. After being heated for 30 min, the suspension was treated with *p*-TsOH·H_2O (90 mg, 0.47 mmol) and heated to reflux for an additional 16 h using a Dean−Stark trap. The precipitate obtained after cooling was collected by filtration and washed with AcOEt to give **34** (170 mg, 0.40 mmol): mp > 300 °C; MS (FAB) m/z 422 (MH^+); ^{1}H NMR (DMSO-d_6) δ 0.86 (t, 3H, J = 7 Hz), 1.82 (q, 2H, J = 7 Hz), 2.04 (s, 3H), 2.55−2.75 (m, 2H), 3.38−3.58 (m, 2H), 4.69 (bs, 2H), 5.35 (s, 2H), 6.39 (bs, 2H), 6.51 (d, 1H, J = 9 Hz), 6.81 (s, 1H), 7.12 (d, 1H, J = 9 Hz).

(*RS*)-9-Ethyl-1,2-dihydro-4,9-dihydroxy-12*H*-pyrano[4,3,2-*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,13(9*H*,15*H*)-dione (33). A mixture of compound **22** (410 mg, 2.12 mmol) and **10a** (560 mg, 2.12 mmol) in AcOH (20 mL) was heated to reflux for 5 h under N_2. The precipitate obtained after cooling was collected by filtration, washed with acetone, and recrystallized (AcOH) to afford (*RS*)-9-ethyl-1,2-dihydro-9-hyroxy-4-methoxy-12*H*-pyrano[4,3,2-*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,13(9*H*,15*H*)-dione (590 mg, 66%): mp 276 °C dec; IR (KBr) 3406, 3094, 1743, 1662, 1608, 1557 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.96 (s, 3H), 5.25 (s, 2H), 5.43 (s, 2H), 6.46 (s, 1H), 7.30 (s, 1H), 7.76 (s, 2H). Anal. ($C_{23}H_{20}N_2O_6 \cdot {}^1/_4H_2O$) C, H, N. A solution of the above compound (412 mg, 0.98 mmol) in 47% aqueous HBr (10 mL) was refluxed for 1 h under N_2. The reaction mixture was poured into ice/water, and the precipitate was collected by filtration, washed successively with water and acetone, and recrystallized ($CHCl_3$/MeOH) to give **33** (390 mg, 98%) as a yellow solid: mp 274 °C dec; IR (KBr) 3400, 3106, 1755, 1656, 1584 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.90 (t, 3H, J = 7 Hz), 1.88 (q, 2H, J = 7 Hz), 4.46 (m, 2H), 5.23 (s, 2H), 5.42 (s, 2H), 6.42 (bs, 1H), 7.29 (s, 1H), 7.47 (d, 1H, J = 9 Hz), 7.64 (d, 1H, J = 9 Hz). Anal. ($C_{22}H_{18}N_2O_6 \cdot {}^1/_2H_2O$) C, H, N.

7-Amino-4-hydroxyindanone (36). To a solution of 4-methoxyindanone (**35**) (8 g, 51.9 mmol) in concentrated H_2SO_4 (48 mL) was added a solution of KNO_3 (5.78 g, 57.1 mmol) in H_2SO_4 (48 mL) at 0 °C. After being stirred at the same temperature for 15 min, the mixture was poured into ice/water and the resulting suspension extracted with a portion of AcOEt. The organic phase was washed with water, dried (Na_2SO_4), and concentrated. The residue was recrystallized ($CHCl_3$/*n*-hexane) to give 4-methoxy-7-nitroindanone (4.10 g, 38%) as yellow needles: mp 158 °C; IR (KBr) 1722, 1581, 1521 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.82 (m, 2H), 3.02 (m, 2H), 4.00 (s, 3H), 7.01 (d, 1H, J = 9 Hz), 7.81 (d, 1H, J = 9 Hz). Anal. ($C_{10}H_9NO_4$) C, H, N. A mixture of this resultant compound (2.0 g, 9.65 mmol) and Raney Ni (3 mL) in a mixture of EtOH (25 mL) and 1,4-dioxane (20 mL) was stirred in an atmosphere of H_2 for 1 h. The catalyst was removed by filtration, and the filtrate was concentrated to give 7-amino-4-methoxyindanone (1.68 g, 98%): mp 96 °C; IR (KBr) 3460, 3358, 1677, 1584, 1497, 1443 cm^{-1}; MS (FAB) m/z 178 (MH^-); ^{1}H NMR ($CDCl_3$) δ 2.50−2.70 (m, 2H), 2.90−3.05 (m, 2H), 3.79 (s, 3H), 4.84 (bs, 2H), 6.43 (d, 1H, J = 9 Hz), 6.89 (d, 1H, J = 9 Hz). Anal. ($C_{10}H_{11}NO_2$) C, H, N. A solution of 7-amino-4-methoxyindanone (2.28 g, 12.9 mmol) in 47% aqueous HBr (110 mL) was refluxed for 3.5 h. The mixture was poured into water and neutralized with $NaHCO_3$. The aqueous mixture was extracted with AcOEt, and the combined extracts were dried (Na_2SO_4) and concentrated to give **36** (2.10 g, 100%): mp 270 °C dec; IR (KBr) 3476, 3356, 3160, 1650, 1610, 1580, 1500, 1474 cm^{-1}; ^{1}H NMR (CD_3OD) δ 2.5−2.7 (m, 2H), 2.9−3.0 (m, 2H), 6.43 (d, 1H, J = 9 Hz), 6.84 (d, 1H, J = 9 Hz).

(8*RS*)-8-Ethyl-1,2-dihydro-3,8-dihydroxy-11*H*-cyclopenta[*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-9,12(8*H*,14*H*)-dione (37). A mixture of compound **36** (1.0 g, 6.13 mmol), **10a** (3.22 g, 12.3 mmol), and *p*-TsOH·H_2O (350 mg, 1.84 mmol) in 1,2-dichloroethane (280 mL) was heated to reflux for 30 min. Then, EtOH (90 mL) was added, and the mixture was heated to reflux for 33 h. The precipitate obtained after cooling was collected by filtration and suspended in a mixture of $CHCl_3$ (100 mL), MeOH (80 mL), and 10% HCl (40 mL). The mixture was heated to reflux for 1 h. The precipitate obtained after cooling was collected by filtration and recrystallized (AcOH) to give **37** (375 mg, 16%): mp 270 °C dec; IR (KBr) 3300, 1749, 1653, 1587, 1497 cm^{-1}; MS (FD) m/z 390 (M^+); ^{1}H NMR (CF_3COOD) δ 1.18 (t, 3H, J = 8 Hz), 2.18 (q, 2H, J = 8 Hz), 3.7−4.1 (m, 4H), 5.75 (s, 2H), 5.65, 5.98 (ABq, 2H, J = 17 Hz), 8.05 (d, 1H, J = 10 Hz), 8.15 (d, 1H, J = 10 Hz), 8.29 (s, 1H). Anal. ($C_{22}H_{18}N_2O_5 \cdot {}^3/_4H_2O$) C, H, N.

4-Amino-6,7,8,9-tetrahydro-5*H*-benzocycloheptan-5-one (39). A mixture of 3- and 4-nitro-6,7,8,9-tetrahydro-5*H*-benzocycloheptane (**38**) (8.7 g, 45.5 mmol; mixture ratio = 3:1) and Raney Ni (10 mL) in a mixture of acetic anhydride (150 mL) and AcOH (50 mL) was stirred in an atmosphere of H_2. The mixture was filtered and concentrated in vacuo. The

residue was then neutralized with saturated aqueous $NaHCO_3$ solution. The aqueous mixture was extracted with $CHCl_3$, and the combined extracts were dried, filtered, and concentrated to give a mixture of 3- and 4-(acetylamino)-6,7,8,9-tetrahydro-5*H*-benzocycloheptane (6.17 g, 67%). Anal. ($C_{13}H_{17}NO \cdot {}^1/_{12}H_2O$) C, H, N. To a solution of the above acetylamino compound (800 mg, 3.94 mmol) in acetone (7.1 mL) and 17% (w/v) aqueous $MgSO_4$ (1.8 mL) was added $KMnO_4$ (950 mg, 6.0 mmol) at 50–55 °C over 2 h. The solution was stirred for 1.5 h and then poured into water and extracted with $CHCl_3$. The combined extracts were washed with water, dried, and concentrated. The residue was subjected to flash chromatography (1% MeOH/$CHCl_3$) to afford 4-(acetylamino)-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-5-one (55 mg, 25%): mp 155–162 °C; MS(FD) *m/z* 217 (M^+); ^1H NMR ($CDCl_3$) δ 1.5–2.0 (m, 6H), 2.17 (s, 3H), 2.6–2.9 (m, 4H), 6.93 (d, 1H, J = 8 Hz), 7.38 (t, 1H, J = 8 Hz), 8.30 (d, 1H, J = 8 Hz). Anal. ($C_{13}H_{15}NO_2 \cdot {}^1/_8H_2O$) C, H, N. A solution of this ketone compound (13 mg, 0.06 mmol) in 6 N HCl (1.2 mL) was heated at 80 °C for 2 h. The mixture was neutralized with 1 N NaOH and extracted with $CHCl_3$. The combined extracts were washed with water, dried, and concentrated to give **39** (9 mg, 86%): MS (FD) *m/z* 175 (M^+); ^1H NMR ($CDCl_3$) δ 1.7–1.9 (m, 4H), 2.6–2.9 (m, 2H), 6.50 (d, 1H, J = 7 Hz), 6.56 (d, 1H, J = 7 Hz), 7.11 (g, 1H, J = 7 Hz).

(10*S*)-10-Ethyl-1,2,3,4-tetrahydro-10-hydroxy-13*H*-cyclopenta[*de*]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-11,14(10*H*,16*H*)-dione (40). A mixture of **10b** (15 mg, 0.06 mmol) and **39** (9 mg, 0.05 mmol) in toluene (5 mL) was heated to reflux for 10 min. Pyridinium *p*-toluenesulfonate (5 mg, 0.02 mmol) was added to the solution, and the resulting mixture was heated to reflux for an additional 10 h. The solvent was removed in vacuo, and the residue was chromatographed (1% MeOH/$CHCl_3$) and recrystallized ($CHCl_3$/MeOH/Et_2O) to give **40** (9 mg, 44%): mp 270–280 °C dec; MS (FD) *m/z* 402 (M^+); ^1H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7 Hz), 1.8–2.0 (m, 2H), 1.9–2.1 (m, 4H), 3.2–3.4 (m, 4H), 5.29 (s, 2H), 5.43 (s, 2H), 6.52 (s, 1H), 7.30 (s, 1H), 7.45 (d, 1H, J = 7 Hz), 7.67 (t, 1H, J = 7 Hz), 7.97 (d, 1H, J = 7 Hz). Anal. ($C_{24}H_{22}N_2O_4 \cdot {}^1/_2H_2O$) C, H, N.

References

(1) (a) Sugimori, M.; Ejima, A.; Ohsuki, S.; Matsumoto, K.; Kawato, Y.; Yasuoka, M.; Tagawa, H.; Terasawa, H. Antitumor Agents. VI. Synthesis and Antitumor Activity of Ring A-, Ring B-, and Ring C-Modified Derivatives of Camptothecin. *Heterocycles* **1994**, *38*, 81–94. (b) Abbreviations: DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ZCl, benzyl chloroformate; ppTs, pyridinium *p*-toluenesulfonate; PPA, polyphosphoric acid; aq, aqueous; conc, concentrated.

(2) Wall M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. Plant Antitumor Agents. I. The Isolation and Structure of Camptothecin, a Novel Alkaloidal Leukemia and Tumor Inhibitor from *Camptotheca acuminata*. *J. Am. Chem. Soc.* **1966**, *88*, 3888–3890.

(3) Giovanella, B. C.; Stehlin, J. S.; Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Liu, L. F.; Silber, R.; Potmesil, M. DNA Topoisomerase I-Targeted Chemotherapy of Human Colon Cancer in Xenografts. *Science* **1989**, *246*, 1046–1048 and references therein.

(4) Hsiang, Y.-H.; Hertzberg, R.; Hecht, S.; Liu, L. F. Camptothecin Induces Protein-linked DNA Breaks via Mammalian DNA Topoisomerase I. *J. Biol. Chem.* **1985**, *260*, 14873–14878.

(5) Sawada, S.; Okajima, S.; Aiyama, R.; Nokata, K.; Furuta, T.; Yokokura, T.; Sugino, E.; Yamaguchi, K.; Miyasaka, T. Synthesis and Antitumor Activity of 20(*S*)-Camptothecin Derivatives: Carbamate-Linked, Water-Soluble Derivatives of 7-Ethyl-10-hydroxycamptothecin. *Chem. Pharm. Bull.* **1991**, *39*, 1446–1454.

(6) Kingsbury, W. D.; Boehm, J. C.; Jakas, D. R.; Holden, K. G.; Hecht, S. M.; Gallagher, G.; Caranfa, M. J.; McCabe, F. L.; Faucette, L. F.; Johnson, R. K.; Hertsberg, R. P. Synthesis of Water-Soluble (Aminoalkyl)camptothecin Analogues: Inhibitor of Topoisomerase I and Antitumor Activity. *J. Med. Chem.* **1991**, *34*, 98–107.

(7) (a) Miyasaka, T.; Sawada, S.; Nokata, K.; Mutai, M. Japanese Patent 59-51289, 1984; *Chem. Abstr.* **1984**, *101*, 91319d. (b) Wani, M. C.; Nicholas, A. W.; Wall, M. E. Plant Antitumor Agents. 23. Synthesis and Antileukemic Activity of Camptothecin Analogues. *J. Med. Chem.* **1986**, *29*, 2358–2363.

(8) Ejima, A.; Terasawa, H.; Sugimori, M.; Ohsuki, S.; Matsumoto, K.; Kawato, Y.; Yasuoka, M.; Tagawa, H. Antitumor Agents. V. Synthesis and Antileukemic Activity of E-Ring-Modified (*RS*)-Camptothecin Analogues. *Chem. Pharm. Bull.* **1992**, *40*, 683–688.

(9) Armarego, W. L. F.; Smith, J. I. C. Quinazolines. Part VII. Steric Effects in 4-Alkylquinazolines. *J. Org. Chem.* **1965**, *30*, 5360–5365.

(10) Wall, M. E.; Wani, M. C.; Natschke, S. M.; Nicholas, A. W. Plant Antitumor Agents. 22. Isolation of 11-Hydroxycamptothecin from *Camptotheca acuminata* Decne: Total Synthesis and Biological Activity. *J. Med. Chem.* **1986**, *29*, 1553–1555.

(11) Tagawa, H.; Terasawa, H.; Ejima, A. Japanese Patent 85-233366, 1985; *Chem. Abstr.* **1987**, *107*, 176302s.

(12) Schwender, C. F.; Pike, R. E.; Shavel, J., Jr. Derivatives of 3,4-Dihydro-1(2*H*)-naphthalenone as β-Adrenergic Blocking Agents. 2. Aromatic-Substituted Analogs of Bunolol. *J. Med. Chem.* **1973**, *16*, 254–257.

(13) Asahina, Y.; Obi, K.; Oomori, Y. European Patent 471358, 1992; *Chem. Abst. 117*, 70111v.

(14) Bossert, F.; Goennert, R. Thiochromones with schistosomicide activity. *Med. Chem., Abh. Med.-Chem. Forschungsstaellen Farbwerke Hoechst A. G.* **1963**, *7*, 367–392.

(15) Ricci, A.; Balucani, D.; Grandolini, G. Thio-1 Chromanones 5, 8-disubstituées et synthèses dans la série du thiéno[4,3,2-*de*]-thiochromanne. (5,8-Disubstituted-1-thiochromanes and syntheses in the thieno[4,3,2-*de*]thiochroman series.) *J. Heterocycl. Chem.* **1974**, *11*, 515–518.

(16) Paul, L. Ueber die Constitution der Guajacol-monosulfosäuren und eines Mononitro-guajacols. (On the constitution of Guaiacol-monosulfonic acid and Mononitro-guaiacols.) *Chem. Ber.* **1906**, *39*, 2773–2782.

(17) Loudon, J. D.; Razdan, R. K. Rearrangements of Chromanones and Dihydrocoumarins by Aluminum Chloride. *J. Chem. Soc.* **1954**, 4299–4303.

(18) Hahn, R. C.; Howard, P. H.; Kong, S.-M.; Lorenzo, G. A.; Miller, N. L. Cyclopropyl Aromatic Chemistry. I. Ultraviolet Spectra of Certain Cyclopropyl Aromatic Systems. *J. Am. Chem. Soc.* **1969**, *91*, 3558–3566.

(19) (a) Sawada, S.; Nokata, K.; Furuta, T.; Yokokura, T.; Miyasaka, T. Chemical Modification of an Antitumor Alkaloid Camptothecin: Synthesis and Antitumor Activity of 7-*C*-Substituted Camptothecins. *Chem. Pharm. Bull.* **1991**, *39*, 2574–2580. (b) Sawada, S.; Matsuoka, S.; Nokata, K.; Nagata, H.; Furuta, T.; Yokokura, T.; Miyasaka, T. Synthesis and Antitumor Activity of 20(*S*)-Camptothecin Derivatives: A-Ring Modified and 7,10-Disubstituted Camptothecins. *Chem. Pharm. Bull.* **1991**, *39*, 3183–3188.

(20) Wani, M. C.; Ronman, P. E.; Lindley, J. T.; Wall, M. E. Plant Antitumor Agents. 18. Synthesis and Biological Activity of Camptothecin Analogues. *J. Med. Chem.* **1980**, *23*, 554–560.

(21) (a) Searle, M. S.; Williams, D. H. The Cost of Conformational Order: Entropy Changes in Molecular Associations. *J. Am. Chem. Soc.* **1992**, *114*, 10690–10697. (b) Williams, D. H. The Molecular Basis of Biological Order. *Aldrichimica* Acta **1991**, *24*, 71–80.

(22) (a) Wani, M. C.; Nicholas, A. W.; Wall, M. E. Plant Antitumor Agents. 23. Synthesis and Antileukemic Activity of Camptothecin Analogues. *J. Med. Chem.* **1986**, *29*, 2358–2363. (b) Jaxel, C.; Kohn, K. W.; Wani, M. C.; Wall, M. E.; Pommier, Y. Structure-Activity Study of the Actions of Camptothecin Derivatives on Mammalian Topoisomerase I: Evidence for a Specific Receptor Site and a Relation to Antitumor Activity. *Cancer Res.* **1989**, *49*, 1465–1469. (c) Wall, M. E.; Wani, M. C.; Nicholas, A. W.; Manikumar, G.; Tele, C.; Moore, L.; Truesdale, A.; Leitner, P.; Besterman, J. M. Plant Antitumor Agents. 30. Synthesis and Structure Activity of Novel Camptothecin Analogs. *J. Med. Chem.* **1993**, *36*, 2689–2700.

(23) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.* **1988**, *48*, 589–601.

(24) Venditti, J. M. The National Cancer Institute Antitumor Drug Discovery Program, Current and Future Perspectives: A Commentary. *Cancer Treat. Rep.* **1983**, *67*, 767–772.