Rigid Analogs of Camptothecin as DNA Topoisomerase I Inhibitors

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Substituted 8-ethyl-2-(2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-triones were synthesized and evaluated as topoisomerase I inhibitors in an *in vitro* cleavable complex assay. The activity of these compounds may be attributed to their rigid, planar geometry, and an attempt was made to correlate the SAR in this series to known attributes of camptothecin.

Topoisomerase I is an enzyme that is required for swiveling and relaxation of DNA during molecular events such as replication and transcription.[1] Researchers have shown that camptothecin (1), an alkaloid originally isolated by Wani and Wall in 1966, is an antitumor agent due to the inhibition of topoisomerase I.[2] Camptothecin stabilizes the complex between DNA and topoisomerase I, thus interfering with the religation process. Studies have shown a correlation between the ability to cause stabilization of a DNA–topoisomerase I intermediate, DNA strand breaks, and antitumor effects of several camptothecin analogs.[3] The elucidation of the mechanism of camptothecin has facilitated the evaluation of natural products in search of new topoisomerase I inhibitors.[4]

The clinical utility of camptothecin as an anticancer agent was limited due to its toxicity and an extremely poor solubility profile.[5] Several researchers have evaluated substituted A- and B-ring camptothecin derivatives and were successful at addressing both of these issues, most notably Topotecan (**2**),[6] Irinotecan (**3**),[7] and 10,-11-(ethylenedioxy)-7-[(*N*-methylpiperazino)methyl]-20-(*S*)-camptothecin (**4**).[8] Many attempts have been made to move away from the camptothecin structure in order to simplify the molecule, reduce its toxicity, and increase the solubility. While these derivatives have not met these criteria, they have offered important information about critical structure features necessary for activity. For example, Wani and Wall demonstrated that analogs of camptothecin in which there was sequential truncation of the rings were devoid of both *in vitro* and *in vivo* activity (tetracyclic B–E-rings, tricyclic C–E-rings, bicyclic D-, E-rings, and monocyclic E-ring analogs), suggesting that all of these rings have key interactions for binding.[9] Kurihara[10] and co-workers described des-C-ring analogs, for example, C-nor-4,6-secocamptothecin (**5**), all of which were devoid of activity possibly due to the lack of planarity. Furthermore, compounds such as **6** in which the D-ring or C- and D-ring of camptothecin have been deleted have recently been described and are also biologically inactive.[11]

We have designed a rigid analog of camptothecin that provides the necessary molecular shape required for activity. In this paper, the synthesis and *in vitro* biological activity of substituted 8(*R*,*S*)-ethyl-2-(2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-triones such as compound **7** are described.

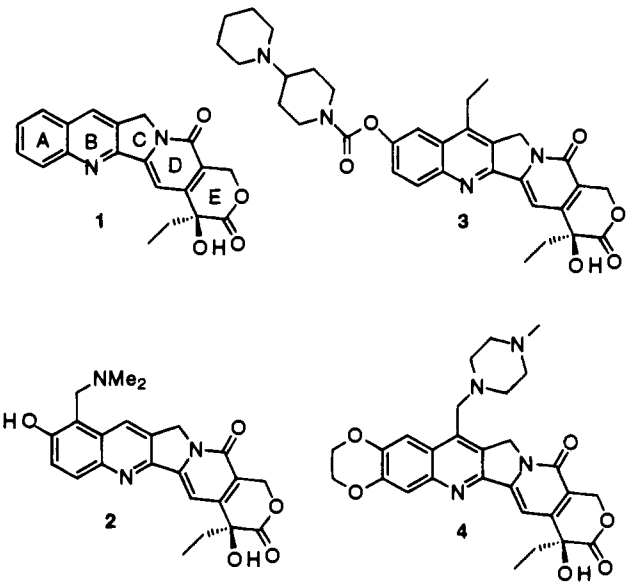

Figure 1. Camptothecin (1), Topotecan (**2**), Irinotecan (**3**), and 10,11-(ethylenedioxy)-7-[(*N*-methylpiperazino)methyl]-20(*S*)-camptothecin (**4**).

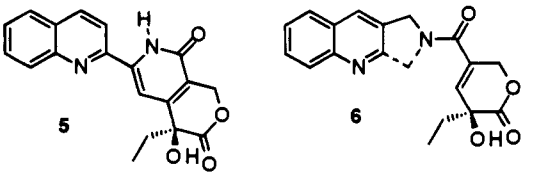

Figure 2.

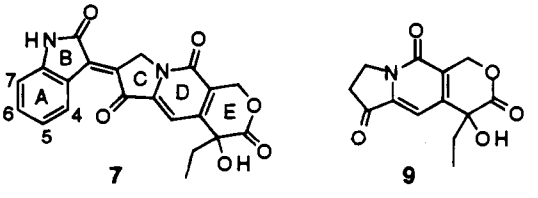

Figure 3.

Chemistry

The synthesis of these compounds was straightforward using an acid-catalyzed aldol reaction (Scheme 1).[12] Most of the commercially available isatins were

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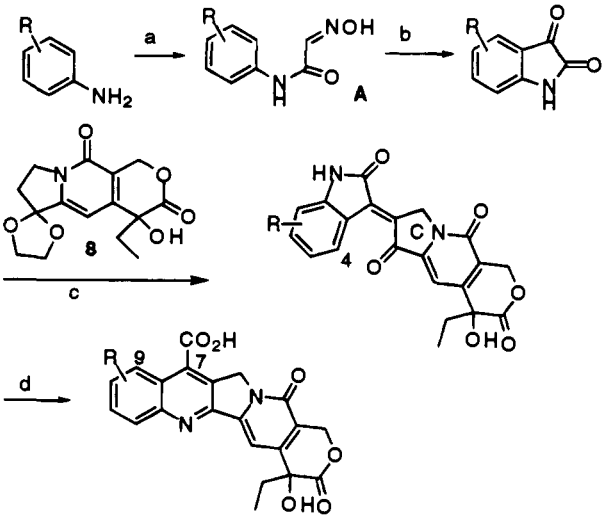
Scheme 1[a]

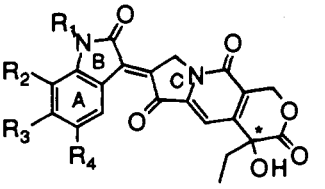


[a] Reagents: (a) chloral hydrate, hydroxylamine, H_2O/HCl; (b) concentrated H_2SO_4, BF_3·Et_2O, or $H_4P_2O_7$/heat; (c) HOAc, HCl, room temperature; (d) HOAc, concentrated HCl, 105 °C.

substituted in the 5-position with electron-withdrawing substituents. In order to investigate a wider range of A-ring substitutions, it was necessary to synthesize isatins via the Sandmeyer procedure whereby the formation of an α-oximinoanilide (generic structure **A** in Scheme 1) by condensation of chloral hydrate, hydroxylamine, and an aniline was cyclized with concentrated sulfuric acid. Equimolar amounts of the tricyclic ketal **8**[13] (or the tricyclic ketone **9**[14]) and substituted isatins were combined in an aqueous acid medium and stirred at room temperature for between 2 and 40 h. The products were collected by filtration and as such were analytically pure after a few organic washes. The oxodihydroindolyidene derivatives are listed in Table 1. If the reactions were run at higher temperatures, the aldol products reacted further to form 7-carboxylic acid camptothecin derivatives. All of these adducts (except **18** and **21**) were converted to their substituted 7-carboxylic acid camptothecin derivatives, since it was important to establish that any *in vitro* activity was due to the oxodihydroindolylidene compounds and not due to trace contamination by the camptothecin derivative. Several examples of the 7-carboxylate camptothecin derivatives are shown in Table 2.

While no attempt was made to control the double-bond geometry during the aldol condensation, only one isomer was obtained. The *E*-geometry was inferred by the downfield shift of the aromatic proton (4H) which presumably is due to it lying in the deshielding region of the C-ring carbonyl. A similar downfield shift of the same aromatic proton (9H) was also observed in the 7-carboxylic acid camptothecin derivatives. Further support for this *E*-geometry was obtained from molecular modeling calculations where a simple energy minimization was applied to both the *E*- and *Z*-isomer. The *Z*-geometry showed an unfavorable oxygen–oxygen electrostatic interaction. It is also possible to propose that the oxodihydroindolylidene molecules are near planar because of the chemical shift equivalence of the C-ring methylene protons. One might expect diastereomers if the molecule was twisted at the exo-cyclic olefin. Unfortunately, all attempts to obtain acceptable crystals for an X-ray structure failed.

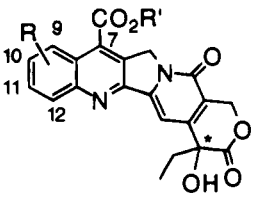
Table 1.



compd no.	R				*	mp[a] (°C)	nom[b] mass	IC_{50} (μM)
	R_4	R_3	R_2	R_1				
7	H	H	H	H	R,S	>265 (d)	393	5.8
10	H	H	H	H	R	>265 (d)	393	>100
11	H	H	H	H	S	>265 (d)	393	4.0
12	F	H	H	H	R,S	>300	411	1.2
13	F	H	H	H	R	300 (d)	411	>100
14	F	H	H	H	S	300 (d)	411	1.1
15	Cl	H	H	H	R,S	>300	427	>100
16	I	H	H	H	R,S	264–266	519	32.0
17	I	H	H	H	S	263	519	12.1
18	F	F	F	H	R,S	>265 (d)	447	>100
19	Me	H	NO_2	H	S	>275 (d)	452	>100
20	NO_2	H	H	H	R,S	>275 (d)	438	>100
21	H	H	H	Me	R,S	>275	407	>100
22	EDO	EDO	H	H	R,S	308 (d)	451	29.6
23	Me	H	H	H	S	326 (d)	407	1.8
24	H	H	Cl	H	S	312 (d)	427	>100
25	H	H	Me	H	S	>325	407	>100
26	Br	H	H	H	S	>300 (d)	481	>100
27	H	CF_3O	H	H	S	>275 (d)	477	>100
28	H	<eS	H	H	S	>275	439	>100
29	H	MeO	H	H	R,S	>250	423	>100
30	H	F	Me	H	R,S	>264 (d)	425	>100
31	F	F	H	H	R,S	281	429	1.5
32	F	F	H	H	S	>300	429	1.3

[a] Melting points are uncorrected. [b] Electrospray MS data reported as *m/z* MH^+.

Table 2.

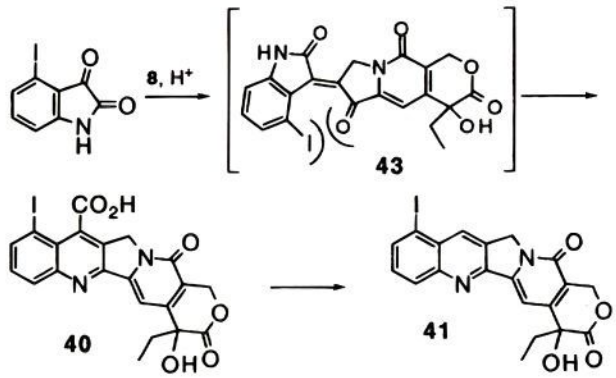


compd no.	R	*	mp[a] (°C)	nom[b] mass	IC_{50} (μM)
33	unsub	S	>300 (d)	393	2.1
34	EDO	R,S	>300	451	24.1
35	EDO 7-CO_2Et	R,S	>365	479	0.7
36	10-F	R,S	>334 (d)	411	3.2
37	10-F	S	>300 (d)	411	3.1
38	10-F	R	>300 (d)	411	>100
39	10-I	R,S	>300	519	2.3
40	9-I	R,S	>250	519	1.3
41	10-methyl	S	>312[c]	407	1.9
42	10,11-difluoro	R,S	305 (d)	429	1.5

[a] Melting points are uncorrected. [b] Electrospray MS data reported as *m/z* MH^+. [c] The material changed to clear needles at 312 °C.

In an attempt to synthesize 4-iodo-2-oxodihydroindolylidene **43**, the appropriate iodoisatin and tricyclic ketal **8** were subjected to the reaction conditions; however, no aldol intermediate could be isolated. The products isolated were 7-carboxylic acid 9-iodocamptothecin (**40**) and 9-iodocamptothecin (**41**) (Scheme 2). The decarboxylation appears to occur after the quinoline ring has formed since subjection of **40** to the aqueous acid conditions at a higher temperature or for a longer reaction time affords **41**. The reaction time to form the quinoline derivative is much shorter, presumably because the isatin aldol intermediate is destabilized due

Scheme 2



to steric interactions. For these reasons, other isatin aldol derivatives substituted in the 4-position were not pursued.

Other compounds with structural similarities to the oxodihydroindolylidenes were briefly evaluated. Several substituted benzaldehydes were condensed with **8** under strongly acidic conditions to form single isomers (for example, **44**) which have been assigned with the trans double-bond geometry on the basis of NOE NMR experiments. In another series of compounds, Schiff base derivatives (for example, **45**) were also synthesized from the corresponding aniline and **9** using catalytic camphorsulfonic acid in refluxing toluene.

In Vitro Pharmacology

The compounds described above were tested in a cleavable complex assay described by Hsiang.[2] DNA and topoisomerase I were incubated with various concentrations of each compound and subjected to a protein denaturation procedure followed by gel electrophoresis. The degree of DNA cleavage, and hence the ability of the compound to stabilize a DNA–topoisomerase I–drug ternary complex, was measured and standardized to that induced by camptothecin.[15] The results are reported as the concentration of compound required to achieve half-maximal cleavage of DNA (see IC_{50}'s in Tables 1 and 2).

Compound **7** ($IC_{50} = 5.8\ \mu m$) represents a rigid analog of a B-ring-modified camptothecin molecule which causes complete DNA fragmentation in the cleavable complex assay. For comparison purposes, the *S*-isomer of camptothecin **1** has an IC_{50} of approximately 0.7 μm, while the *R*-isomer is essentially inactive in this assay. Since the C-, D-, and E-rings of the oxodihydroindolylidene derivative resemble the camptothecin C-, D-, and E-rings, it was important to determine if this compound could be active by a similar mechanism to camptothecin. This question was investigated by synthesizing the 8*R*-isomer **10** as well as the 8*S*-isomer **11**. Within the limits of the assay, it was determined that the *S*-isomer **11** was slightly more active ($IC_{50} = 4.0\ \mu m$) than the racemic compound **7**, the and *R*-isomer **10** was totally inactive. A similar comparison was done with one of the most active derivatives, the 5-fluoro-2-oxodihydroindolylidene compound. The racemate **12** was slightly less active than the 5-fluoro 8*S*-isomer **14**, and again the 5-fluoro 8*R*-isomer **13** was inactive.

The molecular shape of the oxodihydroindolylidene derivatives as manifested in the double-bond geometry appears to be critical for the activity. Compounds **44** and **45** were completely inactive in the cleavable complex assay. These data suggest that it is necessary to have an aromatic ring in the region corresponding to the camptothecin A-ring as was suggested by Wani/Wall with their truncated camptothecin analogs. They had concluded that it was necessary to have the conjugated ring system in the A- and B-rings.[9]

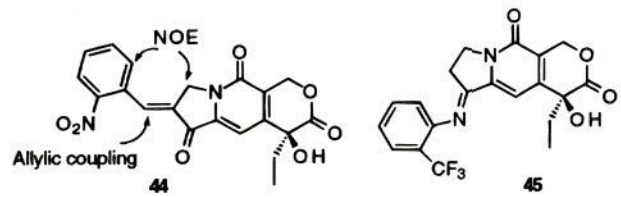


Figure 4.

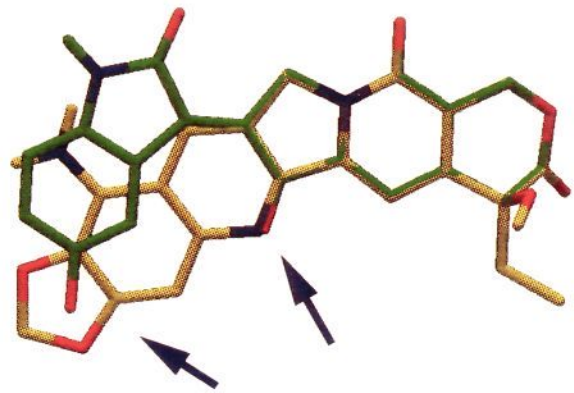


Figure 5. Superposition of **46** (yellow) and **14** (green) demonstrating how well the N in the B-ring of **46** overlaps the carbonyl oxygen of **14** and how the 11-position oxygen of **46** comes very close to the 5-fluoro of **14**.

A series of A-ring substitutions were examined that spanned electron-withdrawing groups to electron-donating groups in positions 5–7. The compounds studied with substitutions in the 7-position demonstrated significantly diminished activity. Of particular interest was the fluorinated series where the monofluoro derivative **12** had an average IC_{50} of 1.2 μm and the 5,6-difluoro derivative **31** was 1.5 μm, while the 5,6,7-trifluoro derivative **18** showed minimal activity. 7-Methyl-6-fluoro-2-oxodihydroindolylidene **30** is virtually inactive, yet the 7-methyl-2-oxodihydroindolylidene **25** shows some fragmentation. When the methyl group is substituted in the 5-position (compound **23**), complete fragmentation is observed affording an IC_{50} of 1.8 μm. It appears from the compounds tested that the substitutions in the 6-position have lower activity relative to the A-ring-unsubstituted oxodihydroindolylidene derivative **7**. The steric constraints in this region are probably the dominant factor in determining the ideal A-ring substitution.

There are some structural similarities between 6-fluoro-2-oxodihydroindolylidene **14** and a known[16] potent substituted camptothecin derivative, 9-amino-10,11-(methylenedioxy)camptothecin (**46**). The two structures were superimposed by aligning the C-, D-, and E-rings of both molecules using the modeling program Macromodel version 3.0[17] (Figure 5). The carbonyl oxygen in the 1-position in structure **14** could be a mimic for the quinoline nitrogen in the B-ring of camptothecin. The Lewis basicity of this quinoline nitrogen appears to be an important factor in the activity of bond-deleted analogs of camptothecin. The isatin portion of the molecule lies in the region where the camptothecin SAR

has shown a tolerance for bulky groups. The fluorine substitution on **14** corresponds to the oxygen of the methylenedioxy substituent on **46.**

Several of the 7-carboxylic acid camptothecin derivatives are listed in Table 2. 7-Carboxylic acid camptothecin (**33**) is approximately 3-fold less active that the parent camptothecin. A 10,11-ethylenedioxy substitution (**34**) lowers the activity even further. However, the corresponding ethyl ester **35** is significantly more active with an IC_{50} of 0.7 μm. The 10-fluoro-substituted derivatives **36** (*R,S*-isomer) and **37** (*S*-isomer) are almost 3-fold less active than the corresponding 5-fluoro-2-oxodihydroindolylidene derivatives **12** (*R,S*-isomer) and **14** (*S*-isomer). 7-Carboxylic acid 10,11-difluorocamptothecin (**42**) is approximately equal in activity to the corresponding difluoro-substituted oxodihydroindolylidene **31**. Therefore, the *in vitro* activity of the oxodihydroindolylidene compounds is not due to trace contamination of the respective camptothecin compound.

Conclusion

We have designed and synthesized novel ring-deleted rigid analogs of camptothecin that inhibit topoisomerase I in a cleavable complex assay described by Hsiang.[9] These derivatives appear to satisfy the stringent geometric requirements for formation of the ternary complex. In particular, the A-ring fluorine compounds **14** and **32** represent the most active compounds in this series.

Experimental Section

Melting points were taken on Mel-Temp II apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. ^{1}H NMR spectra were acquired using a Varian Unity 300 MHz spectrometer in DMSO-d_6 and are reported in parts per million (ppm) internally referenced to residual DMSO (2.49 ppm) unless otherwise noted. ^{13}C NMR spectra were acquired using a Varian Unity spectrometer at 75.4 MHz with Waltz composite decoupling in DMSO and are reported in parts per million internally referenced to the solvent peak (39.5 ppm) unless otherwise noted. Mass spectra were recorded on either a Perkin-Elmer Sciex API III or a JEOL SX102 mass spectrometer. The chirality of the final compound was synthesized by using the appropriate tricyclic ketone **9** precursor (*R*, *S*, and *R,S*), all of which were supplied by the Synthetic Organic Chemistry group at Glaxo Research Institute. All of the oxodihydroindolylidene compounds were isolated as amorphous solids. Each of the syntheses employed commercially available substituted isatins except where noted.

Synthesis of a Novel Isatin. 3-(Trifluoromethoxy)-α-oximinoanilide (47). The following solutions were prepared at room temperature: (1) chloral hydrate (415 mg, 2.51 mmol) and Na_2SO_4 (3.61 g, 18.8 mmol) were dissolved in H_2O (11 mL); (2) hydroxyamine hydrogen chloride (495 mg, 7.12 mmol) was dissolved in H_2O (5 mL); (3) 3-(trifluoromethoxy)aniline (400 mg, 2.26 mmol) and HCl (concentrated, 0.19 mL) were dissolved in H_2O (6 mL). Solution 1 was warmed to 60° C in an open flask, and to this was added solution 2. The reaction mixture was stirred for 10 min before the addition of solution 3. The temperature of the reaction mixture was increased to 95 °C and stirred for 30 min. Upon cooling to room temperature, white needle crystals precipitated from the reaction solution. These crystals were collected by filtration, washed with H_2O (2 × 5 mL each), and dried under high vacuum (485 mg, 87%): mp 140 °C. ^{1}H NMR: δ 7.04 (d, 1H), 7.42 (t, 1H), 7.61 (s, 1H), 7.62 (d, 1H), 7.83 (s, 1H), 10.44 (s, 1H), 12.27 (s, 1H). CI MS: *m/z* 249 (MH^+). Anal. ($C_9H_7F_3N_2O_3$) C, H, N.

6-(Trifluoromethoxy)isatin (48). Sulfuric acid (concentrated, 0.55 mL) was stirred at 90 °C for 5 min before 3-(trifluoromethoxy)-α-oximinoanilide (45.0 mg, 0.18 mmol) was added. The reaction mixture was stirred for 45 min and then cooled to room temperature. Water (5 mL) was added dropwise, and the mixture was extracted with EtOAc (3 × 5 mL each). The organics were combined and the volatiles removed. The desired product, 6-(trifluoromethoxy)isatin (25 mg, 60%), was isolated by column chromatography using 9/1 $CHCl_3$/MeOH as the eluent (R_f = 0.68): mp 169−170 °C. ^{1}H NMR: δ 6.60 (s, 1H), 6.99 (d, 1H), 7.64 (d, 1H), 11.21 (s, 1H, NH). FAB MS: *m/z* 232 (MH^+). Anal. ($C_9H_4F_3NO_3$) C, H, N.

Synthesis of Oxodihydroindolylidene Compounds. 8-(*R,S*)-Ethyl-2-(2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (7). Isatin (50 mg, 0.34 mmol), racemic tricyclic ketone (104 mg, 0.34 mmol), and glacial AcOH (2.0 mL) were combined at room temperature, and the reaction mixture was placed in a preheated oil bath set to 75 °C. The reaction mixture was stirred for 5 min before the addition of concentrated HCl (0.5 mL). After the reaction mixture stirred for 1 h, the oil bath was removed and H_2O (5.0 mL) was added. The orange-red precipitate was collected by filtration and washed with EtOH (5.0 mL) and Et_2O (10 mL). The solid was dried under high vacuum to afford the title compound (125 mg, 94%). ^{1}H NMR: δ 0.98 (t, 3H), 1.95 (m, 2H), 5.13 (s, 2H), 5.41 (dd, 2H), 6.58 (s, 1H, OH), 6.94 (d, 1H, H-8), 7.03 (s, 1H), 7.10 (m, 1H), 7.42 (m, 1H), 8.95 (d, 1H, H-5), 11.02 (s, 1H, NH). Anal. ($C_{21}H_{16}N_2O_6 \cdot 0.5H_2O$) C, H, N. **8*R*-Isomer 10.** Anal. ($C_{21}H_{16}N_2O_6 \cdot 2H_2O$) C, H, N. **8*S*-Isomer 11.** ^{13}C NMR (DMSO-d_6): δ 10.91, 33.91, 52.88, 68.86, 75.33, 103.23, 113.66, 124.0, 125.25, 128.11, 131.32, 134.01, 137.31, 137.33, 145.3, 148.3, 152.4, 159.34, 172.1, 175.44, 189.97. Anal. ($C_{21}H_{16}N_2O_6 \cdot 1.5H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-(5-fluoro-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (12). ^{1}H NMR: δ 0.91 (t, 3H, CH_3), 1.90 (m, 2H, CH_2), 5.15 (s, 2H, H-3), 5.41 (dd, 2H, H-5), 6.59 (bs, 1H, OH), 6.92 (dd, 1H, H-7′), 7.04 (s, 1H, H-9), 7.31 (td, 1H, H-6′), 8.72 (dd, 1H, H-4′), 11.07 (s, 1H, NH). Anal. ($C_{21}H_{15}FN_2O_6$) C, H, N. **8R-Isomer 13.** Anal. ($C_{21}H_{15}FN_2O_6 \cdot 0.5H_2O$) C, H, N. **8*S*-Isomer 14.** ^{1}H NMR (acetone-d_6): δ 0.99 (t, 3H, CH_3), 1.98 (m, 2H, CH_2), 5.38 (s, 2H, H-3), 5.46 (dd, 2H, H-5), 7.06 (dd, 1H, H-7′), 7.24 (s, 1H, H-9), 7.30 (td, 1H, H-6′), 8.90 (dd, 1H, H-4′), 10.02 (bs, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 7.63 (q), 30.48 (t), 49.07 (t), 65.35 (t), 71.50 (s), 100.25 (d), 111.13 (d), 114.17 (d), 114.53 (d), 120.13 (d), 120.45 (d), 124.61 (s), 129.56 (s), 134.90 (s), 140.67 (s), 140.93 (s), 148.68 (s), 155.42 (s), 158.23 (s), 168.01 (s), 171.38 (s), 186.16 (s). Anal. ($C_{21}H_{15}FN_2O_6 \cdot 1.5H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-(5-chloro-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[b]naphthalene-1,4,7-trione (15). ^{1}H NMR: δ 0.91 (t, 3H), 1.91 (m, 2H), 5.12 (s, 2H), 5.42 (dd, 2H), 6.58 (bs, 1H, OH), 6.94 (d, 1H, H-7), 7.05 (s, 1H, H-9), 7.48 (dd, 1H, H-6), 8.94 (d, 1H, H-4), 11.18 (s, 1H, NH). IR (Nujol): 3560, 3210, 2915, 2860, 1755, 1705, 1655, 1600, 1462, 1380, 1300, 1239, 1172 cm^{-1}. Anal. ($C_{21}H_{15}N_2O_6Cl \cdot 2H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-(5-iodo-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[b]naphthalene-1,4,7-trione (16). ^{1}H NMR: δ 0.98 (t, 3H), 1.97 (m, 2H), 5.15 (s, 2H), 5.42 (dd, 2H), 6.79 (d, 1H), 7.04 (s, 1H), 7.86 (d, 1H), 9.25 (s, 1H), 11.19 (s, 1H, NH). Anal. ($C_{21}H_{15}N_2O_6I \cdot 0.25H_2O$) C, H, N. **8*S*-Isomer 17:** Anal. ($C_{21}H_{15}N_2O_6I \cdot 0.75H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-(5,6,7-trifluoro-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (18). This compound was prepared by the procedure of **7** except an equivalent amount of 5,6,7-trifluoroisatin[18] was used in place of isatin. ^{1}H NMR: δ 0.90 (t, 3H), 1.90 (m, 2H), 5.17 (s, 2H), 4.41 (dd, 2H), 7.09 (s, 1H), 8.84 (m, 1H), 11.90 (s, 1H). HR MS ($C_{21}H_{13}F_3N_2O_6$): calcd, 447.0804; found, 447.0800.

8(*S*)-Ethyl-2-(5-methyl7-nitro-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (19). ^{1}H NMR: δ 0.90 (t, 3H), 1.90 (m, 2H), 5.20 (s, 2H), 5.42 (dd, 2H), 7.08 (s, 1H), 8.05 (s, 1H), 9.20 (s, 1H), 11.65 (s, 1H). Anal. ($C_{22}H_{17}N_3O_8 \cdot 1H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-(5-nitro-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]napthalene-1,4,7-trione (20). ^{1}H NMR: δ 0.80 (t, 3H), 1.81 (m, 2H), 5.08 (s, 2H), 5.40 (s, 2H), 6.58 (bs, 1H), 7.01 (s, 1H), 7.04 (d, 1H); 8.37 (d, 1H), 9.80 (s, 1H), 11.76 (s, 1H). Anal. ($C_{21}H_{15}N_3O_8 \cdot 0.5H_2O$) C, H, N.

1-Methyl-8(*R,S*)-ethyl-2-(2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (21). ^{1}H NMR: δ 0.85 (t, 3H), 1.83 (m, 2H), 3.22 (s, 3H), 5.14 (s, 2H), 5.41 (dd, 2H), 6.60 (bs, 1H), 7.04 (s, 1H), 7.16 (m, 2H), 7.51 (dd, 1H), 8.96 (d, 1H). Anal. ($C_{22}H_{18}N_2O_6 \cdot 1H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-[5,6-(ethylenedioxy)-2-oxo-1,2-dihydroindol-3-ylidene]-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]napthalene-1,4,7-trione (22). This compound was prepared by the procedure of **7** except an equivalent amount of 5,6-(ethylenedioxy)isatin[12] was used in place of isatin. ^{1}H NMR: δ 0.85 (t, 3H), 1.81 (m, 2H), 4.22 (m, 2H), 4.39 (m, 2H), 5.10 (s, 2H), 5.40 (s, 2H), 6.44 (bs, 1H), 7.06 (s, 1H), 7.32 (s, 1H), 8.58 (s, 1H), 10.81 (s, 1H). Anal. ($C_{23}H_{18}N_2O_8 \cdot 1H_2O$) C, H, N.

8(*S*)-Ethyl-2-(5-methyl-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (23). ^{1}H NMR: δ 0.82 (t, 3H), 1.80 (m, 2H), 2.31 (s, 3H), 5.15 (s, 2H), 5.42 (s, 2H), 6.58 (bs, 1H), 6.80 (d, 1H), 7.02 (s, 1H), 7.24 (d, 1H), 8.79 (s, 1H), 10.98 (s, 2H). Anal. ($C_{22}H_{18}N_2O_6 \cdot 0.25H_2O$) C, H, N.

8(*S*)-Ethyl-2-(7-chloro-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]-naphthalene-1,4,7-trione (24). ^{1}H NMR: δ 0.83 (t, 3H), 1.81 (m, 2H), 5.18 (s, 2H), 5.42 (dd, 2H), 7.02 (s, 1H), 7.13 (t, 1H), 7.55 (d, 1H), 8.91 (d, 1H), 11.49 (s, 1H). Anal. ($C_{21}H_{15}N_2O_6Cl \cdot 0.5H_2O$) C, H, N.

8(*S*)-Ethyl-2-(7-methyl-2-oxo-1,2-dihydroindol-3ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6oxa-3a-azacyclopenta[*b*]-naphthalene-1,4,7-trione (25). ^{1}H NMR: δ 0.85 (t, 3H), 1.84 (m, 2H), 2.28 (s, 3H), 5.16 (s, 2H), 5.44 (s, 2H), 7.01 (s, 1H), 7.34 (m, 1H), 7.40 (d, 1H), 8.80 (d, 1H), 11.03 (s, 1H). Anal. ($C_{22}H_{18}N_2O_6 \cdot 0.5H_2O$) C, H, N.

8(*S*)-2-(5-bromo-2-oxo-1,2-dihydroindol-3ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (26). ^{1}H NMR: δ 0.81 (t, 3H), 1.82 (m, 2H), 5.17 (s, 2H), 5.44 (s, 2H), 6.60 (bs, 1H); 6.92 (d, 1H), 7.02 (s, 1H), 7.63 (d, 1H), 9.11 (d, 1H), 11.20 (s, 1H). Anal. ($C_{21}H_{15}N_2O_6Br \cdot 1H_2O$) C, H, N.

8(*S*)-Ethyl-2-[6-(trifluoromethoxy)-2-oxo-1,2-dihydroindol-3-ylidene]-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (27). This compound was prepared by the procedure of **7** except an equivalent amount of 6-(trifluoromethoxy)isatin (**48**) was used in place of isatin. ^{1}H NMR: δ 0.84 (t, 3H), 1.80 (m, 2H), 5.10 (s, 2H), 5.42 (s, 2H), 6.91 (s, 1H), 7.06 (m, 2H), 9.00 (d, 1H), 11.22 (s, 1H). Anal. ($C_{22}H_{15}F_3N_2O_7 \cdot 1H_2O$) C, H, N.

8(*S*)-Ethyl-2-[6-(methylthio)-2-oxo-1,2-dihydroindol-3ylidene]-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (28). This compound was prepared by the procedure of **7** except an equivalent amount of 6-(methylthio)isatin[19] was used in place of isatin. ^{1}H NMR: δ 0.82 (t, 3H), 1.80 (m, 2H), 2.51 (s, 3H), 5.11 (s, 2H), 5.41 (s, 2H), 6.64 (bs, 1H), 6.72 (s, 1H), 6.94 (d, 1H), 7.02 (s, 1H), 8.85 (d, 1H), 11.06 (s, 1H). Anal. ($C_{22}H_{17}N_2O_6S \cdot 1H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-(6-methoxy-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (29). This compound was prepared by the procedure of **7** except an equivalent amount of 6-methoxyisatin[20] was used in place of isatin. ^{1}H NMR: δ 0.84 (t, 3H), 1.80 (m, 2H), 3.82 (s, 3H), 5.10 (s, 2H), 5.42 (s, 2H), 6.55 (bs, 1H), 6.61 (s, 1H), 6.70 (d, 1H), 7.02 (s, 1H), 8.93 (d, 1H), 11.12 (s, 1H). Anal. ($C_{22}H_{18}N_2O_7 \cdot 1H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-(6-fluoro-7-methyl-2-oxo-1,2-dihydroindol-3-ylidene)-8-hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (30). This compound was prepared by the procedure of **7** except an equivalent amount of 6-fluoro-7-methylisatin[21] was used in place of isatin. ^{1}H NMR: δ 0.82 (t, 3H), 1.81 (m, 2H), 2.07 (s, 3H), 5.06 (s, 2H), 5.42 (s, 2H), 6.59 (bs, 1H), 6.88 (m, 1H), 7.03 (s, 1H), 8.82 (m, 1H), 11.35 (s, 1H). Anal. ($C_{22}H_{17}FN_2O_6 \cdot 0.5H_2O$) C, H, N.

8(*R,S*)-Ethyl-2-(5,6-difluoro-2-oxo-1,2-dihydroindol-3-ylidene)-8hydroxy-2,3,5,8-tetrahydro-6-oxa-3a-azacyclopenta[*b*]naphthalene-1,4,7-trione (31). This compound was prepared by the procedure of **7** except an equivalent amount of 5,6-difluoroisatin[22] was used in place of isatin. ^{1}H NMR: δ 0.84 (t, 3H), 1.82 (m, 2H), 5.07 (s, 2H), 5.41 (dd, 2H), 6.60 (s, 1H), 7.00 (m, 1H), 7.04 (s, 1H), 8.90 (m, 1H), 11.22 (s, 1H). Anal. ($C_{21}H_{14}F_2N_2O_6 \cdot 0.33H_2O$) C, H, N. **8*S*-Isomer 32.** Anal. ($C_{21}H_{14}F_2N_2O_6 \cdot 0.25H_2O$) C, H, N.

Synthesis of 7-Carboxylic Acid Camptothecin Derivatives. 7-Carboxylic Acid 20(*R,S*)-Camptothecin (33). Isatin (50 mg, 0.34 mmol), racemic tricyclic ketone (104 mg, 0.34 mmol), and glacial AcOH (2.0 mL) were combined at room temperature, and the reaction mixture was placed in a preheated oil bath set to 105 °C. The reaction mixture was stirred for 5 min before the addition of concentrated HCl (0.5 mL). The reaction mixture was stirred for 15 h, the oil bath was removed, and H_2O (5.0 mL) was added. The yellow-orange precipitate was collected by filtration and washed with EtOH (5.0 mL) and Et_2O (10 mL). The solid was dried under high vacuum to afford the title compound (132 mg, 100%). ^{1}H NMR: δ 0.93 (t, 3H), 1.95 (m, 2H), 5.42 (s, 4H), 6.59 (bs, 1H), 7.28 (s, 1 H), 7.81 (t, 1H), 7.92 (t, 1H), 8.24 (d, 1H), 8.85 (d, 1H). Anal. ($C_{21}H_{16}N_2O_6 \cdot 0.5H_2O$) C, H, N.

7-Carboxylic Acid 10,11-(Ethylenedioxy)-20(*R,S*)-camptothecin (34). ^{1}H NMR: δ 0.90 (t, 3H), 1.89 (m, 2H), 4.46 (s, 4H), 5.32 (s, 2H), 5.41 (s, 2H), 6.50 (bs, 1H), 7.27 (s, 1H), 7.62 (s, 1H), 8.35 (s, 1H). Anal. ($C_{23}H_{18}N_2O_8 \cdot 1H_2O$) C, H, N.

7-Ethyl Carboxylate 10,11-(Ethylenedioxy)-20(*R,S*)-camptothecin (35). Compound **34** (45 mg, 0.10 mmol) was slurried in ethanol (absolute, 3 mL), and boron trifluoride etherate (1 drop) was added under N_2 at room temperature. The reaction mixture was warmed to reflux for 41 h, at which point the reaction mixture was completely in solution. Upon cooling to room temperature, diethyl ether (5 mL) was added to precipitate a tan solid that was washed with Et_2O (3 × 5 mL) and dried under high vacuum (41 mg, 86%). ^{1}H NMR: δ 0.84 (t, 3H), 1.41 (t, 3H), 1.88 (m, 2H), 4.42 (s, 4H), 4.52 (m, 2H), 5.37 (s, 2H), 5.42 (s, 2H), 6.54 (bs, 1H), 7.22 (s, 1H), 7.61 (s, 1H), 8.20 (s, 1H). Anal. ($C_{25}H_{22}N_2O_8 \cdot 2H_2O$) C, H, N.

7-Carboxylic Acid 10-Fluoro-20(*R,S*)-camptothecin (36). ^{1}H NMR: δ 0.83 (t, 3H), 1.85 (m, 2H), 5.39 (s, 2H), 5.42 (s, 2H), 6.53 (bs, 1H), 7.32 (s, 1H), 7.88 (td, 1H), 8.30 (dd, 1H), 8.64 (dd, 1H). Anal. ($C_{21}H_{15}FN_2O_6 \cdot 2H_2O$) C, H, N. **20*S*-Isomer 37.** Anal. ($C_{21}H_{15}FN_2O_6 \cdot 1H_2O$) C, H, N. **20*R*-Isomer 38.** Anal. ($C_{21}H_{15}FN_2O_6 \cdot 2H_2O$) C, H, N.

7-Carboxylic Acid 10-Iodo-20(*R,S*)-camptothecin (39). ^{1}H NMR: δ 0.90 (t, 3H), 1.89 (m, 2H), 5.37 (s, 2H), 5.41 (s, 2H), 6.56 (bs, 1H), 7.33 (s, 1H), 7.95 (d, 1H), 8.14 (d, 1H), 9.30 (s, 1H). Anal. ($C_{21}H_{15}N_2O_6I \cdot 0.5H_2O$) C, H, N.

7-Carboxylic Acid 9-Iodo-20(*R,S*)-camptothecin (40). ^{1}H NMR: δ 0.83 (t, 3H), 1.87 (m, 2H), 5.40 (s, 2H), 5.44 (s, 2H), 6.58 (bs, 1H), 7.38 (s, 1H), 8.04 (d, 1H), 8.62 (m, 2H). Anal. ($C_{21}H_{15}N_2O_6I \cdot 0.25H_2O$) C, H, N.

7-Carboxylic Acid 10-Methyl-20(*S*)-camptothecin (41). ^{1}H NMR: δ 0.84 (t, 3H), 1.85 (m, 2H), 2.60 (s, 3H), 5.38 (s, 2H), 5.43 (s, 2H), 6.57 (bs, 1H), 7.31 (s, 1H), 7.75 (d, 1H), 8.13 (d, 1H), 8.61 (s, 1H). Anal. ($C_{22}H_{18}N_2O_6 \cdot 2H_2O$) C, H, N.

7-Carboxylic Acid 10,11-Difluoro-20(*R,S*)-camptothecin (42). ^{1}H NMR: δ 0.86 (t, 3H), 1.82 (m, 2H), 5.42 (s, 2H), 5.44 (s, 2H), 6.56 (bs, 1H), 7.34 (s, 1H), 8.32 (dd, 1H), 8.90 (dd, 1H). Anal. ($C_{21}H_{14}F_2N_2O_6 \cdot 1H_2O$) C, H, N.

Synthesis of Related Derivatives. 9-Iodo-20(*R,S*)-camptothecin (41). This compound was isolated by prolonged heating of compound **40**: mp 272 °C dec. ^{1}H NMR: δ 0.84 (t, 3H), 1.87 (m, 2H), 5.33 (s, 2H), 5.45 (s, 2H), 6.58 (bs, 1H), 7.38 (s, 1H), 7.62 (dd, 1H), 8.21 (d, 1H), 8.31 (d, 1H), 8.76 (s, 1H). FAB MS 475 (MH^+). Anal. ($C_{20}H_{15}IN_2O_4$) C, H, N.

Compound 44. Tricyclic ketone **9** (87 mg, 0.33 mmol), 2-nitrobenzaldehyde (50 mg, 0.33 mmol), and acetic acid (0.75 mL) were combined at room temperature. Hydrochloric acid (concentrated, 0.75 mL) was added, and the reaction mixture was placed in an oil bath set to 105 °C for 4 h. The reaction mixture was then cooled to less than 60 °C, and water (4 mL) was added which precipitated a green-yellow solid. This solid

was washed with EtOAc (3 mL) and Et_2O (5 mL) and dried under high vacuum (120 mg, 92%): mp 231 °C. ^1H NMR: δ 0.84 (t, 3H), 1.82 (m, 2H), 5.02 (s, 2H), 5.41 (q, 2H), 7.04 (s, 1H), 7.78 (m, 2H), 7.80 (s, 1H), 7.82 (m, 1H), 7.93 (m, 1H), 8.21 (d, 1H). Electrospray MS: 397 (MH^+). Anal. ($C_{20}H_{15}N_2O_7$) C, H, N.

Compound 45. Tricyclic ketone **9** (100 mg, 0.38 mmol), 2-(trifluoromethyl)aniline (61 mg, 0.38 mmol), and toluene (1.0 mL) were combined under N_2 and placed in a preheated oil bath adjusted to 65 °C. Camphorsulfonic acid (catalytic) was added, and the reaction mixture was warmed to reflux for 15 h. The reaction mixture was cooled to room temperature, and the light pink solid was collected by filtration. The solid was washed with Et_2O (3 × 5 mL) and dried under vacuum (94 mg, 61%): mp 179 °C dec. ^1H NMR: δ 0.82 (t, 3H), 1.79 (m, 2H), 2.88 (t, 2H), 4.13 (t, 2H), 5.40 (s, 2H), 6.48 (bs, 1H), 6.80 (d, 1H), 6.83 (s, 1H); 7.17 (m, 2H), 7.24 (d, 1H). Electrospray MS: 407 (MH^+). Anal. ($C_{20}H_{17}F_3N_2O_4$) C, H, N.

Gel-Based Cleavable Complex Assay for Topoisomerase I Inhibition. The ability of camptothecin analogs to inhibit topoisomerase I was quantified in the cleavable complex assay as previously described.[2,23] Topoisomerase I was isolated from calf thymus to a high degree of purity and was devoid of topoisomerase II.[24] All reactions were carried out in 10 μL volumes of reaction buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EDTA, 30 mg/mL BSA) in microtiter plates. The camptothecin analogs were dissolved in DMSO at 10 mg/mL and serially diluted in 96-well microtiter plates to which the ^{32}P-end-labeled pBR322 DNA and topoisomerase enzyme were added. The reaction mixture was incubated at room temperature for 30 min and then the reaction stopped by adding 2 μL of a mixture of sodium dodecyl sulfate and proteinase K (Boehringer Mannheim, Indianapolis, IN) (1.6% and 0.14 mg/mL final concentrations, respectively). The plates were heated at 50 °C for 30 min, 10 μL of standard stop mixture containing 0.45 N NaOH was added in order to generate single-stranded DNA, and the samples were electrophoresed in 1.5% agarose gels in TBE buffer. Gels were blotted on nitrocellulose paper (BioRad, Richmond, CA) dried, and exposed to X-ray film. The units of cleavage were calculated from the autoradiographs and plotted against the log drug concentration using the Nonlin84 software package from SCI Software (Lexington, KY). The IC_{50}'s for each drug were determined as an average of multiple runs.

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