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Investigation of the Structure-Activity Relationships of Aza-A-Ring Indenoisoquinoline Topoisomerase I Poisons

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Abstract

Several indenoisoquinolines have shown promise as anticancer agents in clinical trials. Incorporation of a nitrogen atom into the indenoisoquinoline scaffold offers the possibility of favorably modulating ligand-binding site interactions, physicochemical properties, and biological activities. Four series of aza-A-ring indenoisoquinolines were synthesized in which the nitrogen atom was systematically rotated to all four possible locations. The resulting compounds were tested to establish the optimal nitrogen position for topoisomerase IB (Top1) enzyme poisoning activity and cytotoxicity to human cancer cells. The 4-aza compounds were the most likely to yield derivatives with high Top1 inhibitory activity. However, the cytotoxicity was more complicated, since the potency was influenced strongly by the side chains on the lactam nitrogen. The most cytotoxic azaindenoisoquinolines **45** and **46** had nitrogen in the 2- or 3-positions and a 3′-dimethylaminopropyl side chain, and they had MGM GI_{50} values that were slightly better than the corresponding indenoisoquinoline **64**.

Graphical abstract

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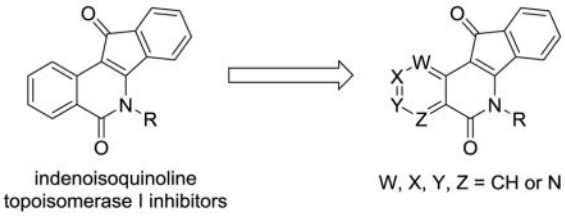
Supporting Information

SMILES molecular formula strings. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare the following competing financial interest(s): Mark Cushman is on the Board of Directors 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(s) to disclose.



INTRODUCTION

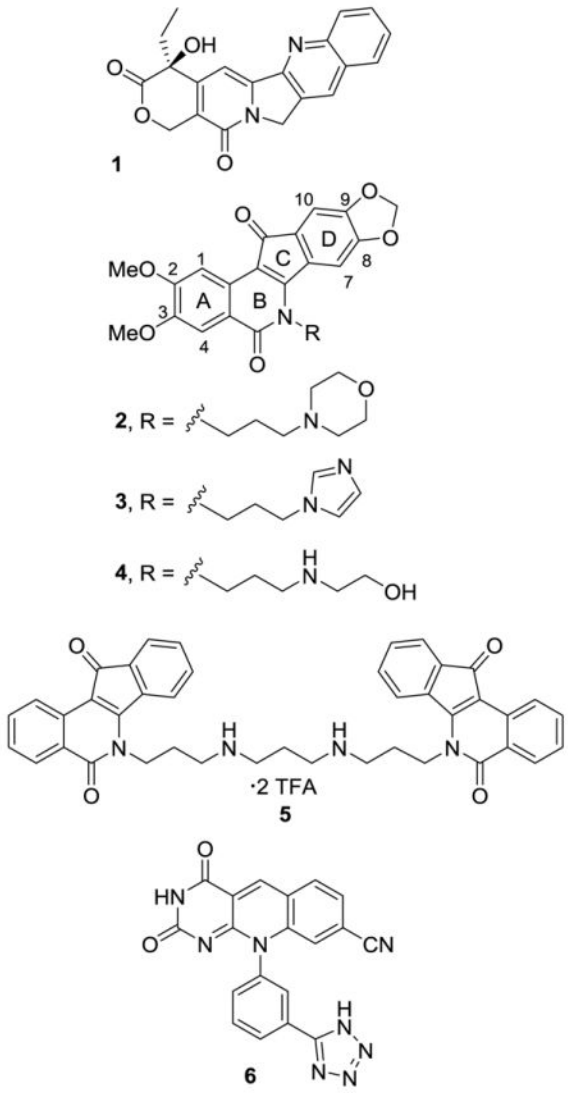
DNA relaxation catalyzed by topoisomerase IB (Top1) is essential for replication and transcription in eukaryotic cells. During this enzyme-mediated process, Tyr723 carries out a nucleophilic attack on a phosphodiester of DNA, resulting in the cleavage of a single strand of DNA and a product in which the enzyme is covalently linked through a 3-phosphodiester.[1, 2] Normally, cleavage complexes reverse rapidly and are undetectable in cells. However, DNA damage and certain cancer chemotherapeutic agents known as Top1 poisons can stabilize the cleavage complexes by preventing their reversal.[3, 4] As a result of the extended lifetimes of the cleavage complexes, advancing replication forks can collide with the DNA cleavage sites and produce DNA double-strand breaks.[5] The DNA damage eventually causes the cell to enter apoptosis.[3, 6]

Several distinct Top1 poison chemotypes have been developed since the discovery of the natural product camptothecin (**1**) and its unique mechanism of action.[7–10] Two derivatives of camptothecin are FDA-approved drugs used for the treatment of solid tumors, and several analogues are being investigated for the treatment of various cancers.[11] The potent anticancer activities of the members of this class are counterbalanced by problems with physicochemical properties, drug resistance, and patient tolerability. The shortcomings of the camptothecins include: (1) poor water solubility; (2) instability of the E-ring lactone at physiological pH, which hydrolyzes to a hydroxyacid that binds to plasma proteins; (3) rapid diffusion from their binding site in the Top1-DNA cleavage complex, which may necessitate longer drug infusion times in order to maintain adequate concentrations of the ternary cleavage complexes; (4) dose-limiting toxicities including bone marrow suppression and severe dose-limiting diarrhea; (5) susceptibility to drug resistance by several Top1 point mutations;[12] and (6) efficient removal from cancer cells by drug efflux pumps that results in drug resistance.[3]

These limitations have resulted in the discovery of improved Top1 poisons. Two compounds first synthesized in our laboratory, the indenoisoquinolines indotecan (LMP 400, **2**) [13] and indimitecan (LMP776, **3**),[13] have been promoted to Phase I clinical trials at the National Cancer Institute.[14, 15] A third, structurally related indenoisoquinoline known as MJ-III-65 (LMP744, **4**)[16, 17] has shown promising preclinical activity. The indenoisoquinolines overcome many of the drawbacks associated with the camptothecins, with improvements that include: (1) greater chemical stability; (2) longer residence times in the binding site;[16] (3) retention of activity versus camptothecin-resistant Top1 mutants;[16] and (4) diminished or completely inhibited ejection by drug efflux pumps.[3]

The structure-activity relationships of the indenoisoquinoline Top1 poisons have not been fully investigated in the area of scaffold atom substitution. Previously, the carbon atoms at positions 7, 8, 9, or 10 were replaced by a nitrogen atom (please see structures **2–4** for position numbering and ring lettering).[18–20] The working hypothesis for the design of these compounds was that the **π**-**π** stacking interactions between the drug and the neighboring base pairs in the ternary DNA-drug-Top1 complex would be strengthened by instillation of electronegative nitrogen atoms in the aromatic system that would facilitate charge transfer complex formation between the neighboring base pairs and the drug. It was found that: (1) certain aza-D-ring indenoisoquinolines have approximately 10-fold increased aqueous solubility relative to indenoisoquinoline analogues without compromising their Top1 and growth inhibitory activities;[19] (2) particular compounds exhibit equal or greater activity than **1** in the Top1-mediated DNA cleavage assay;[20] and (3) cancer cell growth inhibition can be achieved at nanomolar concentrations, as measured by mean graph midpoint (MGM) GI_{50} values in the NCI-60 assay.[20] In the present investigation, the pharmacological effects of carbon-to-nitrogen substitution at positions 1, 2, 3, or 4 of the indenoisoquinoline A-ring were systematically investigated. This necessitated the creation of viable synthetic pathways to make sufficient quantities of the desired compounds using practical methods. The target "azaindenoisoquinolines" compounds are formally novel indenonaphthyridinediones. The 3-azaindenoisoquinoline system has recently been reported,[21] while the 1-, 2-, and 4-azaindenoisoquinolines are novel heterocyclic systems that have never been synthesized and studied before.

A second motivation for this study was to investigate the structural requirements for potent tyrosyl DNA phosphodiesterase 1 and 2 (TDP1 and TDP2) inhibition by indenoisoquinolines. TDP1 and TDP2 are DNA repair enzymes that process Top1- and Top2-mediated DNA lesions, respectively. TDP1 catalyzes the hydrolysis of the 3′-phosphotyrosyl-DNA linkages that result from degradation of Top1-DNA cleavage complexes. TDP2 catalyzes the hydrolysis of the 5′-phosphotyrosyl-DNA linkages that result from degradation of Top2-DNA cleavage complexes and it also displays weak activity against 3′-phosphotyrosyl-DNA linkages. There are currently no promising TDP2 inhibitor series. A series of deazaflavins (e.g. **6**) with low nanomolar TDP2 inhibitory potencies was recently reported. However, the authors remarked that the chemical series is marred by poor cellular permeability.[22] TDP1 and TDP2 can serve as mutual backups for the repair of stalled Top1-DNA cleavage complexes,[23] which would make dual TDP1 and TDP2 inhibition a significant advancement. Bis(indenoisoquinoline) **5** displays potent Top1 inhibitory activity and its IC_{50} values versus purified and whole cell extract-containing TDP1 are each approximately 1 μM,[24, 25] and a number of monomeric indenoisoquinolines have also displayed TDP1 inhibitory activity, suggesting that the azaindenoisoquinolines should also be investigated for TDP1 and TDP2 inhibitory activity.[26–28]



CHEMISTRY

The strategy used to synthesize aza-A-ring indenoisoquinolines centered on the preparation of key tetracyclic lactone precursors, such as **13**. An advantage of this approach is that several azaindenoisoquinolines that differ at the lactam nitrogen side chain can be prepared using a divergent pathway from a single common intermediate. It was anticipated that the location of the nitrogen atom in the azaphthalide intermediates (e.g. **8**) could be controlled by regioselective reactions of carbonyl groups attached to C-2 and C-4 as opposed to C-3 of the pyridine ring system.[29–31] This would ultimately dictate the locations of the nitrogen in the final products.

The lactone **13** with a nitrogen atom in the 1-position was made by the route outlined in Scheme 1. Quinolinic acid anhydride **7** was regioselectively reduced with $NaBH_4$ in THF-AcOH, and the intermediate hydroxyacid was cyclized under the acidic reaction conditions to yield 4-azaphthalide (**8**). The 55% yield of **8** obtained this way was superior to the 15–

25% yields reported by LAH reduction of **7** followed by sublimation.[30, 32] The latter was monobrominated with NBS to afford **9**,[32] which was then hydrolyzed to provide 4-aza-3-hydroxyphthalide (**10**). Condensation of **10** with phthalide (**11**) under basic conditions involving a Dieckmann condensation sequence[33, 34] generated indanedione intermediate **12**, which was treated with refluxing Ac_2O to close the B-ring, yielding the lactone **13**.

The synthesis of lactone **21** began with isonicotinic acid (**14**, Scheme 2). After the formation of *N*-phenyl amide **15**, lithiation and functionalization with DMF provided lactam **16**. Reduction of lactam **16** with $NaBH_4$ in MeOH delivered **17**, and lactonization in aqueous HCl yielded azapthalide **18**. From this point, oxidation with NBS to afford **19**, hydrolysis to yield **20**, and condensation of **20** with **11** followed by cyclization of the resulting intermediate in refluxing Ac_2O produced the final product **21**.

The synthesis of lactone **26** was accomplished according to our previously published procedure (Scheme 3).[21]

The final lactone **31** was produced starting from anhydride **7** (Scheme 4). Heating the latter to reflux in MeOH yielded half-ester **27**.[30] Activation of the carboxylic acid present in **27** with CDI, followed by selective reduction of the mixed anhydride and cyclization yielded 7-azaphthalide **28**. Oxidation with NBS afforded **29**, followed by hydrolysis of the bromide to yield **30**.[32] Condensation of **30** with phthalide (**11**) as before gave the desired lactone **31**. The regioselectivities of the key carbonyl reactions observed in Schemes 1, 3, and 4 (**7** → **8**, **22** → **23**, and **7** → **27**) are the result of the greater reactivity of carbonyl substituents at C-2 and C-4 vs. C-3 on the pyridine ring, which is a consequence of the greater electronegativity of the nitrogen atom vs. a carbon atom.[29–31]

Lactones **13**, **21**, **26**, and **31** were condensed with primary amines **32**–**35** in $CHCl_3$, with or without MeOH as a co-solvent, to yield aza-A-ring indenoisoquinolines **36**–**39**, **40**–**43**, **44**–**47**, and **48**–**51** (Scheme 5). The syntheses of compounds **38**, **42**, and **46** were previously published.

Lastly, lactones **13**, **21**, **26**, and **31** were each condensed with *N*-Boc-1,3-diaminopropane (**52**), and the resulting intermediates **53**–**56** were deprotected with 5 N HCl in MeOH and $CHCl_3$ to yield aza-A-ring indenoisoquinoline dihydrochloride salts **57**–**60** (Scheme 6).[21] The selection of a three-carbon polymethylene linker between the lactam nitrogen and the side chain nitrogen in the final products is consistent with prior studies indicating that the optimal length is 2–4 atoms in comparable systems.[35]

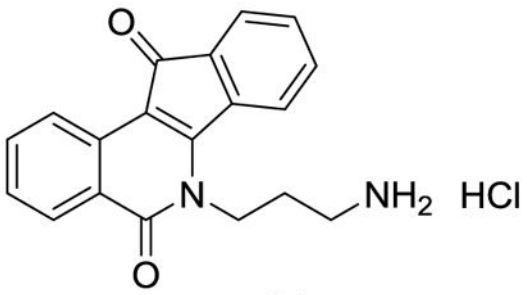
BIOLOGICAL RESULTS AND DISCUSSION

The Top1-mediated DNA cleavage assay was used to measure Top1 inhibitory activity. This assay measures the ability of the compound to stabilize DNA cleavage in a 3′-[^{32}P]-labeled DNA substrate via Top1 poisoning. Putative Top1 poisons are tested at 0.1, 1, 10, and 100 μM concentrations, alongside positive controls camptothecin (**1**) and **4** at 1 μM concentration. In the absence of a Top1 poison, Top1 can execute its DNA relaxation mechanism and Top1-DNA cleavage complexes are not trapped. However, in the presence of

a Top1 poison, Top1-DNA cleavage complexes are stabilized through intercalation of the Top1 poisons between the base pairs at the cleavage site and, following denaturation and gel electrophoresis, the cleaved DNA strands can be visualized as cleavage bands on the gel. The intensity and amount of cleavage bands observed are used to assign a semiquantitative score to a test agent's Top1 inhibitory activity. A representative gel is shown in Figure 1. The footnote under Figure 1 provides further detail on the scoring system used in this assay, and the Experimental Section gives a description of the experimental protocol.[36]

Top1 scores were generally moderate (++) for 1-, 2-, and 3-azaindenoisoquinolines, with only five exceptions out of these 15 compounds (Table 1). The 4-azaindenoisoquinolines performed best in the Top1 assay, and these five compounds (**37**, **42**, **48**, **49**, and **59**) have a greater average Top1 score (2.4) than any of the other azaindenoisoquinolines series prepared in this study. The worst side chain for Top1 activity appears to be 2′*S*-propanediol: three of the four compounds with this moiety were the worst in their respective series. The 2′*S*-propanediol side chain is the most polar of the five side chains, both by computed polar surface area and effect on ClogP (calculated in ChemBioDraw[37]). This would be to the detriment of the hydrophobic effect that helps to drive the binding of an indenoisoquinoline to the Top1-DNA cleavage complex.

TDP1 is involved in the repair of DNA damage that results from the action of Top1 poisons, which induce the formation of DNA-protein adducts that include 3′-phosphotyrosyl linkages. The TDP1 gel-based assay assesses the ability of a compound to inhibit the enzyme-induced cleavage of the phosphotyrosyl-DNA bond at the 3′ end of the DNA (N14Y, Fig. 2). Cleavage of the phosphodiester bond between the 3′-phosphate and tyrosine generates a 3′-phosphate DNA product (N14P, Fig. 2). Compounds are tested in a concentration-dependent manner and inhibition of TDP1 is represented by disappearance of the gel band corresponding to the N14P product (Fig. 2). A schematic representation of the assay and a representative gel are shown in Figure 2, and the IC_{50} values for the inhibition of TDP1 are listed in Table 1.



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TDP2, as opposed to TDP1, cleaves the phosphotyrosyl linkage at the 5′-end of DNA. The latter DNA-protein adducts are produced when topoisomerase II (Top2) is trapped on the DNA in a cleavage complex. Correspondingly, the TDP2 gel-based assay measures the ability of a drug to inhibit the cleavage of a 5′-phosphotyrosyl-DNA bond by TDP2 (Y19, Fig. 3). Upon catalytic cleavage of the phosphotyrosyl linkage, a 5′-phosphate DNA product

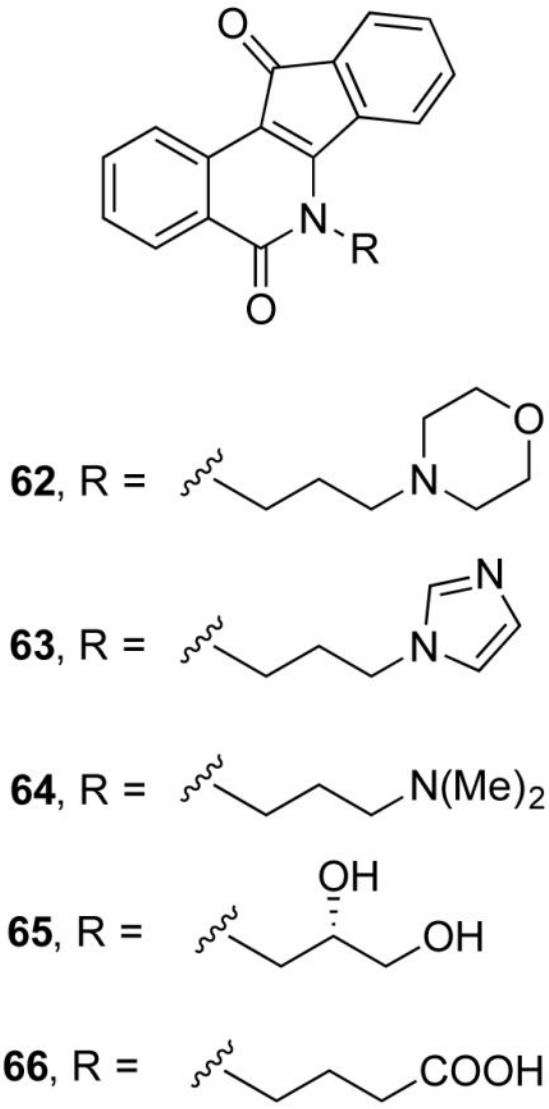
is generated (p19, Fig. 3). Both a schematic representation of the assay and a representative gel are displayed in Figure 3.

TDP1 and TDP2 inhibitory activity was observed only for analogues with sterically undemanding 3′-dimethylaminopropyl or 3′-aminopropyl side chains. This observation is consistent with our previous findings.[21, 26]

The new indenoisoquinolines were evaluated for cancer cell growth inhibitory activities in The National Cancer Institute's human cytotoxicity assay, which measures the ability of test agents to inhibit the growth of approximately 60 different cancer cell lines. In the initial single-dose assay, cells are treated with a 10 μM dose of test agent. The growth of treated cells versus untreated cells is then compared. If a test agent induces a sufficiently low mean growth percent, it is promoted to five-dose testing to determine a mean graph midpoint (MGM) GI_{50} value. In five-dose testing, cells are treated with test agent concentrations ranging from 10^{-8} to 10^{-4} M. The concentration required to achieve 50% growth inhibition in a particular cell line is calculated. In situations in which this value is greater than 100 μM or less than 0.01 μM, it is recorded as 100 μM and 0.01 μM, respectively. The values for each of the tested cell lines are averaged to obtain a mean graph midpoint (MGM) GI_{50} value. MGM GI_{50} values are often, but not always, determined from two separate rounds.[38]

The mean growth percentages observed after 48-hour incubation at 10 micromolar concentration at are listed in Table 1. On the basis of this one-concentration testing, the most active compounds are **45** (−8.83%), **46** (−9.25%), **47** (5.45%), and **60** (12.2%). These four of the compounds met the NCI criteria for testing in the five-dose cytotoxicity assay. Compounds **45**–**47** contain the 3′-dimethylaminopropyl side chain and compound **60** contains the aminopropyl side chain. Compounds **46**, **47**, and **60** displayed single-digit micromolar GI_{50} against most of the cell lines, and compound **45** had sub-micromolar activity (Table 2).

The regular (non-aza) indenoisoquinoline analogues **61**–**65** were previously studied[39, 40] and their relevant biological data are shown in Table 3. Their average Top1 score is +++, and **63** was scored at ++++. Although the non-aza compounds are superior in terms of Top1 activity, their MGM GI_{50} values are not substantially greater than those of the tested azaindenoisoquinolines. In fact, the 3′-dimethylaminopropyl non-aza compound **64** (MGM 1.86 μM) is slightly less cytotoxic than the three identically substituted azaindenoisoquinolines **45** (2-aza, MGM 0.437 μM), **46** (3-aza, MGM 0.977 μM), and **47** (4-aza, MGM 1.26 μM). Most of the compounds in Table 3 show MGM GI_{50} values that are greater than 1 μM, and only one that is submicromolar. Although many of these compounds are more potent versus Top1 than their azaindenoisoquinolines counterparts, this did not always translate to more potent antiproliferative activities.



The docking model for **43** bound to the Top1-DNA cleavage complex indicates that the ligand adopts nearly the same binding orientation as **66** in its co-crystal structure with Top1 and DNA.[41] The nitrogen in the 4-position is oriented toward the major groove, and faces toward the solvent. The clustering of hydrophilic functionality in this position may contribute to the 4-azaindenoisoquinolines' greater average Top1 scores compared to the other regioisomers. The clustering theoretically decreases the extent of desolvation that must occur for these ligands to bind the target or pass through the cell membrane interior. No specific interactions between the side chain imidazole and the surrounding DNA and enzyme were observed, and the side chain projects into the solvent-filled major groove of the DNA. The ligand's aromatic ring system is calculated to form van der Waals interactions with the flanking DNA base pairs, and the ligand's C-ring carbonyl oxygen is calculated to engage Arg364 in a hydrogen bonding interaction.

An attempt was also made to correlate the calculated DNA binding energies with the Top1 inhibitory activities using an MP2 quantum mechanics model that had previously been successful in predicting indenoisoquinoline and camptothecin DNA binding site orientations and DNA binding site selectivities,[42–44] as well as the the Top1 inhibitory activities of camptothecin isomers and camptothecin-lactam.[45] However, this approach proved to be of no value in correlating calculated binding energies with Top1 inhibitory potencies (data not shown).

CONCLUSION

Practical synthetic pathways were successfully pioneered to the 1-, 2-, 3-, and 4-azaindenoisoquinolines and the resulting series of compounds were tested as Top1 poisons, TDP1 and TDP2 inhibitors, and as cytotoxic agents in human cancer cell cultures. These compounds allowed the determination of the most favorable location for the nitrogen atom in the A-ring. The average Top1 scores were greatest for the 4-azaindenoisoquinoline series. The aza-A-ring indenoisoquinolines were generally inactive against TDP1 and TDP2, although sterically undemanding aminopropyl side chains were found to impart some degree of activity. Comparisons against regular (non-aza) indenoisoquinolines show that Top1 inhibition scores are diminished by nitrogen atom incorporation, although MGM GI_{50} values for the most cytotoxic compounds were slightly better for the azaindenoisoquinolines in most cases. The incorporation of nitrogen in the 2-, 3-, or 4-position led to moderate improvement in cytotoxicity in human cancer cell cultures. The anticancer activities were highly dependent on the side chain attached to the lactam nitrogen, with the 3′-dimethylaminopropyl substituent being the most favorable. A molecular modeling study showed that a nitrogen in the 4-position is clustered with other polar functionality at the edge of the indenoisoquinoline that faces the solvent-filled major groove. These molecules might therefore require less desolvation upon cleavage site binding.

EXPERIMENTAL SECTION

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 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 areas, which were ≥ 95% of the combined total peak areas. Compounds **23**–**26**, **38**, **42**, and **46** were prepared according to previously reported procedures.[21]

4-Azaphthalide (8)

$NaBH_4$ (1.14 g, 33.5 mmol) was added to a solution of quinolinic acid anhydride (**7**, 5.0 g, 33.5 mmol) in THF (35 mL) at 15 °C under argon. Acetic acid (4 g, 67 mmol) was added dropwise and the resulting mixture was stirred at 15 °C for 4 h. The solvent was removed in vacuo. The residue was dissolved in acetic acid (13.5 mL) and acetic anhydride (13.5 mL) and the resulting solution was stirred for 3 h at 100 °C. The mixture was concentrated in vacuo and the residue was dissolved in a solution of H_2O (35 mL) + NaCl (6.7 g). The water phase was extracted with $CHCl_3$ (3 × 40 mL) and the combined organic layers were concentrated. Recrystallization from *i*-PrOH yielded compound **8** (2.5 g, 55%) as a light yellow solid: mp 123–125 °C. IR (KBr) 1778, 1567, 1423, 1355, 1000, 743 $cm^{-1;}$ ^1H NMR (300 MHz, $CDCl_3$) δ 8.87 (dd, J = 1.5, 4.9 Hz, 1 H), 8.22 (dd, J = 1.5, 7.7 Hz, 1 H), 7.50 (dd, J = 5.0, 7.8 Hz, 1 H), 5.33 (s, 2 H); ESIMS *m/z* (rel intensity) 136 (MH^+, 100).

4-Aza-3-bromophthalide (9)

Compound **8** (1.0 g, 7.40 mmol) was heated at reflux with NBS (1.44 g, 8.1 mmol) and AIBN (20 mg) in dry CCl_4 (40 mL) for 2 h. The reaction mixture was cooled to room temperature, the succinate salts were filtered off, and the filtrate was concentrated and purified by silica gel flash column chromatography (75:25 EtOAc-hexanes) to afford product **9** (1.4 g 87%) as a colorless syrup. IR (film) 1782, 1594, 1428, 986, 667 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 8.99 (dd, J = 1.4, 4.9 Hz, 1 H), 8.26 (dd, J = 1.4, 7.8 Hz, 1 H), 7.59 (dd, J = 4.8, 7.8 Hz, 1 H), 7.38 (s, 1 H); CIMS *m/z* (rel intensity) 214 (MH^+, 100).

4-Aza-3-hydroxyphthalide (10)

Compound **9** (1.4 g, 6.5 mmol) was heated at reflux in H_2O (40 mL) for 2 h before the solvent was evaporated to afford compound **10 (**0.9 g, 91%) as a light yellow solid: mp 209–211 °C. IR (KBr) 3145, 1784, 1721, 1619, 1214, 1076, 765 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ 8.94 (dd, J = 1.5, 4.9 Hz, 1 H), 8.29 (dd, J = 1.4, 7.8 Hz, 1 H), 7.69 (dd, J = 4.9, 7.7 Hz, 1 H), 6.61 (s, 1 H); ESIMS *m/z (*rel intensity) 152 (MH^+, 100).

General Procedure A

The appropriate aza-3-hydroxyphthalides (0.5 g, 3 mmol) and phthalide (**11**, 0.41 g, 3.1 mmol) were diluted in EtOAc (15 mL). Sodium metal (0.35 g, 15 mmol) was dissolved in MeOH (30 mL) and the solution was added to reaction mixture. The solution was heated at reflux for 24 h, cooled to room temperature, acidified with 37% HCl (3–4 mL), and concentrated. The obtained solid was diluted with Ac_2O (20 mL) and the mixture was heated at reflux for 6 h. The solution was concentrated, diluted with $CHCl_3$ (100 mL), and washed with sat. $NaHCO_3$ (3 × 50 mL). The organic layer was washed with sat. NaCl (75 mL), dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (9:1 $CHCl_3$-hexanes) to yield product **13**, **21**, or **31**.

1-Azaindeno[1,2-*c*]isochromene-5,11-dione (13)

Following general procedure A, **13** (0.125 g, 15%) was obtained as an orange-red solid: mp 167–168 °C. IR (KBr) 1754, 1712, 1492, 998, 695 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ

8.94 (d, J = 4.5 Hz, 1 H), 8.24 (d, J = 7.0 Hz, 2 H), 7.53 (m, 4 H); ESIMS m/z (rel intensity) 250 (MH^+, 100); HRESIMS calcd for $C_{15}H_8NO_3$ 250.0504 (MH^+), found 250.0501.

N-Phenylisonicotinamide (15)

A solution of isonicotinic acid (**14**, 5.0 g, 40.6 mmol) in thionyl chloride (40 mL) was heated at reflux for 2 h. After completion of the reaction, thionyl chloride was removed under reduced pressure. THF (50 mL), K_2CO_3 (16.8 g, 121.9 mmol) and aniline (3.78 g, 40.6 mmol) were added to the residue and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layer was concentrated and the residue was recrystallized from EtOAc-hexanes (60:40) to afford the product **15** (8.0 g, 99%) as a light yellow solid: mp 166–168 °C. IR (KBr) 1344, 1653, 1465, 665 $cm^{-1;}$ ^1H NMR (DMSO-d_6, 300 MHz) δ 10.49 (s, 1 H), 8.78 (m, 2 H), 7.86 (m, 2 H), 7.78 (m, 2 H), 7.39 (t, J = 8.0 Hz, 2 H), 7.15 (t, J = 6.5 Hz, 1 H).

3-Hydroxy-2-phenyl-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridin-1-one (16)

A 2.5 M *n*-BuLi solution in hexanes (17.8 mL, 44.4 mmol) was added to a solution of **15** (4.0 g, 20 mmol) in dry THF (120 mL) at −78 °C. The solution was held at −78 °C for 0.5 h and then allowed to rise to 0 °C and kept at 0 °C for 6 min. The mixture was cooled to −78 °C and DMF (2.94 mL, 40.4 mmol) was added. After 1 h at −78 °C, the reaction mixture was warmed to 0 °C and kept at 0 °C for 1 h. H_2O (40 mL) was added, the organic layer was separated, and the water layer was extracted with $CHCl_3$ (2 × 40 mL). The combined organic layer was dried over Na_2SO_4, concentrated, and purified by silica gel flash column chromatography (60:40 EtOAc-hexanes) to afford compound **16** (3.0 g, 67%) as an off-white solid: mp 209–211 °C. IR (KBr) 3356, 1721, 776 $cm^{-1;}$ ^1H NMR (DMSO-d_6, 300 MHz) δ 8.97 (s, 1 H), 8.86 (d, J = 4.9 Hz, 1 H), 7.77 (m, 3 H), 7.48 (t, J = 6.1 Hz, 2 H), 7.28 (t, J = 7.5 Hz, 1 H), 7.10 (br s, 1 H), 6.69 (s, 1 H).

3-(Hydroxymethyl)-*N*-phenylisonicotinamide (17)

Compound **16** (3.0 g, 13 mmol) was dissolved in MeOH (65 mL), $NaBH_4$ (0.902 g, 26.4 mmol) was added and the mixture was stirred at room temperature for 5 h. MeOH was evaporated under vacuum and water (28 mL) was added to the residue. This mixture was extracted with EtOAc (2 × 100 mL). The combined organic layer was dried over Na_2SO_4, concentrated and purified by silica gel flash column chromatography (70:30 EtOAc-hexanes) to afford compound **17** (2.5 g, 82%) as a light yellow syrup. IR (KBr) 3451, 1675, 666 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ 9.77 (s, 1 H), 8.60 (d, J = 5.1 Hz, 1 H), 8.46 (s, 1 H), 7.67 (m, 3 H), 7.38 (t, J = 7.5 Hz, 2 H), 7.17 (t, J = 6.6 Hz, 1 H), 4.72 (s, 2 H).

5-Azaphthalide (18)

Compound **17** (2.5 g, 11 mmol) was added to hydrochloric acid (15%, 22 mL), and the reaction mixture was heated at 60 °C for 2 d. The mixture was adjusted to pH 5–6 ($NaHCO_3$). The solution was extracted with $CHCl_3$ (3 × 75 mL). The combined organic layer was dried over Na_2SO_4, concentrated and purified by silica gel flash column chromatography (60:40 EtOAc-hexanes) to afford compound **18** (1.0 g, 68%) as a light

yellow solid: mp 101–103 °C. IR (KBr) 1778, 1589, 1423, 1003, 741 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ 8.94 (s, 1 H), 8.85 (d, *J* = 4.9 Hz, 1 H), 7.79 (d, *J* = 4.9 Hz, 1 H), 5.43 (s, 2 H); ESIMS *m/z* (rel intensity) 136 (MH^+, 100).

5-Aza-3-bromophthalide (19)

Compound **18** (1.0 g, 7.4 mmol) was combined with NBS (1.4 g, 8.1 mmol) and AIBN (20 mg) in dry CH_2Cl_2-CCl_4 (10:50 mL) and the mixture was heated to reflux for 48 h. The reaction mixture was cooled to room temperature, solids were filtered off, and the filtrate was concentrated and purified by silica gel flash column chromatography (50:50 EtOAc-hexanes) to afford **19** (0.750 g, 51%) as a light brown syrup. IR (film) 1784, 1587, 1422, 667 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.05 (s, 1 H), 8.94 (d, *J* = 5.0 Hz, 1 H), 7.82 (d, *J* = 5.1 Hz, 1 H), 7.48 (s, 1 H); CIMS *m/z* (rel intensity) 214 (MH^+, 100).

5-Aza-3-hydroxyphthalide (20)

Compound **19** (0.75 g, 3.5 mmol) was heated to reflux in H_2O (25 mL) for 2 h before the solvent was evaporated to afford compound **20 (**0.500 g, 95%) as a light yellow thick syrup: IR (film) 3215, 1726, 1622, 1078, 756 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ 9.01 (s, 1 H) 8.78 (d, *J* = 3.7 Hz, 1 H), 8.22 (d, *J* = 7.7 Hz, 1 H), 6.67 (s, 1 H); ESIMS *m/z* (rel intensity) 152 (MH^+, 100).

2-Azaindeno[1,2-*c*]isochromene-5,11-dione (21)

Following general procedure A, **21** (0.400 g, 48%) was obtained as an orange-red solid: mp 208–209 °C. IR (KBr) 1755, 1702, 1490, 996, 691 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ 9.69 (s, 1 H), 8.80 (d, *J* = 5.1 Hz, 1 H), 8.04 (d, *J* = 5.1 Hz, 1 H), 7.63 (dd, *J* = 1.0, 7.4 Hz, 1 H), 7.49 (m, 3 H); ESIMS *m/z* (rel intensity) 250 (MH^+, 100); HRESIMS calcd for $C_{15}H_8NO_3$ 250.0504 (MH^+), found 250.0503.

2-(Methoxycarbonyl)nicotinic Acid (27)

Compound **7** (5.0 g, 34 mmol) was dissolved in MeOH (25 mL). The mixture was heated to reflux for 2 h and the solvent was then removed. The resulting white solid was dissolved in EtOAc (25 mL) at reflux and filtered to remove insoluble byproducts. The filtrate was concentrated in vacuo and recrystallized from EtOAc to provide **27** (4.5 g, 71%) as a white solid: mp 157–159 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.84 (dd, *J* = 1.7, 5.0 Hz, 1 H), 8.34 (dd, *J* = 1.6, 7.9 Hz, 1 H), 7.57 (dd, *J* = 5.0, 8.1 Hz, 1 H), 4.00 (s, 3 H).

7-Azaphthalide (28)

A solution of compound **27** (4.5 g, 25 mmol) in THF (100 mL) was treated with CDI (5.35 g, 33.1 mmol) at 0 °C. After 1 h, $NaBH_4$ (1.40 g, 41.2 mmol) was added in portions. The mixture was stirred for 2 h and then quenched carefully with MeOH. EtOAc (50 mL) was added. The organic layer was washed with water (75 mL) and brine (25 mL), dried over dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash chromatography (80:20 EtOAc-hexanes) to give **28** (1.5 g, 45%) as a white solid: mp 142–144 °C. IR (KBr) 1785, 1577, 1005, 754 $cm^{-1;}$ ^1H NMR (300 MHz, $CDCl_3$) δ 8.87 (d, *J* =

4.6 Hz, 1 H), 7.95 (d, J = 8.4 Hz, 1 H), 7.59 (dd, J = 4.7, 7.3 Hz, 1 H), 5.38 (s, 2 H); ESIMS *m/z* (rel intensity) 136 (MH^+, 100).

7-Aza-3-bromophthalide (29)

Compound **28** (1.0 g, 7.4 mmol) was heated to reflux with NBS (2.10 g, 12.1 mmol) and AIBN (30 mg) in dry CCl_4 (60 mL) for 24 h. The reaction mixture was cooled to room temperature, solids were filtered off, and the filtrate was concentrated and purified by silica gel flash column chromatography (60:40 hexanes-EtOAc) to afford product **29** (0.550 g 42%) as a white solid: mp 112–113 °C. IR (KBr) 1782, 1590, 982, 665 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ 8.96 (d, J = 3.5 Hz, 1 H), 8.03 (dd, J = 1.3, 8.0 Hz, 1 H), 7.69 (dd, J = 4.8 Hz, 8.0 Hz, 1 H), 7.43 (s, 1 H); CIMS *m/z* (rel intensity) 214 (MH^+, 100).

7-Aza-3-hydroxyphthalide (30)

Compound **29** (0.75 g, 3.5 mmol) was heated at reflux in H_2O (25 mL) for 2 h before the solvent was evaporated to afford compound **30 (**0.530 g, 99%) as a light yellow thick syrup. IR (film) 3165, 1729, 1622, 1075, 758 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ 8.89 (d, J = 3.7 Hz, 1 H), 8.19 (d, J = 7.7 Hz, 1 H), 7.77 (m, 1 H), 6.71 (s, 1 H); ESIMS *m/z* (rel intensity) 152 (MH^+, 100).

4-Azaindeno[1,2-*c*]isochromene-5,11-dione (31)

Following general procedure A, **31** (0.350 g, 42%) was obtained as an orange-red solid: mp 262–263 °C. IR (KBr) 1756, 1710, 1367, 998, 692 $cm^{-1;}$ ^1H NMR ($CDC1_3$, 300 MHz) δ 8.86 (dd, J = 1.5, 4.5 Hz, 1 H), 8.70 (dd, J = 1.5, 8.2 Hz, 1 H), 7.71 (m, 1 H), 7.62 (d, J = 6.8 Hz, 1 H), 7.52 (m, 3 H); CIMS (*m/z* rel intensity) 250 (MH^+, 100); HRESIMS calcd for $C_{15}H_8NO_3$ 250.0504 (MH^+), found 250.0506.

General Procedure B

3-Aminopropylmorpholine (**32**, 0.043 g, 0.301 mmol) was added to a solution of the corresponding lactone (0.050 g, 0.2 mmol) in $CHCl_3$ (30 mL). The solution was allowed to stir at reflux temperature for 15 h, diluted with $CHCl_3$ (45 mL) and washed with H_2O (3 × 25 mL) and sat. NaCl (25 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to provide a crude solid. The solid was purified by silica gel flash column chromatography (1% MeOH in $CHCl_3$) to provide the product, **36**, **37**, or **39**.

1-Aza-5,6-dihydro-6-(3-morpholinopropyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (36)

Following general procedure B, **36** (0.057 g, 85%) was obtained as an orange solid: mp 223–224 °C. IR (KBr) 1702, 1658, 1494, 763 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.09 (dd, J = 1.7, 4.5 Hz, 1 H), 8.59 (dd, J = 1.7 Hz, 8.0 Hz, 1 H), 7.82 (m, 1 H), 7.73 (m, 1 H), 7.47 (m, 2 H), 7.39 (m, 1 H), 4.66 (t, J = 7.8 Hz, 2 H), 3.72 (m, 4 H), 2.59 (t, J = 6.3 Hz, 2 H), 2.49 (m, 4 H), 2.11 (m, 2 H); CIMS (*m/z* rel intensity) 376 (MH^+, 100); HRESIMS calcd for $C_{22}H_{22}N_3O_3$ 376.1662 (MH^+), found 376.1667; HPLC purity, 99.39% (1% TFA in MeOH:H_2O, 90:10).

2-Aza-5,6-dihydro-6-(3-morpholinopropyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (37)

Following general procedure B, **37** (0.066 g, 90%) was obtained as an orange-red solid: mp 218–219 °C. IR (KBr) 1723, 1678, 789 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 10.0 (s, 1 H), 8.68 (d, *J* = 5.4 Hz, 1 H), 8.06 (d, *J* = 5.8 Hz, 1 H), 7.78 (d, *J* = 7.8 Hz, 1 H), 7.66 (dd, *J* = 1.3, 6.2 Hz, 1 H), 7.46 (m, 2 H), 4.63 (t, *J* = 7.8 Hz, 2 H), 3.72 (t, *J* = 4.2 Hz, 4 H), 2.60 (t, *J* = 5.9 Hz, 2 H), 2.50 (m, 4 H), 2.08 (m, 2 H); CIMS (*m/z* rel intensity) 376 (MH^+, 100); HRESIMS calcd for $C_{22}H_{22}N_3O_3$ 376.1662 (MH^+), found 376.1663; HPLC purity, 95.54% (1% TFA in MeOH:H_2O, 90:10).

4-Aza-5,6-dihydro-6-(3-morpholinopropyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (39)

Following general procedure B, **39** (0.067 g, 90%) was obtained as an orange-red solid: mp 177–178 °C. IR (KBr) 1707, 1685, 1494, 765 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.03 (dd, *J* = 1.6, 8.3 Hz, 1 H), 8.83 (dd, *J* = 1.6, 4.3 Hz, 1 H), 7.81 (d, *J* = 6.5 Hz, 1 H), 7.64 (m, 2 H), 7.59 (m, 2 H), 4.70 (t, *J* = 7.9 Hz, 2 H), 3.69 (t, *J* = 4.6 Hz, 4 h), 2.59 (t, *J* = 6.2 Hz, 2 H), 2.48 (m, 4 H), 2.09 (m, 2 H); CIMS (*m/z* rel intensity) 376 (MH^+, 100); HRESIMS calcd for $C_{22}H_{22}N_3O_3$ 376.1662 (MH^+), found 376.1664; HPLC purity, 99.49% (1% TFA in MeOH:H_2O, 90:10).

General Procedure C

3-Aminopropylimidazole (**33**, 0.037 g, 0.301 mmol) was added to a solution of the corresponding lactone (0.050 g, 0.2 mmol) in $CHCl_3$ (30 mL). The solution was allowed to stir at reflux temperature for 15 h, diluted with $CHCl_3$ (50 mL) and washed with H_2O (3 × 20 mL) and sat. NaCl (20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to provide a crude solid. The solid was purified by silica gel flash column chromatography (3% MeOH in $CHCl_3$) to provide the product, **40**, **41**, or **43**.

1-Aza-5,6-dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (40)

Following general procedure C, **40** (0.052 g, 82%) was obtained as an orange-red solid: mp 242–243 °C. IR (KBr) 1702, 1667, 1495, 761, 665 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.09 (dd, *J* = 1.9, 4.5 Hz, 1 H), 8.58 (dd, *J* = 1.9, 8.2 Hz, 1 H), 7.71 (d, *J* = 7.0 Hz, 1 H), 7.64 (s, 1 H), 7.43 (m, 3 H), 7.19 (s, 1 H), 7.07 (s, 1 H), 6.75 (d, *J* = 7.5 Hz, 1 H), 4.58 (t, *J* = 7.4 Hz, 2 H), 4.27 (t, *J* = 6.2 Hz, 2 H), 2.43 (m, 2 H); ESIMS (*m/z* rel intensity) 357 (MH^+, 100); HRESIMS calcd for $C_{21}H_{17}N_4O_2$ 357.1352 (MH^+), found 357.1359; HPLC purity, 98.36% (1% TFA in MeOH:H_2O, 90:10).

2-Aza-5,6-dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (41)

Following general procedure C, **41** (0.063 g, 87%) was obtained as an orange-red solid: mp 274–275 °C. IR (KBr) 1708, 1689, 768, 656 $cm^{-1;}$ ^1H NMR ($CDCl_3$ + CD_3OD, 300 MHz) δ 9.86 (s, 1 H), 8.56 (d, *J* = 5.5 Hz, 1 H), 8.02 (d, *J* = 5.5 Hz, 1 H), 7.69 (s, 1 H), 7.57 (dd, *J* = 1.2, 6.8 Hz, 1 H), 7.36 (m, 2 H), 7.06 (d, *J* = 4.2 Hz, 1 H), 6.81 (d, *J* = 7.3 Hz, 1 H), 4.49 (t, *J* = 7.5 Hz, 2 H), 4.21 (t, *J* = 6.5 Hz, 2 H), 2.33 (m, 2 H); ESIMS (*m/z* rel intensity) 357 (MH^+, 100); HRESIMS calcd for $C_{21}H_{17}N_4O_2$ 357.1352 (MH^+), found 357.1354; HPLC purity, 96.57% (1% TFA in MeOH:H_2O, 90:10).

4-Aza-5,6-dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (43)

Following general procedure C, **43** (0.061 g, 86%) was obtained as an orange-red solid: mp 251–252 °C. IR (KBr) 1712, 1687, 778, 666 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.00 (dd, *J* = 1.6, 8.3 Hz, 1 H), 8.84 (dd, *J* = 1.7, 4.4 Hz, 1 H), 7.64 (s, 1 H), 7.62 (m, 2 H), 7.58 (m, 2 H), 7.18 (s, 1 H), 7.07 (s, 1 H), 6.66 (d, *J* = 7.3 Hz, 1 H), 4.60 (t, *J* = 7.5 Hz, 2 H), 4.28 (t, *J* = 6.2 Hz, 2 H), 2.28 (m, 2 H); ESIMS (*m/z* rel intensity) 357 (MH^+, 100); HRESIMS calcd for $C_{21}H_{17}N_4O_2$ 357.1352 (MH^+), found 357.1358; HPLC purity, 99.80% (1% TFA in MeOH:H_2O, 90:10).

General Procedure D

N,N-Dimethylaminopropylamine (**34**, 0.030 g, 0.301 mmol) was added to a solution of corresponding lactone (0.050 g, 0.2 mmol) in $CHCl_3$ (30 mL). The solution was allowed to stir at reflux temperature for 15 h, diluted with $CHCl_3$ (60 mL) and washed with H_2O (3 × 30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to provide a crude solid. The solid was purified by silica gel flash column chromatography (4% MeOH in $CHCl_3$) to afford the product, **44**, **45**, or **47**.

1-Aza-5,6-dihydro-6-(3-(dimethylamino)propyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (44)

Following general procedure D, **44** (0.055 g, 84%) was obtained as an orange-red solid: mp 187–188 °C. IR (KBr) 1704, 1659, 1554, 764 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.09 (dd, *J* = 1.8, 4.6 Hz, 1 H), 8.61 (dd, *J* = 1.8, 8.0 Hz, 1 H), 7.88 (m, 1 H), 7.85 (m, 1 H), 7.49 (m, 2 H), 7.46 (m, 1 H), 4.64 (m, 2 H), 2.52 (t, *J* = 6.5 Hz, 2 H), 2.29 (s, 6 H), 2.06 (m, 2 H); CIMS (*m/z* rel intensity) 334 (MH^+, 100); HRESIMS calcd for $C_{20}H_{20}N_3O_2$ 334.1556 (MH^+), found 334.1562; HPLC purity, 98.32% (1% TFA in MeOH:H_2O, 90:10).

2-Aza-5,6-dihydro-6-(3-(dimethylamino)propyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (45)

Following general procedure D, **45** (0.059 g, 86%) was obtained as an orange-red solid: mp 177–178 °C. IR (KBr) 1709, 1667, 1492, 768 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 10.0 (s, 1 H), 8.68 (d, *J* = 5.2 Hz, 1 H), 8.07 (d, *J* = 5.3 Hz, 1 H), 7.81 (d, *J* = 7.2 Hz, 1 H), 7.66 (d, *J* = 7.0 Hz, 1 H), 7.47 (m, 2 H), 4.61 (m, 2 H), 2.54 (t, *J* = 6.5 Hz, 2 H), 2.06 (m, 2 H); CIMS (*m/z* rel intensity) 334 (MH^+, 100); HRESIMS calcd for $C_{20}H_{20}N_3O_2$ 334.1556 (MH^+), found 334.1560; HPLC purity, 98.21% (1% TFA in MeOH:H_2O, 90:10).

4-Aza-5,6-dihydro-6-(3-(dimethylamino)propyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (47)

Following general procedure D, **47** (0.056 g, 84%) was obtained as an orange-red solid: mp 183–184 °C. IR (KBr) 1712, 1678, 1489, 767 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.03 (dd, *J* = 1.5 Hz, 8.2 Hz, 1 H), 8.82 (dd, *J* = 2.7, 4.2 Hz, 1 H), 7.86 (d, *J* = 7.1 Hz, 1 H), 7.62 (m, 2 H), 7.58 (m, 2 H), 4.67 (m, 2 H), 2.53 (t, *J* = 6.5 Hz, 2 H), 2.28 (s, 6 H), 2.07 (m, 2 H); CIMS (*m/z* rel intensity) 334 (MH^+, 100); HRESIMS calcd for $C_{20}H_{20}N_3O_2$ 334.1556 (MH^+), found 334.1559; HPLC purity, 99.62% (1% TFA in MeOH:H_2O, 90:10).

(2′*S*)-1-Aza-5,6-dihydro-6-(2′,3′-dihydroxypropyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (48)

Lactone **13** (54 mg, 0.22 mmol) was suspended with stirring in $CHCl_3$ (10 mL) and MeOH (2.5 mL). Amine **35** (32 mg, 0.35 mmol) dissolved in MeOH (0.5 mL) was added to the

suspension and it was stirred with heating to reflux for 17.5 h. The mixture was cooled to room temperature and concentrated in vacuo. H_2O (5 mL) was added and the suspension was filtered to collect the solid. Compound **48** (27 mg, 39%) was obtained as a red-orange solid: mp 245–250 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.00 (dd, J = 4.5, 1.9 Hz, 1 H), 8.50 (dd, J = 8.1, 1.9 Hz, 1 H), 8.14 (d, J = 7.3 Hz, 1 H), 7.67 – 7.42 (m, 4 H), 5.15 (d, J = 5.0 Hz, 1 H), 5.00 (t, J = 5.6 Hz, 1 H), 4.58 (d, J = 6.7 Hz, 2 H), 4.10 – 3.90 (m, 1 H), 3.60 (t, J = 5.4 Hz, 2 H); ESIMS *m/z* (rel intensity) 345 (MNa^+, 100); HRESIMS *m/z* calcd for $C_{18}H_{14}N_2O_4Na$ 345.0852 (MNa^+), found 345.0861; HPLC purity, 100% (MeOH, 100).

(2′*S*)-2-Aza-5,6-dihydro-6-(2′,3′-dihydroxypropyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (49)

Lactone **21** (50 mg, 0.20 mmol) was suspended with stirring in $CHCl_3$ (10 mL) and MeOH (2.5 mL). Amine **35** (26 mg, 0.29 mmol) dissolved in MeOH (0.5 mL) was added to the suspension and it was stirred with heating to reflux for 23 h. The mixture was cooled to room temperature and concentrated in vacuo. H_2O (1 mL) was added and the suspension was filtered to provide a collect solid. Compound **49** (27 mg, 42%) was obtained as a red-orange solid: mp 229–232 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.82 (d, J = 1.0 Hz, 1 H), 8.66 (d, J = 5.3 Hz, 1 H), 8.07 (d, J = 7.4 Hz, 1 H), 8.01 (dd, J = 5.4, 1.0 Hz, 1 H), 7.61 – 7.46 (m, 3 H), 5.14 (d, J = 5.0 Hz, 1 H), 5.01 (t, J = 5.6 Hz, 1 H), 4.60 – 4.46 (m, 2 H), 4.05 – 3.92 (m, 1 H), 3.65 – 3.53 (m, 2 H); CIMS *m/z* (rel intensity) 323 (MH^+, 100); HRESIMS *m/z* calcd for $C_{18}H_{15}N_2O_4$ 323.1032 (MH^+), found 323.1045; HPLC purity, 100% (MeOH, 100).

(2′*S*)-3-Aza-5,6-dihydro-6-(2′,3′-dihydroxypropyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (50)

Lactone **26** (11 mg, 0.044 mmol) was suspended with stirring in $CHCl_3$ (1 mL) and a solution of the amine **35** (7 mg, 0.08 mmol) in MeOH (1 mL) was added. The mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with 95:5 $CHCl_3$-MeOH, to yield **50** (6 mg, 42%) as a yellow-orange solid: mp 237–238 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.32 (s, 1 H), 8.79 (d, J = 5.5 Hz, 1 H), 8.32 (dd, J = 5.5, 0.9 Hz, 1 H), 8.17 (d, J = 7.3 Hz, 1 H), 7.68 – 7.48 (m, 3 H), 5.17 (d, J = 5.0 Hz, 1 H), 5.02 (t, J = 5.6 Hz, 1 H), 4.65 – 4.46 (m, 2 H), 4.07 – 3.93 (m, 1 H), 3.60 (t, J = 5.3 Hz, 2 H); ESIMS *m/z* (rel intensity) 323 (MH^+, 100); HRESIMS *m/z* calcd for $C_{18}H_{15}N_2O_4$ 323.1032 (MH^+), found 323.1024; HPLC purity, 100% (MeOH, 100).

(2′*S*)-4-Aza-5,6-dihydro-6-(2′,3′-dihydroxypropyl)-5,11-dioxo-indeno[1,2-*c*]isoquinoline (51)

Lactone **31** (46 mg, 0.18 mmol) was suspended with stirring in $CHCl_3$ (10 mL) and MeOH (2.5 mL). A solution of the amine **35** (21 mg, 0.23 mmol) in MeOH (0.5 mL) was added to the suspension and the mixture was stirred at room temperature for 2 h and with heating to reflux for 23 h. The mixture was cooled to room temperature and concentrated in vacuo. H_2O (1 mL) was added and the suspension was filtered to yield a solid. Compound **51** (27 mg, 45%) was obtained as a yellow-orange solid: mp 247–255 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.90 (dd, J = 8.3, 1.7 Hz, 1 H), 8.78 (dd, J = 4.3, 1.7 Hz, 1 H), 8.09 (d, J = 7.5 Hz, 1 H), 7.77 (dd, J = 8.3, 4.3 Hz, 1 H), 7.62 – 7.41 (m, 3 H), 5.14 (d, J = 5.0 Hz, 1 H), 5.00 (t, J = 5.6 Hz, 1 H), 4.65 – 4.43 (m, 2 H), 4.09 – 3.94 (m, 1 H), 3.60 (t, J = 5.3 Hz, 2 H);

ESIMS *m/z* (rel intensity) 323 (MH^+, 100); HRESIMS *m/z* calcd for $C_{18}H_{15}N_2O_4$ 323.1032 (MH^+), found 323.1038; HPLC purity, 100% (MeOH, 100).

General Procedure E

A solution of *N*-Boc-1,3-diaminopropane (**52**, 0.068 g, 0.4 mmol) in $CHCl_3$ (10 mL) was added to the appropriate lactone (0.050 g, 0.2 mmol) in $CHCl_3$ (25 mL). The reaction mixture was heated at reflux for 24 h. After completion of the reaction, $CHCl_3$ was removed under reduced pressure. The crude product was purified by silica gel flash column chromatography (95:5 $CHCl_3$-MeOH) to afford Boc-protected compounds **53**–**56**.

1-Aza-6-(*N*-Boc-3-aminopropyl)-5,6-dihydro-5,11-dioxo-indeno[1,2-*c*]isoquinoline (53)

Following general procedure E, **53** (0.075 g, 92%) was obtained as an orange solid: mp 202–203 °C. IR (KBr) 1711, 1698, 1676, 665 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.13 (dd, *J* = 1.7, 4.5 Hz, 1 H), 8.62 (dd, *J* = 1.7, 8.1 Hz, 1 H), 7.75 (d, *J* = 8.1 Hz, 1 H), 7.63 (d, *J* = 8.0 Hz, 1 H), 7.50 (m, 2 H), 7.41 (m, 1 H), 4.66 (t, *J* = 7.0 Hz, 2 H), 3.29 (m, 2 H), 2.13 (m, 2 H), 1.45 (s, 9 H); CIMS (*m/z* rel intensity) 406 (MH^+, 100).

2-Aza-6-(*N*-Boc-3-aminopropyl)-5,6-dihydro-5,11-dioxo-indeno[1,2-*c*]isoquinoline (54)

Following general procedure E, **54** (0.072 g, 90%) was obtained as an orange-red solid: mp 184–185 °C. IR (KBr) 1715, 1685, 1675, 666 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 10.0 (s, 1 H), 8.70 (d, *J* = 5.4 Hz, 1 H), 8.10 (d, *J* = 5.4 Hz, 1 H), 7.69 (d, *J* = 6.5 Hz, 1 H), 7.59 (d, *J* = 6.8 Hz, 1 H), 7.49 (m, 2 H), 4.63 (t, *J* = 7.0 Hz, 2 H), 3.29 (m, 2 H), 2.12 (m, 2 H), 1.45 (s, 9 H); CIMS (*m/z* rel intensity) 406 (MH^+, 100).

3-Aza-6-(*N*-Boc-3-aminopropyl)-5,6-dihydro-5,11-dioxo-indeno[1,2-*c*]isoquinoline (55)

Following general procedure E, **55** (0.070 g, 86%) was obtained as an orange-red solid: mp 176–177 °C. IR (KBr) 1708, 1694, 1671, 665 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.50 (s, 1 H), 8.75 (d, *J* = 5.5 Hz, 1 H), 8.40 (d, *J* = 5.6 Hz, 1 H), 7.89 (m, 1 H), 7.68 (m, 1 H), 7.50 (m, 2 H), 4.61 (m, 2 H), 2.53 (t, *J* = 6.4 Hz, 2 H), 2.10 (m, 2 H), 1.46 (s, 9 H); CIMS (*m/z* rel intensity) 406 (MH^+, 100).

4-Aza-6-(*N*-Boc-3-aminopropyl)-5,6-dihydro-5,11-dioxo-indeno[1,2-*c*]isoquinoline (56)

Following general procedure E, **56** (0.077 g, 95%) was obtained as an orange solid: mp 197–198 °C. IR (KBr) 1721, 1659, 1634, 656 $cm^{-1;}$ ^1H NMR ($CDCl_3$, 300 MHz) δ 9.06 (d, *J* = 8.3 Hz, 1 H), 8.86 (dd, *J* = 1.5 Hz, 4.3 Hz, 1 H), 7.66 (m, 3 H), 7.58 (m, 2 H), 4.69 (t, *J* = 6.6 Hz, 2 H), 3.26 (m, 2 H), 2.12 (m, 2 H), 1.43 (s, 9 H); CIMS (*m/z* rel intensity) 406 (MH^+, 100).

General Procedure F

Appropriate Boc-protected azaindenoisoquinolines **53–56 (**0.065 g, 0.160 mmol) in $CHCl_3$ (20 mL) were treated with 5 N HCl in MeOH (4 mL). The reaction mixture was stirred at room temperature for 6 h. After completion of the reaction, the solvents were removed under reduced pressure and the crude product was washed with 10% MeOH in $CHCl_3$ (20 mL) and filtered to afford the dihydrochloride salts.

6-(3-Aminopropyl)-1-aza-5,6-dihydro-5,11-dioxo-indeno[1,2-*c*]isoquinoline Dihydrochloride (57)

Following general procedure F, **57** (0.053 g, 88%) was obtained as an orange-red solid: mp 226–228 °C. IR (KBr) 3370, 1696, 1676, 763, 666 $cm^{-1;}$ ^1H NMR (DMSO-d_6, 300 MHz) δ 9.01 (d, J = 4.3 Hz, 1 H), 8.57 (d, J = 8.0 Hz, 1 H), 8.04 (br s, 3 H), 7.91 (d, J = 7.7 Hz, 1 H), 7.64 (m, 4 H), 4.60 (t, J = 7.0 Hz, 2 H), 2.99 (m, 2 H), 2.16 (m, 2 H); ESIMS (*m/z* rel intensity) 306 (MH^+, 100); HRESIMS calcd for $C_{18}H_{16}N_3O_2$ 306.1243 (MH^+), found 306.1247; HPLC purity, 96.16% (1% TFA in MeOH:H_2O, 90:10).

6-(3-Aminopropyl)-2-aza-5,6-dihydro-5,11-dioxo-indeno[1,2-*c*]isoquinoline Dihydrochloride (58)

Following general procedure F, **58** (0.052 g, 86%) was obtained as an orange-red solid: mp 272–274 °C. IR (KBr) 3376, 1698, 1685, 765, 665 $cm^{-1;}$ ^1H NMR (DMSO-d_6, 300 MHz) δ 9.78 (s, 1 H), 8.69 (d, J = 5.4 Hz, 1 H), 8.08 (d, J = 5.3 Hz, 1 H), 7.86 (d, J = 7.8 Hz, 1 H), 7.64 (m, 3 H), 4.93 (br s, 3 H), 4.56 (t, J = 7.1 Hz, 2 H), 2.99 (m, 2 H), 2.15 (m, 2 H); ESIMS (*m/z* rel intensity) 306 (MH^+, 100); HRESIMS calcd for $C_{18}H_{16}N_3O_2$ 306.1243 (MH^+), found 306.1249; HPLC purity, 99.62% (1% TFA in MeOH:H_2O, 90:10).

6-(3-Aminopropyl)-3-aza-5,6-dihydro-5,11-dioxo-indeno[1,2-*c*]isoquinoline Dihydrochloride (59)

Following general procedure F, **59** (0.054 g, 89%) was obtained as an orange-red solid: mp 253–254 °C. IR (KBr) 3410, 1702, 1695, 784, 655 $cm^{-1;}$ ^1H NMR (DMSO-d_6, 300 MHz) δ 9.88 (s, 1 H), 8.78 (d, J = 5.6 Hz, 1 H), 8.18 (d, J = 5.5 Hz, 1 H), 7.94 (d, J = 8.0 Hz, 1 H), 7.74 (m, 3 H), 5.03 (br s, 3 H), 4.68 (t, J = 7.5 Hz, 2 H), 3.05 (m, 2 H), 2.10 (m, 2 H); ESIMS (*m/z* rel intensity) 306 (MH^+, 100); HRESIMS calcd for $C_{18}H_{16}N_3O_2$ 306.1243 (MH^+), found 306.1244; HPLC purity, 96.81% (1% TFA in MeOH:H_2O,90:10).

6-(3-Aminopropyl)-4-aza-5,6-dihydro-5,11-dioxo-indeno[1,2-*c*]isoquinoline Dihydrochloride (60)

Following general procedure F, **60** (0.050 g, 83%) was obtained as an orange solid: mp 284–286 °C. IR (KBr) 3395, 1710, 1698, 765, 666 $cm^{-1;}$ ^1H NMR (D_2O, 300 MHz) δ 8.31 (s, 1 H), 8.13 (br s, 1 H), 7.28 (m, 3 H), 7.10 (br s, 1 H), 6.87 (br s, 1 H), 4.20 (t, J = 6.8 Hz, 2 H), 3.13 (t, J = 6.8 Hz, 2 H), 2.12 (m, 2 H); ESIMS (*m/z* rel intensity) 306 (MH^+, 100); HRESIMS calcd for $C_{18}H_{16}N_3O_2$ 306.1243 (MH^+), found 306.1245; HPLC purity, 98.75% (1% TFA in MeOH:H_2O, 80:20).

Topoisomerase I-Mediated DNA Cleavage Reactions

A 3′-[^{32}P]-labeled 117-bp DNA oligonucleotide was prepared as previously described. The oligonucleotide contains previously identified Top1 cleavage sites in 161-bp pBluescript SK(–) phagemid DNA. Approximately 2 nM 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). Cleavage sites are numbered to reflect actual sites on the 117 bp oligonucleotide.[36]

Recombinant TDP1 Assay

A 5′-[^{32}P]-labeled single-stranded DNA oligonucleotide containing a 3′-phosphotyrosine (N14Y)[46] 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[25]. 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).

Recombinant TDP2 Assay

TDP2 reactions were carried out as described previously[47] 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).

and Modeling Studies

The 1SC7 X-ray crystal structure file was prepared for molecular modeling as previously described.[48] Selected indenoisoquinolines were constructed and optimized using the Tripos forcefield with default parameters in SYBYL.[49] Ligands were docked into the prepared crystal structure using GOLD 3.2.[50] The centroid of the binding site was defined by the crystallized ligand. Ten GOLD algorithm runs were executed per ligand, and default parameters were used. The top ten docking poses per ligand were inspected visually following the docking runs. Energy minimizations were performed for selected ligands in SYBYL. Ligand SYBYL atom types were inspected and corrected as necessary prior to minimization. Minimization was executed by allowing only the ligand to move while freezing the surrounding crystal structure, which was defined as a static set. The details of the minimization were set as follows: Powell method; MMFF94s force field;[51] MMFF94 charges; and 0.05 kcal/mol·Å energy gradient convergence criterion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

CPT	camptothecin
Top1	topoisomerase IB
TDP1	tyrosyl-DNA phosphodiesterase 1
TDP2	tyrosyl-DNA phosphodiesterase 2
CDI	carbonyl diimidazole

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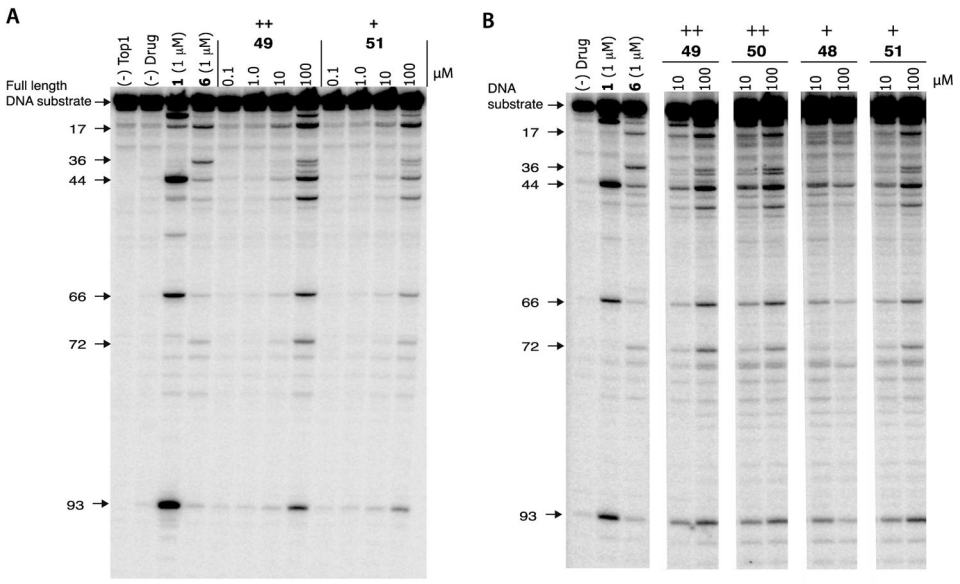


Figure 1.

Representative Top1-mediated DNA cleavage assays. Two independent experiments are shown for the indicated compounds at the indicated concentrations. **A**. Concentration-response for **49** and **51. B**. Comparison of four selected compounds **48**–**5**1 (the gel was cropped to only show the selected concentrations). The numbers and arrows at the left indicate cleavage site positions (see Experimental Section). Gel-based assays include positive controls (i.e., **1** and **6**). The combined intensities of the bands observed at different drug concentrations in the DNA cleavage electrophoresis gels are used to estimate the abilities of the topoisomerase I poisons to stabilize the cleavage complexes through inhibition of the religation reaction at several different DNA cleavage sites. The “+”-based scoring system is based on the activity of 1 μM camptothecin (**1**): 0, no activity; +, between 20 and 50% activity; ++, between 50 and 75% activity at the most effective drug concentration.

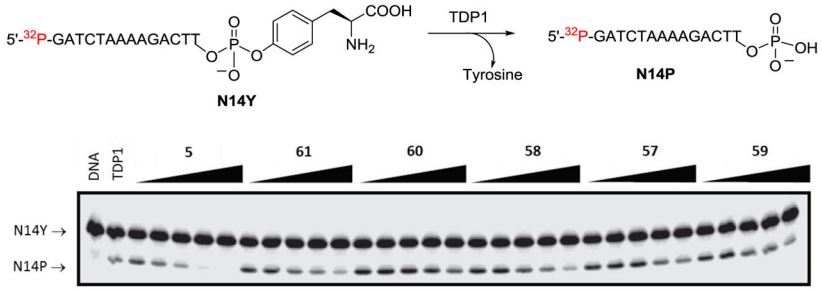


Figure 2.

TDP1 inhibition assay gel. The concentrations of positive controls **5** and **61** and test compounds were 1.4, 4.1, 12.3, 37, and 111 μM (left to right). The 5′-labeled N14Y TDP1 DNA substrate corresponds to a 3′-phosphotyrosyl 14-mer oligonucleotide and the 5′-labeled N14P DNA product corresponds to a 3′-phosphate 14-mer oligonucleotide (see Experimental Section). Gel-based assays are usually run in two independent experiments.

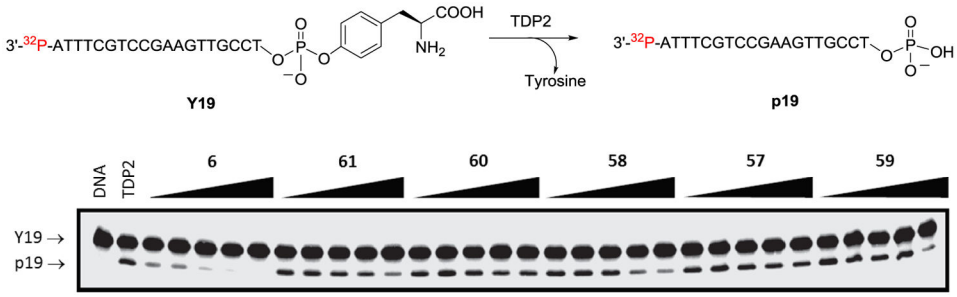


Figure 3.

TDP2 inhibition assay gel. The concentrations of positive control **6** were 0.006, 0.017, 0.05, 0.15, and 0.46 μM and the concentrations of **61** and the test compounds were 0.46, 1.4, 4.1, 12.3, 37, 111 μM (left to right). The 3′-labeled Y19 TDP2 DNA substrate corresponds to a 5′-phosphotyrosyl 19-mer oligonucleotide and the 3′-labeled p19 TDP2 DNA product corresponds to a 5′-phosphate 19-mer oligonucleotide (see Experimental Section).

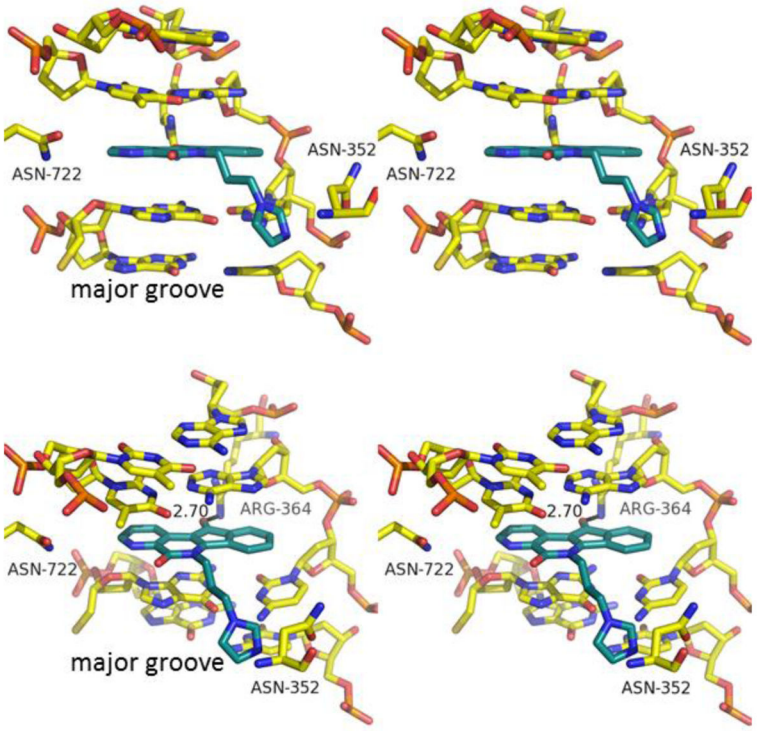
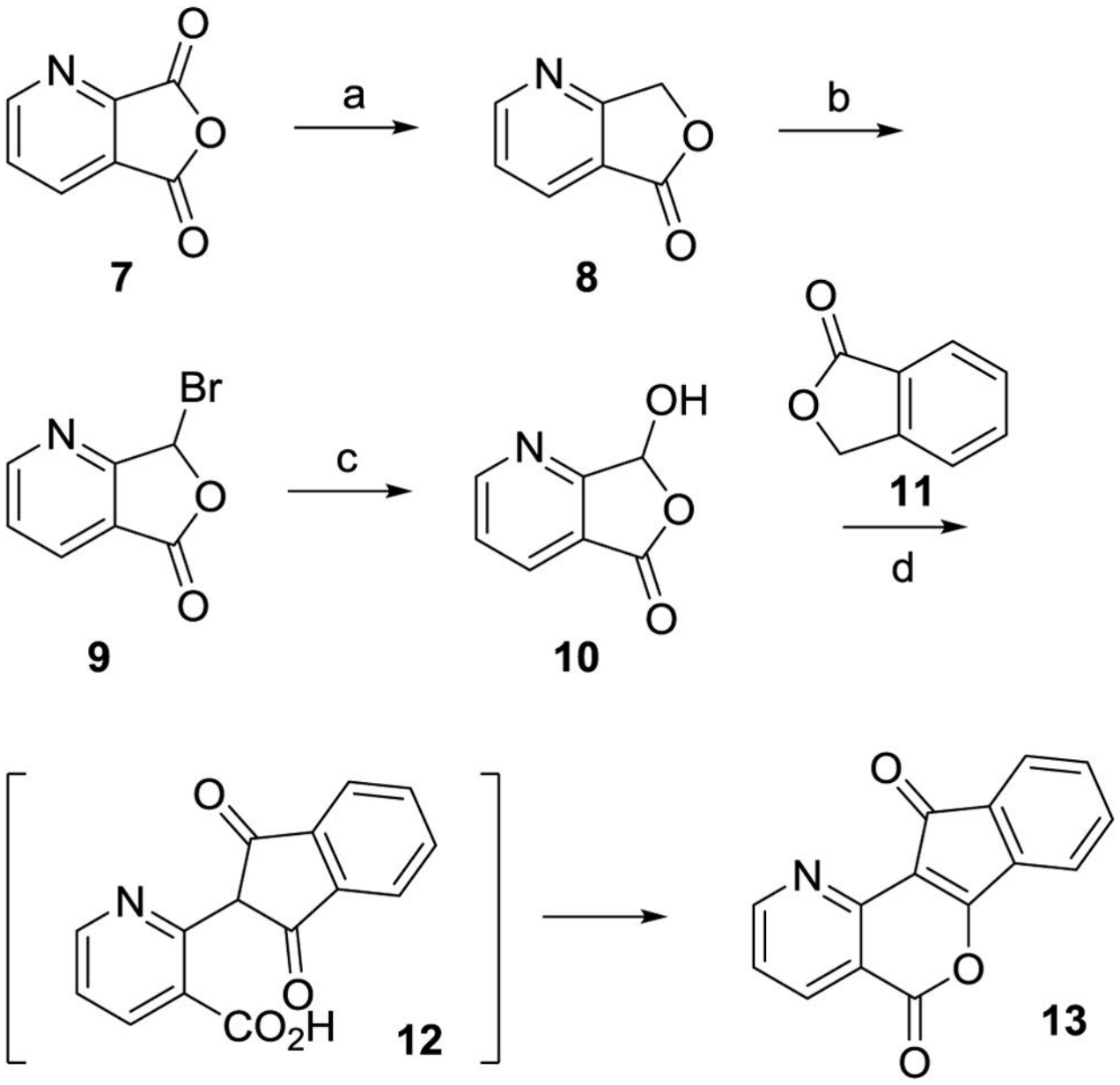


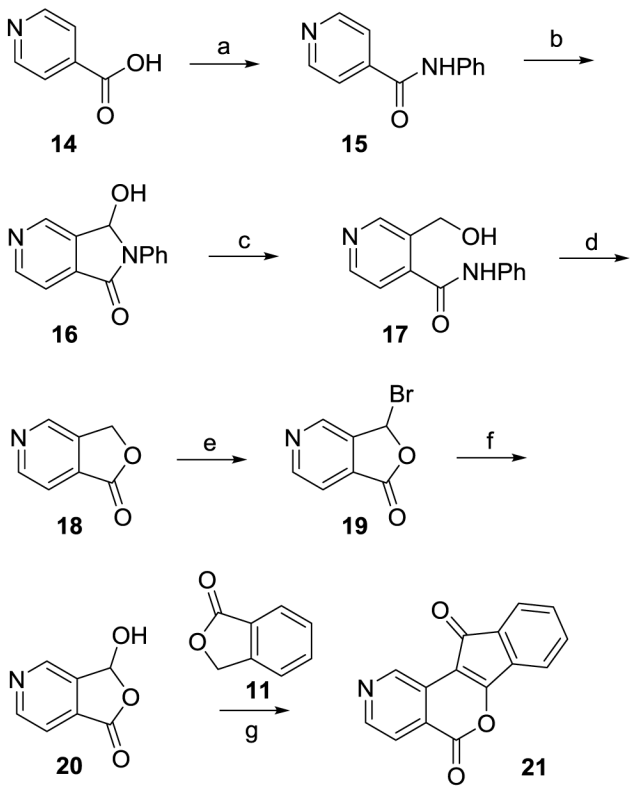
Figure 4.

Energy-minimized hypothetical binding pose of **43** (green) within the X-ray crystal structure of a stalled Top1-DNA cleavage complex co-crystallized with an indenoisoquinoline (**66**, PDB ID 1SC7).[41] The major groove of the DNA is always oriented toward the viewer. Heavy atom distance (in Å) appears next to the dashed line. The stereoviews are programmed for wall-eyed (relaxed) viewing.



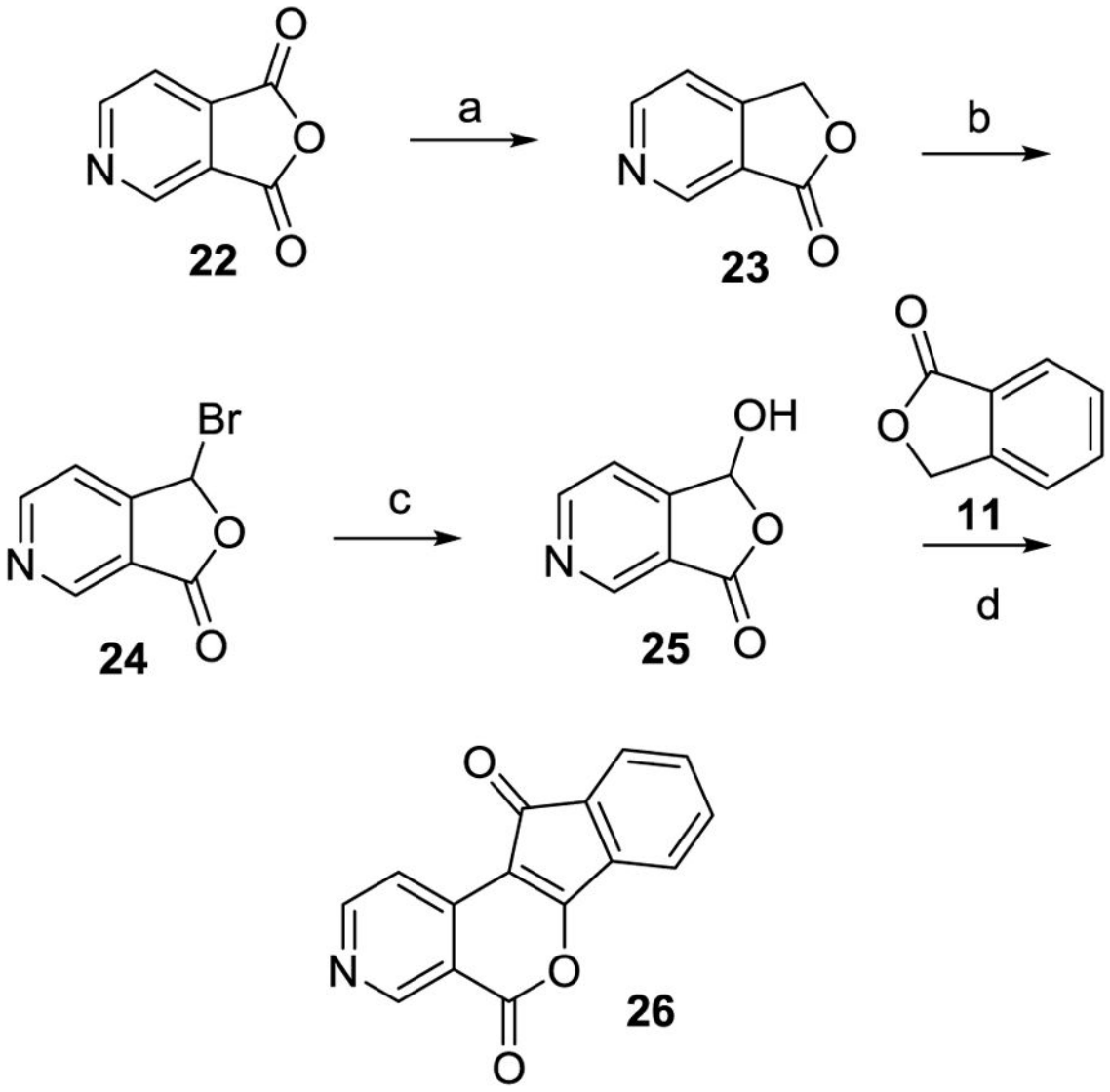
Scheme 1[a]

[a] Reagents and conditions: (a) i. $NaBH_4$, THF, AcOH, 15 °C, 4 h; ii. Ac_2O, AcOH, 100 °C, 3 h; (b) NBS, AIBN, CCl_4, reflux, 2 h; (c) H_2O, reflux, 2 h; (d) i. NaOMe, MeOH, EtOAc, reflux, 15 h, then HCl; ii. Ac_2O, reflux, 6 h.



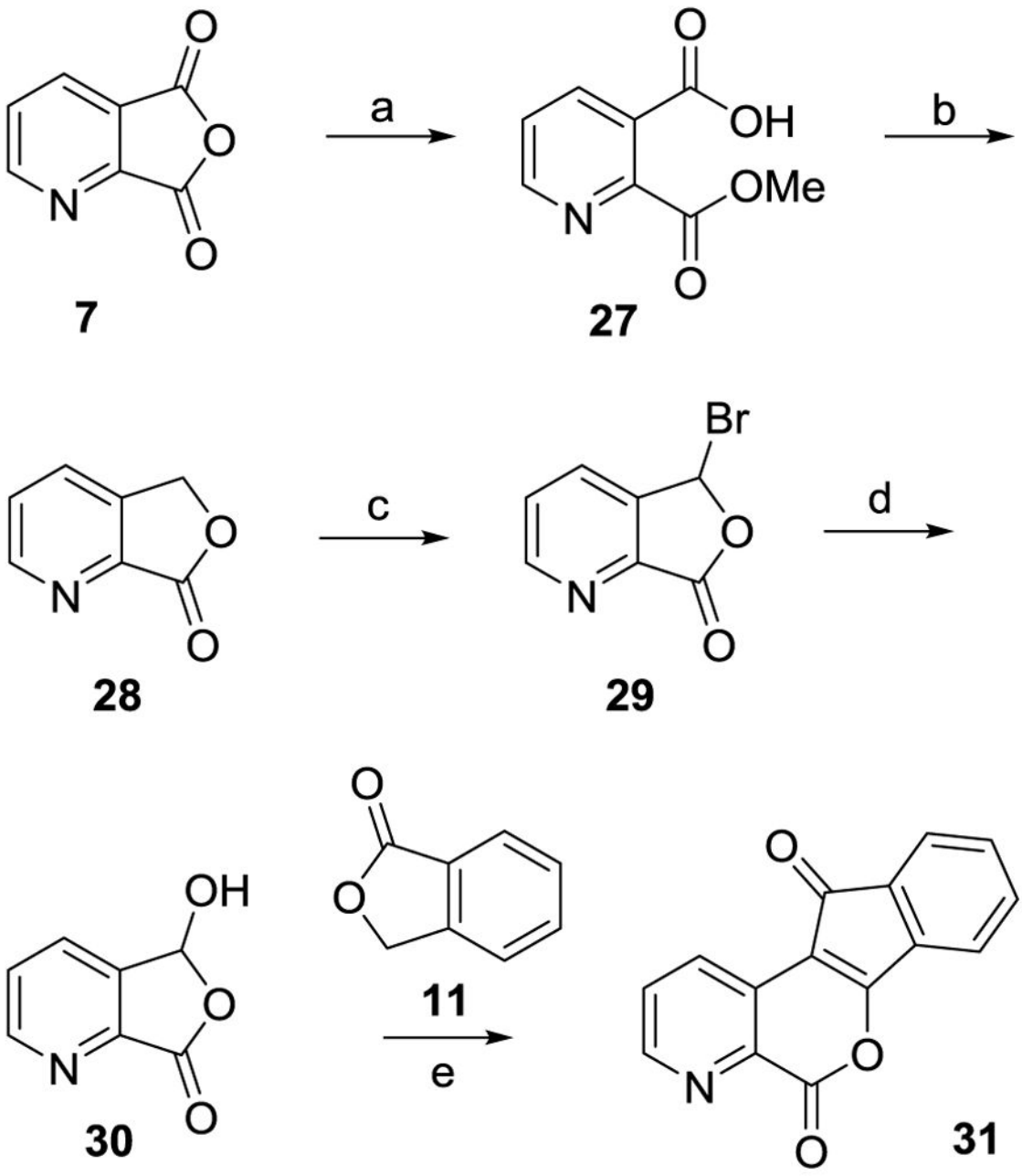
Scheme 2[a]

[a] Reagents and conditions: (a) i. $SOCl_2$, reflux, 2 h; ii. $PhNH_2$, K_2CO_3, THF, room temp., 24 h; (b) i. *n*-BuLi, THF, −78 °C (0.5 h) to 0 °C (0.5 h); ii. DMF, −78 °C (1 h) to 0 °C (1 h); (c) $NaBH_4$, MeOH, room temp., 5 h; (d) 15% HCl, 60 °C, 2 d; (e) NBS, AIBN, CCl_4, CH_2Cl_2, reflux, 2 d; (f) H_2O, reflux, 2 h; (g) i. NaOMe, MeOH, EtOAc, reflux, 15 h, then HCl; ii. Ac_2O, reflux, 6 h.



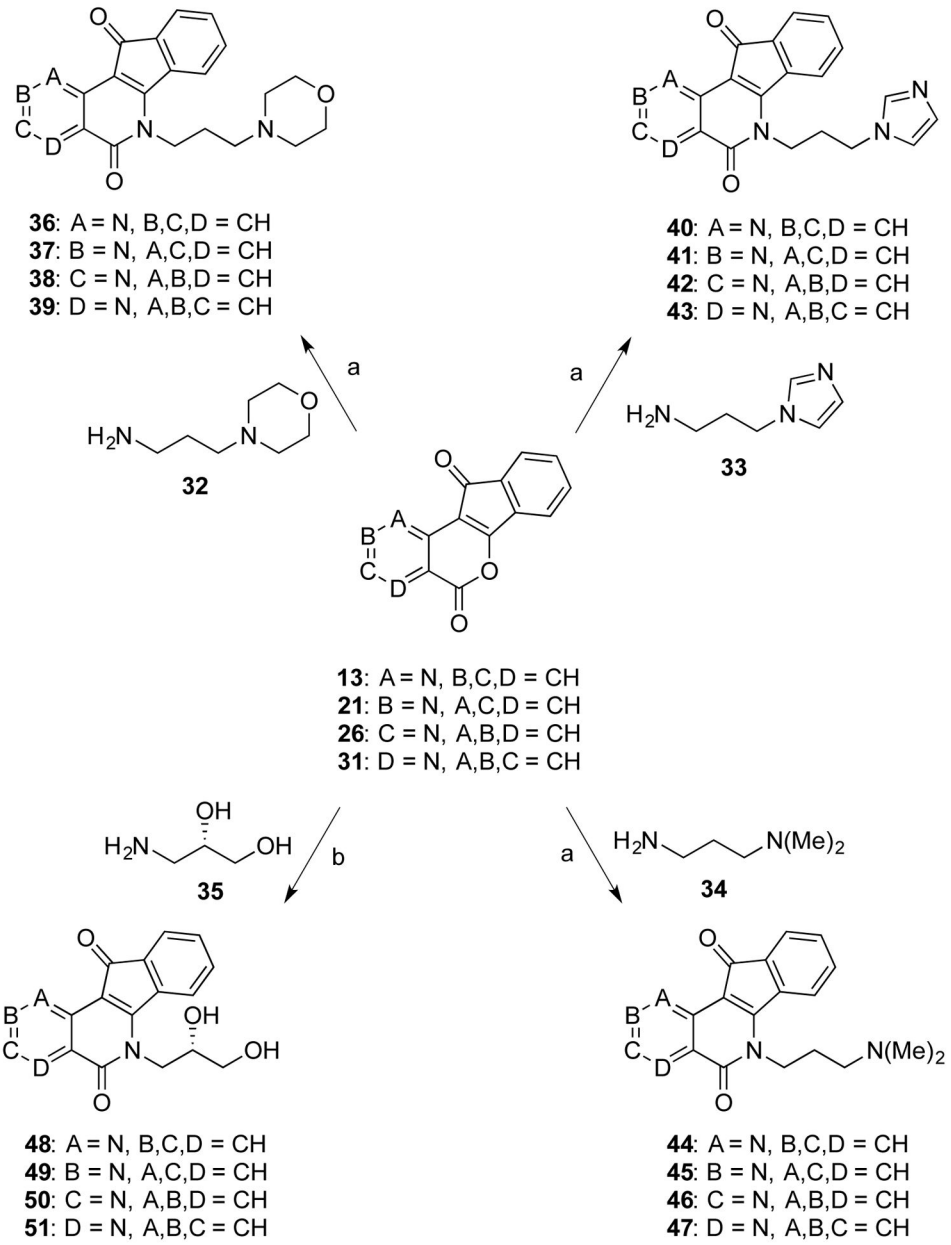
Scheme 3[a]

[a] Reagents and conditions: (a) i. $NaBH_4$, PhMe, DMF, −20 °C to 35 °C, 2 h; ii. 5 M HCl, reflux, 0.5 h; (b) NBS, AIBN, CCl_4, reflux, 2 h; (c) H_2O, reflux, 2 h; (d) i. NaOMe, MeOH, EtOAc, reflux, 24 h; ii. Ac_2O, reflux, 6 h.



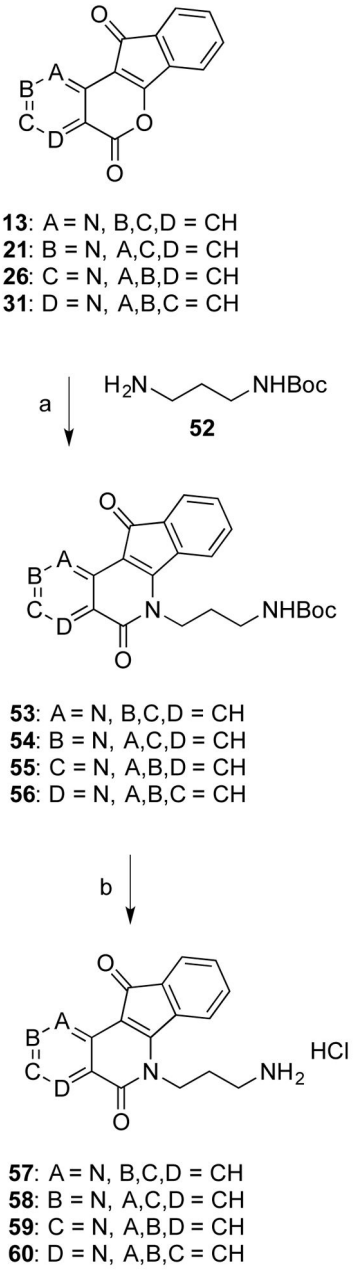
Scheme 4[a]

[a] Reagents and conditions: (a) MeOH, reflux, 2 h; (b) CDI, THF, room temp., 1 h, then $NaBH_4$, MeOH, room temp., 2 h; (c) NBS, AIBN, CCl_4, reflux, 24 h; (d) H_2O, reflux, 2 h; (e) i. NaOMe, MeOH, EtOAc, reflux, 15 h, then HCl; ii. Ac_2O, reflux, 6 h.



Scheme 5[a]

[a] Reagents and conditions: (a) $CHCl_3$, reflux, 15 h; (b) $CHCl_3$, MeOH, reflux or room temperature.



Scheme 6[a]

[a] Reagents and conditions: (a) $CHCl_3$, reflux, 24 h; (b) 5 N HCl in MeOH, $CHCl_3$, 6 h.

Table 1

Top1 Poisoning and TDP1 and TDP2 Inhibitory Activities of Aza-A-Ring Indenoisoquinolines.

	Compound	Top1[a]	TDP1[b]	TDP2[c]	Mean Growth %[d]	Side Chain
	CPT	++++	**N.A.**[e]	**N.A.**	**N.A.**	
	36	++	>111	>111	87.9	morpholinopropyl
	40	++	>111	>111	83.6	imidazolylpropyl
1-aza	**44**	++	90	>111	44.4	dimethylaminopropyl.
	48	+	>111	>111	100	dihydroxypropyl
	57	++	43, 63 (n=2)	>111	N.T.	aminopropyl
	37	+++	>111	>111	N.T.	morpholinopropyl
	41	++	>111	>111	67.8	imidazolylpropyl
2-aza	**45**	++	63	37	−8.83	dimethylaminopropyl
	49	+	>111	>111	85.5	dihydroxypropyl
	58	++	19, 30 (n=2)	~111	N.T.	aminopropyl
	38	++	>111	>111	N.T.	morpholinopropyl
	42	+	>111	>111	47.5	imidazolylpropyl
3-aza	**46**	++	63	80	−9.25	dimethylaminopropyl
	50	++	>111	>111	N.T.	dihydroxypropyl
	59	0/+	30, 48 (n=2)	~111	N.T.	aminopropyl
	39	+++	>111	>111	42.9	morpholinopropyl
	43	+++	>111	>111	49.6	imidazolylpropyl
4-aza	**47**	+++	~111	>111	5.45	dimethylaminopropyl
	51	+	>111	>111	61.7	dihydroxypropyl
	60	++	60, >111 (n=2)	>111	12.2	aminopropyl

[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]IC_{50} values for the inhibition of TDP1 (μM).

[c]IC_{50} values for the inhibition of TDP2 (μM).

[d]The mean-graph midpoint of the percent growth of 60 human cancer cell lines treated with 10 μM drug concentration for 48 h relative to no-drug control, and relative to the time zero number of cells.

[e] Not available.

[f] Not tested because the compound was not accepted for evaluation by the National Cancer Institute, Developmental Therapeutics Program.

Table 2

Antiproliferative Activities of Aza A-Ring Indenoisoquinolines

compd	Cytotoxicity (GI_{50}, μM)								
	Lung, HOP-62	**Colon, HCT-116**	**CNS, SF-539**	**Melanoma, UACC-62**	**Ovarian, OVCAR-3**	**Renal, SN12C**	**Prostate, DU-145**	**Breast, MCF7**	**MGM[a]**
CPT	0.01	0.03	0.01	0.01	0.22	0.02	0.01	0.01	0.0405
45	0.398	0.245	0.380	0.309	1.10	0.288	0.490	0.245	0.437
46	0.871	0.380	0.708	1.23	2.00	0.955	0.977	0.331	0.977
47	1.26	0.437	0.851	1.29	2.40	1.12	1.91	0.363	1.26
60	1.45	0.537	1.38	1.35	2.29	1.12	1.91	0.417	1.51

[a]Mean graph midpoint GI_{50} from the NCI-60 five-concentration assay.

Table 3

Top1 Poisoning and Antiproliferative Activities of Indenoisoquinolines **62–66**

compd	Top1	MGM GI_{50} (μM)[a]
62	++	15.1
63	++++	1.86
64	+++	1.86
65	+++	8.71
66	+++	0.32 ± 0.23

[a]Mean graph midpoint GI_{50} from the NCI-60 five-concentration assay.