HHS Public Access

Author manuscript

Bioorg Med Chem. Author manuscript; available in PMC 2017 April 01.

Published in final edited form as:

Bioorg Med Chem. 2016 April 1; 24(7): 1469–1479. doi:10.1016/j.bmc.2016.02.015.

Synthesis and Biological Evaluation of New Fluorinated and Chlorinated Indenoisoquinoline Topoisomerase I Poisons

Daniel E. Beck[†], **Wei Lv**[†], **Monica Abdelmalak**[‡], **Caroline B. Plescia**[‡], **Keli Agama**[‡], **Christophe Marchand**[‡], **Yves Pommier**[‡], and **Mark Cushman**[†,*]

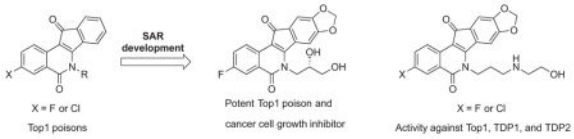
[†]Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, and the Purdue Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907, United States

[‡]Developmental Therapeutics Branch and Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 21892, United States

Abstract

Fluorine and chlorine are metabolically stable, but generally less active replacements for a nitro group at the 3-position of indenoisoquinoline topoisomerase IB (Top1) poisons. A number of strategies were employed in the present investigation to enhance the Top1 inhibitory potencies and cancer cell growth inhibitory activities of halogenated indenoisoquinolines. In several cases, the new compounds' activities were found to rival or surpass those of similarly substituted 3-nitroindenoisoquinolines, and several unusually potent analogues were discovered through testing in human cancer cell cultures. A hydroxyethylaminopropyl side chain on the lactam nitrogen of two halogenated indenoisoquinoline Top1 inhibitors was found to also impart inhibitory activity against tyrosyl DNA phosphodiesterases 1 and 2 (TDP1 and TDP2), which are enzymes that participate in the repair of DNA damage induced by Top1 poisons.

Graphical Abstract



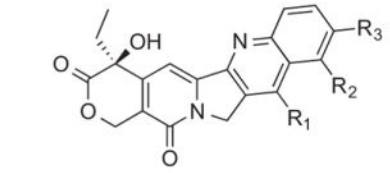
[*]Corresponding Author: Phone: 765-494-1465. Fax: 765-494-6970. cushman@purdue.edu.

The authors declare the following competing financial interest: Mark Cushman is on the Board of Directors of and is an investor in Linus Oncology, Inc., which has licensed indenoisoquinoline intellectual property owned by Purdue University. Neither Linus Oncology, Inc., nor any other commercial company sponsored or provided other direct financial support to the author or his laboratory for the research reported in this article. The remaining authors have no competing and/or relevant financial interest to disclose.

1. Introduction

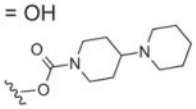
Topoisomerase IB (Top1) is a nuclear enzyme that catalyzes the relaxation of supercoiled DNA. To do so, it cleaves one strand of the DNA duplex and forms a transient, covalent complex with the cut strand.[1, 2] Top1 poisons intercalate between the base pairs at the DNA cleavage site and thereby trap the covalent Top1-DNA intermediate by preventing its reversal.[3] DNA replication forks that encounter a trapped cleavage complex are unable to proceed past it and leave behind a DNA double-strand break and a Top1-DNA adduct. Cells that are unable to efficiently repair this DNA damage ultimately undergo cell death.[4] Cancer cells are especially vulnerable to the cytotoxic effects of Top1 poisons because they express higher levels of Top1 to support rapid cell division,[5] while having compromised DNA repair and checkpoint capabilities.[4]

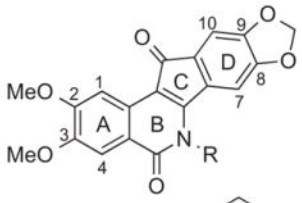
Safer and more effective Top1 poisons outside of the camptothecin (**1**) structural class are needed for cancer chemotherapy. The approved drugs topotecan (**2**) and irinotecan (**3**) suffer from a number of problems related to the development of resistance, chemical instability, drug-DNA-Top1 ternary cleavage complex instability, and dose-limiting side effects, and these might be mitigated by the indenoisoquinoline class of Top1 poisons.[4, 6, 7] Some of the advantages of the indenoisoquinolines include chemical stability, longer drug residence times in the cleavage complex, and greater activity vs. camptothecin-resistant enzyme mutants. The limitations observed for the approved drugs have stimulated the design and synthesis of a variety of camptothecin analogues, including gimatecan, belotecan, lurtotecan, exatecan, and diflomotecan, as well as non-camptothecin analogues such as the indolocarbazole edotecarin and the azabenzophenanthridine topovale, but none have obtained FDA approval.[6, 8] The present report details the development of highly active analogues of the indenoisoquinoline Phase 1 clinical trial drugs indotecan (LMP400, **4**) and indimitecan (LMP776, **5**), as well as the clinical trial candidate MJ-III-65 (LMP744, **6**).[7, 9–14] The new compounds display potent cancer cell growth and Top1 enzyme inhibitory activities and their halogenated A-rings are likely to produce less genotoxic metabolites than the corresponding nitro compounds.[15] In addition, the halogenated Top1 inhibitors were screened for inhibition of the DNA repair enzymes tyrosyl DNA phosphodiesterase 1 and 2 (TDP1 and 2). Because many cancer cell types have an already compromised capacity for DNA repair, inhibition of TDP1 and/or TDP2 may selectively reduce the ability of cancer cells to overcome the cytotoxic effects of Top1 poisons, and triple Top1/TDP1/TDP2 inhibitors would therefore be especially interesting.[16] A limited number of indenoisoquinolines, such as dimer **7**,[17] are already known to inhibit Top1 as well as one or more of these DNA repair enzymes.[18]

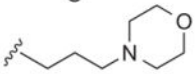


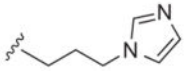
1, $R_1 = R_2 = R_3 = H$

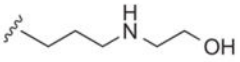
2, $R_1 = H$; $R_2 = CH_2N(Me)_2$; $R_3 = OH$

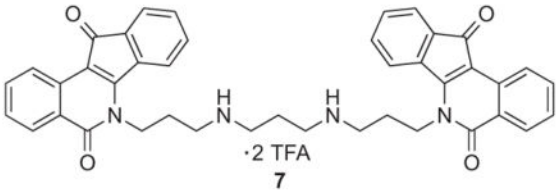
3, $R_1 = CH_2CH_3$; $R_2 = H$; $R_3 =$ 



4, R = 

5, R = 

6, R = 



·2 TFA

7

2. Chemistry

Structural modifications of fluorinated and chlorinated indenoisoquinolines[19] were implemented to enhance their Top1 poisoning activities. The first was the fusion of a dioxolane ring to the 8- and 9-positions of the scaffold. Previous S.A.R. studies documented a modest improvement in Top1 poisoning activity with this substitution.[20] A second modification was made to the 2-position. Molecular modeling indicated that a second fluorine or chlorine atom at this position would be tolerated by the surrounding environment. It was hypothesized that the addition of a second electronegative halogen atom could improve the "π-π-stacking" interactions between the ligand and the flanking base pairs in the ternary drug-DNA-Top1 cleavage complex by facilitating charge transfer complex formation.

A series of pentacyclic lactone intermediates (e.g. **13** and **14**, Scheme 1) were therefore prepared that could be used to probe the effects of having different A-ring substitution patterns and different side chains on the lactam nitrogen. 3-Hydroxyphthalides **8**[19] and **9**[19] were each condensed with phthalide **10** under basic conditions.[21] The 1,3-indanedione

intermediates **11** and **12** were each cyclized in situ in refluxing Ac_2O to yield lactones **13** and **14**,[22] which were condensed with primary amines **15**–**20** to yield the new indenoisoquinolines **21**–**30**. The carbohydrate-derived primary amines **16** and **17** were prepared in two steps from D-xylose or D-ribose, respectively, according to published procedures.[23, 24] The amines **15**–**17** were selected because they contributed to the synthesis of very active carbohydrate-substituted indenoisoquinolines,[25] while **18**, **19**, and **20** were chosen because they would lead to compounds with side chains present in the indotecan (LMP400), indimitecan (LMP776), and MJ-III-65 (LMP744).[7, 11, 13]

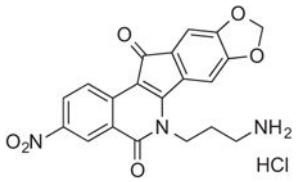
2,3-Difluoroindenoisoquinolines **37** and **38** were synthesized starting from the benzoic acid derivative **31** (Scheme 2). Reduction of **31** with BH_3 provided the benzylic alcohol **32**, which was subjected to a Rosenmund-von Braun reaction followed by hydrolysis and lactonization to yield phthalide **33**. Oxidation of the methylene carbon of phthalide **33** was carried out using NBS, and the resulting 3-bromophthalide **34** was hydrolyzed following column chromatography purification to deliver the 3-hydroxyphthalide **35**. Condensation of precursors **35** and **10** under basic conditions, and treatment of the unisolated 1,3-indanedione intermediate (not shown, similar to **11** and **12**) in heated Ac_2O yielded lactone **36**. Lactone **36** was condensed with primary amines **15** or **18** to yield **37** and **38**, respectively.

3,4-Dichloroindenoisoquinolines **45** and **46** were synthesized starting from phthalic acid **39** (Scheme 3), which was converted to its anhydride **40** with AcCl. Anhydride **40** was reduced with excess $NaBH_4$ and the unisolated intermediate was treated with catalytic pTSA in refluxing PhMe to yield phthalide **41**. Oxidation of phthalide **41** with NBS, and hydrolysis of 3-bromophthalide **42** gave them 3-hydroxypthalide **43**. 3-Hydroxyphthalide **43** and phthalide **10** were used to make lactone **44** by the two-step sequence described before. Derivatization of lactone **44** with primary amines **15** or **18** provided **45** and **46**, respectively.

3. Results and discussion

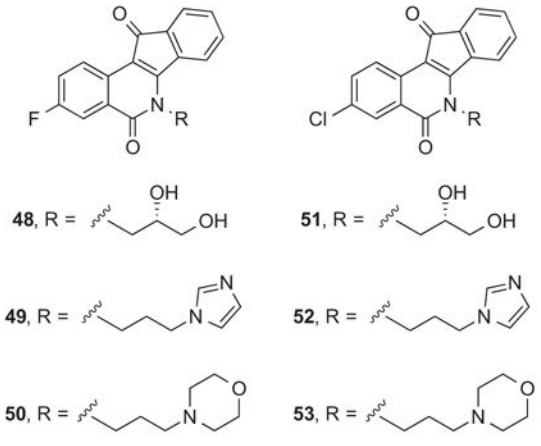
A Top1-mediated DNA cleavage assay[26] was used to evaluate the Top1 poisoning activities of the compounds, which were tested at 0.1, 1, 10, and 100 μM concentrations in the presence of a 3′-[^{32}P]-labeled, double-stranded DNA fragment and recombinant Top1 enzyme. When a compound poisons Top1-DNA cleavage complexes, the labeled cleaved strands are detected as shorter strands in the electrophoretic assay.[26] To measure the activity of a new compound in the Top1-mediated DNA cleavage assay, the number and intensity of the bands observed in test agent lanes is compared to that seen with the positive control, camptothecin (**1**), at 1 μM. The relative activity is estimated and used to assign a semiquantitative score, which ranges from 0 (no activity) to ++++ (activity equal to that of 1 μM **1**; see Table 1 footnotes for the complete rubric). A representative gel is shown in Figure 1. Aside from the determination of relative Top1 poisoning activities, competing Top1 suppression activity can be observed. For example, compound **25** appears to suppress Top1-mediated DNA cleavage at high drug concentration (100 μM), because the band density at several cleavage sites decreases. Suppression may be caused by the compound binding to free DNA, which makes the DNA a poorer substrate for the Top1 cleavage reaction.[13]

The NCI-60 human tumor cell line screen ("NCI-60")[27] was used to evaluate the cytotoxicities of the new compounds. The NCI-60 triages new compounds after first testing them at 10 μM. If the averaged growth percent across the cell lines is sufficiently low, the compound is promoted to a five-concentration assay. The five testing concentrations range from 10^{-8} to 10^{-4} μM, and the resulting concentration-response curves are used to calculate the concentration required for 50% growth inhibition relative to control, or GI_{50}, for each cell line. In cases where a GI_{50} falls below or above the testing range, it is recorded as either the minimum (10^{-8} μM) or maximum (10^{-4} μM) testing concentration. The average GI_{50} for all 60 cell lines tested under these conditions is called the GI_{50} mean graph midpoint (MGM). Cytotoxicity data including MGM values and GI_{50} values for a limited number of specific cell lines, as well as Top1-mediated DNA cleavage assay scores for the tested compounds are displayed in Table 1. Compounds **4–6** and **47**[28] are included in the table for easy referencing.

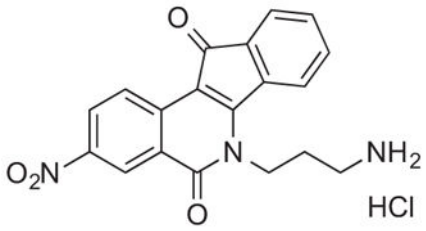


47

Contrary to expectation, the second generation of bioisosteric indenoisoquinolines was not enriched in potent Top1 poisons (i.e., Top1 scores at or above ++++). Of the fourteen new compounds, one achieved a ++++ score, and two achieved a +++ score. Although all of the compounds displayed some Top1 poisoning activity, most were clustered at + or ++ scores. In contrast, the six fluorinated and chlorinated compounds in the first generation (**48**–**53**) either achieved a +++ score (**49**, **50**, and **52**) or a ++ score (**48**, **51**, and **53**). The major structural difference between these two generations was at the D-ring, to which an 8,9-methylenedioxy substitution was added in the second generation. The effect of installation of this group on indenoisoquinoline Top1 poisoning activity was not consistent with observations from previous studies, which have shown that the group can increase activity moderately.[20, 29]



On the other hand, the second generation compounds reported here generally have improved (10-fold or better) GI_{50} MGM values versus the first generation. Twelve of the fourteen compounds have GI_{50} MGM values below 100 nM. The remaining two compounds also have low GI_{50} MGM values (105 nM and 229 nM). It was previously established that particular alkoxy substituents at the 8- and/or 9-positions could enhance antiproliferative potency of indenoisoquinolines. For example, compound **47** has an GI_{50} MGM value of 90 nM, while its analogue **54**, which lacks the 8,9-methylenedioxy group, has an GI_{50} MGM value of 245 nM.[28] The most cytotoxic compound of the present series was the 3-fluoro derivative **25** having an imidazole-containing side chain, which displayed a GI_{50} MGM of 11 nM. It should be recognized that the actual average GI_{50} is significantly lower than MGM value of 11 nM, since cases in which the GI_{50} values were lower than 10 nM were recorded as 10 nM, the lowest concentration tested in the standard NCI-60 assay, for calculation of the GI_{50} MGM value. The indenoisoquinoline **25** is an attractive analogue of LMP776 (**5**), which has been shown to undergo oxidative demethylation of the 2-methoxy group in the presence of human liver microsomes.[30]

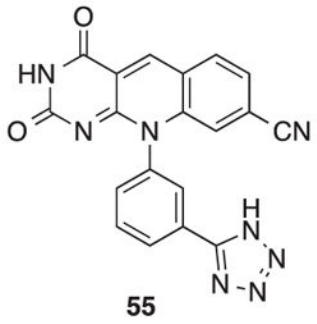


54

The indenoisoquinolines substituted with a 2,3-difluoro (**37** and **38**) or 2,3-dichloro pattern (**45** and **46**) were expected to have improved Top1 poisoning activity relative to 3-fluoro- or 3-chloro-substituted analogues. Instead, these four compounds displayed relatively weak Top1 scores (+ or ++). It is not clear from molecular modeling why 2-position substitution with a fluorine or chlorine should be detrimental to Top1 poisoning activity. On the other hand, the 13–69 nM GI_{50} MGM values for these four compounds indicate very potent cytotoxic activity in human cancer cells.

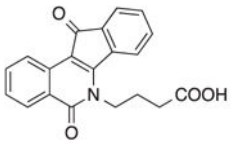
Each of the new compounds was tested in biochemical assays for tyrosyl DNA phosphodiesterase 1[31] and 2[32] inhibitory activity, with the rationale being that combining Top1 poisoning with TDP1 and TDP2 inhibition could potentially enhance the therapeutic usefulness of these drugs. TDP1 hydrolyzes 3′-phosphotyrosyl linkages between peptides and DNA that result from degradation of topoisomerase I (Top1)-DNA adducts produced by the action of Top1 inhibitors. TDP2 hydrolyzes 5′-phosphotyrosyl linkages between peptides and DNA that result from degradation of topoisomerase II (Top2)-DNA adducts produced by the action of Top2 inhibitors and to a lesser extent in Top1-DNA adducts produced by Top1 inhibitors.[16, 33] TDP2 can also hydrolyze 3′-phosphotyrosyl linkages, like TDP1, and so it can also participate in Top1 poison-induced DNA damage repair. Recent evidence shows that TDP1 and TDP2 may serve as mutual backups in DNA damage repair.[16, 33]

Two Top1 inhibitors prepared in the current study were found to be active in both recombinant TDP1 and TDP2 inhibition assays (Figures 2 and 3). Indenoisoquinolines **29** and **30** both inhibited TDP1 enzyme with IC_{50} values of 8.7 and 6.3 μM, respectively (Figure 2 and Table 2). Compounds **29** and **30** also inhibited recombinant TDP2 enzyme with IC_{50} values of 10.2 and 9.1 μM, respectively (Figure 3 and Table 2). Compounds **29** and **30** are structurally similar, with each possessing a hydroxyethylaminopropyl side chain. This side chain was not previously known to confer TDP1 or TDP2 inhibitory activity to the indenoisoquinolines. This observation is significant, because the same side chain is known to confer Top1 inhibitory activity.[7, 12] Compound **29** achieved a ++ score, while compound **30** achieved a +++ score in the Top1 assay, and both compounds are therefore novel triple inhibitors of all three enzymes, Top1, TDP1, and TDP2.



55

A molecular modeling study was carried out to examine the potential interactions between indenoisoquinoline **25** and its binding site in the Top1-DNA cleavage complex (Figure 4). The fluorine atom is calculated to engage Asn722 in a hydrogen-bonding interaction, and its 11-position ketone oxygen engages Arg364 in a second direct hydrogen-bonding interaction. The binding mode is nearly identical to that observed for indenoisoquinoline **56** in its co-crystal structure with the Top1-DNA cleavage complex.[35]



56

4. Conclusion

Installation of 8,9-methylenedioxy substitution on the D-ring of 3-position fluorinated and chlorinated indenoisoquinolines and exploration of varied side chains at the lactam nitrogen resulted in compounds with extremely potent growth inhibitory activities, although Top1 poisoning activities were not generally enhanced. Attachment of a fluorine or chlorine atom in the 2-position of 3-halogenated indenoisoquinolines was attempted because of observations made from molecular modeling. It was suspected that addition of a small electronegative halogen atom in this position would be tolerated and allow modulation of the "π-π-stacking" interaction by facilitating charge transfer complex formation. The resulting 2,3-dihalogenated compounds displayed robust cytotoxic activities in cancer cell cultures,

although they had relatively weak Top1 poisoning activities. The second generation includes two 3-fluoroindenoisoquinolines **21** and **25** that showed significant growth inhibitory and Top1 poisoning activities. Both compounds compare favorably to the nitrated analogue **47**, and are expected to be less genotoxic than **47** because they lack the nitro group.[15] It was also found that hydroxyethylaminopropyl side chains can impart inhibitory activity against tyrosyl DNA phosphodiesterase 1 and 2 (TDP1 and 2) without eliminating Top1 poisoning activity. At present, there are few reported dual inhibitors of both TDP1 and TDP2,[19, 34] and none that are potent triple inhibitors of both of these enzymes as well as poisons of Top1. Generally speaking, triple inhibitors of Top1, TDP1, and TDP2 are attractive because they not only damage DNA, they also inhibit the repair of the damage.

5. Experimental section

5.1 Chemistry

Reactions were monitored by silica gel analytical thin-layer chromatography, and 254 nm UV light was used for visualization. All yields refer to isolated compounds. Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification. Melting points were determined using capillary tubes and are uncorrected. ^{1}H Nuclear magnetic resonance spectroscopy was performed using a 300 MHz or 500 MHz spectrometer. Infrared spectra were obtained using an FTIR spectrometer. Mass spectral analyses were performed at the Purdue University Campus-Wide Mass Spectrometry Center. HPLC analyses were performed on a Waters 1525 binary HPLC pump/Waters 2487 dual λ absorbance detector system, using a 5 μm C18 reversed phase column and UV detection at 254 nm. HPLC purities of all tested compounds were estimated from the major peak area and were ≥ 95% of the combined total peak area.

5.1.1 3-Fluoro-8,9-methylenedioxyindeno[1,2-*c*]isochromene-5,12-dione (13)—

3-Hydroxyphthalide **8**[19] (506 mg, 3.01 mmol) and phthalide **10** (538 mg, 3.02 mmol) were suspended in EtOAc (10 mL) and a freshly prepared solution of NaOMe (0.27 g, 12 mmol of Na) in MeOH (20 mL) was added. The solution was heated at reflux with stirring under an argon atmosphere for 23 h. The mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (1.5 mL), concentrated in vacuo, and azeotroped with PhMe (2 × 10 mL). Ac_2O (20 mL) was added to the residue and the mixture was heated at reflux with stirring under an argon atmosphere for 1 h 20 min. The suspension was azeotroped with PhMe (4 × 10 mL) to provide a purple residue, which was sequentially washed with hexanes (50 mL) and H_2O (50 mL) and hexanes (20 mL). The impure solid residue was purified by silica gel column chromatography, eluting with $CHCl_3$, to give lactone **13** (140 mg, 15%) as a dull purple solid: mp 324–330 °C. IR (film) 1738, 1715, 1504, 1375, 1160 $cm^{-1;}$ ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.17 (dd, J = 9.0 Hz, J = 5.6 Hz, 1 H), 7.91 (dd, J = 9.0 Hz, J = 2.9 Hz, 1 H), 7.87 – 7.75 (m, 1 H), 7.29 – 7.22 (m, 2 H), 6.20 (s, 2 H); EIMS *m/z* 310 (M^+, 100); HRCIMS calcd for $C_{17}H_8FO_5$ 311.0350 (MH^+), found 311.0353.

5.1.2 3-Chloro-8,9-methylenedioxyindeno[1,2-*c*]isochromene-5,12-dione (14)—

3-Hydroxyphthalide **9**[19] (570 mg, 3.09 mmol) and phthalide **10** (561 mg, 3.15 mmol) were suspended in EtOAc (10 mL) and a freshly prepared solution of NaOMe (0.30 g, 13 mmol

of Na) in MeOH (20 mL) was added. The solution was heated at reflux with stirring under an argon atmosphere for 18 h. The mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (1.5 mL), concentrated in vacuo, and azeotroped with PhMe (2 mL). Ac_2O (20 mL) was added to the residue and the mixture was heated at reflux with stirring under an argon atmosphere for 18 h. The suspension was azeotroped with PhMe (3 × 5 mL) to provide a purple residue that was partitioned between saturated $NaHCO_3$ (150 mL) and $CHCl_3$ (50 mL). The aqueous layer was removed. H_2O (25 mL) and hexanes (10 mL) were added to the organic layer, and the mixture was filtered. The solid residue that was collected was washed with 1:1 hexanes-EtOAc (10 mL) and dried under high vacuum to provide lactone **14** (557 mg, 55%) as a purple solid: mp 335–336 °C. IR (film) 1746, 1711, 1490, 1376, 1240, 1025, 838 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 8.20 – 8.05 (m, 2 H), 8.01 – 7.88 (m, 1 H), 7.26 (d, *J* = 8.9 Hz, 2 H), 6.21 (s, 2 H); CIMS *m/z* (rel intensity) 327/329 (MH^+, 100/42); HRCIMS calcd for $C_{17}H_8ClO_5$ 327.0055 (MH^+), found 327.0051.

5.1.3 (*S*)-3-Fluoro-6-(2,3-dihydroxypropyl)-8,9-methylenedioxy-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (21)—Lactone **13** (46 mg, 0.15 mmol) was suspended in $CHCl_3$ (15 mL) with stirring and a solution of (*S*)-2,3-dihydroxypropylamine (**15**, 33 mg, 0.36 mmol) in MeOH (10 mL) was added. The mixture was heated at reflux for 16 h, cooled to room temperature, and concentrated in vacuo. $CHCl_3$ (5 mL) and hexanes (1 mL) were added to the residue, and the suspension was filtered. The product was washed with hexanes (5 mL) and MeOH (5 mL) and dried under high vacuum to provide **21** (38 mg, 66%) as a purple solid: mp 174 °C (dec). ^1H NMR (300 MHz, DMSO-d_6) δ 8.50 (t, *J* = 6.8 Hz, 1 H), 7.79 (d, *J* = 9.7 Hz, 1 H), 7.67 (s, 1 H), 7.08 (s, 1 H), 6.17 (s, 2 H), 5.14 (d, *J* = 5.0 Hz, 1 H), 5.05 (t, *J* = 5.3 Hz, 1 H), 4.56 – 4.29 (m, 2 H), 3.95 (s, 1 H), 3.57 (t, *J* = 5.0 Hz, 2 H); CIMS *m/z* (rel intensity) 384 (MH^+, 100); HRESIMS calcd for $C_{20}H_{14}FNO_6Na$ 406.0703 (MNa^+), found 406.0705; HPLC purity, 95.14% (MeOH, 100%).

5.1.4 (*S*)-3-Chloro-6-(2,3-dihydroxypropyl)-8,9-methylenedioxy-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (22)—Lactone **14** (73 mg, 0.22 mmol) was suspended in MeOH (10 mL) and $CHCl_3$ (15 mL), and a solution of (*S*)-2,3-dihydroxypropylamine (**15**, 40 mg, 0.44 mmol) in MeOH (1 mL) was added with stirring. The mixture was heated at reflux with stirring under an argon atmosphere for 14.5 h, cooled to room temperature, and filtered. The solid residue that collected was washed with hexanes (2 mL) and dried under high vacuum to yield **22** (67 mg, 75%) as a brownish-purple solid: mp 303–304 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.44 (d, *J* = 8.7 Hz, 1 H), 8.04 (d, *J* = 2.3 Hz, 1 H), 7.78 (dd, *J* = 8.7, 2.4 Hz, 1 H), 7.70 (s, 1 H), 7.09 (s, 1 H), 6.18 (s, 2 H), 5.13 (d, *J* = 5.0 Hz, 1 H), 5.11 – 4.97 (m, 1 H), 4.53 – 4.31 (m, 2 H), 4.00 – 3.86 (m, 1 H), 3.57 (t, *J* = 5.1 Hz, 2 H); MALDI *m/z* (rel intensity) 400/402 (MH^+, 100/38); HRESIMS calcd for $C_{23}H_{17}ClN_3O_4$ 400.0588 (MH^+), found 400.0580; HPLC purity, 100% (MeOH, 100%).

5.1.5 (2′*S*,3′*R*,4′*R*)-3-Fluoro-5,6-dihydro-6-(2′,3′,4′,5′-tetrahydroxypentyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (23)—D-Xylitylamine (**16**, 48 mg, 0.32 mmol) was dissolved in H_2O (1 mL), and the solution was added to a stirring suspension of lactone **13** (88 mg, 0.28 mmol) in $CHCl_3$ (20 mL) and MeOH (15 mL). The reaction mixture was heated at reflux with stirring for 24 h. Additional D-

xylitylamine (**16**, 20 mg, 0.13 mmol) dissolved in H_2O (0.25 mL) was added and heating to reflux was continued for 16 h. The mixture was concentrated in vacuo until ~2 mL of solvent remained, and the suspension was filtered to get a solid, which was dried under high vacuum with heating. Compound **23** (39 mg, 31%) was obtained as a red-violet solid: mp 260–272 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.50 (dd, *J* = 9.0, 5.4 Hz, 1 H), 7.83 (s, 1 H), 7.78 (dd, *J* = 9.4, 2.9 Hz, 1 H), 7.65 (td, *J* = 8.8, 2.9 Hz, 1 H), 7.07 (s, 1 H), 6.14 (s, 2 H), 5.03 (d, *J* = 5.2 Hz, 1 H), 4.94 (d, *J* = 5.7 Hz, 1 H), 4.64 (d, *J* = 5.5 Hz, 1 H), 4.59 – 4.49 (m, 2 H), 4.42 (dd, *J* = 14.2, 3.6 Hz, 1 H), 4.11 – 4.03 (m, 1 H), 3.69 – 3.62 (m, 1 H), 3.61 – 3.54 (m, 1 H), 3.50 – 3.43 (m, 1 H), 3.42 – 3.35 (m, 1 H); ESIMS *m/z* (rel intensity) 466 (MNa^+, 100); HRESIMS calcd for $C_{22}H_{19}FNO_8$ 444.1095 (MH^+), found 444.1086; HPLC purity, 100% (MeOH, 100%).

5.1.6 (2′*S*,3′*S*,4′*R*)-3-Chloro-5,6-dihydro-6-(2′,3′,4′,5′-tetrahydroxypentyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (24)—D-Ribitylamine (**17**, 78 mg, 0.24 mmol) was dissolved in H_2O (0.5 mL), and the solution was added to a stirring suspension of lactone **14** (41 mg, 0.27 mmol) in $CHCl_3$ (20 mL) and MeOH (15 mL). The reaction mixture was heated at reflux with stirring for 24 h. The mixture was concentrated in vacuo to ~2 mL solvent remained and then filtered. The collected solid was sonicated in $CHCl_3$-hexanes (2:1, 2 mL). After sitting for 10 min, additional solid had precipitated from the filtrate. Both of the suspensions were filtered. The combined solid was dried under high vacuum with heating to ~100 °C. Product **24** (15 mg, 12%) was obtained as a purple solid: mp 231 °C (dec). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 (d, *J* = 8.6 Hz, 1 H), 8.00 (d, *J* = 2.4 Hz, 1 H), 7.86 (s, 1 H), 7.74 (dd, *J* = 8.6, 2.4 Hz, 1 H), 7.04 (s, 1 H), 6.14 (d, *J* = 3.3 Hz, 2 H), 5.26 (d, *J* = 5.5 Hz, 1 H), 4.97 (d, *J* = 5.2 Hz, 1 H), 4.69 (d, *J* = 5.2 Hz, 1 H), 4.59 – 4.27 (m, 3 H), 4.13 (s, 1 H), 3.58 (m, 3 H), 3.41 (m, 1 H); ESIMS *m/z* (rel intensity) 482/484 (MNa^+, 100/40); HRESIMS calcd for $C_{22}H_{19}ClNO_8$ 460.0800 (MH^+), found 460.0801; HPLC purity, 100% (MeOH, 100%).

5.1.7 3-Fluoro-6-(3-(1*H*-imidazol-1-yl)propyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (25)—Lactone **13** (53 mg, 0.17 mmol) was dissolved in $CHCl_3$ (20 mL) with stirring and 3-imidazolylpropylamine (**18**, 43 mg, 0.34 mmol) was added. The mixture was heated at reflux for 16 h, cooled, and concentrated to ~5 mL. Hexanes (1 mL) were added and the suspension was filtered. The product was washed with hexanes (5 mL) and MeOH (5 mL) and dried under high vacuum with heating to 125 °C to furnish **25** (54 mg, 76%) as a purple solid: mp 235 °C (dec). IR (film) 1667, 1503, 1377, 1310, 1027 $cm^{-1;}$ ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (dd, *J* = 8.9, 5.4 Hz, 1 H), 7.83 (dd, *J* = 9.5, 2.8 Hz, 1 H), 7.78 – 7.64 (m, 2 H), 7.26 (s, 1 H), 7.15 (s, 1 H), 7.10 (s, 1 H), 6.91 (s, 1 H), 6.20 (s, 2 H), 4.41 (t, *J* = 7.4 Hz, 2 H), 4.18 (t, *J* = 6.8 Hz, 2 H), 2.32 – 2.09 (m, 2 H); EIMS *m/z* (rel intensity) 417 (M^+, 100); HRESIMS calcd for $C_{23}H_{17}FN_3O_4$ 418.1203 (MH^+), found 418.1208; HPLC purity, 100% (MeOH, 100%).

5.1.8 3-Chloro-6-(3-(1*H*-imidazol-1-yl)propyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (26)—Lactone **14** (77 mg, 0.24 mmol) was dissolved in $CHCl_3$ (20 mL) with stirring and a solution of 3-imidazolylpropylamine (**18**, 59 mg, 0.47 mmol) in $CHCl_3$ (1 mL) was added. The mixture was heated at reflux with stirring

under an argon atmosphere for 14.5 h, cooled to room temperature, and filtered. The product was washed with hexanes (2 mL) and dried under high vacuum at 60 °C to provide **26** (81 mg, 79%) as a dark purple solid: mp 286–287 °C. IR (film) 1668, 1537, 1482, 1304, 826 $cm^{-1;}$ ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 (d, J = 8.8 Hz, 1 H), 8.31 (s, 1 H), 8.09 (d, J = 2.3 Hz, 1 H), 7.82 (dd, J = 8.6, 2.3 Hz, 1 H), 7.69 (s, 1 H), 7.24 (d, J = 1.1 Hz, 1 H), 7.18 (s, 1 H), 7.13 (s, 1 H), 6.89 (d, J = 1.4 Hz, 1 H), 6.21 (s, 2 H), 4.41 (t, J = 7.3 Hz, 2 H), 4.17 (t, J = 6.9 Hz, 2 H), 2.33 – 2.09 (m, 2 H); MALDI *m/z* (rel intensity) 434/436 (MH^+, 100/83); HRESIMS calcd for $C_{23}H_{17}ClN_3O_4$ 434.0908 (MH^+), found 434.0887; HPLC purity, 100% (MeOH, 100%).

5.1.9 3-Fluoro-5,6-dihydro-6-(3-morpholinopropyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (27)—Lactone **13** (29 mg, 0.093 mmol) was dissolved in $CHCl_3$ (9 mL) with stirring and a solution of 3-morpholinopropylamine (**19**, 14 mg, 0.096 mmol) in $CHCl_3$ (1 mL) was added. The mixture was heated at reflux with stirring under an argon atmosphere for 20 h and concentrated in vacuo until ~1 mL of solvent remained. Hexanes (1 mL) were added and the suspension was filtered to provide the free base (15 mg, 37%) as a red-violet solid. The trifluoroacetate salt was prepared by treating the free base with neat TFA (5 mL) for 5 min at room temperature with stirring and then evaporating the TFA. Chemical characterization data pertain to the free base: mp 294–296 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (dd, J = 9.0, 5.3 Hz, 1 H), 7.94 (dd, J = 9.3, 2.9 Hz, 1 H), 7.49 (s, 1 H), 7.43 (td, J = 8.6, 2.8 Hz, 1 H), 7.12 (s, 1 H), 6.11 (s, 2 H), 4.60 – 4.46 (m, 2 H), 3.85 – 3.65 (m, 4 H), 2.64 – 2.40 (m, 6 H), 2.12 – 1.92 (m, 2 H); ESIMS *m/z* (rel intensity) 437 (MH^+, 100); HRESIMS calcd for $C_{24}H_{22}FN_2O_5$ 437.1513 (MH^+), found 437.1493; HPLC purity, 99.35% (MeOH, 100%).

5.1.10 3-Chloro-5,6-dihydro-6-(3-morpholinopropyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (28)—Lactone **14** (34 mg, 0.10 mmol) was dissolved in $CHCl_3$ (9 mL) with stirring and a solution of 3-morpholinopropylamine (**19**, 15 mg, 0.11 mmol) in $CHCl_3$ (1 mL) was added. The mixture was heated at reflux with stirring under an argon atmosphere for 20 h and concentrated in vacuo until ~1 mL solvent remained. Hexanes (1 mL) was added and the suspension was filtered. The product was dried under high vacuum to provide the free base (15 mg, 33%) as a dark purple solid. The trifluoroacetate salt was prepared by treating the free base with neat TFA (5 mL) for 5 min at room temperature with stirring and then evaporating the TFA. Chemical characterization data pertain to the free base: mp 290–294 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 8.7 Hz, 1 H), 8.26 (d, J = 2.3 Hz, 1 H), 7.62 (dd, J = 8.7, 2.3 Hz, 1 H), 7.50 (s, 1 H), 7.13 (s, 1 H), 6.12 (s, 2 H), 4.59 – 4.46 (m, 2 H), 3.76 (t, J = 4.7 Hz, 4 H), 2.62 – 2.44 (m, 6 H), 2.08 – 1.93 (m, 2 H); ESIMS *m/z* (rel intensity) 453/455 (MH^+, 100/29); HRESIMS calcd for $C_{24}H_{22}ClN_2O_5$ (MH^+) 453.1217, found 453.1211; HPLC purity, 95.04% (MeOH, 100%).

5.1.11 3-Fluoro-5,6-dihydro-6-(3-((2-hydroxyethyl)amino)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (29)—Lactone **13** (69 mg, 0.22 mmol) was dissolved in $CHCl_3$ (15 mL) with stirring and a solution of 3-(2-hydroxyethyl)propylamine (**20**, 36 mg, 0.30 mmol) in $CHCl_3$ (1 mL) was added. The

mixture was heated at reflux with stirring under an argon atmosphere for 3.5 h and concentrated in vacuo until ~1 mL of solvent remained. Hexanes (1 mL) were added and the suspension was filtered. The product was dried under high vacuum to provide **29** (69 mg, 76%) as a dull purple solid: mp 170 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.45 (dd, J = 9.0, 5.4 Hz, 1 H), 7.77 (dd, J = 9.5, 2.8 Hz, 1 H), 7.71 – 7.53 (m, 2 H), 7.10 (s, 1 H), 6.17 (s, 2 H), 4.56 – 4.27 (m, 3 H), 3.43 (d, J = 6.3 Hz, 2 H), 2.73 – 2.51 (m, 2 H), 1.92 – 1.65 (m, 2 H); ESIMS *m/z* (rel intensity) 411 (MH^+, 100); HRESIMS calcd for $C_{22}H_{20}FN_2O_5$ 411.1357 (MH^+), found 411.1363; HPLC purity, 95.00% (0.1% TFA in MeOH, 100%).

5.1.12 3-Chloro-5,6-dihydro-6-(3-((2-hydroxyethyl)amino)propyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (30)—Lactone **14** (84 mg, 0.26 mmol) was dissolved in $CHCl_3$ (15 mL) with stirring and a solution of 3-(2-hydroxyethyl)propylamine (**20**, 35 mg, 0.30 mmol) in $CHCl_3$ (1 mL) was added. The mixture was heated at reflux with stirring under an argon atmosphere for 22 h and concentrated in vacuo until ~1 mL of solvent remained. Hexanes (1 mL) were added and the suspension was filtered and dried under high vacuum to provide **30** (93 mg, 84%) as a purple solid: mp 198 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.38 (d, J = 8.7 Hz, 1 H), 8.02 (d, J = 2.4 Hz, 1 H), 7.76 (dd, J = 8.7, 2.4 Hz, 1 H), 7.63 (s, 1 H), 7.10 (s, 1 H), 6.18 (s, 2 H), 4.59 – 4.27 (m, 3 H), 3.44 (t, J = 5.7 Hz, 2 H), 2.67 (t, J = 6.4 Hz, 2 H), 2.58 (t, J = 5.8 Hz, 2 H), 1.92 – 1.71 (m, 2 H); ESIMS *m/z* (rel intensity) 427/429 (MH^+, 100/31); HRESIMS calcd for $C_{22}H_{20}ClN_2O_5$ 427.1061 (MH^+), found 427.1067; HPLC purity, 98.60% (0.1% TFA in MeOH, 100%).

5.1.13 (2-Bromo-4,5-difluorophenyl)methanol (32)—Benzoic acid derivative **31** (5.05 g, 21.3 mmol) was dissolved with stirring in THF (25 mL). A solution of BH_3 in THF (1.0 M, 21.3 mL, 21.3 mmol) was added dropwise at room temperature with frequent pausing to allow violent bubbling to subside. The mixture was heated at reflux with stirring for 4 h. A solution of aqueous KOH (2 M, 11 mL) was added and the mixture was cooled to room temperature with stirring for 30 min. The mixture was extracted with Et_2O (6 × 25 mL). The combined organic layers were washed with brine (1 × 25 mL) and dried over Na_2SO_4. The organic solution was concentrated in vacuo to yield a yellow-tinted liquid. Drying under high vacuum with heating to ca. 100 °C afforded **32** (4.01 g, 84%) as an off-white solid: mp 59–66 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 – 7.32 (m, 2 H), 4.68 (d, J = 1.3 Hz, 2 H), 2.12 (br s, 1 H); EIMS *m/z* (rel intensity) 222/224 (M^+, 70/70), 143 [$(M - Br)^+$, 93], 115 [$(M - Br - H_2O)^+$, 100].

5.1.14 5,6-Difluorophthalide (33)—Alcohol **32** (4.01 g, 18.0 mmol) and CuCN (3.33 g, 37.2 mmol) were suspended with stirring in DMF (20 mL) with heating at reflux under Ar atmosphere for 2.5 h. The mixture was cooled to room temperature, H_2O (2 mL) was added, and the mixture was heated at 100 °C for 1.5 h. After cooling to room temperature, H_2O (80 mL) and EtOAc (100 mL) were added to the mixture, and it was filtered through a Celite plug. The organic layer of the filtrate was removed. The aqueous layer of the filtrate was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (3 × 50 mL), dried over $MgSO_4$, and concentrated in vacuo. The orange, semisolid residue was purified by silica gel column chromatography, eluting with $CHCl_3$, and the pure

product-containing fractions were pooled and concentrated in vacuo. The residue was washed with hexanes (~10 mL) to provide **33** (1.17 g, 38%) as an off-white solid, R_f (SiO_2, $CHCl_3$) 0.31: mp 109–112 °C. IR (film) 3062, 1774, 1498, 1455, 1314, 1023 $cm^{-1;}$ ^1H NMR (300 MHz, $CDCl_3$) δ 7.72 (dd, *J* = 8.2, 7.0 Hz, 1 H), 7.32 (dd, *J* = 8.6, 6.3 Hz, 1 H), 5.29 (s, 2 H); CIMS *m/z* (rel intensity) 171 (MH^+, 100).

5.1.15 5,6-Difluoro-3-hydroxyphthalide (35)—Phthalide **33** (1.00 g, 5.88 mmol), NBS (1.06 g, 5.96 mmol), and AIBN (0.05 g) were suspended with stirring in CCl_4 and the mixture was heated at reflux under Ar atmosphere for 1 h 10 min. The suspension was cooled to room temperature and filtered. The filtered solids were washed with CCl_4 (10 mL). The combined filtrates were concentrated in vacuo to yield a yellow oil. The oily residue was purified by silica gel column chromatography, eluting with $CHCl_3$. The pure product-containing fractions were pooled and concentrated in vacuo, and the obtained residue was heated at reflux with stirring in H_2O (20 mL) for 1 h. The mixture was cooled to room temperature and acidified with $NaHSO_4$ (1.45 g). The product was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL) and dried over Na_2SO_4. The organic solution was concentrated in vacuo and dried under high vacuum with heating to yield **35** (727 mg, 66%) as a white solid, R_f (SiO_2, $CHCl_3$) 0: mp 104–107 °C. IR (film) 3401, 1764, 1500, 1353, 1319 $cm^{-1;}$ ^1H NMR (300 MHz, DMSO-d_6) δ 7.81 (dd, *J* = 8.9, 7.0 Hz, 1 H), 7.66 (dd, *J* = 9.2, 6.8 Hz, 1 H), aldehydic acid peaks not observed; CIMS *m/z* (rel intensity) 187 (MH^+, 94), 169 ($MH^+ - H_2O$, 100).

5.1.16 2,3-Difluoro-8,9-methylenedioxyindeno[1,2-*c*]isochromene-5,12-dione (36)—3-Hydroxyphthalide derivative **35** (408 mg, 2.19 mmol) and phthalide **10** (393 mg, 2.21 mmol) were suspended in EtOAc (10 mL) and a freshly prepared solution of NaOMe (0.21 g, 9.1 mmol of Na) in MeOH (20 mL) was added. The solution was heated at reflux with stirring under Ar atmosphere for 24 h. The mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (2 mL), concentrated in vacuo, and azeotroped with PhMe (10 mL). Ac_2O (20 mL) was added to the residue and the mixture was heated at 90 °C with stirring under an argon atmosphere for 2 h. The mixture was cooled to room temperature, H_2O (20 mL) was added, and the suspension was filtered. The solid was purified by silica gel column chromatography, eluting with $CHCl_3$, to provide lactone **36** (86 mg, 12%) as a dark purple solid, R_f (SiO_2, $CHCl_3$) 0.60: mp 300–301 °C. IR (film) 1744, 1722, 1433, 780 $cm^{-1;}$ ^1H NMR (300 MHz, $CDCl_3$) δ 8.12 – 7.99 (m, 2 H), 7.10 (s, 1 H), 6.97 (s, 1 H), 6.12 (s, 2 H); CIMS *m/z* (rel intensity) 329 (MH^+, 100); HRESIMS calcd for $C_{17}H_7F_2O_5$ 329.0256 (MH^+), found 329.0244.

5.1.17 (*S*)-2,3-Difluoro-6-(2,3-dihydroxypropyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (37)—Lactone **36** (28 mg, 0.085 mmol) was suspended in $CHCl_3$ (5 mL), and a solution of (*S*)-2,3-dihydroxypropylamine (**15**, 14 mg, 0.15 mmol) in MeOH (2 mL) was added with stirring. The mixture was heated at reflux with stirring under Ar atmosphere for 18.5 h. The suspension was cooled to room temperature, concentrated in vacuo until ~1 mL of solvent remained, and hexanes (0.5 mL) were added. The suspension was filtered and the solid residue that collected was dried under high vacuum to yield **37** (32 mg, 94%) as a red-violet solid: mp 256–261 °C. ^1H NMR (300

MHz, DMSO-d_6) δ 8.28 (dd, J = 11.6, 7.8 Hz, 1 H), 8.06 (dd, J = 11.1, 8.2 Hz, 1 H), 7.74 (s, 1 H), 7.14 (s, 1 H), 6.19 (dd, J = 2.4, 1.0 Hz, 2 H), 5.16 (d, J = 5.0 Hz, 1 H), 5.06 (t, J = 5.3 Hz, 1 H), 4.50 – 4.37 (m, 2 H), 3.94 (s, 1 H), 3.57 (t, J = 5.0 Hz, 2 H); APCIMS *m/z* (rel intensity) 402 (MH^+, 100); HRESIMS calcd for $C_{20}H_{13}F_2NO_6Na$ 424.0609 ($M + Na^+$), found 424.0606; HPLC purity, 100% (MeOH, 100%).

5.1.18 2,3-Difluoro-6-(3-(1*H*-imidazol-1-yl)propyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (38)—Lactone **36** (26 mg, 0.079 mmol) was dissolved in $CHCl_3$ (5 mL) with stirring and a solution of 3-imidazolylpropylamine (**18**, 13 mg, 0.10 mmol) in $CHCl_3$ (1 mL) was added. The mixture was heated at reflux with stirring under Ar atmosphere for 18.5 h, cooled to room temperature, and filtered. The product was washed with $CHCl_3$ (1 mL) and dried under high vacuum to provide **38** (37 mg, 100%) as a dark purple solid: mp > 400 °C. IR (film) 1673, 1386, 1313 $cm^{-1;}$ ^1H NMR (300 MHz, DMSO-d_6) δ 8.25 (dd, J = 11.5, 7.7 Hz, 1 H), 8.07 (dd, J = 11.0, 8.2 Hz, 1 H), 7.71 – 7.67 (m, 1 H), 7.25 (t, J = 1.2 Hz, 1 H), 7.17 (s, 1 H), 7.13 (s, 1 H), 6.95 – 6.85 (m, 1 H), 6.21 (s, 2 H), 4.39 (t, J = 7.2 Hz, 2 H), 4.17 (t, J = 6.8 Hz, 2 H), 2.26 – 2.12 (m, 2 H); ESIMS *m/z* (rel intensity) 436 (MH^+, 100); HRESIMS calcd for $C_{23}H_{16}F_2N_3O_4$ 436.1109 (MH^+), found 436.1107; HPLC purity, 98.41% (0.1% TFA in MeOH, 100%).

5.1.19 5,6-Dichlorophthalic Anhydride (40)—Diacid **39** (10.00 g, 42.55 mmol) was suspended in AcCl (50 mL) and the mixture was heated at reflux with stirring for 2 h. The obtained suspension was concentrated in vacuo and azeotroped with hexanes (2 × 10 mL) and PhMe (2 × 10 mL) to provide anhydride **40** (9.23 g, 100%) as a beige powder, R_f (SiO_2, $CHCl_3$) 0.64: mp 185–189 °C. ^1H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 2 H); CIMS *m/z* (rel intensity) 217/219/221 (MH^+, 100/69/12).

5.1.20 5,6-Dichlorophthalide (41)—Anhydride **40** (9.53 g, 42.6 mmol) was dissolved with stirring in THF (80 mL) and the solution was cooled to 0 °C under an Ar atmosphere. $NaBH_4$ (1.63 g, 43.1 mmol) was added slowly in a single portion, and the mixture was stirred with cooling to 0 °C for 50 min. The mixture was removed from the ice-water bath and warmed to room temperature for 17 h. MeOH (20 mL) was added slowly and cautiously to the obtained suspension. The mixture was concentrated in vacuo. Dilute, aqueous HCl (0.1 M, 24 mL) was added to the residue. After stirring the mixture for 10 min, it was filtered. The collected solids were vigorously heated at reflux in PhMe (100 mL) with pTsOH·H_2O (0.15 g), using a Dean-Stark trap to collect H_2O, for 30 h 15 min. The mixture was concentrated in vacuo. The residue was washed with H_2O (50 mL) and the solid was azeotroped with PhMe (10 mL) and dried under high vacuum to provide **41** (6.41 g, 74%) as a white solid, R_f (SiO_2, $CHCl_3$) 0.55: mp 157–160 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.14 (s, 1 H), 8.04 (s, 1 H), 5.40 (s, 2 H); CIMS *m/z* (rel intensity) 203/205/207 (MH^+, 100/71/14).

5.1.21 5,6-Dichloro-3-hydroxyphthalide (43)—Phthalide **41** (1.05 g, 5.17 mmol), NBS (0.93 g, 5.22 mmol), and AIBN (0.10 g) were suspended with stirring in CCl_4 (25 mL) and the mixture was heated at reflux under Ar atmosphere for 3 h 15 min. The suspension was cooled to room temperature and filtered. The filtered solids were washed with CCl_4 (10

mL). The combined filtrates were concentrated in vacuo to yield a yellow oil. The oily product was purified by silica gel column chromatography, eluting with $CHCl_3$. The pure product-containing fractions were pooled and concentrated in vacuo, and the residue was heated at reflux with stirring in a solution of KOH (0.59 g, 11 mmol) in H_2O (15 mL) for 2 h. The mixture was cooled to room temperature and $NaHSO_4$ (1.7 g) was added to acidify. The product was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (15 mL) and dried over Na_2SO_4. The organic solution was concentrated in vacuo and dried under high vacuum at room temperature to yield **43** (574 mg, 51%) as a yellow-white solid, R_f (SiO_2, $CHCl_3$) 0.03: mp 163 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (br s, 0.2 H), 8.41 (d, J = 7.7 Hz, 1 H), 8.05 (s, 1 H), 7.97 (s, 0.3 H), 6.66 (d, J = 7.8 Hz, 1 H); CIMS *m/z* (rel intensity) 219/221/223 (MH^+, 50/32/6), 201/203/205 ($MH^+ - H_2O$, 100/72/14).

5.1.22 2,3-Dichloro-8,9-methylenedioxyindeno[1,2-*c*]isochromene-5,12-dione (44)—3-Hydroxyphthalide derivative **43** (494 mg, 2.26 mmol) and phthalide **10** (405 mg, 2.27 mmol) were suspended in EtOAc (10 mL) and a freshly prepared solution of NaOMe (0.24 g, 10 mmol of Na) in MeOH (20 mL) was added. The solution was heated at reflux with stirring under Ar atmosphere for 4 d. The mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (1.5 mL), concentrated in vacuo, and azeotroped with PhMe (10 mL). Ac_2O (20 mL) was added to the residue and the mixture was heated at 90 °C with stirring under Ar atmosphere for 3 h. The suspension was azeotroped with PhMe (10 mL) and H_2O (20 mL) was added to the residue. The mixture was filtered. The residue was washed sequentially with 3:2 $CHCl_3$-hexanes (2 × 30 mL), 1:1 hexanes-EtOAc (25 mL) and 3:2 $CHCl_3$-Et_2O (25 mL). The solid was dried under high vacuum to provide lactone **44** (448 mg, 55%) as a dark purple solid, R_f (SiO_2, $CHCl_3$) 0.63: mp 301–302 °C. IR (film) 1760, 1714, 1483, 1386, 1317, 780 $cm^{-1;}$ ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1 H), 8.31 (s, 1 H), 7.11 (s, 1 H), 6.96 (s, 1 H), 6.12 (s, 2 H); CIMS *m/z* (rel intensity) 361/363/365 (MH^+, 100/76/20); HRCIMS calcd for $C_{17}H_7Cl_2O_5$ 360.9665 (MH^+), found 360.9670.

5.1.23 (*S*)-2,3-Dichloro-6-(2,3-dihydroxypropyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (45)—Lactone **44** (77 mg, 0.21 mmol) was suspended in MeOH (10 mL) and $CHCl_3$ (15 mL), and a solution of (*S*)-2,3-dihydroxypropylamine (**15**, 44 mg, 0.48 mmol) in MeOH (1 mL) was added with stirring. The mixture was heated at reflux with stirring under an argon atmosphere for 19.5 h, cooled to room temperature, and filtered. The solid residue that collected was washed with hexanes (2 mL) and dried under high vacuum to yield **45** (77 mg, 84%) as a brownish purple solid: mp 314–315 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 (s, 1 H), 8.15 (s, 1 H), 7.67 (s, 1 H), 7.06 (s, 1 H), 6.18 (s, 2 H), 5.18 (d, J = 4.9 Hz, 1 H), 5.13 (t, J = 5.2 Hz, 1 H), 4.52 – 4.23 (m, 2 H), 4.01 – 3.85 (m, 1 H); CIMS *m/z* (rel intensity) 434/436/438 (MH^+, 100/82/28); HREIMS calcd for $C_{20}H_{13}Cl_2NO_6$ 433.0114 (M^+), found 433.0112; HPLC purity, 100% (MeOH, 100%).

5.1.24 2,3-Dichloro-6-(3-(1*H*-imidazol-1-yl)propyl)-8,9-methylenedioxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (46)—Lactone **44** (82 mg, 0.23 mmol) was dissolved in $CHCl_3$ (20 mL) with stirring and a solution of 3-imidazolylpropylamine (**18**, 56

mg, 0.45 mmol) in $CHCl_3$ (1 mL) was added. The mixture was heated at reflux with stirring under an argon atmosphere for 19.5 h, cooled to room temperature, and filtered. The product was washed with hexanes (2 mL) and dried under high vacuum to provide **46** (90 mg, 84%) as a brownish purple solid: mp 309–310 °C. IR (film) 1670, 1603, 1478, 1308, 1293, 1031, 830, 756 $cm^{-1;}$ ^1H NMR (300 MHz, DMSO-d_6) δ 8.54 (s, 1 H), 8.32 (s, 1 H), 8.25 (s, 1 H), 7.69 (s, 1 H), 7.25 (s, 1 H), 7.19 (s, 1 H), 7.16 (s, 1 H), 6.91 (s, 1 H), 6.23 (s, 2 H), 4.40 (t, *J* = 7.0 Hz, 2 H), 4.18 (t, *J* = 6.9 Hz, 2 H), 2.27 – 2.14 (m, 2 H); CIMS *m/z* (rel intensity) 468/470/472 (MH^+, 100/92/31); HRESIMS calcd for $C_{23}H_{16}Cl_2N_3O_4$ 468.0518 (MH^+), found 468.0521; HPLC purity, 99.90% (MeOH, 100%).

5.2 Topoisomerase I-Mediated DNA Cleavage Reactions

A 3′-[^{32}P]-labeled 117-bp DNA oligonucleotide was prepared as previously described.[26] The oligonucleotide contains previously identified Top1 cleavage sites in 161-bp pBluescript SK(−) phagemid DNA. Approximately 2 nM of radiolabeled DNA substrate was incubated with recombinant Top1 in 20 μL of reaction buffer [10 mM Tris-HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 15 μg/mL BSA] at 25 °C for 20 min in the presence of various concentrations of test 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% bromophenol 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). The numbers in Figure 1 represent actual cleavage site positions on the 117 bp oligonucleotide substrate.

5.3 Recombinant TDP1 Assay

A 5′-[^{32}P]-labeled single-stranded DNA oligonucleotide containing a 3′-phosphotyrosine (N14Y)[31] was incubated at 1 nM with 10 pM recombinant TDP1 in the absence or presence of inhibitor for 15 min at room temperature in the LMP1 assay buffer containing 50 mM Tris HCl, pH 7.5, 80 mM KCl, 2 mM EDTA, 1 mM DTT, 40 μg/mL BSA, and 0.01% Tween-20.[17] Reactions were terminated by the addition of 1 volume of gel loading buffer [99.5% (v/v) formamide, 5 mM EDTA, 0.01% (w/v) xylene cyanol, and 0.01% (w/v) bromophenol blue]. Samples were subjected to a 16% denaturing PAGE with multiple loadings at 12-min intervals. Gels were dried and exposed to a PhosphorImager screen (GE Healthcare). Gel images were scanned using a Typhoon 8600 (GE Healthcare), and densitometry analyses were performed using ImageQuant software (GE Healthcare).

5.4 Recombinant TDP2 Assay

TDP2 reactions were carried out as described previously[32] with the following modifications. The 19-mer single-stranded oligonucleotide DNA substrate containing a 5′ phosphotyrosine (Y19, α^{32}P-cordycepin-3′-labeled) was incubated at 1 nM with 25 pM recombinant human TDP2 in the absence or presence of inhibitor for 15 min at room temperature in the LMP2 assay buffer containing 50 mM Tris-HCl, pH 7.5, 80 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 40 μg/mL BSA, and 0.01% Tween 20. Reactions were terminated and treated similarly to recombinant TDP1 reactions (see above).

5.5 Cytotoxicity Testing

The cytotoxicity data reported in Table 1 were generated in the National Cancer Institutes' collection of sixty human cancer cell lines (NCI-60). The assay procedures have been reported in detail.[27, 36]

5.6 Molecular Mechanics Calculations

The 1SC7[35] X-ray crystal structure file was prepared for molecular modeling by correcting several atom types, removing all of the water molecules, and adding hydrogens. The hydrogens were energy minimized while the remaining atoms were frozen in aggregate within SYBYL-X.[37] The details of the minimizations for Top1 models were set as follows: Powell method; MMFF94s force field[38]; MMFF94 charges; and 0.05 kcal/mol·Å energy gradient convergence. Top1 ligands were docked into the prepared 1SC7 crystal structure using GOLD 3.2 software.[39] The binding site was defined by the crystallized ligand, **56**. Ten GOLD algorithm runs were executed per ligand, using default parameters. The top ten docking poses per ligand were inspected visually following the docking runs. The docking poses that had a favorable GOLD score and similar binding mode to the crystallized ligand were selected for further analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was made possible by the National Institutes of Health (NIH) through support with Research Grants U01CA089566 and P30CA023168. In vitro cytotoxicity testing was performed by the Developmental Therapeutics Program at the National Cancer Institute, under contract NO1-CO-56000. Work done in the NCI Intramural Program, Center for Cancer Research was supported by the NIH Intramural Program (Z01 BC006161).

Abbreviations

CPT	camptothecin
Top1	topoisomerase IB
TDP1	tyrosyl-DNA phosphodiesterase 1
TDP2	tyrosyl-DNA phosphodiesterase 2
PAGE	polyacrylamide gel electrophoresis
SDS	sodium dodecyl sulfate

References

1. Stewart L, Redinbo MR, Qiu X, Hol WGJ, Champoux JJ. A Model for the Mechanism of Human Topoisomerase I. Science. 1998; 279:1534–1541. [PubMed: 9488652]
2. Redinbo MR, Stewart L, Kuhn P, Champoux JJ, Hol WGJ. Crystal Structures of Human Topoisomerase I in Covalent and Noncovalent Complexes with DNA. Science. 1998; 279:1504–1513. [PubMed: 9488644]

3. Staker BL, Hjerrild K, Feese MD, Behnke CA, Burgin AB, Stewart L. The Mechanism of Topoisomerase I Poisoning by a Camptothecin Analogue. Proc Natl Acad Sci USA. 2002; 99:15387–15392. [PubMed: 12426403]

4. Pommier Y. Topoisomerase I Inhibitors: Camptothecins and Beyond. Nat Rev Cancer. 2006; 6:789–802. [PubMed: 16990856]

5. Thomas CJ, Rahier NJ, Hecht SM. Camptothecin: Current Perspectives. Bioorg Med Chem. 2004; 12:1585–1604. [PubMed: 15028252]

6. Pommier Y. Drugging Topoisomerases: Lessons and Challenges. ACS Chem Biol. 2013; 8:82–95. [PubMed: 23259582]

7. Antony S, Jayaraman M, Laco G, Kohlhagen G, Kohn KW, Cushman M, Pommier Y. Differential Induction of Topoisomerase I-DNA Cleavage Complexes by the Indenoisoquinoline MJ-III-65 (NSC 706744) and Camptothecin: Base Sequence Analysis and Activity against Camptothecin-Resistant Topoisomerase I. Cancer Res. 2003; 63:7428–7435. [PubMed: 14612542]

8. Pommier Y. DNA Topoisomerase I Inhibitors: Chemistry, Biology, and Interfacial Inhibition. Chem Rev. 2009; 109:2894–2902. [PubMed: 19476377]

9. [accessed July 13, 2015] 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

10. [accessed July 13, 2015] Indenoisoquinoline LMP400 for Advanced Solid Tumors and Lymphomas. http://clinicaltrials.gov/ct2/show/NCT01794104

11. Nagarajan M, Morrell A, Ioanoviciu A, Antony S, Kohlhagen G, Agama K, Hollingshead M, Pommier Y, Cushman M. Synthesis and Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Substituted with Nitrogen Heterocycles. J Med Chem. 2006; 49:6283–6289. [PubMed: 17034134]

12. Antony S, Kohlhagen G, Agama K, Jayaraman M, Cao S, Durrani FA, Rustum YM, Cushman M, Pommier Y. Cellular Topoisomerase I Inhibition and Antiproliferative Activity by MJ-III-65 (NSC 706744), an Indenoisoquinoline Topoisomerase I Poison. Mol Pharmacol. 2005; 67:523–530. [PubMed: 15531731]

13. Cushman M, Jayaraman M, Vroman JA, Fukunaga AK, Fox BM, Kohlhagen G, Strumberg D, Pommier Y. Synthesis of New Indeno[1,2-*c*]isoquinolines: Cytotoxic Non-Camptothecin Topoisomerase I Inhibitors. J Med Chem. 2000; 43:3688–3698. [PubMed: 11020283]

14. Antony S, Agama KK, Miao ZH, Takagi K, Wright MH, Robles AI, Varticovski L, Nagarajan M, Morrell A, Cushman M, Pommier Y. Novel Indenoisoquinolines NSC 725776 and NSC 724998 Produce Persistent Topoisomerase I Cleavage Complexes and Overcome Multidrug Resistance. Cancer Res. 2007; 67:10397–10405. [PubMed: 17974983]

15. Smith, GF. Designing Drugs to Avoid Toxicity. In: Lawton, G.; Witty, DR., editors. Prog Med Chem. Vol. 50. 2011. p. 1-47.

16. Pommier Y, Huang S-yN, Gao R, Das BB, Murai J, Marchand C. Tyrosyl-DNA-phosphodiesterases (TDP1 and TDP2). DNA Repair. 2014; 19:114–129. [PubMed: 24856239]

17. Nguyen TX, Morrell A, Conda-Sheridan M, Marchand C, Agama K, Bermingam A, Stephen AG, Chergui A, Naumova A, Fisher R, O'Keefe BR, Pommier Y, Cushman M. Synthesis and Biological Evaluation of the First Dual Tyrosyl-DNA Phosphodiesterase I (Tdp1)-Topoisomerase I (Top1) Inhibitors. J Med Chem. 2012; 55:4457–4478. [PubMed: 22536944]

18. Lv PC, Agama K, Marchand C, Pommier Y, Cushman M. Design, Synthesis, and Biological Evaluation of O-2-Modified Indenoisoquinolines as Dual Topoisomerase I-Tyrosyl-DNA Phosphodiesterase I Inhibitors. J Med Chem. 2014; 57:4324–4336. [PubMed: 24800942]

19. Beck DE, Abdelmalak M, Lv W, Reddy PVN, Tender GS, O'Neill E, Agama K, Marchand C, Pommier Y, Cushman M. Discovery of Potent Indenoisoquinoline Topoisomerase I Poisons Lacking the 3-Nitro Toxicophore. J Med Chem. 2015; 58:3997–4015. [PubMed: 25909279]

20. Nagarajan M, Morrell A, Fort BC, Meckley MR, Antony S, Kohlhagen G, Pommier Y, Cushman M. Synthesis and Anticancer Activity of Simplified Indenoisoquinoline Topoisomerase I Inhibitors Lacking Substituents on the Aromatic Rings. J Med Chem. 2004; 47:5651–5661. [PubMed: 15509164]

21. Shapiro SL, Freedman L, Youlus J, Geiger K. Indandiones 2. Modified Dieckmann Reaction. J Org Chem. 1961; 26:3580.
22. Pailer M, Worthen H, Meller A. Some Reactions of 2-Aryl-1,3-indandiones. Monatsh Chem. 1961; 92:1037–1047.
23. Peterson KE, Cinelli MA, Morrell AE, Mehta A, Dexheimer TS, Agama K, Antony S, Pommier Y, Cushman M. Alcohol-, Diol-, and Carbohydrate-Substituted Indenoisoquinolines as Topoisomerase I Inhibitors: Investigating the Relationships Involving Stereochemistry, Hydrogen Bonding, and Biological Activity. J Med Chem. 2011; 54:4937–4953. [PubMed: 21710981]
24. Weinstock C, Plaut GWE. Synthesis and Properties of Certain Substituted Lumazines. J Org Chem. 1961; 26:4456–4462.
25. Beck DE, Agama K, Marchand C, Chergui A, Pommier Y, Cushman M. Synthesis and Biological Evaluation of New Carbohydrate-Substituted Indenoisoquinoline Topoisomerase I Inhibitors and Improved Syntheses of the Experimental Anticancer Agents Indotecan (LMP400) and Indimitecan (LMP776). J Med Chem. 2014; 57:1495–1512. [PubMed: 24517248]
26. Dexheimer TS, Pommier Y. DNA Cleavage Assay for the Identification of Topoisomerase I Inhibitors. Nat Protoc. 2008; 3:1736–1750. [PubMed: 18927559]
27. Shoemaker RH. The NCI60 Human Tumour Cell Line Anticancer Drug screen. Nat Rev Cancer. 2006; 6:813–823. [PubMed: 16990858]
28. Morrell A, Antony S, Kohlhagen G, Pommier Y, Cushman M. Synthesis of Nitrated Indenoisoquinolines as Topoisomerase I Inhibitors. Bioorg Med Chem Lett. 2004; 14:3659–3663. [PubMed: 15203138]
29. Morrell A, Placzek M, Parmley S, Antony S, Dexheimer TS, Pommier Y, Cushman M. Nitrated Indenoisoquinolines as Topoisomerase I Inhibitors: A Systematic Study and Optimization. J Med Chem. 2007; 50:4419–4430. [PubMed: 17696418]
30. Cinelli MA, Reddy PVN, Lv PC, Liang JH, Chen L, Agama K, Pommier Y, van Breemen RB, Cushman M. Identification, Synthesis, and Biological Evaluation of Metabolites of the Experimental Cancer Treatment Drugs Indotecan (LMP400) and Indimitecan (LMP776) and Investigation of Isomerically Hydroxylated Indenoisoquinoline Analogues as Topoisomerase I Poisons. J Med Chem. 2012; 55:10844–10862. [PubMed: 23215354]
31. Marchand C, Lea WA, Jadhav A, Dexheimer TS, Austin CP, Inglese J, Pommier Y, Simeonov A. Identification of Phosphotyrosine Mimetic Inhibitors of Human Tyrosyl-DNA Phosphodiesterase I by a Novel AlphaScreen High-throughput Assay. Mol Cancer Ther. 2009; 8:240–248. [PubMed: 19139134]
32. Gao R, Huang S-yN, Marchand C, Pommier Y. Biochemical Characterization of Human Tyrosyl-DNA Phosphodiesterase 2 (TDP2/TTRAP) A Mg2//Mn2+-dependent Phosphodiesterase Specific for the Repair of Topoisomerase Cleavage Complexes. J Biol Chem. 2012; 287:30842–30852. [PubMed: 22822062]
33. Zeng Z, Sharma A, Ju L, Murai J, Umans L, Vermeire L, Pommier Y, Takeda S, Huylebroeck D, Caldecott KW, El-Khamisy SF. TDP2 promotes repair of topoisomerase I-mediated DNA damage in the absence of TDP1. Nucleic Acids Res. 2012; 40:8371–8380. [PubMed: 22740648]
34. Raoof A, Depledge P, Hamilton NM, Hamilton NS, Hitchin JR, Hopkins GV, Jordan AM, Maguire LA, McGonagle AE, Mould DP, Rushbrooke M, Small HF, Smith KM, Thomson GJ, Turlais F, Waddell ID, Waszkowycz B, Watson AJ, Ogilvie DJ. Toxoflavins and Deazaflavins as the First Reported Selective Small Molecule Inhibitors of Tyrosyl-DNA Phosphodiesterase II. J Med Chem. 2013; 56:6352–6370. [PubMed: 23859074]
35. Staker BL, Feese MD, Cushman M, Pommier Y, Zembower D, Stewart L, Burgin AB. Structures of Three Classes of Anticancer Agents Bound to the Human Topoisomerase I-DNA Covalent Complex. J Med Chem. 2005; 48:2336–2345. [PubMed: 15801827]
36. Holbeck SL, Collins JM, Doroshow JH. Analysis of Food and Drug Administration-approved Anticancer Agents in the NCI60 Panel of Human Tumor Cell Lines. Mol Cancer Ther. 2010; 9:1451–1460. [PubMed: 20442306]
37. SYBYL-X. Tripos, Inc; St. Louis, MO: 2009.
38. Halgren TA. Merck Molecular Force Field. 1. Basis, Form, Scope, Parameterization, and Performance of MMFF94. J Comput Chem. 1996; 17:490–519.

39. Verdonk ML, Cole JC, Hartshorn MJ, Murray CW, Taylor RD. Improved Protein-Ligand Docking Using GOLD. Protein Struct Funct Genet. 2003; 52:609–623.

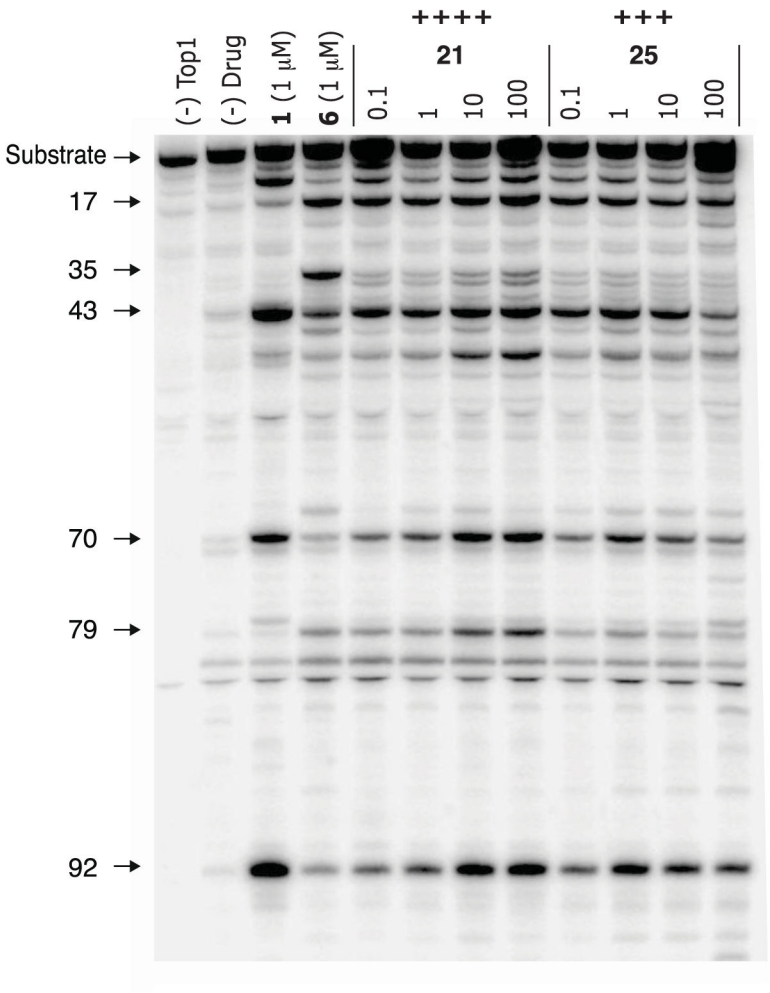


Figure 1.

Top1-mediated DNA cleavage assay gel. From left: lane 1, DNA alone; lane 2, DNA and Top1; lane 3, DNA and Top1 and 1 μM **1**; lane 4, DNA and Top1 and 1 μM **6**; remaining lanes, DNA and Top1 and indicated concentration (μM) of test compound. The numbers and arrows at the left indicate cleavage site positions on the DNA substrate (see Experimental Section). Gel-based assays are performed twice for each active compound, and they are always run with positive controls (i.e., **1** and **6**).

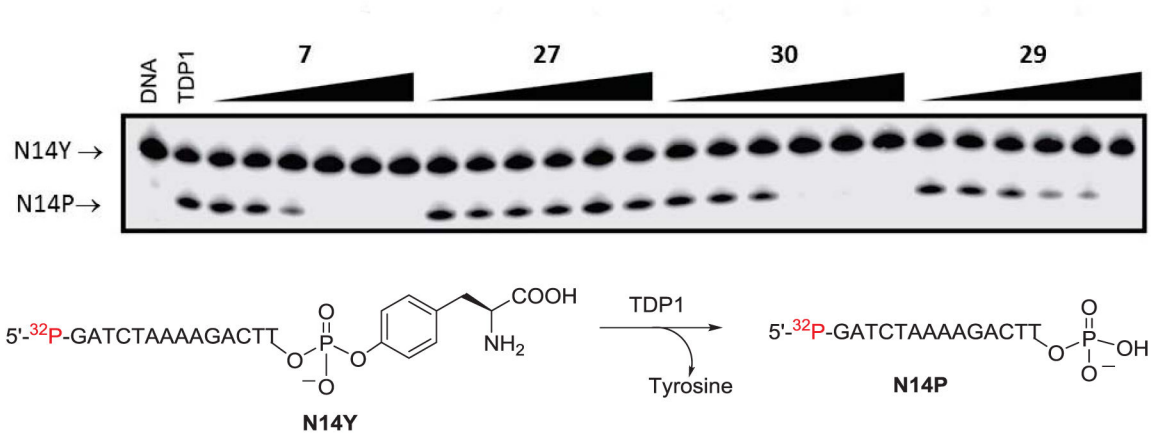


Figure 2.

TDP1 inhibition assay gel. The concentrations of positive control **7** and test compounds were 0.46, 1.4, 4.1, 12.3, 37, 111 μM (left to right). N14Y is 5′-end labeled DNA oligonucleotide with 3′ phosphotyrosyl, and N14P is 5′-end labeled DNA oligonucleotide with a 3′-phosphate group (see Experimental Section).

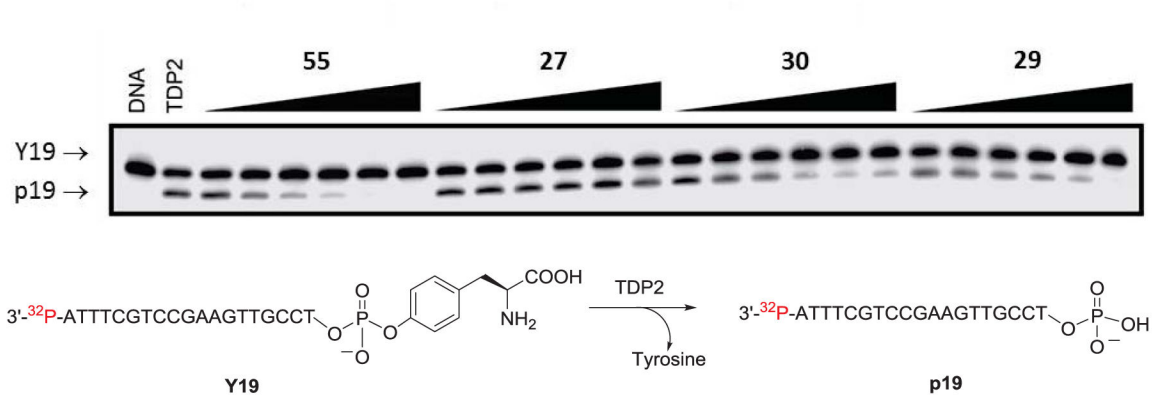


Figure 3.

TDP2 inhibition assay gel. The concentrations of positive control **55**[34] were 0.005, 0.017, 0.05, 0.15, 0.46, 1.4 μM, and the concentrations for the test compounds were 0.46, 1.4, 4.1, 12.3, 37, 111 μM (left to right). The TDP2 substrate Y19 corresponds to a 3′-end labeled DNA oligonucleotide with a 5′ phosphotyrosyl, and the TDP2 product p19 corresponds to a 3′-end labeled DNA oligonucleotide with a 5′ phosphate group (see Experimental Section).

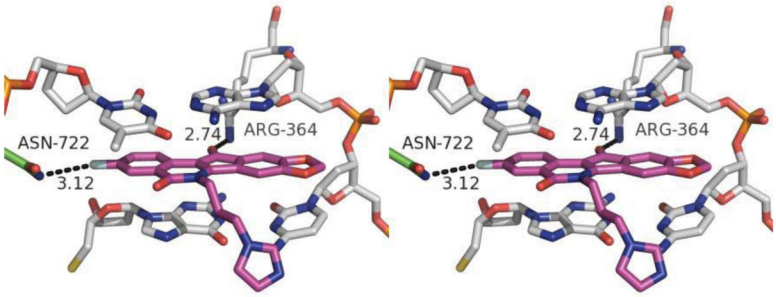
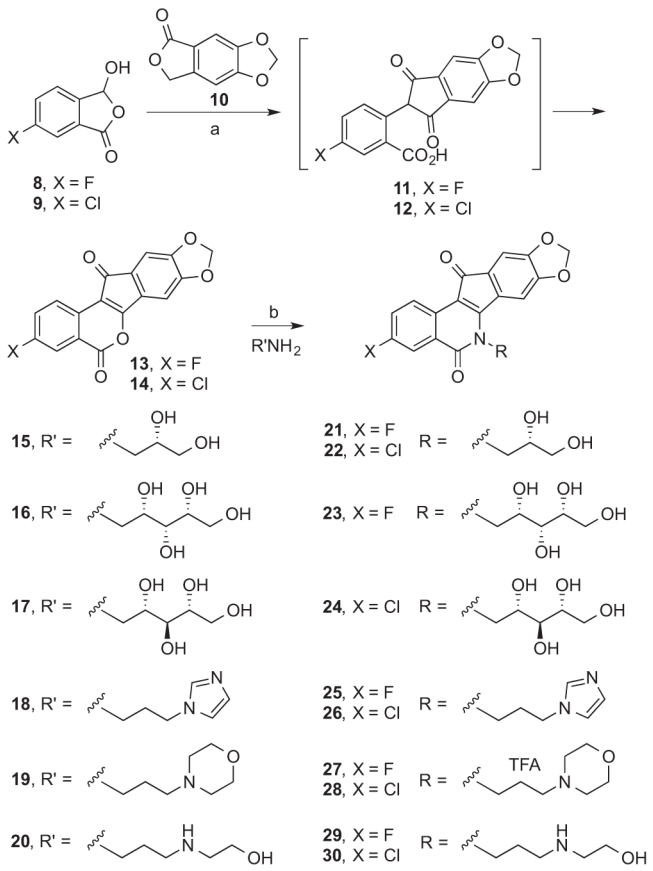


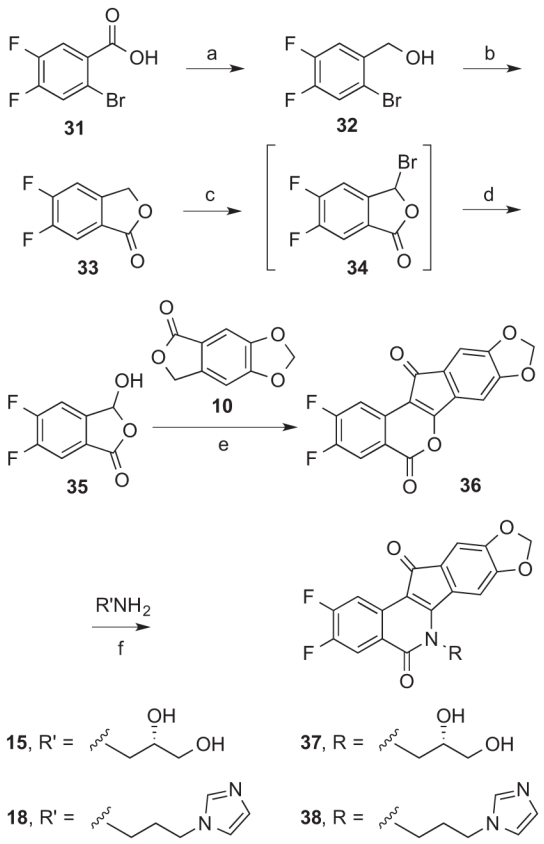
Figure 4.

Energy-minimized hypothetical binding pose of **25** (purple) within the X-ray crystal structure of a stalled Top1-DNA cleavage complex co-crystallized with **56** (PDB ID 1SC7).[35] Potential hydrogen-bonding interactions are indicated by dashed lines. Heavy atom distances (in Å) appear next to the dashed lines. The stereoview is programmed for wall-eyed (relaxed) viewing.



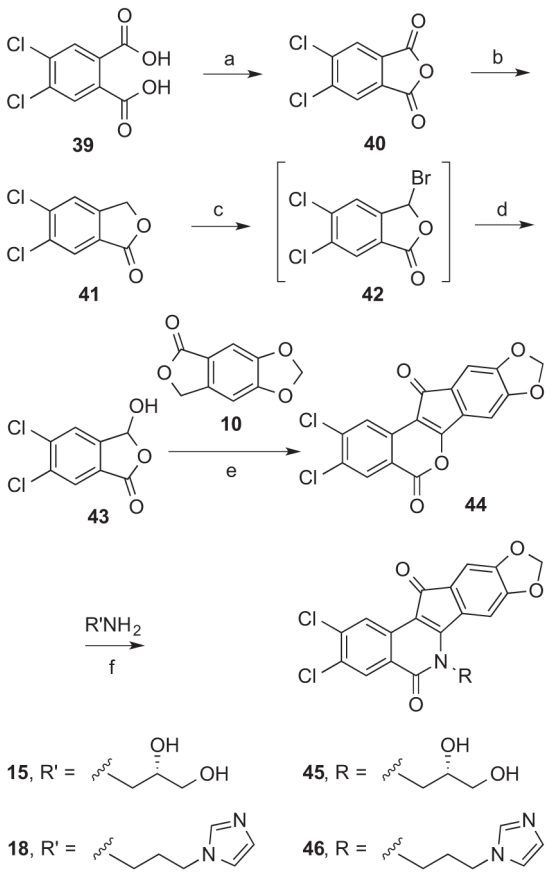
Scheme 1.

[a.] [a]Reagents and conditions: (a) i. NaOMe, MeOH, EtOAc, reflux; ii. Ac_2O, reflux; (b) $CHCl_3$, MeOH, reflux.



Scheme 2.

[a.] [a]Reagents and conditions: (a) BH_3, THF, reflux; (b) i. CuCN, DMF, reflux; ii. H_2O, reflux; (c) i. NBS, AIBN, CCl_4, reflux; (d) H_2O, reflux; (e) i. NaOMe, MeOH, EtOAc, reflux; ii. Ac_2O, 90 °C; (f) $CHCl_3$, MeOH, reflux.



Scheme 3.

[a.] [a]Reagents and conditions: (a) AcCl, reflux; (b) $NaBH_4$, THF, 0 °C to room temp.; (c) NBS, AIBN, CCl_4, reflux; (d) i. KOH in H_2O, reflux; ii. $NaHSO_4$ (e) i. NaOMe, MeOH, EtOAc, reflux; ii. Ac_2O, 90 °C; (f) $CHCl_3$, MeOH, reflux.

Table 1

Antiproliferative and Top1 Poisoning Activities of Indenoisoquinolines

			Cytotoxicity (GI_{50}, μM)								
	compd	**Top1 Cleavage**[a]	**Lung HOP-62**	**Colon HCT-116**	**CNS SF-539**	**Melanoma UACC-62**	**Ovarian OVCAR-3**	**Renal SN12C**	**Prostate DU-145**	**Breast MCF7**	**MGM**[b]
	4[11]	++++	1.78	1.15	0.040	0.030	74.1	0.813	0.155	N.D.[c]	4.64 ± 1.25
	5[11]	++++	<0.010	<0.010	0.037	<0.010	0.085	<0.010	<0.010	N.D.	0.079 ± 0.023
	6[13]	++++	0.01	0.15	0.09	0.03	1.50	0.01	0.02	N.D	0.21 ± 0.19
3-NO_2	**47**[28]	++++	< 0.010	< 0.010	< 0.010	0.017	0.302	< 0.010	< 0.010	0.025	0.090 ± 0.04
	21	++++	0.049	0.079	0.051	0.045	0.182	0.076	0.102	0.032	0.105
	23	++	0.032	0.035	0.029	0.025	0.100	0.079	0.051	0.012	0.078
3-F	**25**	+++	< 0.010	< 0.010	< 0.010	< 0.010	0.019	< 0.010	< 0.010	< 0.010	0.011
	27	+									N.S.[d]
	29	++	0.036	0.019	0.041	0.028	0.076	0.107	0.033	< 0.010	0.055
	22	++	0.022	0.020	0.025	0.012	0.062	0.027	0.032	< 0.010	0.042
	24	+									N.T.[e]
3-Cl	**26**	++	< 0.010	< 0.010	< 0.010	< 0.010	0.040	< 0.010	< 0.010	< 0.010	0.024
	28	+									N.S.
	30	+++	0.071	0.105	0.240	0.043	1.32	1.07	0.059	0.018	0.229
2,3-F	**37**	++	0.022	0.037	0.029	0.015	0.224	0.033	0.041	< 0.010	0.068
	38	+	< 0.010	< 0.010	< 0.010	< 0.010	0.115	< 0.010	< 0.010	< 0.010	0.030
2,3-Cl	**45**	++	< 0.010	< 0.010	< 0.010	< 0.010	0.016	< 0.010	< 0.010	< 0.010	0.013
	46	+	< 0.010	< 0.010	< 0.010	< 0.010	0.043	< 0.010	< 0.010	< 0.010	0.017
	48[d, 19]	++									
	49[19]	+++	0.490	0.309	0.417	0.245	1.29	0.437	0.575	0.135	0.692
2.75	**50**[19]	+++	2.09	1.29	2.95	1.38	4.27	2.34	2.95	0.347	2.75
	51[19]	++	0.692	0.437	0.417	0.398	2.24	0.977	2.09	0.257	1.12
	52[19]	+++	0.186	0.107	0.204	0.074	0.407	0.110	0.309	0.031	0.229

		Cytotoxicity (GI_{50}, μM)								
compd	**Top1 Cleavage**[a]	**Lung HOP-62**	**Colon HCT-116**	**CNS SF-539**	**Melanoma UACC-62**	**Ovarian OVCAR-3**	**Renal SN12C**	**Prostate DU-145**	**Breast MCF7**	**MGM**[b]
53[19]	++	2.14	1.62	2.40	3.31	6.03	5.01	4.07	0.372	4.17

[a] Compound-induced DNA cleavage due to Top1 poisoning, with scores given according to the following system based on the activity of 1 μM **1**: 0, no activity; +, between 20 and 50% activity; ++, between 50 and 75% activity; +++, between 75 and 95% activity; ++++, equal activity; ++++(+), greater activity.

[b] Mean graph midpoint of growth inhibition from the 5 concentration assay, ranging from 10^{-8}–10^{-4} M, for all 60 cell lines tested.

[c] N.D. = not determined.

[d] N.S. = not selected for 5 concentration assay.

[e] N.T. = not tested in the NCI-60.

Table 2

IC_{50} Determinations for Indenoisoquinolines **29** and **30** versus TDP1 and TDP2

Compound	TDP1 IC_{50}, μM	TDP2 IC_{50}, μM
29	6.2, 11.2 (n = 2)	6.3, 14.1 (n = 2)
30	6.3 ± 1.4 (n = 3)	8.9, 9.3 (n = 2)