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# Dibenzo[*c*,*h*][1,5]naphthyridinediones as Topoisomerase I Inhibitors: Design, Synthesis, and Biological Evaluation

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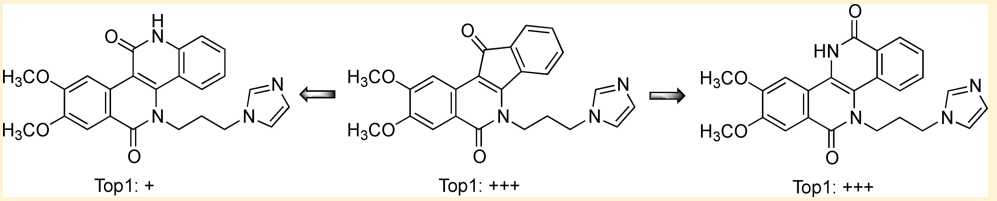
Supporting Information




**ABSTRACT:** Dibenzo[*c*,*h*][1,5]naphthyridinediones were prepared via a novel synthetic pathway. The compounds were designed as topoisomerase I (Top1) inhibitors based on the indenoisoquinoline series of drugs. The results of biological evaluation demonstrate that, unlike very closely related dibenzo[*c*,*h*][1,6]naphthyridinediones, dibenzo[*c*,*h*][1,5]-naphthyridinediones retain the Top1 inhibitory activity of similarly substituted indenoisoquinolines.

Topoisomerase type I (Top1) has been recognized as an important target for cancer chemotherapy since the discovery of the plant alkaloid camptothecin (**1**) and its synthetic analogues.[1,2] Depite the camptothecins being the only chemical class of Top1 inhibitors approved for clinical use, their application and efficiency is hampered by a number of factors such as instability of the lactone at physiological pH and quick reversibility of ternary complexes formed by these drugs.[3] To overcome these limitations, a number of noncamptothecin Top1 inhibitor classes have emerged, including indenoisoquinolines such as **2**, LMP776 (**3a**, indimitecan), LMP400 (**3b**, indotecan), and MJ-III-65 (**3c**) (Figure 1).[4,5] Indenoisoquinolines offer both greater chemical stability and slower reversibility of the ternary complexes.[6] As a result, two members of the indenoisoquinoline class of Top1 inhibitors, **3a** and **3b**, are currently undergoing phase 1 clinical trials.[7]

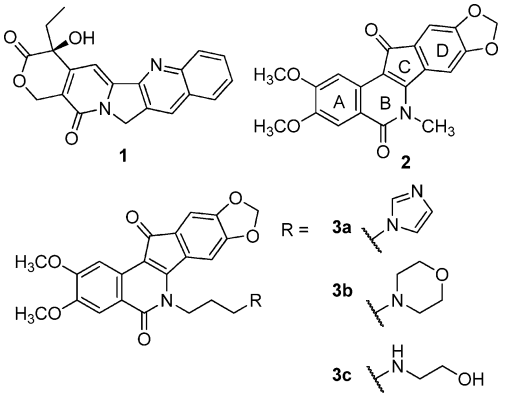



**Figure 1.** Representative Top1 inhibitors.

In an effort to expand structure−activity relationship knowledge of indenoisoquinolines as Top1 inhibitors, dibenzo-[*c*,*h*][1,6]naphthyridinediones such as **4** and **5** were designed and synthesized (Figure 2).[8] Compounds **4** and **5** possess a six-membered lactam fragment in place of the five-membered C-ring of the indenoisoquinolines (Figures 1 and 2). A number of closely related, fused tetracyclic systems have previously been described. This list includes molecules such as nitidine chloride (**6**),[9,10] topovale (**7**),[11] benzo[*c*]phenanthrolinone **8**,[12] and dibenzo[*c*,*h*][1,5]naphthyridine **9**.[13] Molecules **6**−**9** and their derivatives were found to be capable of binding to the Top1−DNA cleavage complex (Top1−DNAcc) or intercalating into the DNA double helix, and their development as anticancer agents and DNA probes have therefore been pursued.[13−16]

Unfortunately, the expansion of the five-membered indenoisoquinoline C-ring into the six-membered lactam ring of dibenzo[*c*,*h*][1,6]naphthyridinediones **4** and **5** adversely affected their Top1 inhibitory and antiproliferative activities.[8] Docking studies have indicated that compounds **4** and **5** bind to the Top1−DNAcc by placing the dimethoxyisoquinolinone fragment into the spatially restricted part of the binding pocket toward the intact DNA strand (Figure 3, left).[8] Therefore, the replacement of the quinolinone moiety of **4** and **5** with the isoquinolinone of dibenzo[*c*,*h*][1,5]naphthyridinediones **20** and **21** was considered (Scheme 1). Modeling of **20** into the Top1−DNAcc suggested that the dimethoxyisoquinolinone

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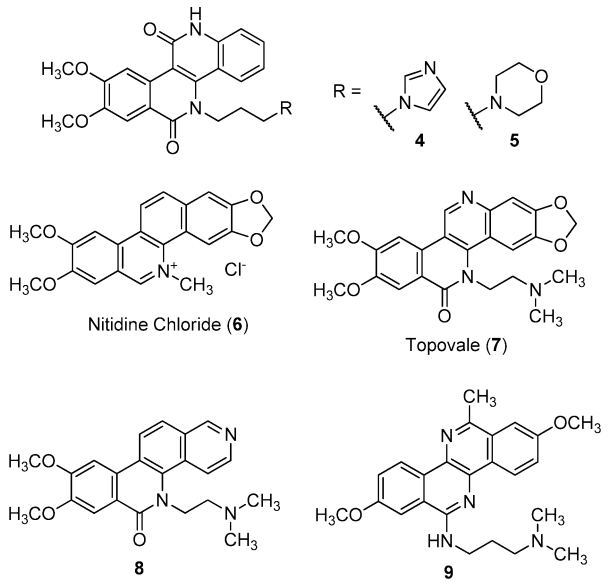


**Figure 2.** Dibenzonaphthyridines and structurally related compounds.

fragment could be placed within the Top1−DNAcc on the more spacious side where the cut DNA strand is located. At the same time the unsubstituted isoquinoline fragment would be facing the intact strand (Figure 3, right). Additionally, the lactam aminoalkyl side chain of **4** was calculated to face the minor groove of the DNA potentially creating unfavorable steric interactions, whereas in the case of **20**, the hypothetical binding mode suggested that the same group would be placed in the less restrictive major groove. The evaluation of the dibenzo[*c*,*h*][1,5]naphthyridinedione series would conclude the study of the effects that size, geometry, and electronic properties of the C-ring have on Top1 inhibitory activity.

At the present time, only one protocol has been reported for the preparation of dibenzo[*c*,*h*][1,5]naphthyridinediones.[17] Here an alternative, efficient synthesis of dibenzo[*c*,*h*][1,5]-naphthyridinediones is reported in which one of the isoquinolinone nitrogens is substituted with an aminopropyl side chain.

The condensation of **10**,[18] obtained from commercially available 2-carboxybenzaldehyde, with 3-chloropropylamine was followed by the reaction of the resulting Schiff base **11** with 4,5-dimethoxyhomophthalic anhydride (**12**)[19] to produce the *cis*-isoquinolonic acid **13**. The cis configuration of **13** was established based on $^1$H NMR analysis. Protons H-3 and H-4 appear as doublets with the coupling constant of 6.9 Hz. For the trans configuration, two singlets would be expected for H-3 and H-4. This type of stereochemical assignment for the Schiff base/homophthalic anhydride condensation products has been previously established through total synthesis of several natural products[20−22] and recently confirmed by crystallographic studies.[23,24] Thermal decarboxylation of **13** led to dihydroisoquinolone **14**. The removal of the carboxy group eliminated the possibility for cis/trans isomerization and associated difficulties encountered during synthesis of dibenzo[*c*,*h*][1,6]-naphthyridinediones[8] and indenoisoquinolines.[25] Oxidation of **14** with DDQ led to the dehydrogenated isoquinolone **15**. Further mild nitration of position 3 of **15** yielded **16**. The nitrogen of the introduced nitro group was intended to be converted to the lactam nitrogen of the final products.

During the synthesis of **4** and **5**, it was noticed that the products with an assembled dibenzonaphthyridinedione polycyclic core were poorly soluble in most organic solvents, making their further derivatization and purification difficult.[8] It was therefore advantageous to introduce the desired amine functionality at the end of the propyl chain at an earlier stage of the synthesis. The Finkelstein reaction of **16** with potassium iodide in acetonitrile provided compound **17**, which was further converted to amines **18** and **19** by reaction with imidazole and morpholine, respectively. The two-step conversion of **16** to **18** and **19** via intermediate iodide **17** provided greater yields of the final products in comparison to the more direct, one-step transformation. Reduction of the nitro group was accomplished

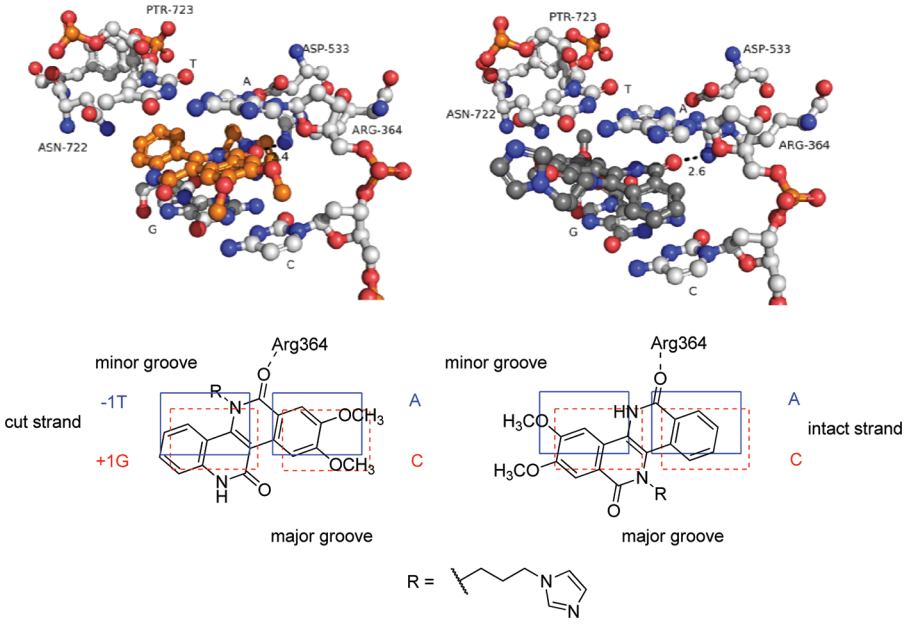


**Figure 3.** Hypothetical binding modes of dibenzo[*c*,*h*][1,6]naphthyridinedione **4** (left) and dibenzo[*c*,*h*][1,5]naphthyridinedione **20** (right) to Top1−DNAcc.


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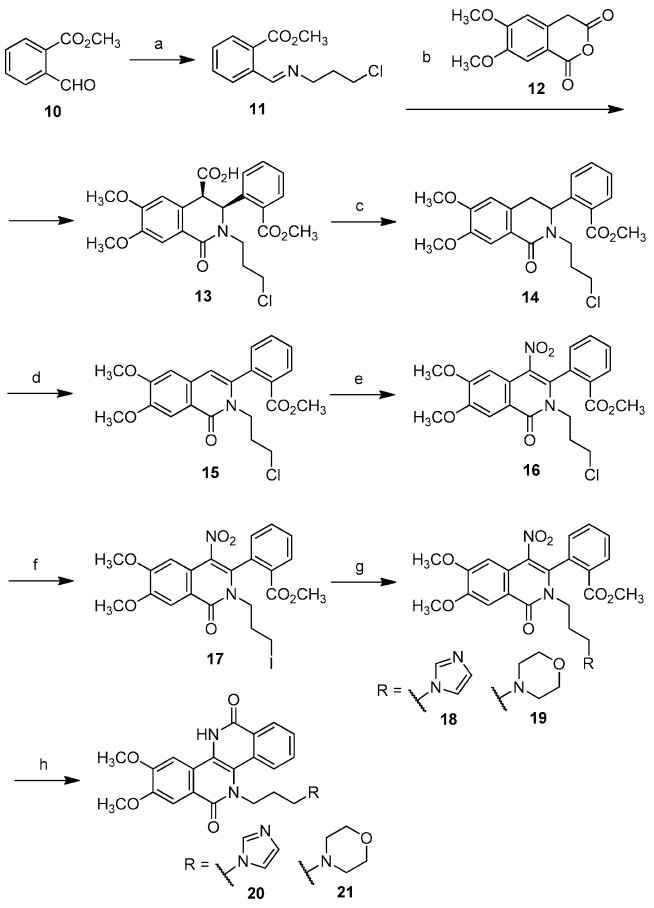
**Scheme 1**[a]




[a]Reagents and conditions: (a) 3-chloropropylamine hydrochloride, $Et_3N$, $MgSO_4$, $CHCl_3$, 23 °C, 2 h (99%); (b) $CHCl_3$, 23 °C, 3 h (82%); (c) *N*-methyl-2-pyrrolidone, 170−190 °C, 30−45 min (54%); (d) DDQ, 1,4-dioxane, reflux, 7 h (70%); (e) $HNO_3$, acetic acid, ethyl acetate, 10−23 °C, 2 h (72%); (f) sodium iodide, acetonitrile, reflux, 24 h (98%); (g) imidazole (or morpholine), $K_2CO_3$, 1,4-dioxane, reflux, 12 h (**18**, 97%; **19**, quant.); (h) $NaHSO_3$, 1,4-dioxane, water, reflux, 48 h [**20**, 64% (14% from **10**); **21**, 51% (12% from **10**)].

with sodium bisulfite in water/dioxane solution. This reduction method allowed for easy removal of inorganic salts and uncyclized byproducts by washing the precipitate with water and dioxane and leaving the less soluble dibenzo[*c*,*h*][1,5]-naphthyridinediones **20** and **21**. The total yields of **20** and **21** over 8 steps from **10** were 14% and 12%, respectively.

The Top1 inhibitory activities of the final compounds were tested by incubating a $^{32}P$ 3′-end-labeled 117-bp DNA fragment with human recombinant Top1 and increasing concentrations of **20** and **21**.[26] After the separation of the DNA fragments on a denaturing gel and visual inspection of the number and intensities of the DNA cleavage bands, the Top1 inhibitory activity was assigned on a semiquantitative scale relative to the Top1 inhibitory activities of compounds **1** and **2**: 0, no detectable activity; +, weak activity; ++, similar activity to compound **2**; +++, greater activity than **2**; ++++, equipotent to **1** (Figure 4).

The antiproliferative activities of compounds **20** and **21** against approximately 60 different human cancer cell lines were determined in the National Cancer Institute (NCI) screen. The concentrations of the test compounds that cause 50% cell growth inhibition ($GI_{50}$) were determined by incubating cells

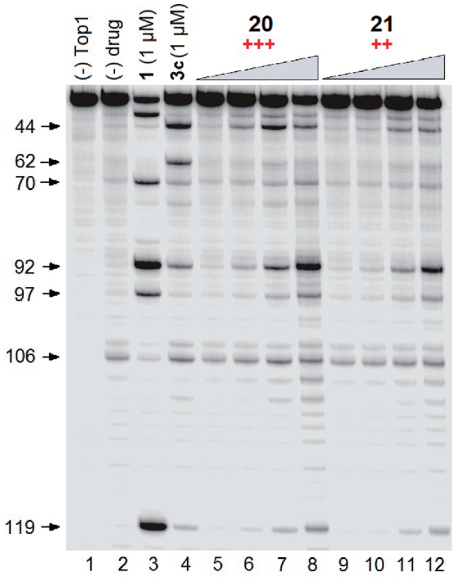



**Figure 4.** Lane 1: DNA alone. Lane 2: Top1 alone. Lane 3: Top1 + **1** (1 μM). Lane 4: Top1 + **3c** (1 μM). Lanes 5−8: Top1 + **20** at 0.1, 1, 10, and 100 μM. Lanes 9−12: Top1 + **21** at 0.1, 1, 10, and 100 μM. Numbers on left and arrows show the cleavage site positions. Top1-mediated DNA cleavage activities are expressed semiquantitatively as follows: 0, no detectable activity; +, weak activity; ++, similar activity as compound **2**; +++, greater activity than compound **2**; ++++, similar activity as 1 μM **1**.

with five 10-fold dilutions of the test compounds down to 10 nM for 48 h, followed by calorimetric quantification of viable cells with sulforhodamine B dye. Unfortunately, compound **21** did not express a sufficient level of toxicity in the preliminary one-concentration test at 10 μM for it to be promoted to five-concentration testing for the $GI_{50}$. Compound **20**, which demonstrated greater Top1 inhibitory activity, was also found to be cytotoxic with a $GI_{50}$ mean-graph midpoint (MGM) of 3.3 μM. Cell lines such as MCF7 and HCT-116, which express particularly high levels of Top1 and Top1 mRNA,[27] were found to be especially sensitive to treatment with **20** with $GI_{50}$ values of 0.54 and 1.8 μM, respectively. Despite the similar of the Top1 inhibitory activity, the MGM value for **20** was determined to be 2 orders of magnitude higher than that of **22** (Figure 5),[28] limiting the prospective use of **20** as an antiproliferative agent.

In conclusion, dibenzo[*c*,*h*][1,5]naphthyridinediones were designed as potential Top1 inhibitors based on the molecular modeling of previously published closely related isomeric dibenzo[*c*,*h*][1,6]naphthyridinediones **4** and **5** as well as indenoisoquinolines **2**, **3** and **22**.[6,8,28] The target compounds were prepared via a novel and efficient synthetic protocol. The Top1 inhibitory and antiproliferative activities of the prepared compounds were evaluated. The imidazolylpropyl analog **20** was found to be cytotoxic with a low-micromolar MGM value whereas morpholinopropyl compound **21** was not cytotoxic enough to warrant an accurate MGM determination. The Top1 activity of the imidazolylpropyl analogues of dibenzonaphthyridinediones rose from + to +++ in the transition from **4** to **20**, matching that of similarly substituted indenoisoquinoline **22**.[28]

## EXPERIMENTAL SECTION

**General Methods.** Melting points were determined using capillary tubes and are uncorrected. The nuclear magnetic resonance ($^1H$ and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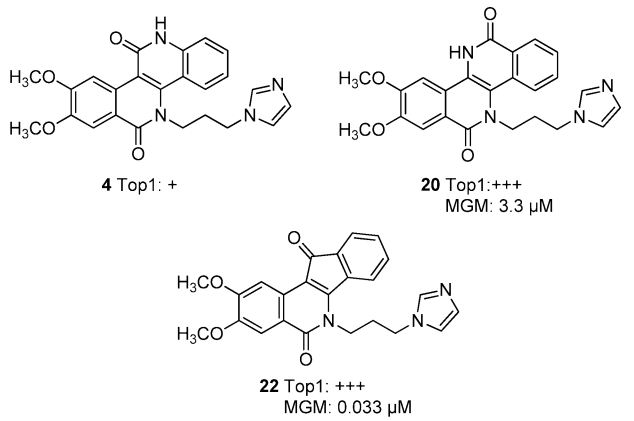


**Figure 5.** Relative Top1 inhibitory activities of compounds are presented as follows: 0, no detectable activity; +, weak activity; ++, similar activity as compound **2**; +++, greater activity than compound **2**; ++++, similar activity as 1 $\mu$M **1**.

$^{13}$C NMR) spectra were recorded using 300 or 500 MHz spectrometers. IR spectra were recorded using an FTIR spectrometer. High-resolution mass spectra were recorded on double-focusing sector mass-spectrometer with magnetic and electrostatic mass analyzers. Purity of all tested compounds was ≥95%, as estimated by HPLC analysis. The major peak of the compounds analyzed by HPLC accounted for ≥95% of the combined total peak area when monitored by a UV detector at 254 nm. Analytical thin-layer chromatography was carried out, and compounds were visualized with UV light at 254 nm. Silica gel flash chromatography was performed using 230−400 mesh silica gel.

**Methyl 2-Formylbenzoate (10).**[18] A solution of 2-formylbenzoic acid (5.0 g, 33 mmol), iodomethane (9.8 g, 69 mmol), and potassium carbonate (2.5 g, 18 mmol) in dry DMF (11 mL) was heated at reflux for 2 h. After the mixture was cooled to room temperature, water (100 mL) was added, and product was extracted with chloroform (3 × 20 mL). The combined extracts were washed with concentrated sodium bicarbonate solution (15 mL), water, (2 × 15 mL), and brine (20 mL), dried with sodium sulfate, and evaporated to dryness providing pure ester **10** as a colorless oil (5 g, 92%): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 10.55 (s, 1 H), 7.92−7.85 (m, 2 H), 7.62−7.58 (m, 2 H), 3.02 (s, 3 H). The $^1$H NMR spectrum is consistent with previously published data.[18]

**Methyl 2-[(3-Chloropropylimino)methyl]benzoate (11).** A mixture of methyl 2-formylbenzoate (**10**, 3.3 g, 20 mmol), 3-chloropropylamine hydrochloride (3.0 g, 23 mmol), triethylamine (3.2 mL) and magnesium sulfate (7 g) in chloroform (40 mL) was stirred at room temperature for 2 h. The precipitate was filtered off and washed with chloroform (3 × 20 mL). Combined filtrates were washed with water (4 × 30 mL) and brine (30 mL), dried with sodium sulfate, and evaporated to dryness to obtain **11** as a yellow oil (4.7 g, 99%): IR (film) 1720, 1639, 1434, 1292, 1263 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.90 (s, 1 H), 7.90 (dd, $J$ = 7.7, 1.4 Hz, 1 H), 7.85 (dd, $J$ = 7.7, 1.4 Hz, 1 H), 7.49 (td, $J$ = 7.5, 1.4 Hz, 1 H), 7.39 (td, $J$ = 7.6, 1.4 Hz, 1 H), 3.85 (s, 3 H), 3.73 (td, $J$ = 6.3, 1.4 Hz, 2 H), 3.60 (t, $J$ = 6.4 Hz, 2 H), 2.13 (p, $J$ = 6.4 Hz, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.2, 161.44, 161.40, 137.1, 132.2, 130.1, 130.0, 129.8, 128.3, 57.8, 52.3, 42.7, 33.3; positive ESIMS $m/z$ (rel intensity) 240/242 ($MH^+$, 100/28), 204 (42); HRMS−ESI $m/z$ $MH^+$, calcd for $C_{12}H_{14}ClNO_2$ 240.0791, found 240.0789.

***cis*-4-Carboxy-2-(3-chloropropyl)-6,7-dimethoxy-3-(2-(methoxycarbonyl)phenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline (13).** 4,5-Dimethoxyhomophthalic anhydride (**12**, 2.8 g, 12 mmol) was slowly added to a solution of imine **11** (2.9 g, 12 mmol) in chloroform (50 mL). The resulting mixture was stirred at room temperature for 3 h. The precipitate was collected by filtration, washed with chloroform, and dried to afford **13** as a white solid (4.5 g, 82%): mp 214−215 °C dec; IR (KBr) 1739, 1710, 1622, 1597, 1577, 1494, 1297, 1277, 1232, 1185, 1171, 1107 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.72 (dd, $J$ = 5.9, 3.3 Hz, 1 H), 7.55 (s, 1 H), 7.42−7.31 (m, 2 H), 7.11−7.00 (m, 1 H), 6.94 (s, 1 H), 6.17 (d, $J$ = 6.9 Hz, 1 H), 4.74 (d, $J$ = 6.9 Hz, 1 H), 3.97−3.86 (m, 1 H), 3.85 (s, 4 H), 3.83 (s, 3 H), 3.73 (s, 3 H), 3.61 (td, $J$ = 6.5, 2.2 Hz, 2 H), 2.97−2.90 (m, 1 H), 2.04−1.90 (m, 2 H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 170.7, 167.7, 162.9, 151.5, 147.7, 138.1, 131.8, 131.1, 129.7, 128.1, 127.8, 127.0, 121.3, 110.4, 109.9, 55.5, 54.9, 52.4, 47.3, 43.3, 43.1, 40.3, 30.6; positive ESIMS $m/z$ (rel intensity) 462/464 ($MH^+$, 100/36); HRMS−ESI $m/z$ $MH^+$, calcd for $C_{23}H_{24}ClNO_7$ 462.1320, found 462.1325.

**2-(3′-Chloropropyl)-6,7-dimethoxy-3-(2′-methoxycarbonylphenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline (14).** A mixture of acid **13** (12.3 g, 26.6 mmol) and degassed 1-methyl-2-pyrrolidinone (70 mL) was heated up to 170−190 °C for 30−45 min. The reaction mixture was quenched by addition of water (300 mL). The water layer was extracted with chloroform (3 × 50 mL). The combined extracts were washed with water (2 × 30 mL), concentrated sodium bicarbonate solution (30 mL), water (30 mL), and brine (40 mL), dried with sodium sulfate, and evaporated to dryness. Separation by means of column chromatography (silica gel), eluting with ethyl acetate−hexanes (1:1), provided pure **14** (6.1 g, 54%): mp 136−138 °C dec; IR (KBr) 1717, 1645, 1602, 1513, 1463, 1432, 1356, 1281, 1244, 1213, 1185, 1132, 1106, 1078, 1031 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.93 (dd, $J$ = 7.2, 2.1 Hz, 1 H), 7.57 (s, 1 H), 7.31−7.16 (m, 3 H), 7.02−6.88 (m, 1 H), 6.33 (s, 1 H), 5.82 (d, $J$ = 7.4 Hz, 1 H), 4.18−4.01 (m, 1 H), 3.89 (s, 2 H), 3.86 (s, 3 H), 3.72 (s, 4 H), 3.64 (dd, $J$ = 16.2, 7.6 Hz, 1 H), 3.59−3.52 (m, 2 H), 2.91 (d, $J$ = 16.5 Hz, 1 H), 2.83−2.74 (m, 1 H), 2.11−2.00 (m, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 167.2, 165.4, 152.0, 147.9, 142.3, 132.3, 131.5, 128.6, 128.0, 127.4, 126.9, 121.4, 110.0, 109.8, 56.7, 55.9, 55.8, 52.2, 44.7, 42.5, 34.6, 31.3; positive ESIMS $m/z$ (rel intensity) 418/420 ($MH^+$, 36/11), 382 (100); HRMS−ESI $m/z$ $MH^+$, calcd for $C_{22}H_{24}ClNO_5$ 418.1421, found 418.1425.

**2-(3′-Chloropropyl)-6,7-dimethoxy-3-(2′-methoxycarbonylphenyl)-1-oxo-1,2-dihydroisoquinoline (15).** A mixture of **14** (2.0 g, 4.8 mmol) and DDQ (2.2 g, 4.8 mmol) in 1,4-dioxane (100 mL) was heated to reflux for 7 h. After completion of the reaction, the solvent was evaporated under reduced pressure, and the residue was suspended in chloroform (200 mL). The organic layer was washed with dilute sodium bicarbonate solution (2 × 100 mL), water (2 × 100 mL), and brine (100 mL), dried with sodium sulfate, and evaporated to dryness. Products were subjected to flash column chromatography (silica gel), eluting with ethyl acetate/hexanes (1:1), to yield **15** as glassy amorphous solid (1.4 g, 70%): IR (film) 1724, 1646, 1601, 1507, 1440, 1406, 1259, 1234, 1172, 1135, 1117, 1083, 1043 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.01 (dd, $J$ = 7.7, 1.5 Hz, 1 H), 7.75 (s, 1 H), 7.59−7.46 (m, 2 H), 7.35 (dd, $J$ = 7.4, 1.5 Hz, 1 H), 6.73 (s, 1 H), 6.15 (s, 1 H), 4.13 (ddd, $J$ = 14.5, 7.3, 3.8 Hz, 1 H), 3.93 (s, 3 H), 3.86 (s, 3 H), 3.64−3.55 (m, 4 H), 3.35 (t, $J$ = 6.3 Hz, 2 H), 2.19−1.77 (m, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 166.3, 161.8, 153.4, 149.1, 141.3, 136.1, 132.2, 131.9, 131.2, 130.6, 130.3, 129.3, 118.9, 107.6, 105.9, 105.8, 56.1, 56.0, 52.3, 43.9, 42.5, 31.2; positive ESIMS $m/z$ (rel intensity) 416/418 ($MH^+$, 100/36); HRMS−ESI $m/z$ $MH^+$, calcd for $C_{22}H_{22}ClNO_5$ 416.1265, found 416.1263.

**2-(3′-Chloropropyl)-6,7-dimethoxy-3-(2′-methoxycarbonylphenyl)-4-nitro-1-oxo-1,2-dihydroisoquinoline (16).** Chloride **15** (470 mg, 11 mmol) was dissolved in a mixture of acetic acid (5 mL) and ethyl acetate (1 mL), and the solution of concentrated nitric acid (70%, 1.5 mL, 24 mmol) was added dropwise to the resulting mixture at 10 °C. The mixture was stirred for 2 h while being allowed to warm to room temperature. The resulting solution was diluted with water (50 mL), and the products were extracted with ethyl acetate (3 × 15 mL). The combined extracts were washed with diluted sodium bicarbonate solution (2 × 10 mL), water (2 × 10 mL), and brine (10 mL), dried with sodium sulfate, and evaporated to dryness. The residue was recrystallized from ethanol to obtain pure **16** as yellow solid (377 mg, 72%): mp 172−174 °C; IR (film) 1724, 1652, 1602, 1514 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.41 (dd, $J$ = 7.7, 1.5 Hz, 1 H), 8.12−7.91 (m, 4 H), 4.29 (ddd, $J$ = 13.5, 10.2, 5.3 Hz, 1 H), 4.21 (s, 3 H), 4.15 (s, 3 H), 4.01 (s, 3 H), 3.78−3.68 (m, 3 H), 2.39−


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1.98 (m, 2 H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 165.3, 159.6, 154.1, 149.8, 140.7, 133.3, 131.0, 130.8, 130.6, 130.4, 130.0, 123.6, 117.3, 107.7, 102.0, 56.0, 55.8, 52.6, 44.4, 42.7, 34.6, 30.2; positive ESIMS $m/z$ (rel intensity) 461/463 ($MH^+$, 12/4), 483/485 [$(M + Na^+)$, 17/6]; HRMS−ESI $m/z$ ($M + Na^+$), calcd for $C_{22}H_{21}ClN_2O_7Na$ 483.0935, found 483.0941.

**6,7-Dimethoxy-3-(2′-methoxycarbonylphenyl)-2-(3′-iodopropyl)-4-nitro-1-oxo-1,2-dihydroisoquinoline (17).** A mixture of **16** (2.45 g, 34.9 mmol), sodium iodide (7 g, 47 mmol), and acetonitrile (30 mL) was heated at reflux for 24 h. After the mixture was cooled to room temperature, the solvent was evaporated under reduced pressure, and the residue was subjected to flash column chromatography (silica gel), eluting with chloroform, to provide **17** as yellow solid (2.9 g, 98%): mp 199−201 °C; IR (film) 1723, 1651, 1602, 1514 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.21 (dd, $J$ = 7.5, 1.8 Hz, 1 H), 7.85 (s, 1 H), 7.79−7.56 (m, 2 H), 7.37 (dd, $J$ = 7.4, 1.8 Hz, 1 H), 7.01 (s, 1 H), 4.13−3.99 (m, 4 H), 3.96 (s, 3 H), 3.82 (s, 3 H), 3.64−3.48 (m, 1 H), 3.12−2.86 (m, 2 H), 2.30−1.78 (m, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 165. 8, 160.8, 154.6, 150.4, 140.4, 133.2, 131.4, 131.0, 130.9, 130.2, 124.4, 118.4, 108.31, 108.27, 102.4, 56.5, 52.8, 48.2, 31.4, 2.0; positive ESIMS $m/z$ (rel intensity) 553 ($MH^+$, 18), 575 [$(M + Na^+)$, 6]; HRMS−ESI $m/z$ ($M + Na^+$), calcd for $C_{22}H_{21}IN_2O_7Na$ 575.0291, found 575.0284.

**2-[3-(1*H*-Imidazol-1-yl)propyl]-6,7-dimethoxy-3-(2′-methoxycarbonylphenyl)-4-nitro-1-oxo-1,2-dihydroisoquinoline (18).** Iodide **17** (500 mg, 0.9 mmol), potassium carbonate (250 mg, 1.8 mol), and imidazole (615 mg, 9 mmol) were dissolved in 1,4-dioxane (50 mL), and the mixture was heated at reflux for 12 h. The solvent was removed under reduced pressure, and the residue was redissolved in chloroform (100 mL). The organic layer was washed water (4 × 100 mL) and brine (50 mL), dried with sodium sulfate, and evaporated to dryness. The residue was subjected to flash column chromatography (silica gel), eluting with 5% methanol in chloroform, to yield **18** as a yellow solid (414 mg, 97%): mp 204−206 °C; IR (film) 1724, 1652, 1602, 1515 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.14 (dd, $J$ = 5.7, 3.4 Hz, 1 H), 7.84 (s, 1 H), 7.64 (dd, $J$ = 5.6, 3.4 Hz, 2 H), 7.49−7.28 (m, 2 H), 6.99 (s, 2 H), 6.94 (s, 1 H), 6.68 (s, 1 H), 4.05 (d, $J$ = 3.0 Hz, 4 H), 4.02−3.86 (m, 9 H), 3.81 (s, 3 H), 3.63−3.46 (m, 1 H), 2.24−1.77 (m, 3 H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 165.6, 160.6, 154.5, 150.3, 139.6, 132.7, 131.0, 130.9, 130.6, 129.9, 128.7, 124.2, 118.3, 118.1, 108.0, 102.1, 56.3, 56.3, 44.7, 44.1, 29.4; positive ESIMS $m/z$ (rel intensity) 493 ($MH^+$, 100); HRMS−ESI $m/z$ $MH^+$, calcd for $C_{25}H_{24}N_4O_7$ 493.1723, found 493.1719.

**6,7-Dimethoxy-3-(2′-methoxycarbonylphenyl)-2-(3-morpholinopropyl)-4-nitro-1-oxo-1,2-dihydroisoquinoline (19).** Iodide **17** (500 mg, 0.9 mmol), potassium carbonate (250 mg, 1.8 mol), and morpholine (790 mg, 9 mmol) were dissolved in 1,4-dioxane (50 mL), and the mixture was heated at reflux for 12 h. The solvent was removed under reduced pressure, and the residue was redissolved in chloroform (100 mL). The organic layer was washed water (4 × 100 mL) and brine (50 mL), dried with sodium sulfate, and evaporated to dryness. The residue was subjected to flash column chromatography (silica gel), eluting with 5% methanol in chloroform, to yield **19** as yellow solid (458 mg, 100%): mp 208−210 °C; IR (film) 1726, 1652, 1602, 1514 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.19 (dd, $J$ = 6.7, 2.4 Hz, 1 H), 7.84 (s, 1 H), 7.74−7.56 (m, 2 H), 7.39 (dd, $J$ = 7.2, 1.8 Hz, 1 H), 7.02 (s, 1 H), 4.18−4.05 (m, 1 H), 4.02 (s, 3 H), 3.95 (s, 3 H), 3.52 (s, 1 H), 3.48−3.35 (m, 1 H), 2.23−2.14 (m, 4 H), 1.90−1.52 (m, 2 H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 165.6, 160.7, 154.5, 150.3, 140.5, 132.9, 131.8, 131.1, 130.9, 130.6, 130.3, 124.4, 118.5, 108.2, 102.3, 67.2, 66.9, 56.5, 56.0, 53.3, 52.8, 45.9, 24.8; positive ESIMS $m/z$ (rel intensity) 512 ($MH^+$, 100); HRMS−ESI $m/z$ $MH^+$, calcd for $C_{26}H_{29}N_3O_8$ 512.2033, found 512.2040.

**11-[3-(1*H*-Imidazol-1-yl)propyl]-2,3-dimethoxydibenzo[*c*,*h*][1,5]naphthyridine-6,12(5*H*,11*H*)-dione (20).** Sodium bisulfite (312 mg, 3 mmol) was added to a solution of **18** (150 mg, 0.30 mmol) in 1,4-dioxane (25 mL) and water (5 mL). The mixture was heated to reflux for 48 h. The precipitate was collected by filtration and washed with hot 1,4-dioxane (20 mL) and water (50 mL). The dried solid was subjected to flash column chromatography (silica gel), eluting with 15% methanol in chloroform, to afford **20** (82 mg, 64%): mp 277 °C; IR (film) 1724, 1646, 1601, 1507, 1440, 1406, 1259, 1234, 1172, 1135, 1117, 1083, 1043 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 11.75 (s, 1 H), 8.33 (dd, $J$ = 8.1, 1.3 Hz, 1 H), 8.05 (s, 1 H), 7.82 (d, $J$ = 8.1 Hz, 1 H), 7.69 (d, $J$ = 6.8 Hz, 2 H), 7.64−7.47 (m, 2 H), 7.15 (s, 1 H), 6.86 (s, 1 H), 4.26 (t, $J$ = 7.5 Hz, 2 H), 3.98 (s, 3 H), 3.96 (t, $J$ = 6.7 Hz, 2 H), 3.91 (s, 3 H), 2.31 (t, $J$ = 7.4 Hz, 2 H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 161.7, 160.4, 153.5, 150.1, 132.0, 130.7, 127.7, 127.1, 124.0, 122.7, 121.3, 119.7, 118.5, 108.3, 103.6, 56.6, 55.7, 47.7, 43.7, 29.6; positive ESIMS $m/z$ (rel intensity) 431 ($MH^+$, 100); HRMS−ESI $m/z$ $MH^+$, calcd for $C_{24}H_{22}N_4O_4$ 431.1719, found 431.1726; HPLC purity 98.46% (C-18 reversed phase, MeOH).

**2,3-Dimethoxy-11-(3-morpholinopropyl)dibenzo[*c*,*h*][1,5]-naphthyridine-6,12(5*H*,11*H*)-dione (21).** Sodium bisulfite (312 mg, 3 mmol) was added to a solution of **19** (150 mg, 0.29 mmol) in 1,4-dioxane (25 mL) and water (5 mL). The mixture was heated to reflux for 48 h. The precipitate was collected by filtration and washed with hot 1,4-dioxane (20 mL) and water (50 mL). The dried solid was subjected to flash column chromatography (silica gel), eluting with 15% methanol in chloroform, to afford **21** as a light-yellow solid (67 mg, 51%): mp 274 °C; IR (film) 1719, 1652, 1609 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 11.74 (s, 2 H), 8.35 (d, $J$ = 8.2 Hz, 3 H), 8.17−7.97 (m, 5 H), 7.82 (t, $J$ = 7.6 Hz, 2 H), 7.76−7.68 (m, 2 H), 7.68−7.56 (m, 3 H), 4.65−4.40 (m, 5 H), 3.98 (s, 2 H), 3.91 (s, 2 H), 3.22 (s, 8 H), 2.04 (s, 7 H), 1.94 (s, 5 H), 1.86 (s, 1 H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 161.7, 160.4, 153.5, 150.1, 132.2, 130.9, 127.6, 127.1, 126.0, 124.4, 122.7, 121.1, 119.7, 108.3, 103.6, 56.6, 55.7, 54.1, 47.5; positive ESIMS $m/z$ (rel intensity) 450 ($MH^+$, 100); HRMS−ESI $m/z$ $MH^+$, calcd for $C_{25}H_{27}N_3O_5$ 450.2029, found 450.2028; HPLC purity: 97.40% (C-18 reversed phase, MeOH).

**Topoisomerase I-Mediated DNA Cleavage Reactions.** Human recombinant Top1 was purified from baculovirus as previously described.[29] DNA cleavage reactions were prepared as previously reported with the exception of the DNA substrate.[26] Briefly, a 117-bp DNA oligonucleotide (Integrated DNA Technologies) encompassing the previously identified Top1 cleavage sites in the 161-bp fragment from pBluescript SK(−) phagemid DNA was employed. This 117-bp oligonucleotide contains a single 5′-cytosine overhang, which was 3′-end-labeled by fill-in reaction with [$\alpha$-$^{32}$P]-dGTP in React 2 buffer (50 mM Tris−HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM NaCl) with 0.5 units of DNA polymerase I (Klenow fragment, New England BioLabs). Unincorporated [$^{32}$P]-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled DNA substrate was collected. Approximately 2 nM of radiolabeled DNA substrate was incubated with recombinant Top1 in 20 $\mu$L of reaction buffer [10 mM Tris−HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 15 $\mu$g/mL BSA] at 25 °C for 20 min in the presence of various concentrations of compounds. The reactions were terminated by adding SDS (0.5% final concentration) followed by the addition of two volumes of loading dye (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromphenol blue). Aliquots of each reaction mixture were subjected to 20% denaturing PAGE. Gels were dried and visualized by using a phosphoimager and ImageQuant software (Molecular Dynamics). For simplicity, cleavage sites were numbered as previously described in the 161-bp fragment.[29]

**Molecular Modeling.** Sybyl 8.1[30] was used to prepare the structures of **4** and **20**. Geometry of the ligands was optimized by energy minimization using MMFF94s force field and MMFF94 charges. The structure of Top1−DNAcc was obtained from the Protein Data Bank (PDB ID: 1SC7). Hydrogen atoms were added to all atoms, and MMFF94 charges were assigned. The positions of hydrogen atoms were optimized with the MMFF94s force field. A 100 docking runs in place of the original ligand were performed for both **4** and **20** using the docking genetic algorithm and GoldScore fitness function within GOLD 3.2.[31] The highest ranked solutions were merged with the structure of the cleavage complex. The GOLD suggested positions of the naphthyridine ligands within newly obtained ternary complexes were refined through 100 iteration steps of geometry optimization with steepest descent minimization followed


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by 200 iterations with conjugate gradient using the MMFF94s force field and MMFF94 charges within Sybyl 8.1.

## ■ ASSOCIATED CONTENT

### Supporting Information

$^{1}H$ and $^{13}C$ NMR spectra for compounds **11** and **13**−**21** and HPLC charts for compounds **20** and **21**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

(1) Wall, M. E.; Wani, M. C.; Cook, C. E.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888−3890.
(2) Pommier, Y.; Leo, E.; Zhang, H. L.; Marchand, C. *Chem. Biol.* **2010**, *17*, 421−433.
(3) Pommier, Y. *Nat. Rev. Cancer* **2006**, *6*, 789−802.
(4) Sheng, C. Q.; Miao, Z. Y.; Zhang, W. N. *Curr. Med. Chem.* **2011**, *18*, 4389−4409.
(5) Drwal, M. N.; Agama, K.; Wakelin, L. P. G.; Pommier, Y.; Griffith, R. *PLoS One* **2011**, *6*, e25150.
(6) Pommier, Y.; Cushman, M. *Mol. Cancer Ther.* **2009**, *8*, 1008−1014.
(7) Clinical study: 10-C-0056, a Phase I Study of Indenoisoquinolines LMP400 and LMP776 in Adults with Relapsed Solid Tumors and Lymphomas. http://clinicalstudies.info.nih.gov/cgi/detail.cgi?A_2010-C-0056.html (accessed May 2012).
(8) Kiselev, E.; Dexheimer, T. S.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2010**, *53*, 8716−8726.
(9) Cushman, M.; Cheng, L. *J. Org. Chem.* **1978**, *43*, 3781−3783.
(10) Cushman, M.; Cheng, L. *J. Org. Chem.* **1978**, *43*, 286−288.
(11) Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X. H.; Yang, J. M.; Li, T. K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2061−2073.
(12) Genes, C.; Michel, S.; Tillequin, F.; Poree, F. H. *Tetrahedron* **2009**, *65*, 10009−10015.
(13) Erve, A.; Saoudi, Y.; Thirot, S.; Guetta-Landras, C.; Florent, J. C.; Nguyen, C. H.; Grierson, D. S.; Popov, A. V. *Nucleic Acids Res.* **2006**, *34*, e43.
(14) Wang, L. K.; Johnson, R. K.; Hecht, S. M. *Chem. Res. Toxicol.* **1993**, *6*, 813−818.
(15) Li, T. K.; Houghton, P. J.; Desai, S. D.; Daroui, P.; Liu, A. A.; Hars, E. S.; Ruchelman, A. L.; LaVoie, E. J.; Liu, L. F. *Cancer Res.* **2003**, *63*, 8400−8407.
(16) Genes, C.; Lenglet, G.; Depauw, S.; Nhili, R.; Prado, S.; David-Cordonnier, M. H.; Michel, S.; Tillequin, F.; Poree, F. H. *Eur. J. Med. Chem.* **2011**, *46*, 2117−2131.
(17) Bisagni, E.; Landras, C.; Thirot, S.; Huel, C. *Tetrahedron* **1996**, *52*, 10427−10440.
(18) Osuka, A.; Nakajima, S.; Maruyama, K. *J. Org. Chem.* **1992**, *57*, 7355−7359.
(19) Potts, K. T.; Robinson, R. *J Chem Soc* **1955**, 2675−2686.
(20) Cushman, M.; Wong, W. C. *J. Org. Chem.* **1984**, *49*, 1278−1280.
(21) Cushman, M.; Abbaspour, A.; Gupta, Y. P. *J. Am. Chem. Soc.* **1983**, *105*, 2873−2879.
(22) Cushman, M.; Choong, T. C.; Valko, J. T.; Koleck, M. P. *J. Org. Chem.* **1980**, *45*, 5067−5073.
(23) Westcott, S. A.; Zhang, H.; Vogels, C. M.; Wheaton, S. L.; Baerlocher, F. J.; Decken, A. *Synthesis* **2005**, 2739−2743.
(24) Vara, Y.; Bello, T.; Aldaba, E.; Arrieta, A.; Pizarro, J. L.; Arriortua, M. I.; Lopez, X.; Cossio, F. P. *Org. Lett.* **2008**, *10*, 4759−4762.
(25) Xiao, X. S.; Cushman, M. *J. Org. Chem.* **2005**, *70*, 6496−6498.
(26) Dexheimer, T. S.; Pommier, Y. *Nat. Protoc.* **2008**, *3*, 1736−1750.
(27) Pfister, T. D.; Reinhold, W. C.; Agama, K.; Gupta, S.; Khin, S. A.; Kinders, R. J.; Parchment, R. E.; Tomaszewski, J. E.; Doroshow, J. H.; Pommier, Y. *Mol. Cancer Ther.* **2009**, *8*, 1878−1884.
(28) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1846−1849.
(29) Pourquier, P.; Ueng, L. M.; Fertala, J.; Wang, D.; Park, H. J.; Essigmann, J. M.; Bjornsti, M. A.; Pommier, Y. *J. Biol. Chem.* **1999**, *274*, 8516−8523.
(30) *SYBYL 8.0*, Tripos Int.: St. Louis.
(31) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. *Proteins* **2003**, *52*, 609−623.