Synthesis and Biological Evaluation of Nitrated 7-, 8-, 9-, and 10-Hydroxyindenoisoquinolines as Potential Dual Topoisomerase I (Top1)−Tyrosyl-DNA Phosphodiesterase I (TDP1) Inhibitors

Trung Xuan Nguyen,[†] Monica Abdelmalak,[‡] Christophe Marchand,[‡] Keli Agama,[‡] Yves Pommier,[‡] and Mark Cushman*,[†]

[†]Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, and the Purdue Center for Cancer Research, Purdue University, 575 Stadium Mall Drive, West Lafayette, Indiana 47907, United States

[‡]Developmental Therapeutics Branch and Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892-4255, United States

(S) *Supporting Information*

ABSTRACT: The structure−activity relationships and hit-to-lead optimization of dual Top1−TDP1 inhibitors in the indenoisoquinoline drug class were investigated. A series of nitrated 7-, 8-, 9-, and 10-hydroxyindenoisoquinolines were synthesized and evaluated. Several compounds displayed potent dual Top1−TDP1 inhibition. The 9-hydroxy series exhibited potencies and cytotoxicities vs Top1 that surpassed those of camptothecin (CPT), the natural alkaloid that is being used as a standard in the Top1-mediated DNA cleavage assay. One member of this series was a more potent Top1 inhibitor at a concentration of 5 nM and produced a more stable ternary drug−DNA−Top1 cleavage complex than CPT.
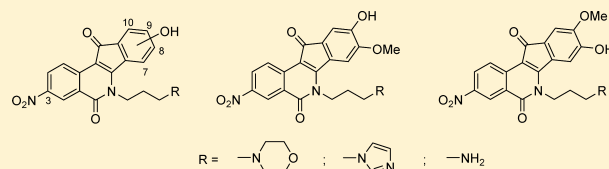
INTRODUCTION

Human topoisomerase type IB (Top1) is an essential enzyme for various cellular processes as it relaxes DNA supercoils and resolves DNA topology so that genetic information can be accessed, and critical events, including DNA replication and transcription, can take place.[1,2] The enzyme cuts a single strand of DNA to form a transient complex that allows supercoils to be dissipated, followed by religation of the broken strand.[3] The cleavage complex can be trapped by Top1 poisons that intercalate between the DNA base pairs at the cleavage site and thereby inhibit the religation reaction. Collision of the replication fork with the single-strand break then generates a double-strand break, causing cell death.[4] Defective repair explains the therapeutic effect of Top1 inhibitors. DNA damage produced by Top1 inhibitors can be reversed by a group of enzymes that includes tyrosyl-DNA phosphodiesterase I (TDP1), which hydrolyzes the phosphotyrosyl linkage between degraded or denatured Top1 and the DNA at the cleavage site to produce DNA 3′-phosphates and free peptides containing terminal tyrosyl residue.[5−8] Polynucleotide kinase phosphatase (PNKP) then hydrolyzes that 3′-phosphate and installs a phosphate on the 5′-end on the other side of the break, and DNA ligase III reseals the DNA.[9] Because TDP1 counteracts the action of Top1 inhibitors, it is possible that TDP1 inhibitors could potentiate the cytotoxic effects of Top1 inhibitors in combination anticancer drug therapy.[9] The discovery of camptothecin (CPT, **1**, Figure 1) as a selective and potent Top1 poison led to the validation of Top1 as an anticancer target,[10] and in turn attracted a considerable amount
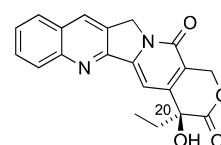
Figure 1. Structure and activity of camptothecin (**1**). (*a*) See Table 1 for the Top1 inhibition grading rubric. (*b*) The MGM is the mean-graph midpoint value obtained from cytotoxicity testing in the National Cancer Institute's panel of 60 human cancer cell lines.[29]

of research effort in the design and synthesis of Top1 inhibitors.[11,12] As TDP1 plays an important role in the maintenance of genomic stability[13] and is a key enzyme in DNA repair machinery,[14−17] TDP1 is also being pursued as a possible anticancer drug target.[9,15,18−26]

TDP1-dependent repair pathways are normally redundant with other DNA damage response pathways that are often compromised in cancer cells. This provides a reason to expect selective TDP1 inhibitor potentiation of Top1 cytotoxicity in cancer cells vs normal cells. Moreover, checkpoint deficiencies are common in cancer cells,[27] and in these cases TDP1
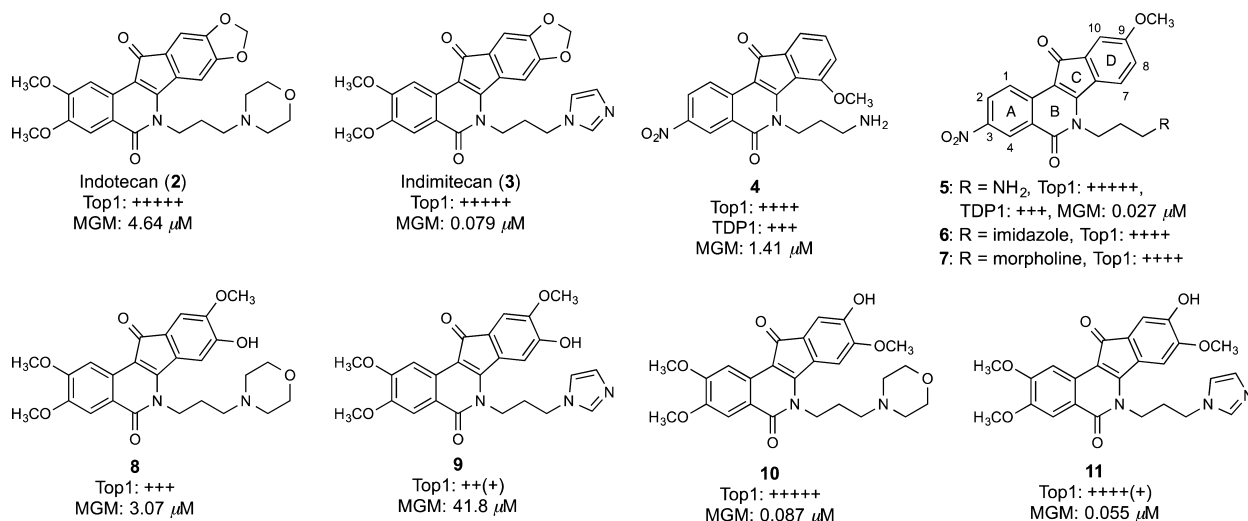
Figure 2. Indenoisoquinoline Top1 inhibitors and their hydroxylated analogues.
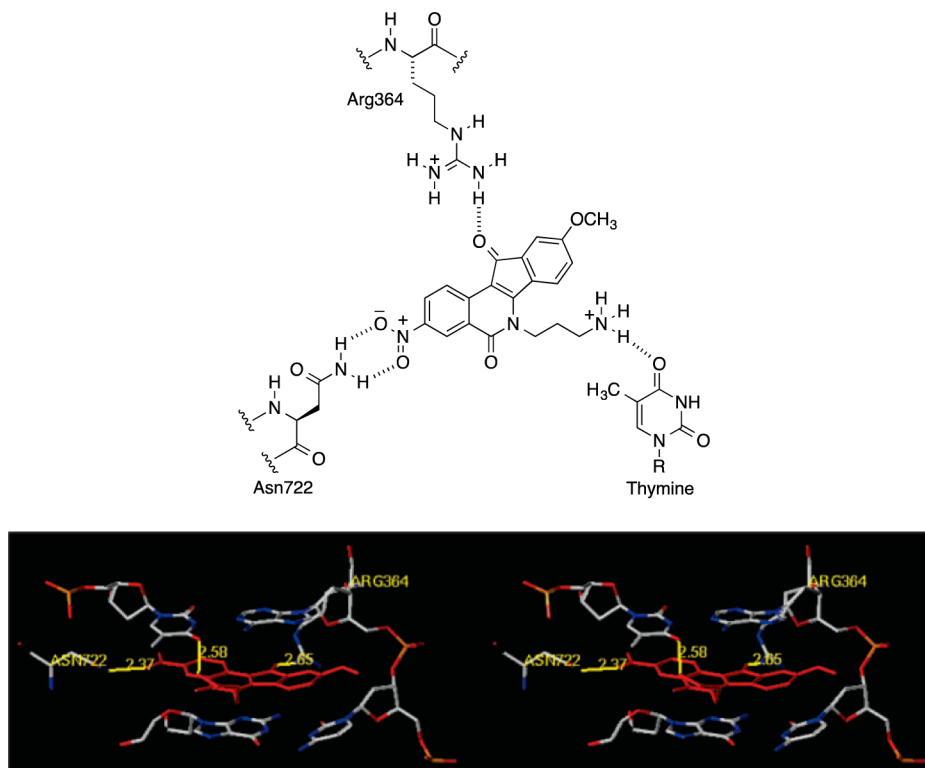

Figure 3. (top) Proposed hydrogen bonding network of indenoisoquinoline **5** (red) in the DNA–Top1–indenoisoquinoline ternary complex. (bottom) Hypothetical model of the ternary complex derived from the PDB ID 1SC7 crystal structure.[35] Hydrogen bonds are shown as yellow lines. The diagram is programmed for wall-eyed (relaxed) viewing.

becomes the main repair mechanism for removal of Top1-mediated DNA damage.[5,9,28] The goal of the present studies was to design and synthesize potential anticancer agents that would possess dual Top1–TDP1 inhibitory activities and thereby potentially enhance anticancer activity and selectivity.

■ DESIGN RATIONALE

Two indenoisoquinoline Top1 inhibitors, indotecan (LMP400, **2**) and indimitecan (LMP776, **3**), are currently in phase I clinical trials at the National Cancer Institute (Figure 2).[30,31] These compounds induce persistent Top1-linked DNA breaks, overcome some of the drug resistance issues associated with the

camptothecins, and have a long half-life in humans.[32] Recently, Cinelli et al. reported the synthesis of some hydroxylated indenoisoquinolines that were utilized as synthetic standards in the metabolism studies of **2** and **3** in human liver microsomes in the presence of NADPH.[33] Four of the synthetic compounds were confirmed as the actual metabolites of the two clinical drugs based on matches in molecular masses and retention times with the most abundant peaks of metabolites detected by LC-MS/MS.[33] Four other hydroxyindenoisoquinolines **8**–**11** (Figure 2) were not identified as actual metabolites, but they exhibited attractive Top1 inhibition and antiproliferative activities, especially the two 9-hydroxy-8-methoxy compounds
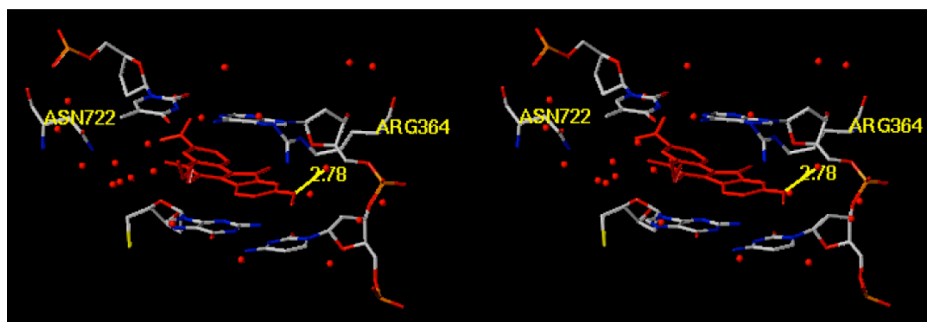
Figure 4. Hypothetical molecular model depicting a water-mediated hydrogen bond from indenoisoquinoline **5** (red) to the backbone of the nonscissile DNA strand in ternary complex derived from the PDB ID 1K4T crystal structure.[36] The diagram is programmed for wall-eyed (relaxed) viewing.

10 and **11**. Through molecular modeling, Cinelli rationalized the lower potencies of their regioisomers (8-hydroxy-9-methoxy, compounds **8** and **9**) as a consequence of possible steric and electronic clashes of the 9-methoxy group with the nonscissile DNA strand in the ternary DNA–Top1-inhibitor complex.[33] However, independent studies reported by Morrell et al. showed that placing a variety of bulky substituents (e.g., methoxycarbonyl, ethoxy, halogen) at the 9-position did not result in a significant attenuation or loss of Top1 inhibition or antiproliferative activity of indenoisoquinolines when a nitro group was present at the 3-position.[34−36] This observation led to the hypothesis that the 3-nitro group exhibits a "steric-rescuing" effect, which is probably facilitated by its hydrogen bonding to the Asn722 residue in the open environment of the cleaved DNA strand, as observed in the hypothetical docking model of compound **5** in the ternary complex (Figure 3),[35] thus moving the inhibitors away from the uncleaved DNA strand so that bulky substituents could be tolerated in this crowded area. Because both the 9-hydroxyl group and the 3-nitro group enhance the anti-Top1 potency, the combination of these two features in a single indenoisoquinoline molecule might create exceptionally potent Top1 inhibitors. Although the nitro group is a toxicophore, one of the objectives in the present case was to synthesize very cytotoxic indenoisoquinolines that could be linked to cancer cell-targeting moieties through the phenolic hydroxyl groups, which would make the nitro group more tolerable.

Morrell et al. reported a series of indenoisoquinolines bearing a 3-nitro group on the A-ring and a 7- or 9-methoxy group on the D-ring (compounds **4−7**) as cytotoxic and potent Top1 inhibitors.[35,36] The 9-methoxy group was shown to typically improve cytotoxicity at the expense of Top1 inhibition, whereas the 3-nitro group enhanced Top1 inhibition to a greater extent than cytotoxicity.[35] Although the importance of the 3-nitro group to the biological activity of this drug class was rationalized in detail by molecular modeling and electrostatic potential calculations,[35] explanations of the role of the 9-methoxy group were not completely confirmed. In particular, electrostatic potential modeling revealed charge complementarity between the electron-rich, 9-position oxygen atom and the outer edges of the nonscissile DNA base pairs in the Top1 cleavage complex, while the hypothetical binding model of **5** derived from the high resolution crystal structure of topotecan (PDB ID: 1K4T, in which water molecules were present in the intercalation site) showed a single bridging hydrogen bond (2.78 Å) between a water molecule and the 9-methoxy group (Figure 4).[36] From these observations, it was concluded that

the optimal place to incorporate a methoxy group on the D-ring was the 9-position (as in compounds **5−7**).[36] The generally lower potencies of compounds in the 7-methoxy series was rationalized as being the result of the 7-methoxy group having deleterious steric interactions with the lactam side chain, limiting the number of planar conformations (relative to the 9-methoxy group) required to confer Top1 inhibition, and increasing steric repulsion with the DNA base pairs of the nonscissile DNA strand.[36] However, Morrell did observe an exception in that the 7-methoxy-3-nitroindenoisoquinoline **4** (Figure 2) was a potent Top1 inhibitor.

A hydroxyl group could possibly serve as a biologically active replacement for the methoxy group. It might also provide a point of attachment for the synthesis of prodrugs so that the pharmacokinetics could be modulated and optimized. Hydroxylated indenoisoquinolines might also offer an advantage in terms of solubility for intravenous formulation, which has been a concern with this drug class. In the present studies, the syntheses of 18 nitrated 7-, 8-, 9-, and 10-hydroxyindenoisoquinolines bearing a 3-nitro substituent were investigated (Figure 5), all of which are analogues of the highly potent dual Top1−TDP1 inhibitors **4−7**,[34,37] so as to optimize the dual Top1−TDP1 inhibitory potency and gain more insight into the importance of the location of the hydroxyl group on the overall biological activities of this drug class.

CHEMISTRY

The indenoisoquinoline system can be constructed by several methods: (1) condensation of indenoisochromenone with a primary amine,[38] (2) Suzuki−Miyaura cross-coupling reaction followed by ring-closing metathesis,[39] and (3) oxidative cyclization of cis acid produced by the condensation of a homophthalic anhydride and a Schiff base.[34−36] The latter methodology was chosen in this case to prepare all of the proposed compounds because of the ease in synthesis of intermediates and manipulation of starting materials to obtain indenoisoquinolines bearing the desired substituents.

A. Synthesis of 8-Hydroxy-9-methoxy-3-nitroindenoisoquinolines and 9-Hydroxy-8-methoxy-3-nitroindenoisoquinolines. The synthesis of nitrated 9-hydroxy-8-methoxyindenoisoquinolines **21**, **23**, and **25** is depicted in Scheme 1, and the preparation of 8-hydroxy-9-methoxyindenoisoquinolines **32**, **34**, and **36** is shown in Scheme 2. Commercially available homophthalic acid **12** was nitrated with fuming HNO_3 to provide the diacid **13**, which underwent dehydration in AcCl to provide anhydride **14**. The reactive hydroxyl groups in vanillin (**15**) and isovanillin (**26**) were protected with a benzyl
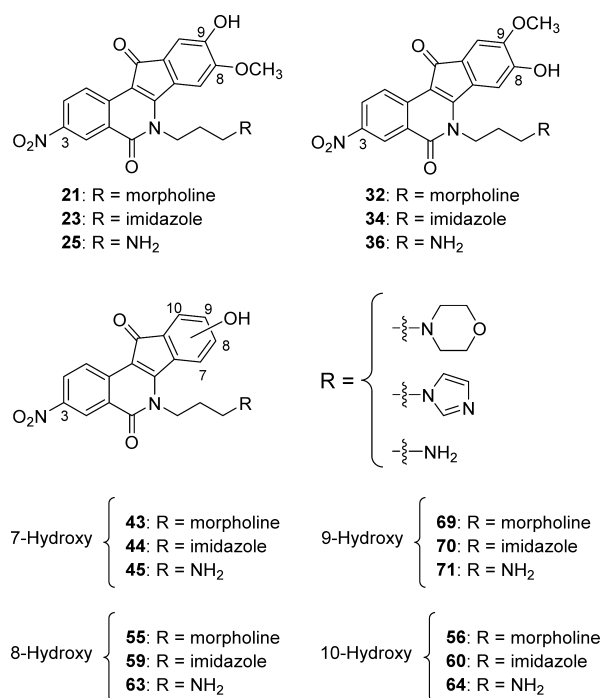
Figure 5. Proposed Top1−TDP1 inhibitors.

group. Benzylvanillin (**16**) and benzylisovanillin (**27**) reacted with 3-bromopropylamine hydrobromide to give Schiff bases **17** and **28**, which upon condensation with anhydride **14** in CHCl₃ furnished cis acids **18** and **29**, respectively, in good yields with excellent diastereoselectivities. Treatment of **18** or **29** with SOCl₂ (neat) provided a mixture of indenoisoquinoline **19** (from **18**) or **30** (from **29**) and their regioisomers, which was evident by ¹H NMR spectroscopy and consideration of the reaction mechanism (Scheme 3). Isomers **19** and **30** appeared as the major products. The structure assignments were based on the distinct splitting patterns of the D-ring hydrogens in the ¹H NMR spectra of the two regioisomers. The very similar R_f values of the regioisomers led to lengthy and difficult purifications by flash column chromatography. The yields of pure **19** and **30** after two chromatographic purifications were low (15−25%) and inconsistent. The low yields may be due to the nitro group activating epimerization to the trans diastereomers, which exist in pseudodiaxial conformations and do not oxidize and cyclize in SOCl₂.[40,41]

Treatment of cis acids **18** and **29** with SOCl₂ alone resulted in conversion to the acid chlorides, dehydrogenation, and intramolecular Friedel−Crafts cyclization to provide the aromatic indenoisoquinoline systems. Purification of crude **19** or **30** by column chromatography (SiO₂) caused decomposition of the products, and therefore the products were used directly in the next step without further purification.

Displacement of the terminal bromide in **19** or **30** with morpholine or imidazole in 1,4-dioxane, or azide in DMSO, yielded the benzyl-protected compounds **20**, **22**, and **24** (from **19**), or **31**, **33**, and **35** (from **30**), respectively. Many attempts were made to optimize the yields of these S$_N$2 reactions. For morpholine derivatives **20** and **31**, the best yields (44−52%) were obtained when the starting materials **19** or **30** were stirred with morpholine for 16 h at room temperature. In contrast, the syntheses of imidazole derivatives **22** and **33** required constant heating at 70 °C for 16 h, and the yields (36−40%) were

slightly lower than those of morpholine compounds (44−52%). Column chromatographic purification of the crude mixtures provided analytically pure products with much ease as compared to the separation of the bromides **19** or **30** from their regioisomers. These results supported the direct use of the crude bromides **19** and **30** from the previous step.

Many attempts were made to achieve the debenzylation of the protected compounds **20**, **22**, **24**, **31**, **33**, and **35**. 1,4-Cyclohexadiene has been shown to be an effective hydrogen donor in catalytic transfer hydrogenation, a method that was utilized to remove *N*-benzyloxycarbonyl, benzyl ester, and benzyl ether protecting groups in peptide chemistry.[42] Disappointingly, attempted implementation of this method on the benzyl-protected morpholine compound **31** in various solvents (CHCl₃, EtOH, or glacial AcOH) at room temperature was not successful in cleaving the benzyl group. In all cases, starting material was recovered. Attempted debenzylation with HBr in acetic acid (33 wt %) not only removed the benzyl group in **31**, but a subsequent Fisher esterification of the resulting phenol **32** also occurred to provide the acetate **37** as the major product while the desired phenol **32** was only obtained in minor amount (Scheme 4).

Treatment of the benzyl-protected starting materials **20**, **22**, **24**, **31**, **33**, and **35** with aqueous HBr at 70 °C for 4−5 h, followed by dilution with acetone and then concentration (iterated three times), afforded a mixture that was suitable for vacuum filtration to provide the desired phenols **21**, **23**, **25**, **32**, **34**, and **36** in high yields (80−100%) and excellent purity. The moderate yield of imidazole **23** (50%) was due to its higher aqueous solubility. All of the starting materials produced reaction mixtures as emulsions from which all of the products precipitated. The products derived from vanillin (**21**, **23**, and **25**) were so finely powdered that the use of an HPLC filter paper was crucial for their recovery, while a regular double-layered filter paper worked well for those derived from isovanillin (**32**, **34**, and **36**). The three-time iteration of dilution with acetone and concentration was imperative for the success of vacuum filtration. For the syntheses of amines **25** and **36**, corresponding azides **24** and **35** were subjected to a Staudinger reduction with P(OEt)₃, followed by treatment with aqueous HBr to achieve acidic hydrolysis and debenzylation simultaneously in one pot.

B. Synthesis of 7-Hydroxy-3-nitroindenoisoquinolines (Scheme 5). Commercially available salicylaldehyde **38** was *O*-benzyl protected to give **39**, which reacted with 3-bromopropyl-amine to afford Schiff base **40**. Condensation of **40** with anhydride **14** in CHCl₃ yielded cis acid **41**, which upon treatment with SOCl₂, followed by AlCl₃ in 1,2-dichloroethane, provided indenoisoquinoline bromide **42** in good yield (63%). The ¹H NMR spectrum of **42** indicated excellent purity without chromatographic purification, and it also indicated that AlCl₃ induced debenzylation to afford the free phenol. Interestingly, the use of AlCl₃ to simultaneously remove the benzyl group was tried in connection with the chemistry described in Schemes 1 and 2, but in those cases it led to complicated mixtures and no debenzylation products could be isolated. Displacement of the bromide in **42** with morpholine, imidazole, or NaN₃, followed by a Staudinger reduction of the azide intermediate and acidic hydrolysis with methanolic HCl, provided the desired amines **43**, **44**, and **45**, respectively. The pure products were isolated without chromatographic purification.

C. Synthesis of 8- and 10-Hydroxy-3-nitroindenoiso-quinolines. A similar approach was implemented to prepare 8-

Scheme 1. Synthesis of Nitrated 9-Hydroxy-8-methoxyindenoisoquinolines[a]
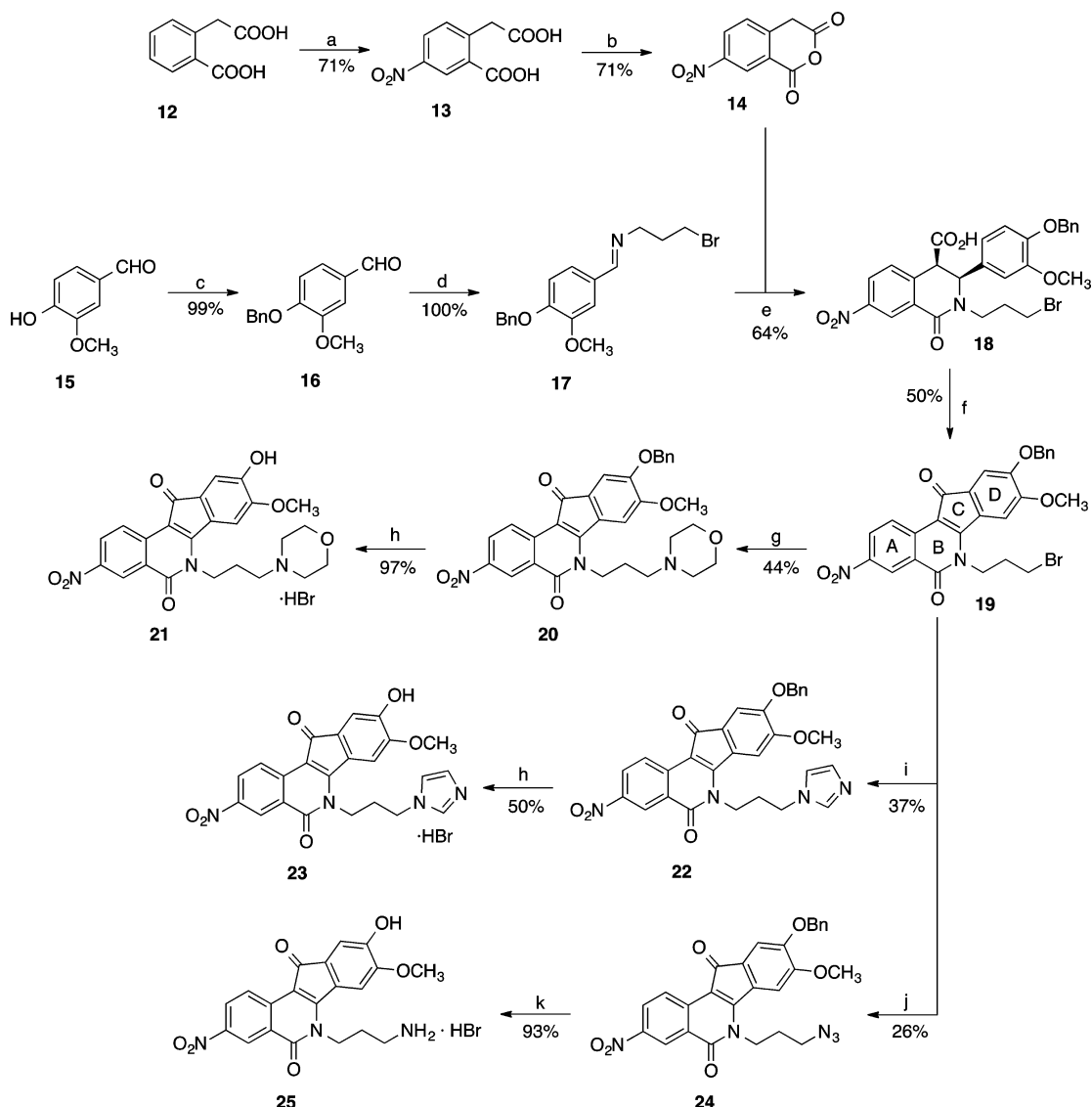
[a]Reagents and conditions: (a) fuming HNO₃, 0−23 °C; (b) AcCl; reflux; (c) BnCl, DMF, K₂CO₃, 23 °C; (d) 3-bromopropylamine hydrobromide, Et₃N, Na₂SO₄, CHCl₃, 23 °C; (e) CHCl₃, 0−23 °C; (f) SOCl₂, 0−23 °C; (g) morpholine, 1,2-dioxane, 23 °C; (h) aqueous HBr, 70 °C; (i) imidazole, 1,4-dioxane, 70 °C; (j) NaN₃, DMSO, 23 °C; (k) (i) P(OEt)₃, benzene, reflux, (ii) aqueous HBr, 70 °C.
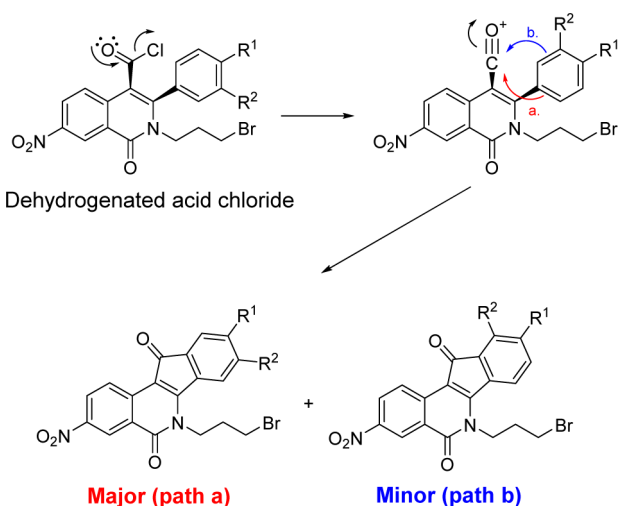
hydroxy-3-nitroindenoisoquinolines (Scheme 6). The commercially available 3-hydroxybenzaldehyde **46** was protected with a benzyl group to give **47**, which was then converted to Schiff base **48** upon treatment with 3-bromopropylamine hydrobromide under basic conditions. Condensation of **48** with anhydride **14** provided a mixture of the desired cis acid **49** and its trans diastereomer. Boiling the mixture in CHCl₃, followed by filtration, helped remove the unwanted trans acid and provide the pure cis acid **49** as a sole product. However, treatment of **49** with SOCl₂ 0 to 23 °C, followed by AlCl₃ in refluxing 1,2-dichloroethane, did not yield the desired bromide **50**. Gratifyingly, a profitable transformation was observed when cis acid **49** was heated with SOCl₂ (neat) at reflux for 4 h, during which the solution turned to clear orange. Removal of SOCl₂, followed by filtering the residue and washing with ether, provided an orange solid whose ¹H NMR spectrum revealed that it was an approximately 2:1, inseparable mixture of 8- and 10-(benzyloxy)indenoisoquinolines **51** and **52**.

Treatment of the mixture of bromides **51** and **52** with morpholine and imidazole, followed by chromatographic purification, allowed the isolation of each pure morpholinyl and imidazolyl 8- and 10-benzyloxy compounds **53**, **54**, **57**, and **58** (Scheme 7). In all cases, the 8-substituted products eluted first (higher R_f value), and the two regioisomers were distinguished based on the differences in ¹H NMR multiplicity of the three aromatic protons in the D-ring: the 8-substituted products have 2 doublets and 1 singlet (or 1 doublet with a small meta coupling constant), while the 10-substituted products have 2 doublets and 1 multiplet. All of the benzyl-protected materials were subjected to a 3 h debenzylation with aqueous HBr (48 wt %) to provide the desired 8- and 10-hydroxyindenoisoquinolines **55**, **56**, **59**, and **60** in good yields and purities. The azides **61** and **62**, prepared by reaction of the bromides **51** and **52** with sodium azide in DMSO at room temperature, were subjected to a Staudinger reduction, followed by simultaneous acidic hydrolysis and deprotection

Scheme 2. Synthesis of Nitrated 8-Hydroxy-9-methoxyindenoisoquinolines[a]
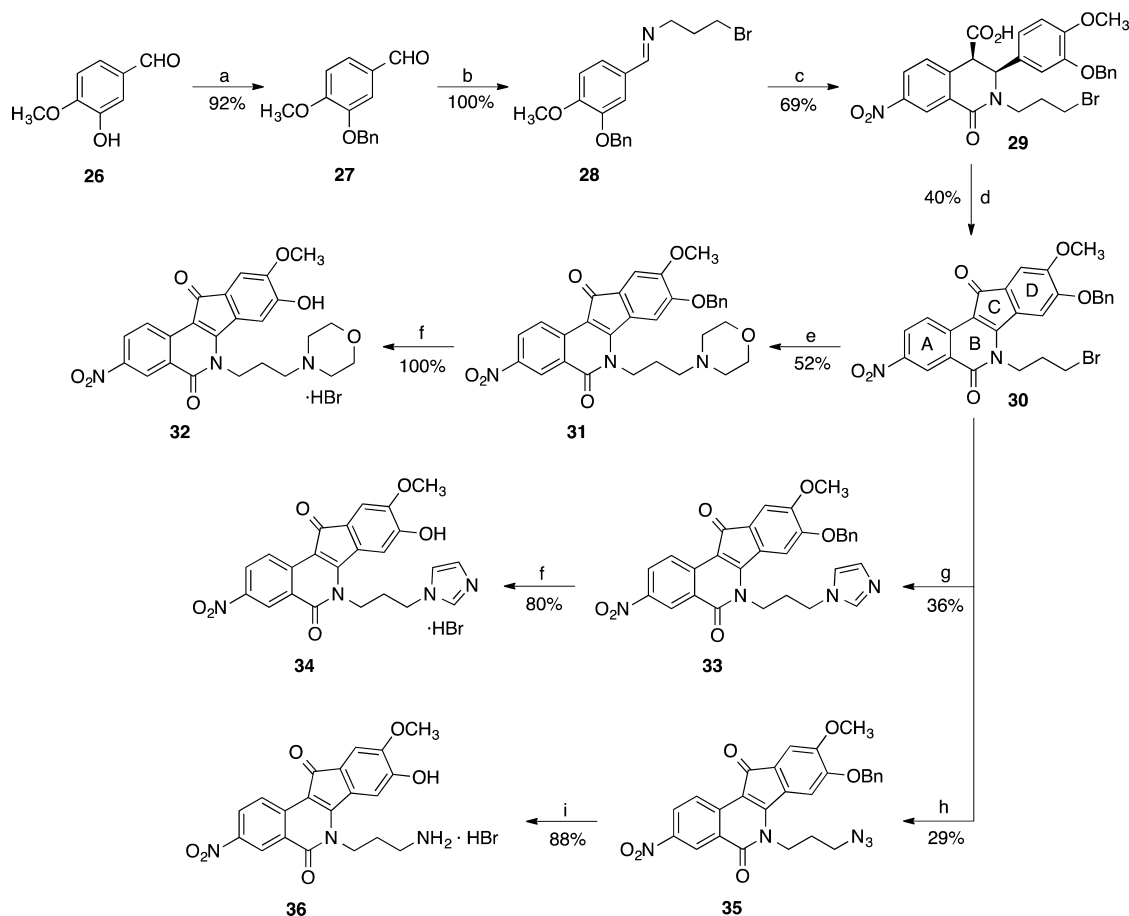
[a]Reagents and conditions: (a) BnCl, DMF, K$_2$CO$_3$, 23 °C; (b) 3-bromopropylamine hydrobromide, Et$_3$N, Na$_2$SO$_4$, CHCl$_3$, 23 °C; (c) anhydride **14**, CHCl$_3$, 0−23 °C; (d) SOCl$_2$, 0 to 23 °C; (e) morpholine, 1,4-dioxane, 23 °C; (f) aqueous HBr, 70 °C; (g) imidazole,1,4-dioxane, 70 °C; (h) NaN$_3$, DMSO, 23 °C; (i) (i) P(OEt)$_3$, benzene, reflux, (ii) aqueous HBr, 70 °C.

Scheme 3. Formation of Two Regioisomers in the Cyclization of Acid Chloride Intermediates

Scheme 4. Debenzylation of 31 with HBr in AcOH (33 wt %)

with aqueous HBr, to provide pure HBr salts of amines **63** and **64** in excellent purity and moderate yields (Scheme 7).

D. Synthesis of 9-Hydroxy-3-nitroindenoisoquinolines (Scheme 8). 4-Benzyloxybenzaldehyde (**65**) is commercially available or can be prepared easily by the methodology
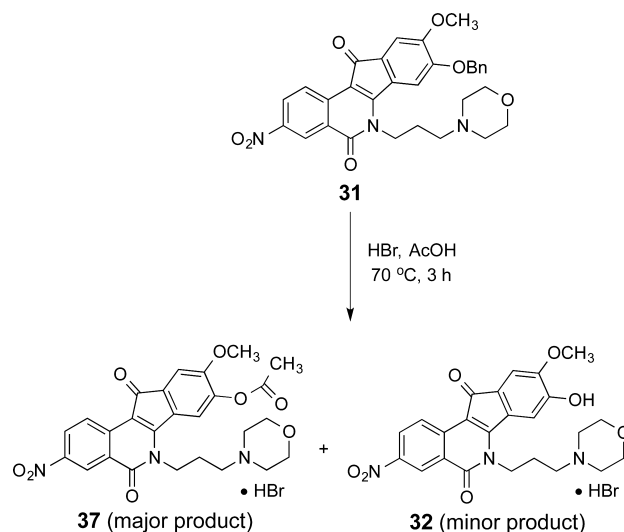
shown in Scheme 5 for 2-benzyloxybenzaldehyde. Treatment of **65** with 3-bromopropylamine hydrobromide and Et$_3$N provided Schiff base **66**, which upon condensation with anhydride **14** produced cis acid **67** in good yield and excellent

Scheme 5. Synthesis of 7-Hydroxy-3-nitroindenoisoquinolines[a]
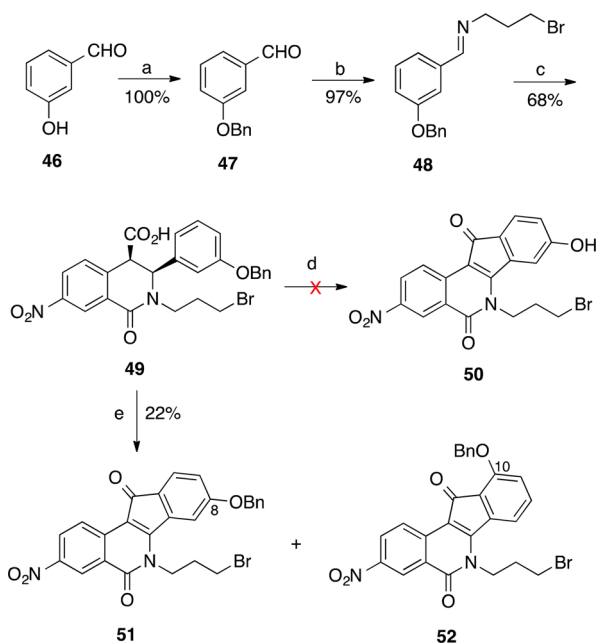
[a]Reagents and conditions: (a) BnBr, DMF, K$_2$CO$_3$, 23 °C; (b) 3-bromopropylamine hydrobromide, Et$_3$N, Na$_2$SO$_4$, CHCl$_3$, 23 °C; (c) anhydride **14**, CHCl$_3$, 0−23 °C; (d) (i) SOCl$_2$, 23 °C, (ii) AlCl$_3$, 1,2-dichloroethane, 0−23 °C; (e) morpholine, 1,4-dioxane, 70 °C; (f) imidazole, 1,4-dioxane, 70 °C; (g) (i) NaN$_3$, DMSO, 23 °C, (ii) P(OEt)$_3$, benzene, reflux, (iii) HCl, MeOH, 70 °C.

Scheme 6[a]

[a]Reagents and conditions: (a) BnBr, DMF, K$_2$CO$_3$, 23 °C; (b) 3-bromopropylamine hydrobromide, Et$_3$N, Na$_2$SO$_4$, CHCl$_3$, 23 °C; (c) anhydride **14**, CHCl$_3$, 0 to 23 °C; (d) (i) SOCl$_2$, 0−23 °C, (ii) AlCl$_3$, 1,2-dichloroethane, reflux; (e) SOCl$_2$, reflux.

purity. Treatment of acid **67** with SOCl$_2$ for 16 h at room temperature yielded a yellow syrup upon removal of SOCl$_2$. The yellow syrup was then treated with AlCl$_3$ in 1,2-

Scheme 7. Synthesis of 8- and 10-Hydroxy-3-nitroindenoisoquinolines[a]

[a]Reagents and conditions: (a) morpholine, 1,4-dioxane, 70 °C; (b) HBr, H$_2$O, 70 °C; (c) imidazole, 1,4-dioxane, 70 °C; (d) NaN$_3$, DMSO, 23 °C; (e) (i) P(OEt)$_3$, benzene, reflux, (ii) HBr, H$_2$O, 70 °C.
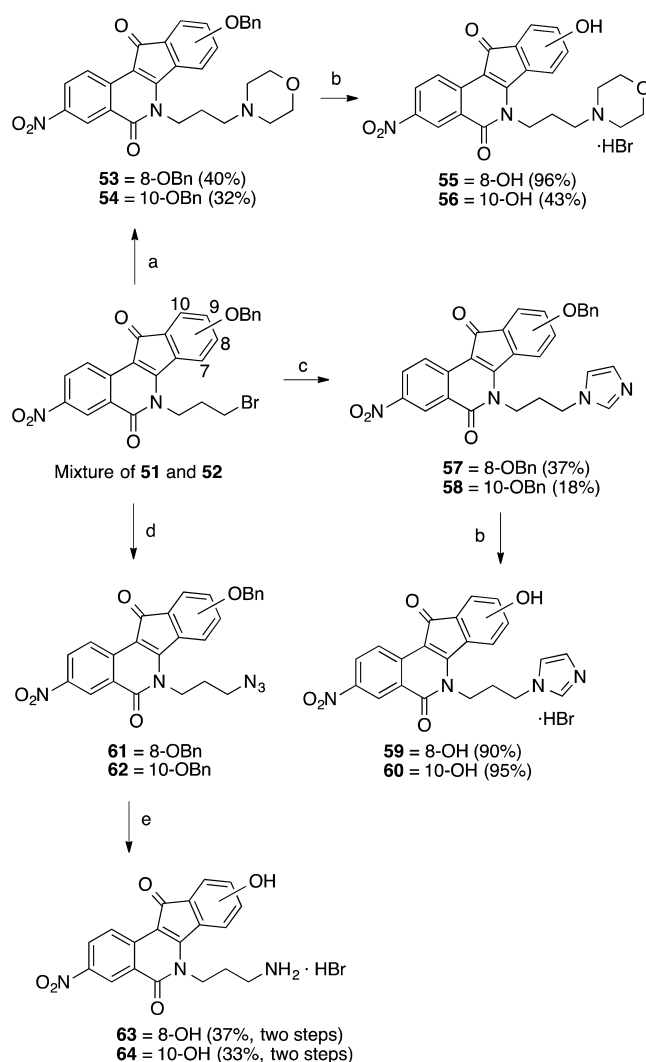
dichloroethane to provide the benzyl-free indenoisoquinoline phenol **68** after aqueous workup. Treatment of **68** with morpholine or imidazole in THF provided the corresponding displacement products, which were then stirred in freshly made methanolic HBr or HCl to afford the HBr and HCl salts **69** and **70**, respectively. This extra step was meant to facilitate later biological testing because the salts were more soluble in DMSO than the neutral compounds. The synthesis of amine **71** by the previous methodology involving reduction of the azide intermediate with P(OEt)$_3$ in benzene (as shown Scheme 5) was not successful due to complications in purification and isolation of the compound in solid form. The Staudinger reaction was therefore reattempted by treating the azide intermediate, obtained from **68**, with PPh$_3$ in THF (instead of P(OEt)$_3$ in benzene), followed by 4 h acidic hydrolysis with methanolic HBr. This modification provided the desired amine **71** in 32% yield with excellent purity.

Scheme 8. Synthesis of 9-Hydroxy-3-nitroindenoisoquinolines[a]
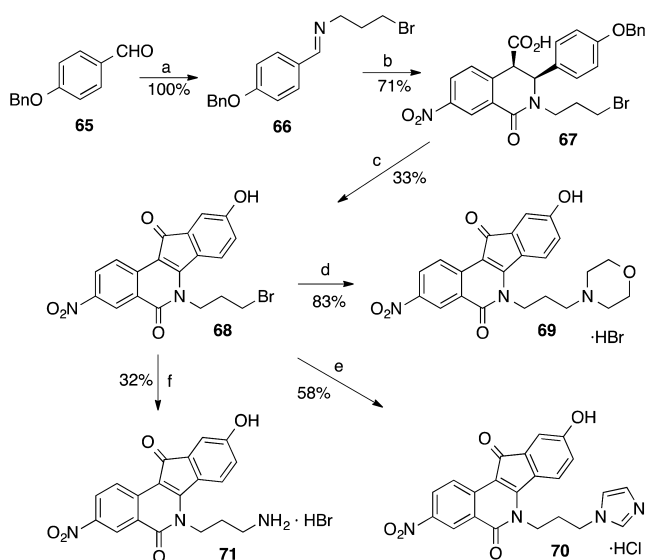
[a]Reagents and conditions: (a) 3-bromopropylamine hydrobromide, Et₃N, Na₂SO₄, CHCl₃, 23 °C; (b) anhydride **14**, CHCl₃, 0−23 °C; (c) (i) SOCl₂, 23 °C, (ii) AlCl₃, 1,2-dichloroethane, 0−23 °C; (d) (i) morpholine, THF, 70 °C, (ii) HBr, MeOH, 23 °C; (e) (i) imidazole, THF, 70 °C, (ii) HCl, MeOH, 23 °C; (f) (i) NaN₃, DMSO, 23 °C, (ii) PPh₃, THF, 70 °C, (iii) HBr, MeOH, 70 °C.

■ BIOLOGICAL RESULTS AND DISCUSSION

All of the proposed compounds (collectively shown in Figure 5) were subjected to determinations of Top1 and TDP1 inhibitory potencies and antiproliferative activities. Top1 inhibition was recorded as the ability of a drug to poison Top1 and induce enzyme-linked DNA breaks in the Top1-mediated DNA cleavage assay.[43] The anti-Top1 potency was graded on the basis of visual inspection of the number and intensities of the bands corresponding to DNA fragments and was recorded on a semiquantitative scale relative to 1 μM camptothecin (CPT, **1**, Figure 1): 0, no inhibitory activity; +, between 20% and 50% activity; ++, between 50% and 75% activity; +++, between 75% and 95% activity; ++++, equipotent; +++++, more potent. Ambiguous scores between two values are designated with parentheses (e.g., ++(+) would be between ++ and +++). The loss of potency at 100 μM of drug concentration, as evidenced by the pale intensity or disappearance of the DNA cleavage bands (Figure 6), results from the drug intercalating into free DNA, rendering it a poorer substrate for the Top1-catalyzed cleavage reaction. Some of the indenoisoquinolines act as Top1 poisons (i.e., inhibiting the DNA religation reaction) at low drug concentrations and as Top1 suppressors (i.e., inhibiting the DNA cleavage reaction) at high drug concentrations. The steric bulk of the substituent on the lactam side chain seems to play a role in determining whether a given indenoisoquinoline poisons or suppresses Top1, with larger substituents showing a preference for DNA intercalation into the binary Top1−DNA complex instead of free DNA.[44] In the present case, the 3-nitro group seems to facilitate intercalation into free DNA so that **55**, **56**, **59**, and **60**, with large substituents on the side chain, all act as Top1 suppressors at high drug concentrations (Figure 6).

TDP1 inhibition was measured as the ability of a drug to inhibit the enzyme-catalyzed hydrolysis of the phosphodiester
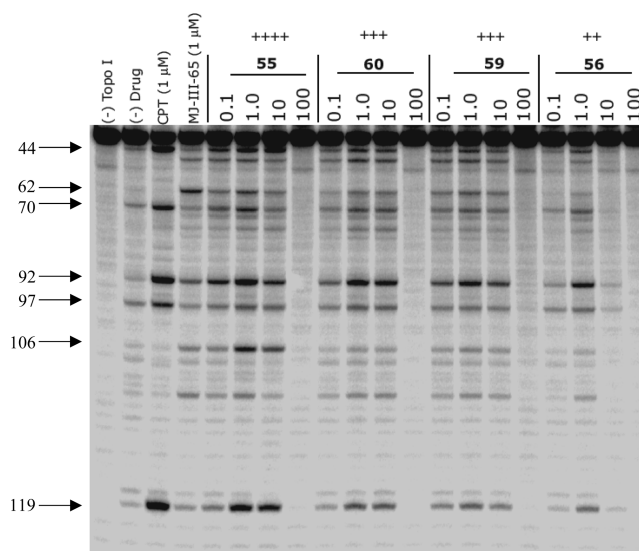

Figure 6. Top1-mediated DNA cleavage induced by indenoisoquinolines **55**, **56**, **59**, and **60**. From left to right: lane 1, DNA alone; lane 2, DNA + Top1; lane 3, CPT (**1**), 1 μM; lane 4, MJ-III-65, 1 μM; lanes 5−20, **55**, **56**, **59**, and **60** at 0.1, 1.0, 10, and 100 μM, respectively. Numbers and arrows on the left indicate arbitrary cleavage site positions. MJ-III-65 is the positive indenoisoquinoline control.[45]

linkage between tyrosine and the 3′-end of a DNA substrate (N14Y), thus preventing the generation of an oligonucleotide with a free 3′-phosphate (N14P).[21] The anti-TDP1 potency, as indicated by the disappearance of the gel band for N14P, was recorded as IC₅₀ based on the following semiquantitative scale: 0, IC₅₀ > 111 μM; +, IC₅₀ between 37 and 111 μM; ++, IC₅₀ between 12 and 37 μM; +++, IC₅₀ between 1 and 12 μM; +++ +, IC₅₀ < 1 μM. Representative gels demonstrating dose-dependent Top1 and TDP1 inhibitory activities of some target indenoisoquinolines are depicted in Figures 6 and 7, respectively.

The antiproliferative activities of all of the target compounds were evaluated in the National Cancer Institute's Developmental Therapeutics Program screen (NCI-DTP) against 60 common human cancer cell lines (the "NCI-60"). All compounds were initially tested in a one-dose prescreening assay at moderately high concentration (10 μM), and those that passed a cutoff threshold were tested at five concentrations ranging from 100 μM to 10 nM. The 50% growth-inhibitory concentration (GI₅₀) in each cell line was determined by interpolation of data points received from the five-dose screen. The cytotoxicity of a compound was reported as a mean-graph midpoint (MGM) of the average GI₅₀ value across the entire panel of 60 cell lines, where GI₅₀ values that fell outside the tested range (100 μM to 10 nM) were assigned as the maximal (100 μM) and minimal (10 nM) drug concentrations, respectively, used in the screening. Top1 and TDP1 inhibitory potencies, and the antiproliferative activities of all target compounds are collectively shown in Table 1. For comparison, activities of compounds **1** (Figure 1), **4**−**5** and **8**−**11** (Figure 2) are included.

As observed in Table 1, all of the compounds exhibited at least some Top1 inhibitory activity. The nitrated 9-hydroxy-8-methoxy series **21**, **23**, and **25** displayed only weak to moderate anti-Top1 potencies (+ or ++) even though their 2,3-dimethoxy analogues **10** and **11** were excellent Top1 inhibitors (+++++). Furthermore, the nitrated 8-hydroxy-9-methoxy
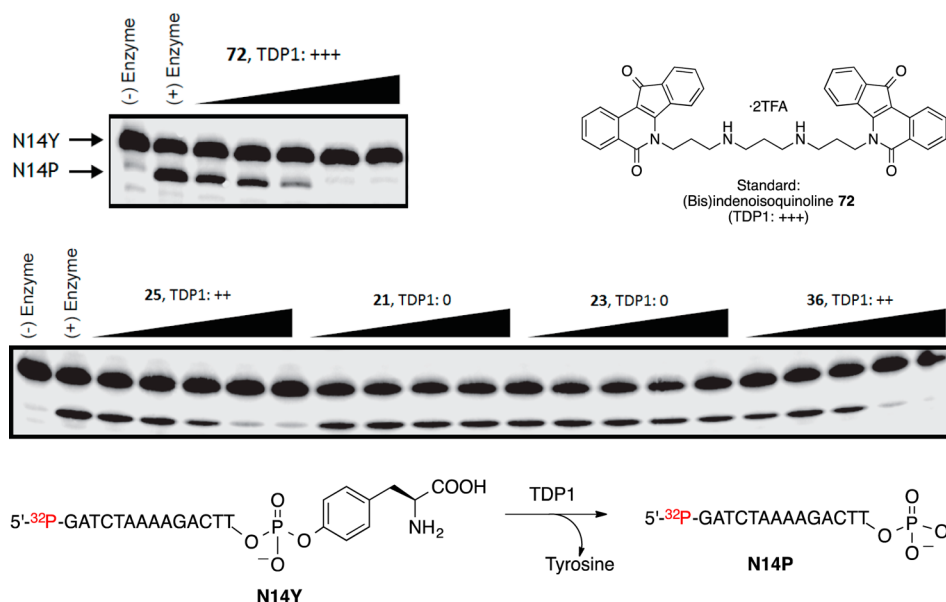
Figure 7. Inhibition of recombinant TDP1 hydrolysis of N14Y to N14P by indenoisoquinolines **21**, **23**, **25**, **36**, and **72**. From left to right: lane 1, DNA alone; lane 2, DNA + TDP1; lanes 3−22, compounds **21**, **23**, **25**, **36**, and **72** (each at 1.4, 4.1, 12.3, 37, and 111 μM). Bis(indenoisoquinoline) **72** (TDP1: +++) was used as the standard.

series **32**, **34**, and **36** showed a similar extent of Top1 inhibition as observed in their 2,3-dimethoxy analogues **8** and **9** (++ or +++). In all cases, the nitrated compounds displayed a significant improvement in terms of cytotoxicity when compared to their corresponding dimethoxy analogues, with the 9-hydroxy-8-methoxy series **21**, **23**, and **25** possessing low nanomolar antiproliferative potencies (MGM values of 16−21 nM). Although the apparent enhancement of cytotoxicity brought about by the presence of the 3-nitro group was in agreement with our initial hypothesis, the stark contrasts in Top1 inhibition among these very similar analogues was rather surprising for several reasons.

First, the electron-withdrawing nature of the 3-nitro group is proposed to confer biological activity to the indenoisoquinolines because it facilitates the $\pi-\pi$ stacking by enhancing charge-transfer interactions between the aromatic indenoisoquinoline ring system and the flanking DNA base pairs, and it also provides a favorable hydrogen bond to the Asn722 residue of Top1 in the spacious area provided by the cleaved DNA strand, thus moving the inhibitor away from the crowded environment of the uncleaved DNA strand. As a result, favorable A-ring electronics may offset the deleterious steric effect of bulky substituents at the 9-position that clash with the unbroken DNA strand.[34−36] As noted above, Morrell et al. reported that 3-nitroindenoisoquinolines bearing bulky groups like methoxycarbonyl, ethoxy, or halogen (bromine, iodine) at the 9-position exhibited excellent Top1 inhibitory potencies (+++ to ++++).[34]

Second, even though this effect did not enhance Top1 inhibition, it was expected to at least retain, instead of drastically attenuate the activity, as shown by the drastic drop in the nitrated 9-hydroxy-8-methoxy series **21**, **23**, and **25** (+ or ++) compared to their dimethoxy analogues **10** and **11** (+++++). Such a decrease in Top1 inhibition is even more striking if one takes into account that for the 2,3-dimethoxy series, the 9-hydroxy-8-methoxy compounds **10** and **11** are much more potent than their 8-hydroxy-9-methoxy regioisomers **8** and **9**. Rationalizing this observation through docking studies led to

the hypothesis that for the 2,3-dimethoxy series, the 9-methoxy group protrudes near the phosphodiester backbone of the uncleaved DNA strand, thus representing a less favorable fit as compared to the 8-methoxy group.[33] However, for the 3-nitro series, the results were completely opposite because the 9-hydroxy-8-methoxy compounds **21**, **23**, and **25** were not better but were even somewhat less active than their 8-hydroxy-9-methoxy regioisomers **32**, **34**, and **36**.

Third, the anti-Top1 potency and antiproliferative activity generally correlate well, with better Top1 inhibitors also displaying higher cytotoxicity. However, this was not the case for the nitrated 9-hydroxy-8-methoxy series **21**, **23**, and **25** because these relatively weak Top1 inhibitors (+ or ++) exhibited cytotoxicities (16−21 nM) 4-fold higher than their strong Top1 inhibitor (+++++) dimethoxy analogues **10** and **11** (55−87 nM). The reason for the lack of a strong correlation between Top1 inhibition and antiproliferative activity observed for the nitrated series remains unknown, but it is probably a complicated consequence of several factors, including pharmacokinetics (cellular permeability, solubility, and metabolism) and possible off-target effects.

All of the nitrated hydroxyindenoisoquinolines that do not possess a D-ring methoxy group displayed similar or better Top-1 inhibitory potency than their analogues with a methoxy group. In contrast to the 9-hydroxy-8-methoxy series, a strong correlation between Top1 inhibition and antiproliferative activity was observed for these compounds. Indeed, the order of Top1 inhibition and cytotoxicity went from the 9-hydroxyl series **68−71** as the most active and cytotoxic (Top1 inhibition ++++ or more, MGM 14−117 nM) to the 8-hydroxyl series **55**, **59**, and **63** (+++ to ++++, 56−407 nM), and finally to the equipotent 7-hydroxyl series **43−45** and the 10-hydroxyl series **56**, **60**, and **64** (++ to +++ for both series, 234 to 3550 nM for the 10-hydroxyl) as the least active and least cytotoxic. The 7-hydroxyl series **43−45** had very low cytotoxicities in the initial one-dose testing at 10 μM that did not pass the predetermined cutoff value to warrant the subsequent five-dose testing required for the GI_{50} determination in the NCI-60.

Table 1. Top1 and TDP1 Inhibitory Potencies and Antiproliferative Activities of Nitrated Hydroxyindenoisoquinolines

Structures:

21: R = morpholine; 23: R = imidazole; 25: R = NH$_2$
32: R = morpholine; 34: R = imidazole; 36: R = NH$_2$
43: R = morpholine; 44: R = imidazole; 45: R = NH$_2$
55: R = morpholine; 59: R = imidazole; 63: R = NH$_2$
68: R = Br; 69: R = morpholine; 70: R = imidazole; 71: R = NH$_2$
56: R = morpholine; 60: R = imidazole; 64: R = NH$_2$

	compd	Top1[b] cleavage	TDP1[c] inhibition	MGM[d] (μM)	cytotoxicity (GI$_{50}$ in μM)[a] lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVACAR-3	renal SN12C	prostate DU-145	breast MCF7
camptothecin	1[34]	++++	–[e]	0.040 ± 19[f]	<0.010	0.030	<0.010	<0.010	0.22	0.020	<0.010	<0.010
7-methoxy	4[34,37]	++++	+++	1.4	1.1	0.72	1.4	2.3	2.3	7.1	1.1	1.6
9-methoxy	5[34,37]	+++++	+++	0.027 ± 8	<0.010	<0.010	<0.010	<0.010	2.8	<0.010	–	3.3
8-hydroxy-9-methoxy	8[33]	+++	+	3.1 ± 0.3	0.18	2.3	0.28	0.070	8.3	0.19	0.20	0.030
9-hydroxy	9[33]	++(+)	0	42 ± 7	>100	>100	–	0.33	>100	–	>100	0.050
9-hydroxy-8-methoxy	10[33]	+++++	0	0.087 ± 63	0.020	0.030	0.020	0.020	0.030	0.020	0.020	0.020
	11[33]	++++(+)	0	0.055 ± 3	<0.010	<0.010	<0.010	<0.010	0.020	<0.010	<0.010	<0.010
9-hydroxy-8-methoxy	21	++	0	0.021 ± 2	<0.010	<0.010	<0.010	<0.010	–	<0.010	<0.010	<0.010
	23	+	0	0.016 ± 2	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
	25	++	++	0.017 ± 2	<0.010	<0.010	<0.010	<0.010	0.027	<0.010	<0.010	<0.010
8-hydroxy-9-methoxy	32	++	0	0.20 ± 1	0.079	0.13	0.13	0.039	0.72	0.063	0.11	0.035
	34	++(+)	++	0.74 ± 5	0.085	0.071	0.049	0.049	0.32	0.051	0.051	0.020
	36	++	++	0.066 ± 2	0.47	0.15	0.66	0.95	1.7	0.45	0.43	0.17
7-hydroxy	43	++	0	–	–	–	–	–	–	–	–	–
	44	+++	0	–	–	–	–	–	–	–	–	–
	45	++	++	–	–	–	–	–	–	–	–	–
8-hydroxy	55	++++	0	0.056 ± 6	0.025	0.026	0.021	0.020	0.041	0.036	0.034	<0.010
	59	+++	0	0.072 ± 6	–	<0.010	<0.010	<0.010	0.023	<0.010	<0.010	<0.010
	63	+++(+)	+++	0.41 ± 1	–	0.22	0.21	0.21	0.27	0.27	0.35	0.12
9-hydroxy	68	+++++	+	0.022 ± 2	–	<0.010	<0.010	<0.010	0.023	<0.010	<0.010	<0.010
	69	+++++	0	0.014 ± 1	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
	70	+++(+)	0	0.023 ± 2	<0.010	<0.010	<0.010	<0.010	0.019	<0.010	<0.010	<0.010
	71	++++	+	0.12 ± 1	0.056	0.078	0.030	0.041	0.22	0.046	0.052	0.026
10-hydroxy	56	++	0	3.5	12	0.55	0.20	0.39	29	–	–	0.064
	60	+++	0	0.23	0.081	0.13	0.15	0.18	0.23	0.17	0.13	0.056
	64	+++	++	2.0	1.9	0.47	1.7	2.5	2.9	3.4	1.2	0.25

[a]The listed cytotoxicity GI$_{50}$ values are concentrations that induce 50% growth inhibition and are the results of single determination. [b]Top1 inhibitory potency in the Top1-mediated DNA cleavage assay is graded relative to 1 μM camptothecin (1): 0, no inhibitory activity; +, 20–50% activity; ++, 50–75% activity; +++, 75–95% activity; ++++, equipotent; +++++, more potent. Ambiguous scores between two values are designated with parentheses (e.g., ++(+) would be between ++ and +++). [c]TDP1 inhibition is reported as IC$_{50}$ based on the following scale (μM): 0, IC$_{50}$ > 111; +, IC$_{50}$ 37–111; ++, IC$_{50}$ 12–37; +++, IC$_{50}$ 1–12; ++++, IC$_{50}$ < 1. [d]Mean-graph midpoint (MGM) is an approximate average of GI$_{50}$ values across the entire panel of 60 human cancer cell lines successfully tested, calculated by assigning MGM GI$_{50}$ values of 0.01 and 100 μM to compounds whose MGM GI$_{50}$ values fall outside the testing range of 0.01–100 μM. [e]–, not tested; for TDP1, the values were not available from previous reports; for MGM, the compound had a low activity in the initial one-dose testing at 10 μM that did not pass the predetermined cutoff value to warrant the five-dose testing required for the GI$_{50}$ determination; for GI$_{50}$ of individual cell lines, no values available in the NCI-60 reports, probably as a result of unsuccessful tests. [f]MGM GI$_{50}$ values with a standard deviation have their individual GI$_{50}$ value for each cell line as an average of two determinations, otherwise one determination was done.

J

Interestingly, the 9-hydroxyl series **68**−**71** displayed potencies and cytotoxicities comparable to or even greater than camptothecin (**1**, ++++, 40 nM) and the 3-nitro-9-methoxy standard **5** (+++++, 27 nM). Therefore, the 9-hydroxyl group fulfilled our initial mission of replacing the 9-methoxy group in compounds **5**−**7** with a reactive functional group that could be derivatized to produce prodrugs or linked to targeting moieties or antibodies for improvement of selectivity for cancer cells vs normal cells. The better overall activities of the 8- and 9-hydroxyl series as compared to the 7- and 10-hydroxyl series suggest that the 8- and 9-positions are optimal for forming favorable interactions between the D-ring hydroxyl group and their surroundings in the ternary cleavage complex. In agreement with the docking results reported by Morrell (Figure 4),[36] the 8- and 9-hydroxyl groups are within a reasonable distance to form a water-mediated hydrogen bond from the oxygen atom to the phosphodiester DNA backbone, and the electron-rich oxygen atom also provides an electrostatic potential that is complementary to the outer edges of the nonscissile DNA strand. Furthermore, because the 9-hydroxyl group can be considered to be directly across from and in resonance with the 3-nitro group, the slightly higher activities observed for the 9-hydroxyl series might result from resonance contributors involving donation of electron density from the phenol to the nitro group. Within each series, the position of the hydroxyl group in the D-ring is a more important determinant of Top1 inhibitory activity than the identity of the amine group in the side chain.

Interestingly, the indenoisoquinoline bromide **68**, a member of the 9-hydroxyl series, was a more potent Top1 poison (+++++) than CPT (**1**, ++++). Indeed, the following two points are apparent from the inspection of the gels in Figure 8: (1) **68** trapped Top1 cleavage complexes at a concentration as low as 5 nM, whereas CPT had lesser activity at that concentration, and (2) in contrast to the other indenoisoquinolines reported in this study, the bromide **68** did not intercalate into free DNA to suppress the cleavage reaction at a concentration as high as 100 μM. It might be reasonable to pose the question of whether the superior potency of **68** came about as a result of its DNA alkylation via displacement of the bromide group in the inhibitor. To answer this question, the kinetics of ternary cleavage complex reversal for **68** were studied, and the results showed that it formed drug−Top1−DNA cleavage complexes that did not reverse as quickly as CPT at their common cleavage sites (70, 92, 97, and 119) (Figure 9). The ternary cleavage complex complexes induced by **68** were quite persistent but did reverse over time, while those induced by CPT reversed much faster. Obviously **68** forms more stable cleavage complexes than CPT. The decrease in intensity of the cleavage bands induced by **68** over time also suggest that the drug did not alkylate or form an irreversible ternary cleavage complex.

Table 1 reveals that most nitrated hydroxyindenoisoquinolines were either inactive or weakly active against TDP1 with 0 or + activity. However, there were also compounds that exhibited good anti-TDP1 potencies (++ to +++), with the 8-hydroxyindenoisoquinoline amine **63** displaying the highest activity (+++). With an excellent anti-Top1 potency of ++++, amine **63** is the most potent dual Top1−TDP1 inhibitor reported in the present study. A thorough inspection of TDP1 inhibition within each series in Table 1 revealed an apparent trend: compounds with a primary amine side chain consistently exhibited higher potencies than their morpholine and imidazole
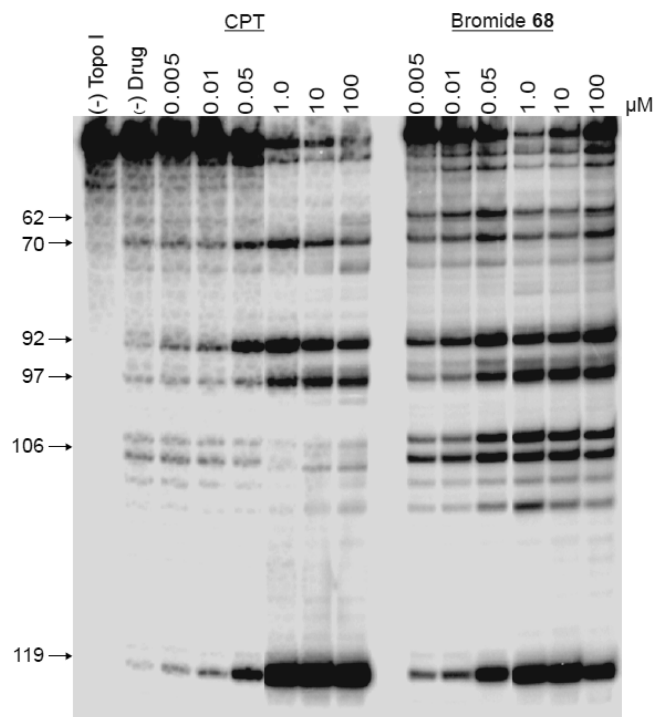

Figure 8. Comparison of Top1 inhibitory potencies between camptothecin (CPT, **1**) and indenoisoquinoline bromide **68** in a dose-dependent Top1-mediated DNA cleavage. From left to right: lane 1, DNA alone; lane 2, DNA + Top1; lanes 3−16, CPT (**1**) and **68** at 0.005, 0.01, 0.05, 0.1, 1.0, 10, and 100 μM, respectively.

analogues, with one single exception of the imidazole **34** possessing an anti-TDP1 potency (++) comparable to its amino analogue **36**. This trend also consistently applied across the whole series of hydroxyindenoisoquinolines, with or without a methoxy. This observation is in agreement with the hypothesis that the positively charged ammonium cation is of importance in orienting the ammonium group toward the hydrophilic region in the TDP1 active site so that the aromatic indenoisoquinoline ring system could reside in close proximity and form favorable contacts with some key residues in the catalytic region (H263, K265, N283, H493, K495, and N516), thus conferring TDP1 inhibitory activities to the indenoisoquinolines.[3] Moreover, it is also apparent from Table 1 that most indenoisoquinolines with a morpholine or imidazole side chain are inactive against TDP1 (0 activity), with the imidazole **34** as the single exception (++ activity). Similar observations regarding indenoisoquinoline TDP1 inhibitors were reported by Conda-Sherian[37] and Lv,[46] in which compounds bearing a bulky group on the lactam side chain (e.g., morpholine, imidazole, Boc-protected amino, N-methylpiperidine) showed no TDP1 inhibition.

When compared to the lead compounds **4**−**5**, the target compounds were, in general, somewhat weaker TDP1 inhibitors. The primary amines **25**, **36**, **45**, **63**, **64**, and **71** were all TDP1 inhibitors. The most advantageous position for a single hydroxyl group in the D-ring for the highest TDP1 inhibitory activity is at C-8, followed by C-7 and C-10, which confer equal activity. Placing the hydroxyl group at C-9 is the least effective.
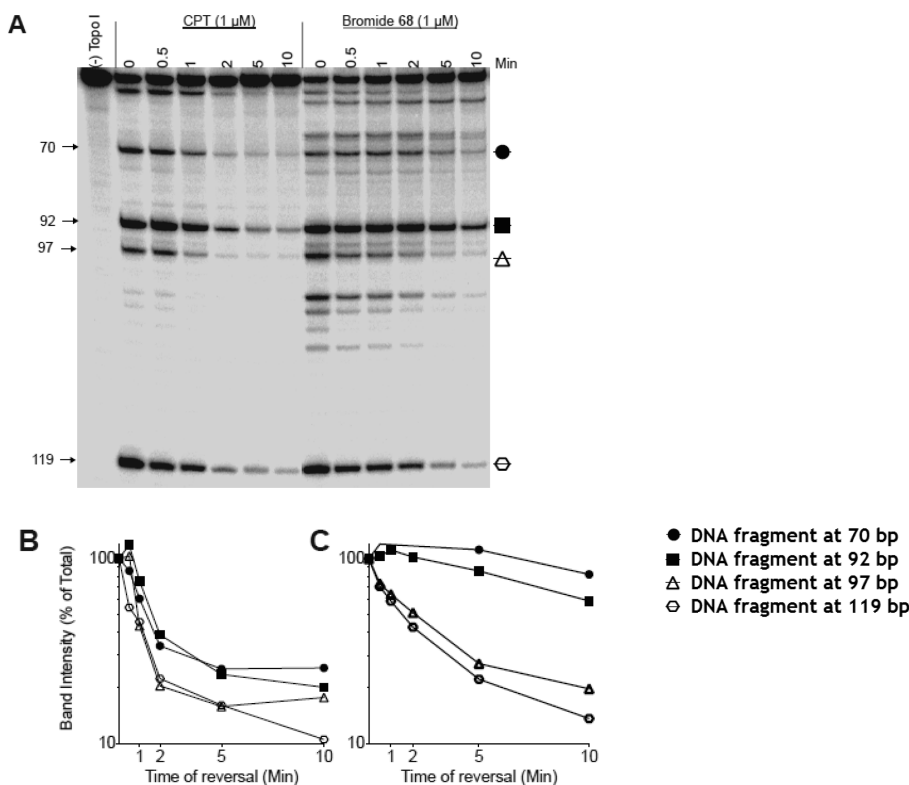
Figure 9. Kinetics of reversal assay of Top1-mediated DNA cleavage complexes induced by CPT vs bromide **68**. The forward reaction was terminated with 0.35 M NaCl.

■ CONCLUSION

A series of 18 nitrated 7-, 8-, 9-, and hydroxyindenoisoquinolines were designed as dual Top1–TDP1 inhibitors based on the ideas that (1) they are the demethylated analogues of the lead compounds **4** and **5**, both of which are potent dual Top1–TDP1 inhibitors, (2) the hydroxyl group might serve as a replacement of the methoxy group in the lead compounds **4** and **5** and provide a point of attachment for prodrug modules, targeting moieties, or antibodies, and (3) both the 3-nitro group in the A-ring and the hydroxyl group in the D-ring of indenoisoquinolines were previously shown separately to enhance both Top1 inhibitory and antiproliferative activities of Top1 poisons. All of the target compounds were synthesized using the condensation of homophthalic anhydride and Schiff bases bearing appropriate substituents, and were evaluated for dual Top1–TDP1 inhibition and antiproliferative activities. The 9-hydroxyl series comprised of compounds **68**–**71** was the most active Top1 inhibitors in the series. Among them, bromide **68** exhibited many unique features: (1) its anti-Top1 potency and cytotoxicity surpassed those of CPT, (2) it acted as a Top1 poison and was not a Top1 suppressor at a concentration as high as 100 μM, whereas many other indenoisoquinolines start to suppress DNA cleavage at this concentration, (3) it induced a drug–DNA–Top1 ternary cleavage complex that reversed slower than that of camptothecin, and (4) there was no evidence of DNA alkylation. All the indenoisoquinolines bearing a terminal amine on their lactam side chains had TDP1 inhibitory activities, while their morpholine and imidazole analogues were generally inactive. The relative TDP1 inhibitory potencies of amines **45** (++), **63** (+++), **64** (++), and **71** (+) indicate that the most advantageous location for installation of a single

hydroxyl group in the D-ring is at C-8. The 8-hydroxyindenoisoquinoline amine **63** is the most potent dual Top1–TDP1 inhibitor reported in the present studies (++++ for Top1 and +++ for TDP1). Its activities are also comparable to the bis(indenoisoquinoline) **72** (Figure 7), which is the most potent dual Top1–TDP1 inhibitor. These results detail successful potency optimization of phenolic indenoisoquinolines as Top1–TDP1 inhibitors.

■ EXPERIMENTAL SECTION

General. Solvents and reagents were purchased from commercial vendors and were used without further purification. Melting points were determined using capillary tubes with a Mel-Temp apparatus and were uncorrected. Infrared spectra were obtained as films on KBr pellets with $CHCl_3$ as the solvent, using a PerkinElmer 1600 series or Spectrum One FTIR spectrometer, and were baseline-corrected. ^{1}H NMR spectra were recorded at 300 or 500 MHz, using Bruker ARX300 or Bruker Avance 500 spectrometers with a QNP probe or TXI 5 mm/BBO probe, respectively. Mass spectral analyses were performed at the Purdue University Campus-Wide Mass Spectrometry Center. ESI-MS studies were performed using a FinniganMAT XL95 (FinniganMAT Corp., Bremen, Germany) mass spectrometer. The instrument was calibrated to a resolution of 10000 with a 10% valley between peaks using the appropriate polypropylene glycol standards. EI/CI-MS studies were performed using a Hewlett-Packard Engine or GCQ FinniganMAT mass spectrometer. APCI-MS studies were performed using an Agilent 6320 ion trap mass spectrometer. Combustion microanalyses were performed at Midwest Microlab, LLC (Indianapolis, IN). All reported values were within 0.4% of calculated values. Analytical thin layer chromatography was carried out on Baker-flex silica gel IB2-F plastic-backed TLC plates. Compounds were visualized with both short and long wavelength UV light and ninhydrin staining unless otherwise specified. Silica gel flash column chromatography was performed using 40–63 μM flash silica gel. Analytical HPLC studies were performed using a Waters 1525 binary

HPLC pump with a Waters 2487 dual wavelength absorbance detector and an injection volume of 10 μL. A Sunrise C_{18} 5 μM 100 Å reverse-phase column with dimensions of 15 cm × 4.6 mm (ES Industries) was used for all analytical HPLC experiments. For purities estimated by HPLC, the major peak accounted for ≥95% of the combined total peak area when monitored by a UV detector at 254 nm unless otherwise specified. All yields refer to isolated compounds.

4-Nitrohomophthalic Acid.[47] Homophthalic acid (**12**, 10.0 g, 55.5 mmol) was slowly added to fuming HNO_3 (35 mL) at 0 °C. After the addition was complete, the clear-yellow solution was warmed to room temperature, and stirring was continued for 5 h, during which a white precipitate was formed. Ice was slowly added to the cloudy mixture until the volume was doubled. The mixture was sonicated until the ice melted, and then it was filtered. The residue was washed with cold H_2O to afford the product **13** as an amorphous white solid (8.83 g, 71%): mp 219–220 °C (lit.[47] 217 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61 (d, J = 2.6 Hz, 1 H), 8.37 (dd, J = 2.6 and 5.6 Hz, 1 H), 7.67 (d, J = 8.4 Hz, 1 H), 4.10 (s, 2 H).

4-Nitrohomophthalic Anhydride (14).[48] Diacid **13** (8.83 g, 39.2 mmol) was diluted in acetyl chloride (30 mL), and the mixture was stirred and heated at reflux for 4 h. The yellow solution was then evaporated to dryness. The resulting residue was filtered and washed with 50% $CHCl_3$ in hexane to provide the product **14** as pale-yellow solid (5.75 g, 71%); mp 147–148 °C (lit.[48] 154–155 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.66 (s, 1 H), 8.55 (d, J = 8.1 Hz, 1 H), 7.73 (d, J = 7.9 Hz, 1 H), 4.41 (s, 2 H).

Benzylvanillin (16).[49] Vanillin (**15**, 5.00 g, 32.9 mmol), benzyl bromide (5.90 g, 34.5 mmol), and K_2CO_3 (9.08 g, 65.7 mmol) were diluted in DMF (50 mL). The yellow mixture was stirred at room temperature for 2 h, poured into a solution of Et_2O–H_2O (200 mL, 1:1), and stirred for 10 min. The ethereal layer was separated. The aqueous layer was extracted with Et_2O (50 mL × 2). The combined extract was washed with H_2O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. The crude residue was washed with hexane to provide the pure product **16** as a white solid (7.91 g, 99%); mp 49–51 °C (lit.[49] 61 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.84 (s, 1 H), 7.44–7.36 (m, 7 H), 7.00 (d, J = 8.2 Hz, 1 H), 5.25 (s, 2 H), 3.95 (s, 3 H).

N-[4′-(Benzyloxy)-3′-methoxybenzylidene]-3-bromopropyl-1-amine (17).[33] Benzylvanillin **16** (3.00 g, 12.4 mmol), 3-bromopropyl-amine hydrobromide (3.12 g, 14.2 mmol), Et_3N (1.39 g, 13.6 mmol), and Na_2SO_4 (3.52 g, 24.8 mmol) were diluted in $CHCl_3$ (100 mL). The mixture was stirred at room temperature for 16 h and then washed with H_2O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to provide the product **17** as a yellow syrup (4.49 g, 100% + residual solvent). IR (film) 2937, 2841, 1646, 1602, 1587, 1512, 1456, 1415, 1270, 1233, 743 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1 H), 7.45–7.30 (m, 6 H), 7.10 (dd, J = 1.7 and 6.4 Hz, 1 H), 6.90 (d, J = 8.2 Hz, 1 H), 5.20 (s, 2 H), 3.95 (s, 3 H), 3.74 (t, J = 6.1 Hz, 2 H), 3.51 (t, J = 6.5 Hz, 2 H), 2.27 (m, 2 H).

cis-3-[4-(Benzyloxy)-3-methoxyphenyl]-N-(3-bromopropyl)-4-car-boxy-3,4-dihydro-7-nitro-1(2H)-isoquinolone (18). Schiff base **17** (7.48 g, 20.7 mmol) was diluted in $CHCl_3$ (50 mL) and cooled to 0 °C, and anhydride **14** (4.28 g, 20.7 mmol) was added. The red mixture was stirred at 0 °C for 2 h and then at room temperature for 3 h. The mixture was filtered, and the residue was washed with 50% $CHCl_3$ in hexane to afford the product **18** as a white solid (7.55 g, 64%); mp 145–146 °C. IR (film) 3079, 1748, 1621, 1520, 1493, 1418, 1349, 1177, 755 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (d, J = 2.4 Hz, 1 H), 8.36 (dd, J = 2.5 and 6.0 Hz, 1 H), 7.87 (d, J = 8.8 Hz), 7.35–7.28 (m, 5 H), 6.71 (d, J = 8.9 Hz, 1 H), 6.50 (m, 2 H), 5.13 (d, J = 6.4 Hz, 1 H), 5.04 (s, 2 H), 4.80 (d, J = 6.4 Hz, 1 H), 4.04 (m, 1 H), 3.63 (s, 3 H), 3.48 (m, 2 H), 3.28 (m, 1 H), 2.31 (m, 1 H), 2.18 (m, 1 H). ESI-MS m/z (rel intensity) 569/571 ([MH]$^+$, 27/28). HRMS (+ESI) calcd for $C_{27}H_{25}BrN_2O_7$ MH$^+$, 569.0923; found. 569.0932.

9-(Benzyloxy)-6-(3-bromopropyl)-8-methoxy-3-nitro-5H-indeno-[1,2-c]isoquinoline-5,11(6H)-dione (19). Cis acid **18** (1.50 g, 2.63 mmol) was diluted in $SOCl_2$ (50 mL) and the mixture was stirred at

room temperature for 16 h. The red solution was evaporated to dryness. The resulting residue was diluted with $CHCl_3$ (50 mL) and treated slowly with saturated aqueous $NaHCO_3$ (100 mL). The mixture was stirred at room temperature for 10 min, and the two layers were separated. The aqueous layer was extracted with $CHCl_3$ (100 mL × 2). The combined extract was washed with H_2O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated, adsorbed onto SiO_2, and purified by flash column chromatography (SiO_2), eluting with $CHCl_3$ to provide the product **19** as a reddish-brown solid (722 mg, 50%); mp 218–220 °C (dec). IR (film) 1677, 1611, 1504, 1427, 1336, 1300, 746 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.15 (d, J = 2.4 Hz, 1 H), 8.78 (d, J = 9.0 Hz, 1 H), 8.47 (dd, J = 2.5 and 6.7 Hz, 1 H), 7.44–7.36 (m, 6 H), 7.31 (d, J = 1.8 Hz, 1 H), 5.26 (s, 2 H), 4.71 (t, J = 7.0 Hz, 2 H), 4.06 (s, 3 H), 3.74 (t, J = 5.7 Hz, 2 H), 2.51 (m, 2 H). ESI-MS m/z (rel intensity) 549/551 ([MH]$^+$, 42/53). HRMS (+ESI) calcd for $C_{27}H_{21}BrN_2O_6$ MH$^+$, 549.0661; found, 549.0672.

9-(Benzyloxy)-8-methoxy-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11[6H]-dione (20). Bromide **19** (320 mg, 0.58 mmol) and morpholine (304 mg, 3.49 mmol) were diluted in 1,4-dioxane (30 mL). The mixture was stirred at room temperature for 16 h and then evaporated to dryness. The resulting residue was diluted with H_2O (100 mL) and extracted with $CHCl_3$ (50 mL × 3). The combined extract was washed with H_2O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated, adsorbed onto SiO_2, and purified by flash column chromatography (SiO_2), eluting with a gradient of MeOH in $CHCl_3$ (2% to 4%) to provide the product **20** as a brown solid (140 mg, 44%); mp 233–234 °C (dec). IR (film) 1673, 1612, 1557, 1507, 1428, 1333, 1300, 667 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.15 (d, J = 2.5 Hz, 1 H), 8.75 (d, J = 9.0 Hz, 1 H), 8.45 (dd, J = 2.4 and 6.5 Hz, 1 H), 7.47–7.35 (m, 5 H), 7.31 (s, 1 H), 7.21 (s, 1 H), 5.25 (s, 2 H), 4.63 (t, J = 7.3 Hz, 2 H), 4.01 (s, 3 H), 3.66 (m, 4 H), 2.60 (t, J = 6.7 Hz, 2 H), 2.46 (m, 4 H), 2.14 (m, 2 H). ESI-MS m/z (rel intensity) 556 ([MH]$^+$, 100). HRMS (+ESI) calcd for $C_{31}H_{29}N_3O_7$ MH$^+$, 556.2084; found, 556.2076.

9-Hydroxy-8-methoxy-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (21). Compound **20** (50 mg, 0.090 mmol) was diluted in aqueous HBr (48 wt %, 35 mL), and the mixture was heated at 70 °C for 5 h, during which it turned to a black emulsion. The cooled mixture was concentrated to remove HBr. The concentrate was then diluted with acetone (10 mL) and concentrated again. This procedure was done three times. The final mixture was filtered through an HPLC filter paper, and the residue was washed with acetone and $CHCl_3$ to provide the desired product **21** as a black solid (47.5 mg, 97%); mp >400 °C. IR (film) 3206, 1697, 1641, 1614, 1558, 1506, 1427, 1335, 792 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.45 (s, 1 H), 9.49 (s, 1 H), 8.87 (s, 1 H), 8.65 (d, J = 8.8 Hz, 1 H), 8.55 (d, J = 8.2 Hz, 1 H), 7.23 (s, 1 H), 7.08 (s, 1 H), 4.63 (m, 2 H), 4.00–3.95 (m, 7 H), 3.61 (m, 4 H), 3.10 (m, 2 H), 2.28 (m, 2 H). ESI-MS m/z (rel intensity) 447 (MH$^+$, 89). HRMS (+ESI) calcd $C_{24}H_{23}N_3O_7$ for MH$^+$, 466.1614; found, 466.1618; HPLC purity: 100% (MeOH, 100%), 97.8% (MeOH–H_2O, 90:10). Anal. Calcd for $C_{24}H_{24}BrN_3O_7$·0.1H_2O: C, 52.59; H, 4.45; N, 7.67. Found: C, 52.28; H, 4.24; N, 7.30.

6-(3-(1H-Imidazol-1-yl)propyl)-9-(benzyloxy)-8-methoxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione (22). Bromide **19** (100 mg, 0.182 mmol) and imidazole (124 mg, 1.82 mmol) were diluted in 1,4-dioxane (30 mL). The mixture was heated at 70 °C for 16 h and then evaporated to dryness. The resulting residue was diluted with H_2O (100 mL) and extracted with $CHCl_3$ (50 mL × 3). The combined extract was washed with H_2O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated, adsorbed onto SiO_2, and purified by flash column chromatography (SiO_2), eluting with 4% MeOH in $CHCl_3$ to provide the product **22** as a brown solid (36.2 mg, 37%); mp 235–236 °C (dec). IR (film) 1662, 1612, 1555, 1424, 1291, 746 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.17 (d, J = 2.3 Hz, 1 H), 8.77 (d, J = 9.0 Hz, 1 H), 8.48 (dd, J = 2.4 and 6.6 Hz, 1 H), 7.61 (s, 1 H), 7.46–7.30 (m, 5 H), 7.12 (s, 1 H), 7.05 (s, 1 H), 6.85 (s, 1 H), 5.24 (s, 2 H), 4.61 (t, J

= 6.8 Hz, 2 H), 4.27 (t, J = 6.5 Hz, 2 H), 3.86 (s, 3 H), 2.42 (m, 2 H). ESI-MS m/z (rel intensity) 537 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{30}H_{24}N_4O_6$ MH$^+$, 537.1774; found, 537.1784.

6-(3-(1H-Imidazol-1-yl)propyl)-9-hydroxy-8-methoxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (23). Compound **22** (50 mg, 0.093 mmol) was diluted in aqueous HBr (48 wt %, 35 mL) and the mixture was heated at 70 °C for 5 h, during which it turned to a brown emulsion. The cooled mixture was concentrated to remove HBr. The concentrate was then diluted with acetone (10 mL) and concentrated again. This procedure was done three times. The final mixture was filtered through an HPLC filter paper, and the residue was washed with acetone and CHCl$_3$ to provide the desired product **23** as a pale-brown solid (24.6 mg, 50%); mp >400 °C. IR (film) 3398, 1680, 1609, 1557, 1492, 1429, 1385, 1338, 859 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.43 (s, 1 H), 9.11 (s, 1 H), 8.86 (s, 1 H), 8.65 (d, J = 9.2 Hz, 1 H), 8.54 (d, J = 6.5 Hz, 1 H), 7.83 (s, 1 H), 7.68 (s, 1 H), 7.23 (s, 1 H), 7.08 (s, 1 H), 4.60 (m, 2 H), 4.37 (m, 2 H), 3.97 (s, 3 H), 2.50 (m, 2 H, under the water peak). ESI-MS m/z rel intensity) 466 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{23}H_{18}N_4O_6$ MH$^+$, 447.1305; found, 447.1303. HPLC purity: 100% (MeOH, 100%), 96.7% (MeOH−H$_2$O, 90:10). Anal. Calcd for $C_{23}H_{19}BrN_4O_6\cdot0.5H_2O$: C, 51.51; H, 3.76; N, 10.45. Found: C, 51.33; H, 3.46; N, 10.30.

6-(3-Azidopropyl)-9-(benzyloxy)-8-methoxy-3-nitro-5H-indeno-[1,2-c]isoquinoline-5,11(6H)-dione (24). Bromide **19** (150 mg, 0.273 mmol) and NaN$_3$ (178 mg, 2.73 mmol) were diluted in DMSO (50 mL), and the mixture was stirred at room temperature for 16 h. The deep-red solution was diluted with H$_2$O (100 mL) and extracted with CHCl$_3$ (50 mL × 3). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated, adsorbed onto SiO$_2$, and purified by flash column chromatography (SiO$_2$), eluting with CHCl$_3$, to provide the product **24** as a deep-red solid (36.2 mg, 26%); mp 205−207 °C (dec). IR (film) 2090, 1673, 1610, 1579, 1502, 1427, 847 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.14 (d, J = 2.3 Hz, 1 H), 8.75 (d, J = 9.0 Hz, 1 H), 8.45 (dd, J = 2.3 and 6.7 Hz, 1 H), 7.48−7.35 (m, 5 H), 7.28 (s, 1 H), 5.26 (s, 2 H), 4.61 (t, J = 6.9 Hz, 2 H), 4.07 (s, 3 H), 3.79 (t, J = 5.7 Hz, 2 H), 2.16 (m, 2 H). ESI-MS m/z (rel intensity) 512 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{27}H_{21}N_5O_6$ MH$^+$, 512.1570; found, 512.1576.

6-(3-Aminopropyl)-9-hydroxy-8-methoxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (25). Azide **24** (30 mg, 0.059 mmol) was diluted in benzene (50 mL), and triethyl phosphite (29.2 mg, 0.176 mmol) was added. The mixture was heated at reflux for 16 h and then allowed to cool to room temperature. Aqueous HBr (48 wt %, 30 mL) was added, and the mixture was heated at 70 °C for 5 h, during which it turned to a brown/red emulsion. The cooled mixture was concentrated to remove benzene and HBr. The concentrate was then diluted with acetone (10 mL) and concentrated again. This procedure was done three times. The final mixture was filtered through an HPLC filter paper, and the residue was washed with acetone and CHCl$_3$ to provide the desired product **25** as a brown solid (26.0 mg, 93%); mp 285−287 °C (dec). IR (film) 3243, 2848, 1705, 1641, 1614, 1562, 1488, 1336, 1207, 1133, 868 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.41 (s, 1 H), 8.83 (d, J = 2.3 Hz, 1 H), 8.60 (d, J = 9.0 Hz, 1 H), 8.51 (dd, J = 2.5 and 6.5 Hz, 1 H), 7.74 (br s, 3 H), 7.19 (s, 1 H), 7.03 (s, 1 H), 4.58 (m, 2 H), 3.98 (s, 3 H), 3.01 (m, 2 H), 2.14 (m, 2 H). ESI-MS m/z (rel intensity) 396 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{20}H_{19}N_3O_6$ MH$^+$, 396.1196; found, 396.1199. HPLC purity: 100% (MeOH, 100%), 98.6% (MeOH−H$_2$O, 90:10). Anal. Calcd for $C_{20}H_{18}BrN_3O_6$: C, 50.44; H, 3.81; N, 8.82. Found: C, 50.13; H, 3.75; N, 8.59.

Benzylisovanillin (27).[50] Isovanillin **26** (5.00 g, 32.9 mmol), benzyl bromide (5.90 g, 34.5 mmol), and K$_2$CO$_3$ (9.08 g, 65.7 mmol) were diluted in DMF (50 mL). The yellow mixture was stirred at room temperature for 2 h and then poured into a solution of Et$_2$O−H$_2$O (200 mL, 1:1) and stirred for 5 min. The ethereal layer was separated. The aqueous layer was extracted with Et$_2$O (50 mL × 2). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and

concentrated to dryness. The crude residue was washed with hexane to provide the pure product **27** as a white solid (7.34 g, 92%); mp 48−49 °C (lit.[50] 61−62 °C). ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.82 (s, 1 H), 7.48−7.46 (m, 4 H), 7.39−7.36 (m, 3 H), 7.01 (d, J = 8.5 Hz, 1 H), 5.20 (s, 2 H), 3.97 (s, 3 H).

N-[3′-(Benzyloxy)-4′-methoxybenzylidene]-3-bromo-1-propyl-amine (28).[33] Benzylisovanillin **27** (3.00 g, 12.4 mmol), 3-bromopropylamine hydrobromide (3.12 g, 14.2 mmol), Et$_3$N (1.39 g, 13.6 mmol), and Na$_2$SO$_4$ (3.52 g, 24.8 mmol) were diluted in CHCl$_3$ (100 mL). The mixture was stirred at room temperature for 16 h and then washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to provide the product **28** as a yellow syrup (4.49 g, 100% + residual solvent). IR (film) 2935, 2837, 1642, 1602, 1583, 1512, 1437, 1265, 1137, 741 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.21 (s 1 H), 7.49−7.44 (m, 3 H), 7.40−7.33 (m, 3 H), 7.21 (dd, J = 6.4 and 1.8 Hz, 1 H), 6.92 (d, J = 8.2 Hz, 1 H), 5.20 (s, 2 H), 3.93 (s, 3 H), 3.73 (t, J = 5.4 Hz, 2 H), 3.51 (t, J = 6.5 Hz, 2 H), 2.26 (m, 2 H).

cis-3-[3-(Benzyloxy)-4-methoxyphenyl]-N-(3-bromopropyl)-4-car-boxy-3,4-dihydro-7-nitro-1(2H)-isoquinolone (29). Schiff base **28** (4.486 g, 12.38 mmol) was diluted in CHCl$_3$ (50 mL), and the mixture was cooled to 0 °C for 10 min. Anhydride **14** (2.56 g, 12.38 mmol) was then added. The red mixture was stirred at 0 °C for 2 h and then at room temperature for 3 h. The mixture was filtered, and the residue was washed with 50% CHCl$_3$ in hexane to provide the product **29** as a white solid (4.867 g, 69%); mp 165−167 °C. IR (film) 3078, 1754, 1630, 1519, 1352, 1259, 1272, 1142, 701 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.99 (d, J = 2.3 Hz, 1 H), 8.28 (dd, J = 8.4 and 2.5 Hz, 1 H), 7.74 (d, J = 8.4 Hz, 1 H), 7.41−7.35 (m, 6 H), 6.70 (d, J = 8.4 Hz, 1 H), 6.48 (d, J = 8.5 Hz, 1 H), 6.38 (d, J = 2.0 Hz, 1 H), 5.23 (d, J = 14 Hz, 1 H), 5.04 (s, 2 H), 4.64 (d, J = 6.5 Hz, 1 H), 3.94 (m, 1 H), 3.83 (s, 3 H), 3.43 (m, 2 H), 3.22 (m, 1 H), 2.20 (m, 1 H), 2.10 (s, 1 H). ESI-MS m/z (rel intensity) 445 ([MH − COOH − Br]$^+$, 100). HRMS (+ESI) calcd for $C_{27}H_{25}BrN_2O_7$ [MH − COOH − Br]$^+$, 445.1763; found, 445.1771.

8-(Benzyloxy)-6-(3-bromopropyl)-9-methoxy-3-nitro-5H-indeno-[1,2-c]isoquinoline-5,11[6H]-dione (30). Cis acid **29** (1.50 g, 2.63 mmol) was diluted in SOCl$_2$ (50 mL) at 0 °C and stirred for 16 h, during which the mixture warmed to room temperature. The red solution was evaporated to dryness, and the residue was diluted with CHCl$_3$ (50 mL) and treated slowly with saturated NaHCO$_3$ (100 mL). The mixture was stirred at room temperature for 10 min, and the two layers were separated. The aqueous layer was extracted with CHCl$_3$ (100 mL × 2). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated, adsorbed onto SiO$_2$, and purified by flash column chromatography (SiO$_2$), eluting with CHCl$_3$ to provide the product **30** as a greenish-brown solid (578 mg, 40%); mp 239−240 °C. IR (film) 1671, 1613, 1557, 1507, 1488, 1335, 1297, 746 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.15 (d, J = 3.3 Hz, 1 H), 8.78 (d, J = 9.0 Hz, 1 H), 8.47 (dd, J = 6.7 and 2.4 Hz, 1 H), 7.44−7.31 (m, 5 H), 6.91−6.88 (m, 2 H), 5.30 (s, 2 H), 4.59 (t, J = 7.9 Hz, 2 H), 4.02 (s, 3 H), 3.62 (t, J = 6.0 Hz, 2 H), 2.35 (m, 2 H). APCI-MS m/z (rel intensity) 549/550 ([MH]$^+$, 100). HRMS (+EI/CI) calcd for $C_{27}H_{21}BrN_2O_6$ MH$^+$, 549.0661; found, 549.0655.

8-(Benzyloxy)-9-methoxy-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11[6H]-dione (31). Bromide **30** (87 mg, 0.16 mmol) and morpholine (83 mg, 5.2 mmol) were diluted in 1,4-dioxane (30 mL). The mixture was stirred at room temperature for 16 h and then evaporated to dryness. The resulting residue was diluted with H$_2$O (100 mL) and extracted with CHCl$_3$ (50 mL × 3). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated, adsorbed onto SiO$_2$, and purified by flash column chromatography (SiO$_2$), eluting with a gradient of MeOH in CHCl$_3$ (0% to 2%), to provide the product **31** as a deep-purple solid (46 mg, 52%) after being triturated with ether; mp 229−230 °C. IR (film) 3399, 1698, 1672, 1614, 1556, 1330, 1304 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.14 (d, J = 2.3 Hz, 1 H), 8.76 (d, J = 9.0 Hz, 1 H), 8.45 (dd, J = 6.7 and 2.4 Hz, 1 H), 7.42−7.35 (m, 5 H), 7.31 (s, 1 H), 7.12 (s, 1

H), 5.30 (s, 2 H), 4.47 (t, J = 7.3 Hz, 2 H), 4.03 (s, 3 H), 3.55 (m, 4 H), 2.41 (m, 6 H), 1.88 (m, 2 H). ESI-MS m/z (rel intensity) 556 ([MH]$^+$, 100). HRMS (+ESI) calcd for $C_{31}H_{29}N_3O_7$ MH$^+$, 556.2084; found, 556.2082. HPLC purity: 99.2% (MeOH, 100%), 99.0% (MeOH–H$_2$O, 90:10).

8-Hydroxy-9-methoxy-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (32). Compound **31** (25 mg, 0.045 mmol) was diluted in aqueous HBr (48 wt %, 30 mL), and the mixture was heated at 70 °C for 5 h, during which it turned to a brown emulsion. The cooled mixture was concentrated to remove HBr. The concentrate was then diluted with acetone (10 mL) and concentrated again. This procedure was done three times. The final mixture was filtered under vacuum, and the residue was washed with acetone and CHCl$_3$ to provide the desired product **32** as a pale plum-colored solid (24.6 mg, 100%); mp 288–289 °C. IR (film) 3259, 1682, 1608, 1556, 1506, 1390, 1334, 1275, 1209, 861 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.28 (s, 1 H), 9.58 (s, 1 H), 8.84 (d, J = 2.4 Hz, 1 H), 8.65 (d, J = 8.9 Hz, 1 H), 8.55 (dd, J = 2.5 and 6.5 Hz, 1 H), 7.30 (s, 1 H), 7.26 (s, 1 H), 4.50 (m, 2 H), 3.92 (s, 3 H), 3.63 (m, 4 H), 3.45 (m, 4 H), 3.10 (m, 2 H), 2.24 (m, 2 H). ESI-MS m/z (rel intensity) 466 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{24}H_{23}N_3O_7$ MH$^+$, 466.1614; found, 466.1606. HPLC purity: 98.0% (MeOH, 100%), 97.2% (MeOH–H$_2$O, 90:10). Anal. Calcd for $C_{24}H_{24}BrN_3O_7$: C, 52.76; H, 4.43; N, 7.69. Found: C, 52.51; H, 4.39; N, 7.35.

6-(3-(1H-Imidazol-1-yl)propyl)-8-(benzyloxy)-9-methoxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione (33). Bromide **30** (100 mg, 0.182 mmol) and imidazole (124 mg, 1.82 mmol) were diluted in 1,4-dioxane (30 mL). The mixture was heated at 70 °C for 16 h and then evaporated to dryness. The resulting residue was diluted with H$_2$O (100 mL) and extracted with CHCl$_3$ (50 mL × 3). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated, adsorbed onto SiO$_2$, and purified by flash column chromatography (SiO$_2$), eluting with 4% MeOH in CHCl$_3$, to provide the product **33** as a brown solid (35.0 mg, 36%); mp 236–237 °C (dec). IR (film) 1671, 1612, 1557, 1507, 1334, 1299 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.14 (d, J = 2.3 Hz, 1 H), 8.76 (d, J = 9.0 Hz, 1 H), 8.47 (dd, J = 2.4 and 6.6 Hz, 1 H), 7.52 (s, 1 H), 7.38–7.36 (m, 4 H), 7.32 (m, 2 H), 7.08 (s, 1 H), 6.94 (s, 1 H), 6.83 (s, 1 H), 5.25 (s, 2 H), 4.40 (t, J = 6.5 Hz, 2 H), 4.03 (s, 3 H), 4.01 (t, J = 7.1 Hz, 2 H), 2.00 (m, 2 H). ESI-MS m/z (rel intensity) 537 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{30}H_{24}N_4O_6$ MH$^+$, 537.1774; found, 537.1780.

6-(3-(1H-Imidazol-1-yl)propyl)-8-hydroxy-9-methoxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (34). Compound **33** (30 mg, 0.056 mmol) was diluted in aqueous HBr (48 wt %, 30 mL) and the mixture was stirred at 70 °C for 5 h, during which it turned to a brown emulsion. The cooled mixture was concentrated to remove HBr. The concentrate was then diluted with acetone (10 mL) and concentrated again. This procedure was done three times. The final mixture was filtered under vacuum, and the residue was washed with acetone and CHCl$_3$ to provide the desired product **34** as a pale plum-colored solid (23.6 mg, 80%); mp 285–286 °C. IR (film) 3301, 1669, 1616, 1562, 1334, 1265, 1165, 854 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.27 (s, 1 H), 9.12 (s, 1 H), 8.80 (d, J = 2.2 Hz, 1 H), 8.62 (d, J = 8.9 Hz, 1 H), 8.52 (dd, J = 2.3 and 6.7 Hz, 1 H), 7.83 (s, 1 H), 7.68 (s, 1 H), 7.22 (s, 2 H), 4.48 (m, 2 H), 4.42 (t, J = 6.7 Hz, 2 H), 3.91 (s, 3 H), 2.41 (m, 2 H). ESI-MS m/z (rel intensity) 447 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{23}H_{18}N_4O_6$ MH$^+$, 447.1305; found, 447.1308. HPLC purity: 97.8% (MeOH, 100%), 97.3% (MeOH–H$_2$O, 90:10). Anal. Calcd for $C_{23}H_{19}BrN_4O_6$·0.1H$_2$O: C, 52.21; H, 3.66; N, 10.59. Found: C, 51.91; H, 3.55; N, 10.32.

6-(3-Azidopropyl)-8-(benzyloxy)-9-methoxy-3-nitro-5H-indeno-[1,2-c]isoquinoline-5,11(6H)-dione (35). Bromide **30** (150 mg, 0.273 mmol) and NaN$_3$ (178 mg, 2.73 mmol) were diluted in DMSO (30 mL), and the mixture was stirred at room temperature for 16 h. The deep-red solution was diluted with H$_2$O (100 mL) and extracted with CHCl$_3$ (50 mL × 3). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over

anhydrous Na$_2$SO$_4$, filtered, and concentrated, adsorbed onto SiO$_2$, and purified by flash column chromatography (SiO$_2$), eluting with CHCl$_3$ to provide the product **35** as a brown solid (40.5, 29%); mp 222–224 °C (dec). IR (film) 2099, 1713, 1666, 1614, 1508, 1340, 751 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.14 (d, J = 2.2 Hz, 1 H), 8.77 (d, J = 9.0 Hz, 1 H), 8.46 (dd, J = 2.4 and 6.5 Hz, 1 H), 7.44–7.37 (m, 5 H), 7.31 (s, 2 H), 5.33 (s, 2 H), 4.52 (t, J = 7.1 Hz, 2 H), 4.02 (s, 3 H), 3.67 (t, J = 5.9 Hz, 2 H), 2.01 (m, 2 H). ESI-MS m/z (rel intensity) 512 (MH$^+$, 28).

6-(3-Aminopropyl)-8-hydroxy-9-methoxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (36). Azide **35** (30 mg, 0.059 mmol) was diluted in benzene (50 mL), and triethyl phosphite (29.2 mg, 0.176 mmol) was added. The mixture was heated at reflux for 16 h and then allowed to cool to room temperature. Aqueous HBr (48 wt %, 30 mL) was added, and the reaction mixture was heated at 70 °C for 5 h, during which it turned to a brown/red emulsion. The cooled mixture was concentrated to remove benzene and HBr. The concentrate was then diluted with acetone (10 mL) and concentrated again. This procedure was done three times. The final mixture was filtered under vacuum, and the residue was washed with acetone and CHCl$_3$ to provide the desired product **36** as a brown solid (24.6 mg, 88%); mp 338–340 °C (dec). IR (film) 3218, 2853, 1678, 1609, 1555, 1472, 1336, 1178, 855 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.34 (s, 1 H), 8.85 (d, J = 2.5 Hz, 1 H), 8.66 (d, J = 9.0 Hz, 1 H), 8.55 (dd, J = 2.5 and 6.4 Hz, 1 H), 7.73 (br s, 3 H), 7.28 (s, 2 H), 4.49 (m, 2 H), 3.92 (s, 3 H), 2.99 (m, 2 H), 2.08 (m, 2 H). ESI-MS m/z (rel intensity) 396 (MH$^+$, 44). HRMS (+ESI) calcd for $C_{20}H_{17}N_3O_6$ MH$^+$, 396.1196; found, 396.1191. HPLC purity: 100% (MeOH, 100%), 98.6% (MeOH–H$_2$O, 90:10). Anal. Calcd for $C_{20}H_{18}BrN_3O_6$·0.5H$_2$O: C, 49.50; H, 3.95; N, 8.66. Found: C, 49.39; H, 3.77; N, 8.48.

2-(Benzyloxy)benzaldehyde (39).[51] Compound **38** (5.00 g, 0.041 mol) was diluted in DMF (30 mL), followed by addition of benzyl bromide (7.70 g, 0.045 mol) and K$_2$CO$_3$ (11.3 g, 0.082 mmol). The yellow mixture was stirred at room temperature for 3 h until it turned cloudy white. The mixture was then diluted in H$_2$O (100 mL) and extracted with ether (50 mL × 3). The combined extract was washed with H$_2$O (50 mL × 3) and brine (50 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to yield the product **39** as a colorless liquid (8.69 g, 100%). ^{1}H NMR (300 MHz, CDCl$_3$) δ 10.57 (s, 1 H), 7.88 (d, J = 2.0 Hz, 1 H), 7.86 (d, J = 1.6 Hz, 1 H), 7.54–7.38 (m, 5 H), 7.07–7.05 (m, 2 H), 5.20 (s, 2 H).

N-[2-(Benzyloxy)benzylidene]-3-bromo-1-propylamine (40). 3-Bromopropylamine hydrobromide (3.56 g, 16.2 mmol) was diluted in CHCl$_3$ (30 mL) and Et$_3$N (1.64 g, 16.2 mmol). The mixture was stirred until the salt dissolved completely, and then compound **39** (3.00 g, 14.1 mmol) and Na$_2$SO$_4$ (4.02 g, 28.3 mmol) were added. The mixture was stirred at room temperature for 16 h, diluted with CHCl$_3$ (100 mL), and then washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to yield the product **40** as a yellow syrup (4.69 g, 100%). IR (film) 2894, 1637, 1599, 1452, 1245, 754 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.82 (s, 1 H), 7.96 (d, J = 7.7 Hz, 1 H), 7.43–7.34 (m, 6 H), 7.00 (m, 2 H), 5.14 (s, 2 H), 3.78 (dt, J = 0.9 and 5.4 Hz, 2 H), 3.52 (t, J = 6.6 Hz, 2 H), 2.28 (m, 2 H). ESI-MS m/z (rel intensity) 354/356 (MNa$^+$, 99/100).

cis-4-Carboxy-3,4-dihydro-N-(3-bromopropyl)-3-[2-(benzyloxy)-phenyl]-7-nitro-1(2H)-isoquinolone (41). Schiff base **40** (4.69 g, 0.014 mmol) was diluted in CHCl$_3$ (50 mL) at 0 °C, and anhydride **14** (2.92 g, 0.014 mmol) was added. The red mixture was stirred at 0 °C for 1 h and then at room temperature for 3 h. The cloudy orange mixture was filtered, and the residue was washed with 50% CHCl$_3$ in hexane to provide the product **41** as a pale-yellow solid (3.48 g, 46%); mp 145–147 °C. IR (film) 3075, 1749, 1621, 1579, 1525, 1486, 1353, 1162, 760 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (d, J = 2.6 Hz, 1 H), 8.38 (dd, J = 2.6 and 5.9 Hz, 1 H), 7.73 (dd, J = 0.7 and 8.0 Hz, 1 H), 7.46–7.31 (m, 5 H), 7.22 (m, 1 H), 7.05 (d, J = 8.3 Hz, 1 H), 6.74 (d, J = 3.9 Hz, 2 H), 5.74 (d, J = 6.9 Hz, 1 H), 5.05 (s, 2 H), 3.88 (m, 1 H), 3.51 (m, 3 H), 3.01 (m, 1 H), 2.11 (m, 1 H), 1.96 (m, 1 H); ESI-MS m/z (rel intensity) 415 ([MH − COOH − Br]$^+$, 100).

HRMS (+ESI) calcd for $C_{26}H_{23}BrN_2O_6$ (MH − COOH − Br)$^+$, 415.1658; found, 415.1654.

6-(3-Bromopropyl)-7-hydroxy-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione (42). Cis acid **41** (0.50 g, 0.93 mmol) was diluted in SOCl$_2$ (30 mL), and the mixture was stirred at room temperature for 5 h. The resulting yellow solution was evaporated to dryness. The yellow syrup was diluted in 1,2-dichloroethane (30 mL) at 0 °C, followed an addition of AlCl$_3$ (0.37 g, 2.78 mmol), and the mixture was stirred for 15 min. Stirring was continued at room temperature for 7 h. The black mixture was then diluted with CHCl$_3$ (50 mL) and washed with cold 6 N HCl (100 mL). The aqueous layer was extracted with CHCl$_3$ (50 mL × 2). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The yellow organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to dryness. The residue was triturated with ether, filtered, and washed with excess ether to provide the product **42** in high purity as a yellow amorphous solid (253 mg, 63%); mp 172−175 °C. IR (film) 1719, 1674, 1606, 1516, 1342 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.58 (d, J = 9.3 Hz, 1 H), 9.29 (d, J = 2.6 Hz, 1 H), 8.61 (dd, J = 2.6 and 6.7 Hz, 1 H), 8.01 (dd, J = 1.2 and 7.1 Hz, 1 H), 7.70 (dt, J = 1.3 and 7.2 Hz, 1 H), 7.53−7.42 (m, 2 H), 4.72 (t, J = 7.2 Hz, 2 H), 3.50 (t, J = 5.9 Hz, 1 H), 2.69 (m, 2 H). ESI-MS *m/z* (rel intensity) 351 ([MH − HBr]$^+$, 100).

7-Hydroxy-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione (43). Bromide **42** (70 mg, 0.16 mmol) and morpholine (71 mg, 0.81 mmol) were diluted in 1,4-dioxane (30 mL). The yellow mixture was heated at 70 °C for 16 h. The solution was then diluted with H$_2$O (100 mL) and extracted with CHCl$_3$ (50 mL × 3). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to dryness. The residue was triturated with acetone, filtered, and washed with ether to provide the product **43** as a brown solid (68.7 mg, 97%); mp 216−218 °C. IR (film) 3413, 1719, 1675, 1606, 1515, 1342, 1117 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (d, J = 9.3 Hz, 1 H), 8.99 (d, J = 2.4 Hz, 1 H), 8.68 (dd, J = 2.6 and 6.5 Hz, 1 H), 8.12 (d, J = 8.3 Hz, 1 H), 7.79 (t, J = 7.5 Hz, 1 H), 7.59 (d, J = 8.3 Hz, 1 H), 7.50 (t, J = 7.5 Hz, 1 H), 4.60 (m, 2 H), 3.17 (m, 4 H), 2.04−1.98 (m, 8 H). ESI-MS *m/z* (rel intensity) 436 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{23}H_{21}N_3O_6$ MH$^+$, 436.1509; found, 436.1505. HPLC purity: 95.0% (MeOH, 100%), 96.7% (MeOH−H$_2$O, 90:10).

6-(3-(1H-Imidazol-1-yl)propyl)-7-hydroxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione (44). Bromide **42** (100 mg, 0.23 mmol) and imidazole (79 mg, 1.16 mmol) were diluted in 1,4-dioxane (30 mL). The yellow mixture was heated at 70 °C for 16 h. The solution was then diluted with H$_2$O (100 mL) and extracted with CHCl$_3$ (50 mL × 3). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to dryness. The residue was triturated with acetone, filtered, and washed with ether to provide the product **44** as a yellow solid (77.2 mg, 79%); mp 173−175 °C. IR (film) 1717, 1675, 1606, 1515, 1342, 1119, 756 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (d, J = 9.3 Hz, 1 H), 8.97 (d, J = 2.5 Hz, 1 H), 8.67 (dd, J = 2.5 and 6.7 Hz, 1 H), 7.85 (d, J = 7.7 Hz, 1 H), 7.76 (t, J = 7.4 Hz, 1 H), 7.56 (d, J = 8.0 Hz, 1 H), 7.38 (t, J = 8.5 Hz, 1 H), 4.37 (m, 2 H), 4.14 (m, 2 H). ESI-MS *m/z* (rel intensity) 417 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{22}H_{16}N_4O_5$ MH$^+$, 417.1199; found, 417.1202.

6-(3-Aminopropyl)-7-hydroxy-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione Hydrochloride (45). Bromide **42** (100 mg, 0.23 mmol) and NaN$_3$ (45 mg, 0.70 mmol) were diluted in DMSO (30 mL). The yellow mixture was stirred at room temperature for 16 h. The solution was then diluted with H$_2$O (100 mL) and extracted with CHCl$_3$ (50 mL × 3). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to dryness. The residue was diluted in benzene (30 mL), and triethyl phosphite (116 mg, 0.70 mmol) was added. The yellow solution was heated at reflux for 16 h and then cooled to room temperature. Methanolic HCl (3 M, 40 mL) was added. The mixture was heated at 70 °C for 3 h and then concentrated to dryness. The residue was triturated with acetone, filtered, and washed with acetone to provide the product **45** as a yellow solid (56.5 mg, 60%); mp 272−274 °C (dec). IR (film) 3283, 1712, 1671, 1603, 1509, 1484, 1341, 1284, 760 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.41 (d, J = 9.3 Hz, 1 H), 9.00 (d, J = 2.6 Hz, 1 H), 8.70 (dd, J = 2.6 and 6.6 Hz, 1 H), 8.03 (d, J = 8.1 Hz, 1 H), 7.86 (br s, 3 H), 7.81 (t, J = 7.4 Hz, 1 H), 7.61 (d, J = 7.3 Hz, 1 H), 7.51 (t, J = 7.3 Hz, 1 H), 4.47 (t, J = 6.5 Hz, 2 H), 2.87 (m, 2 H), 2.32 (m, 2 H). ESI-MS *m/z* (rel intensity) 366 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{19}H_{15}N_3O_5$ MH$^+$, 366.1090; found, 366.1093. HPLC purity: 96.4% (MeOH, 100%), 98.1% (MeOH−H$_2$O, 90:10).

3-(Benzyloxy)benzaldehyde (47).[52] Compound **46** (5.00 g, 0.041 mol) was diluted in DMF (50 mL), followed by addition of benzyl bromide (7.70 g, 0.045 mol) and K$_2$CO$_3$ (11.3 g, 0.082 mmol). The yellow mixture was stirred at room temperature for 3 h until it turned cloudy white. The mixture was then diluted in H$_2$O (100 mL) and extracted with ether (50 mL × 3). The combined extract was washed with H$_2$O (50 mL × 3) and brine (50 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to yield a pale-yellow syrup, which solidified upon standing at room temperature. The solid was washed with hexane and filtered to provide the product **47** as a white solid (8.69 g, 100%); mp 43−45 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ 9.98 (s, 1 H), 7.49−7.35 (m, 8 H), 7.28 (d, J = 2.5 Hz, 1 H), 5.13 (s, 2 H).

N-[3-(Benzyloxy)benzylidene]-3-bromo-1-propylamine (48). 3-Bromopropylamine hydrobromide (6.45 g, 29.4 mmol) was diluted in CHCl$_3$ (50 mL) and Et$_3$N (2.98 g, 29.4 mmol). The mixture was stirred for 5 min, and then compound **47** (5.00 g, 23.6 mmol) and Na$_2$SO$_4$ (6.69 g, 4.71 mmol) were added. The mixture was stirred at room temperature for 16 h and then washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to yield the product **48** as a pale-yellow syrup (7.57 g, 97%). IR (film) 2847, 1646, 1580, 1445, 1263, 689 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.31 (s, 1 H), 7.46−7.28 (m, 8 H), 7.08 (dd, J = 1.0 and 1.4 Hz, 1 H), 5.11 (s, 2 H), 3.78 (dt, J = 0.9 and 5.3 Hz, 2 H), 3.52 (t, J = 6.5 Hz, 2 H), 2.29 (m, 2 H). ESI-MS *m/z* (rel intensity) 332/334 (MH$^+$, 100/99).

cis-3-[3-(Benzyloxy)phenyl]-N-(3-bromopropyl)-4-carboxy-3,4-dihydro-7-nitro-1(2H)-isoquinolone (49). Schiff base **48** (4.57 g, 13.8 mmol) was diluted in CHCl$_3$ (50 mL) at 0 °C, and anhydride **14** (2.85 g, 13.8 mmol) was added. The red mixture was stirred at 0 °C for 1 h and then at room temperature for 2 h. The cloudy orange mixture was filtered, and the residue was diluted in CHCl$_3$ (50 mL) and heated at reflux for 20 min. The cloudy mixture was then filtered and washed with hot CHCl$_3$ to provide the product **49** as an off-white solid (5.08 g, 68%); mp 166−167 °C. IR (film) 3082, 1749, 1628, 1586, 1524, 1268, 1181, 698 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.59 (d, J = 2.4 Hz, 1 H), 8.25 (dd, J = 2.4 and 5.9 Hz, 1 H), 7.46 (d, J = 8.4 Hz, 1 H), 7.36−7.26 (m, 5 H), 7.20 (t, J = 7.9 Hz, 1 H), 6.87 (dd, J = 2.2 and 5.9 Hz, 1 H), 6.71 (s, 1 H), 6.63 (d, J = 7.8 Hz, 1 H), 5.10 (d, J = 5.4 Hz, 1 H), 5.00 (s, 2 H), 4.14 (m, 1 H), 3.81 (q, J = 6.7 H, 1 H), 3.59 (t, J = 7.1 Hz, 2 H), 2.96 (m, 1 H), 2.21 (m, 2 H). ESI-MS *m/z* (rel intensity) 495/497 ([MH − COOH]$^+$, 91/97). HRMS (+ESI) calcd for $C_{26}H_{23}BrN_2O_6$ (MH − COOH)$^+$, 495.0919; found, 495.0911.

Mixture of 8- and 10-(Benzyloxy)-6-(3-bromopropyl)-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione (51 and 52). Cis acid **49** (1.48 g, 2.86 mmol) was diluted in SOCl$_2$ (50 mL), and the mixture was heated at reflux for 4 h. The resulting red solution was evaporated to dryness. The residue was triturated with ether and filtered to provide an approximately 2:1 (by ^{1}H NMR) mixture of 8- and 10-(benzyloxy)indenoisoquinolines (**51** and **52**, respectively) as an orange solid (0.32 g, 22%). Identities of the products were determined by ^{1}H NMR. The product mixture was subjected to the next S$_N$2 reaction without further purification.

8-(Benzyloxy)-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione (53) and 10-(Benzyloxy)-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione (54). A mixture of bromides **51** and **52** (150 mg, 0.29 mmol) and morpholine (151 mg, 1.73 mmol) was diluted in 1,4-dioxane (30 mL),

and the mixture was heated at 70 °C for 16 h. The mixture was then evaporated to dryness. The resulting residue was diluted with CHCl$_3$ (100 mL) and washed with H$_2$O (100 mL × 4) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated, adsorbed onto SiO$_2$, and purified by flash column chromatography (SiO$_2$), eluting with a gradient of MeOH in CHCl$_3$ (0% to 1%), to provide the 8-benzyloxy product (**53**) as an orange solid (60.3 mg, 40%) and the 10-benxyloxy product (**54**) as a red solid (48.6 mg, 32%).

8-Benzyloxy (**53**): mp 250−252 °C. IR (film) 1669, 1615, 1560, 1508, 1337, 1114, 841 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.17 (d, J = 2.4 Hz, 1 H), 8.87 (d, J = 8.9 Hz, 1 H), 8.48 (dd, J = 2.4 and 6.5 Hz, 1 H), 7.65 (d, J = 8.0 Hz, 1 H), 7.45−7.41 (m, 6 H), 6.94 (dd, J = 1.7 and 6.4 Hz, 1 H), 5.16 (s, 2 H), 4.62 (t, J = 7.2 Hz, 2 H), 3.58 (t, J = 4.4 Hz, 4 H), 2.56 (t, J = 6.1 Hz, 2 H), 2.44 (m, 4 H), 2.06 (m, 2 H). ESI-MS m/z (rel intensity) 526 (MH$^+$, 100). HRMS (+ESI) calcd for C$_{30}$H$_{27}$N$_3$O$_6$ MH$^+$, 526.1978; found, 526.1984.

10-Benzyloxy (**54**): mp 254−256 °C. IR (film) 1678, 1610, 1558, 1332, 1117, 746 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.18 (d, J = 2.4 Hz, 1 H), 8.95 (d, J = 9.0 Hz, 1 H), 8.49 (dd, J = 2.5 and 6.5 Hz, 1 H), 7.54−7.48 (m, 3 H), 7.45−7.34 (m, 4 H), 7.10 (d, J = 8.5 Hz, 1 H), 5.35 (s, 2 H), 4.65 (t, J = 7.9 Hz, 2 H), 3.71 (t, J = 4.4 Hz, 4 H), 2.60 (t, J = 6.2 Hz, 2 H), 2.49 (m, 4 H), 2.08 (m, 2 H). ESI-MS m/z (rel intensity) 526 (MH$^+$, 100). HRMS (+ESI) calcd C$_{30}$H$_{27}$N$_3$O$_6$ for MH$^+$, 526.1978; found, 526.1974.

8-(Hydroxy)-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione (55). Compound **53** (55 mg, 0.10 mmol) was diluted with aqueous HBr (48 wt %, 30 mL), and the mixture was heated at 70 °C for 3 h. The red mixture was diluted with CHCl$_3$ (10 mL) and acetone (10 mL) and then concentrated in vacuo. This procedure was done three times. The final concentrate was filtered through an HPLC filter paper, and the residue was washed with acetone and CHCl$_3$ to provide the desired product **55** as an orange solid (51.6 mg, 96%); mp >400 °C. IR (film) 3353, 1668, 1615, 1562, 1506, 1435, 1338, 1258, 847 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.87 (s, 1 H), 9.48 (br s, 1 H), 8.90 (d, J = 2.4 Hz, 1 H), 8.77 (d, J = 9.0 Hz, 1 H), 8.60 (dd, J = 2.4 and 6.6 Hz, 1 H), 7.56 (d, J = 8.1 Hz, 1 H), 7.25 (s, 1 H), 6.90 (d, J = 8.0 Hz, 1 H), 4.56 (m, 2 H), 3.98 (m, 2 H), 3.64 (m, 2 H), 3.44 (m, 4 H), 3.09 (m, 2 H), 2.25 (m, 2 H). ESI-MS m/z (rel intensity) 436 (MH$^+$, 100). HRMS (+ESI) calcd for C$_{23}$H$_{21}$N$_3$O$_6$ MH$^+$, 436.1509; found, 436.1514. HPLC purity: 98.0% (MeOH, 100%), 97.4% (MeOH−H$_2$O, 90:10). Anal. Calcd for C$_{23}$H$_{22}$BrN$_3$O$_6$·0.75H$_2$O: C, 52.14; H, 4.47; N, 7.93. Found: C, 51.79; H, 4.19; N, 7.64.

10-(Hydroxy)-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione (56). Compound **54** (45 mg, 0.086 mmol) was diluted with aqueous HBr (48 wt %, 30 mL), and the mixture was heated at 70 °C for 3 h. The red mixture was diluted with CHCl$_3$ (10 mL) and acetone (10 mL) and then concentrated in vacuo. This procedure was done 3 times. The final concentrate was filtered through an HPLC filter paper, and the residue was washed with acetone and CHCl$_3$ to provide the desired product **56** as a brown solid (19 mg, 43%); mp 314−316 °C (dec). IR (film) 2917, 1688, 1611, 1557, 1506, 1430, 1344, 1172, 802 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.72 (s, 1 H), 9.48 (br s, 1 H), 8.91 (d, J = 2.4 Hz, 1 H), 8.81 (d, J = 8.9 Hz, 1 H), 8.61 (dd, J = 2.5 and 6.5 Hz, 1 H), 7.50 (t, J = 7.8 Hz, 1 H), 7.39 (d, J = 7.7 Hz, 1 H), 7.09 (d, J = 8.4 Hz, 1 H), 4.56 (m, 2 H), 3.98 (m, 2 H), 3.63 (m, 2 H), 3.43 (m, 4 H), 3.09 (m, 2 H), 2.26 (m, 2 H). ESI-MS m/z (rel intensity) 436 (MH$^+$, 100). HRMS (+ESI) calcd for C$_{23}$H$_{21}$N$_3$O$_6$ MH$^+$, 436.1509; found, 436.1500. HPLC purity: 98.8% (MeOH, 100%), 98.3% (MeOH−H$_2$O, 90:10). Anal. Calcd for C$_{23}$H$_{22}$BrN$_3$O$_6$: C, 53.50; H, 4.29; N, 8.14. Found: C, 53.24; H, 4.29; N, 7.91.

6-(3-(1H-Imidazol-1-yl)propyl)-8-(benzyloxy)-3-nitro-5H-indeno-[1,2-c]-isoquinoline-5,11(6H)-dione (57) and 6-(3-(1H-Imidazol-1-yl)propyl)-10-(benzyloxy)-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione (58). A mixture of bromides **51** and **52** (100 mg, 0.19 mmol) and imidazole (131 mg, 1.9 mmol) was diluted in 1,4-dioxane (30 mL), and the mixture was heated at 70 °C for 16 h. The mixture was then concentrated to dryness. The resulting residue was diluted in

CHCl$_3$ (100 mL) and washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated, adsorbed onto SiO$_2$, and purified by flash column chromatography (SiO$_2$), eluting with a gradient of MeOH in CHCl$_3$ (0−2%) to provide the 8-benzyloxy product (**57**) as an orange solid (35.6 mg, 37%) and the 10-benxyloxy product (**58**) as an orange solid (17.8 mg, 18%).

8-Benzyloxy (**57**): mp 265−267 °C. IR (film) 1670, 1613, 1560, 1505, 1339, 1229, 842 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.89 (d, J = 2.4 Hz, 1 H), 8.76 (d, J = 9.0 Hz, 1 H), 8.59 (d, J = 9.1 Hz, 1 H), 7.78 (s, 1 H), 7.64 (d, J = 8.2 Hz, 1 H), 7.52 (d, J = 7.0 Hz, 2 H), 7.44−7.35 (m, 3 H), 7.26 (m, 2 H), 7.17 (d, J = 8.0 Hz, 1 H), 6.90 (m, 1 H), 5.28 (s, 2 H), 4.51 (m, 2 H), 4.16 (m, 2 H), 2.26 (m, 2 H). ESI-MS m/z (rel intensity) 507 (MH$^+$, 100). HRMS (+ESI) calcd for MH$^+$, 507.1668; found, 507.1662.

10-Benzyloxy (**58**): mp 260−261 °C. IR (film) 1671, 1612, 1562, 1502, 1454, 1331, 1264, 840 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.88 (m, 1 H), 8.78 (d, J = 9.1 Hz, 1 H), 8.54 (dd, J = 2.4 and 6.6 Hz, 1 H), 7.80 (br s, 1 H), 7.55 (d, J = 7.6 Hz, 2 H), 7.47−7.31 (m, 6 H), 6.94 (m, 2 H), 5.33 (s, 2 H), 4.48 (t, J = 7.6 Hz, 2 H), 4.26 (t, J = 6.6 Hz, 2 H), 2.24 (m, 2 H). ESI-MS m/z (rel intensity) 507 (MH$^+$, 100). HRMS (+ESI) calcd for C$_{29}$H$_{22}$N$_4$O$_5$ MH$^+$, 507.1668; found, 507.1673.

6-(3-(1H-Imidazol-1-yl)propyl)-8-hydroxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (59). Compound **57** (41 mg, 0.081 mmol) was diluted in aqueous HBr (30 mL, 48 wt %), and the mixture was heated at 70 °C for 3 h. The red mixture was diluted in CHCl$_3$ (10 mL) and acetone (10 mL) and then concentrated in vacuo. This procedure was done 3 times. The final concentrate was filtered through an HPLC filter paper, and the residue was washed with acetone and CHCl$_3$ to provide the product **59** as a bright-orange solid (36.3 mg, 90%); mp 329−331 °C (dec). IR (film) 3064, 1669, 1615, 1562, 1506, 1340, 1255, 835 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.84 (s, 1 H), 9.04 (s, 1 H), 8.89 (d, J = 2.4 Hz, 1 H), 8.76 (d, J = 9.0 Hz, 1 H), 8.60 (dd, J = 2.5 and 6.5 Hz, 1 H), 7.80 (s, 1 H), 7.63 (s, 1 H), 7.55 (d, J = 8.0 Hz, 1 H), 7.18 (s, 1 H), 6.89 (d, J = 9.5 Hz, 1 H), 4.54 (m, 2 H), 4.40 (t, J = 6.6 Hz, 2 H), 2.42 (m, 2 H). ESI-MS m/z (rel intensity) 417 (MH$^+$, 100). HRMS (+ESI) calcd for C$_{22}$H$_{16}$N$_4$O$_5$ MH$^+$, 417.1199; found, 417.1195. Anal. Calcd for C$_{22}$H$_{17}$BrN$_4$O$_5$·0.5H$_2$O: C, 52.19; H, 3.58; N, 11.07. Found: C, 51.82; H, 3.44; N, 10.67.

6-(3-(1H-Imidazol-1-yl)propyl)-10-hydroxy-3-nitro-5H-indeno-[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (60). Compound **58** (27 mg, 0.053 mmol) was diluted in aqueous HBr (30 mL, 48 wt %), and the mixture was heated at 70 °C for 3 h. The red mixture was diluted in CHCl$_3$ (10 mL) and acetone (10 mL) and then concentrated in vacuo. This procedure was done 3 times. The final concentrate was filtered through an HPLC filter paper, and the residue was washed with acetone and CHCl$_3$ to provide the product **60** as a bright-orange solid (25.3 mg, 95%); mp 337−339 °C (dec). IR (film) 3347, 2946, 1694, 1666, 1615, 1563, 1503, 1428, 1336, 1287, 852 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.70 (s, 1 H), 9.11 (s, 1 H), 8.90 (d, J = 2.4 Hz, 1 H), 8.79 (d, J = 8.9 Hz, 1 H), 8.60 (dd, J = 2.5 and 6.5 Hz, 1 H), 7.83 (s, 1 H), 7.68 (s, 1 H), 7.46 (t, J = 7.9 Hz, 1 H), 7.28 (d, J = 7.4 Hz, 1 H), 7.08 (d, J = 8.5 Hz, 1 H), 4.54 (m, 2 H), 4.42 (t, J = 7.1 Hz, 2 H), 2.39 (m, 2 H). ESI-MS m/z (rel intensity) 417 (MH$^+$, 100). HRMS (+ESI) calcd for C$_{22}$H$_{16}$N$_4$O$_5$ MH$^+$, 417.1199; found, 417.1203. Anal. Calcd for C$_{22}$H$_{17}$BrN$_4$O$_5$·0.25H$_2$O: C, 52.66; H, 3.52; N, 11.16. Found: C, 52.81; H, 3.49; N, 10.79.

6-(3-Aminopropyl)-8-hydroxy-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione Hydrobromide (63) and 6-(3-Amino-propyl)-10-hydroxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione Hydrobromide (64). A mixture of bromides **51** and **52** (100 mg, 0.19 mmol) and NaN$_3$ (63 mg, 0.96 mmol) was diluted in DMSO (30 mL), and the mixture was stirred at room temperature for 16 h. The mixture was then diluted with H$_2$O (100 mL) and extracted with CHCl$_3$ (50 mL × 3). The combined extract was washed with H$_2$O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, concentrated, and purified by flash column chromatography (SiO$_2$), eluting with CHCl$_3$ to provide azides

61 and **62** as orange solids. Both compounds were diluted separately in benzene (20 mL) and triethyl phosphite (36 mg, 0.22 mmol) and heated at reflux for 16 h. Aqueous HBr (48 wt %, 20 mL) was added to the cooled solutions, and stirring was continued at reflux for 4 h, during which orange precipitates formed. The two mixtures were concentrated, washed with acetone (10 mL) and $CHCl_3$ (10 mL), and concentrated again. The mixtures were then filtered and the residues were washed thoroughly with acetone and $CHCl_3$ to provide the amines **63** (32 mg, 37% in two steps) and **64** (28 mg, 33% in two steps) as orange solids.

Amine **63**: mp 353−355 °C (dec). IR (film) 3248, 1666, 1613, 1595, 1507, 1480, 1388, 1341, 1295, 864 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.86 (s, 1 H), 8.87 (d, J = 2.4 Hz, 1 H), 8.74 (d, J = 9.0 Hz, 1 H), 8.58 (dd, J = 2.6 and 6.4 Hz, 1 H), 7.73 (br s, 3 H), 7.53 (d, J = 8.0 Hz, 1 H), 7.24 (d, J = 1.5 Hz, 1 H), 6.88 (dd, J = 1.5 and 6.5 Hz, 1 H), 4.55 (t, J = 6.7 Hz, 2 H), 3.00 (m, 2 H), 2.11 (m, 2 H). ESI-MS m/z (rel intensity) 366 (MH$^+$, 14); HRMS (+ESI) calcd for $C_{19}H_{15}N_3O_5$ MH$^+$, 366.1090; found, 366.1087. HPLC purity: 97.1% (MeOH, 100%).

Amine **64**: mp 337−339 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.69 (s, 1 H), 8.90 (d, J = 2.4 Hz, 1 H), 8.79 (d, J = 8.9 Hz, 1 H), 8.60 (dd, J = 2.6 and 6.4 Hz, 1 H), 7.71 (br s, 3 H), 7.49−7.44 (m, 1 H), 7.39−7.35 (m, 1 H), 7.08 (d, J = 8.3 Hz, 1 H), 4.55 (t, J = 6.8 Hz, 2 H), 3.01 (m, 2 H), 2.10 (m, 2 H). MALDI-MS m/z (rel intensity) 366 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{19}H_{15}N_3O_5$ MH$^+$, 366.1090; found, 366.1109. HPLC purity: 96.5% (MeOH, 100%).

N-[4-(Benzyloxy)benzylidene]-3-bromo-1-propylamine (66). 3-Bromopropylamine hydrobromide (3.56 g, 16.2 mmol) was diluted in $CHCl_3$ (50 mL) and Et$_3$N (1.64 g, 16.2 mmol). The mixture was stirred for 5 min, and then compound **65** (3.00 g, 14.1 mmol) and Na$_2$SO$_4$ (4.02 g, 28.3 mmol) were added. The mixture was stirred at room temperature for 16 h and then washed with H_2O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to yield the product **66** as a pale-yellow syrup (4.69 g, 100% + residual solvent). IR (film) 2839, 1645, 1605, 1509, 1246, 1166, 830 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.26 (s, 1 H), 7.68 (dd, J = 1.8 and 6.9 Hz, 2 H), 7.45−7.33 (m, 5 H), 7.02 (dd, J = 1.9 and 6.9 Hz, 2 H), 5.11 (s, 2 H), 3.74 (dt, J = 0.9 and 6.2 Hz, 2 H), 3.51 (t, J = 6.6 Hz, 2 H), 2.27 (m, 2 H). ESI-MS m/z (rel intensity) 332/334 (MH$^+$, 100/97).

cis-4-Carboxy-3,4-dihydro-N-(3-bromopropyl)-3-[4-(benzyloxy)-phenyl]-7-nitro-1(2H)-isoquinolone (67). Schiff base **66** (4.69 g, 14.1 mmol) was diluted in $CHCl_3$ (50 mL) at 0 °C, and anhydride **14** (2.80 g, 13.5 mmol) was added. The red mixture was stirred at 0 °C for 2 h, and then at room temperature for 2 h. The cloudy orange mixture was filtered, and the residue was washed with 50% $CHCl_3$ in hexane to provide the product **67** as an off-white solid (5.18 g, 71%); mp 140−141 °C. IR (film) 3076, 1727, 1630, 1525, 1347, 1187, 738 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.71 (d, J = 2.6 Hz, 1 H), 8.39 (dd, J = 2.6 and 6.0 Hz, 1 H), 7.92 (d, J = 8.2 Hz, 1 H), 7.40−7.30 (m, 5 H), 6.92−6.83 (m, 4 H), 5.19 (d, J = 6.4 Hz, 1 H), 5.03 (d, J = 6.3 Hz, 1 H), 4.98 (s, 2 H), 3.90 (m, 1 H), 3.59 (m, 2 H), 3.03 (m, 1 H), 2.16 (m, 1 H), 2.04 (m, 1 H). ESI-MS m/z (rel intensity) 415 ([MH − COOH − Br]$^+$, 100). HRMS (+ESI) calcd for $C_{26}H_{23}BrN_2O_6$ MH$^+$, 539.0818; found, 539.0812.

6-(3-Bromopropyl)-9-hydroxy-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione (68). Cis acid **67** (1.00 g, 1.85 mmol) was diluted in SOCl$_2$ (50 mL), and the mixture was stirred at room temperature for 5 h. The resulting yellow solution was evaporated to dryness. The yellow syrup was diluted in 1,2-dichloroethane (40 mL) at 0 °C, followed an addition of AlCl$_3$ (0.75 g, 5.56 mmol), and the mixture was stirred for 15 min. Stirring was continued at room temperature for 8 h. The black mixture was then diluted with $CHCl_3$ (50 mL) and THF (30 mL) and washed with cold 6 N HCl (100 mL). The aqueous layer was extracted with $CHCl_3$ (50 mL × 2). The combined extract was washed with H_2O (100 mL × 3) and brine (100 mL). The red organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to dryness. The residue was triturated with $CHCl_3$, filtered, and washed with excess $CHCl_3$ to provide the product **68** in high purity as a deep-red solid (262 mg, 33%); mp 281−283 °C (dec).

IR (film) 3273, 1659, 1613, 1531, 1345, 1270, 755 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.82 (s, 1 H), 8.83 (d, J = 2.1 Hz, 1 H), 8.61 (d, J = 9.0 Hz, 1 H), 8.51 (d, J = 9.2 Hz, 1 H), 7.74 (d, J = 8.6 Hz, 1 H), 6.99 (s, 1 H), 6.89 (d, J = 8.6 Hz, 1 H), 4.54 (m, 2 H), 3.78 (t, J = 6.3 Hz, 2 H), 2.33 (m, 2 H). ESI-MS m/z (rel intensity) 428/430 (M$^+$, 99/100). HRMS (+ESI) calcd for $C_{19}H_{13}BrN_2O_5$ M$^+$, 428.0008; found, 428.0000.

9-Hydroxy-6-(3-morpholinopropyl)-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione Hydrobromide (69). Phenol **68** (50 mg, 0.12 mmol) and morpholine (51 mg, 0.58 mmol) were diluted in THF (20 mL). The red mixture was heated at 70 °C for 16 h. The cooled solution was diluted with 3 M methanolic HBr (20 mL) and stirred at room temperature for 4 h. The deep-red solution was concentrated and filtered through an HPLC filter paper. The residue was washed with $CHCl_3$ to provide the product **69** as a deep-brown solid (49 mg, 83%); mp 295−297 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.87 (s, 1 H), 9.53 (br s, 1 H), 8.86 (d, J = 2.4 Hz, 1 H), 8.66 (d, J = 9.0 Hz, 1 H), 8.56 (dd, J = 2.4 and 6.5 Hz, 1 H), 7.70 (d, J = 8.4 Hz, 1 H), 7.04 (d, J = 2.4 Hz, 1 H), 6.93 (dd, J = 2.3 and 6.0 Hz, 1 H), 4.52 (m, 2 H), 3.98 (m, 2 H), 3.64 (m, 2 H), 3.38 (m, 4 H), 3.08 (m, 2 H), 2.21 (m, 2 H). ESI-MS m/z (rel intensity) 436 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{23}H_{21}N_3O_6$ MH$^+$, 436.1509; found, 436.1508. Anal. Calcd for $C_{23}H_{22}BrN_3O_6$: C, 53.50; H, 4.29; N, 8.14. Found: C, 53.51; H, 4.23; N, 7.98.

6-(3-(1H-Imidazol-1-yl)propyl)-7-hydroxy-3-nitro-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione hydrochloride (70). Bromide **68** (70 mg, 0.16 mmol) and imidazole (67 mg, 0.98 mmol) were diluted in THF (20 mL). The red mixture was heated at 70 °C for 16 h. The cooled solution was diluted with 3 M methanolic HCl (20 mL) and stirred at room temperature for 4 h. The deep-red mixture was concentrated and filtered through an HPLC filter paper. The residue was washed with $CHCl_3$ to afford the product **70** as a brown solid (42.6 mg, 58%); mp 323−325 °C (dec). IR (film) 1668, 1611, 1503, 1428, 1334, 1263 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (d, J = 9.3 Hz, 1 H), 8.97 (d, J = 2.5 Hz, 1 H), 8.67 (dd, J = 2.5 and 6.7 Hz, 1 H), 7.85 (d, J = 7.7 Hz, 1 H), 7.76 (t, J = 7.4 Hz, 1 H), 7.56 (d, J = 8.0 Hz, 1 H), 7.38 (t, J = 8.5 Hz, 1 H), 4.37 (m, 2 H), 4.14 (m, 2 H). ESI-MS m/z (rel intensity) 417 (MH$^+$, 100). HRMS (+ESI) calcd for $C_{22}H_{16}N_4O_5$ MH$^+$, 417.1199; found, 417.1202. HPLC purity: 100% (MeOH, 100%), 100% (MeOH−H_2O, 70:30).

6-(3-Aminopropyl)-9-hydroxy-3-nitro-5H-indeno[1,2-c]-isoquinoline-5,11(6H)-dione Hydrobromide (71). Bromide **68** (100 mg, 0.23 mmol) and NaN$_3$ (75 mg, 1.15 mmol) were diluted in DMSO (30 mL) and stirred at room temperature for 16 h. The solution was then diluted with H_2O (100 mL) and extracted with $CHCl_3$ (50 mL × 3). The combined extract was washed with H_2O (100 mL × 3) and brine (100 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated to provide the crude intermediate azide. The compound was then diluted in THF (20 mL), followed by addition of PPh$_3$ (181 mg, 0.69 mmol), and the mixture was heated at reflux for 16 h. The cooled deep-brown solution was diluted with 3 M methanolic HBr (20 mL), and stirring was continued at 70 °C for 4 h. The bright-red solution was evaporated, rediluted in $CHCl_3$ (10 mL), and let sit for 16 h at 0 °C, during which a precipitate formed. The solution was then filtered through an HPLC filter paper, and the residue was washed thoroughly with $CHCl_3$ to provide the product **71** (33.1 mg, 32%) as a deep-red solid; mp 360−362 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.83 (s, 1 H), 8.83 (d, J = 2.3 Hz, 1 H), 8.61 (d, J = 9.0 Hz, 1 H), 8.52 (dd, J = 2.5 and 6.4 Hz, 1 H), 7.74−7.67 (m, 4 H), 6.99 (d, J = 2.3 Hz, 1 H), 6.91 (dd, J = 2.2 and 6.1 Hz, 1 H), 4.52 (t, J = 7.0 Hz, 2 H), 2.99 (m, 2 H), 2.08 (m, 2 H). APCI-MS m/z (rel intensity) 366 ([MH − NH$_3$]$^+$, 100). HRMS (+ESI) calcd for $C_{19}H_{15}N_3O_5$ MH$^+$, 366.1090; found, 366.1082; HPLC purity: 99.6% (MeOH, 100%).

Topoisomerase I-Mediated DNA Cleavage Reactions. Human recombinant Top1 was purified from baculovirus as previously described.[53] DNA cleavage reactions were performed as previously reported with the exception of the DNA substrate.[31] Briefly, a 117-bp DNA oligonucleotide (Integrated DNA Technologies) encompassing the previously identified Top1 cleavage sites in the 161-bp fragment

from pBluescript SK(−) phagemid DNA was employed. This 117-bp oligonucleotide contains a single 5′-cytosine overhang, which was 3′-end-labeled by fill-in reaction with $[\alpha\text{-}^{32}P]$dGTP in React 2 buffer [50 mM Tris-HCl (pH 8.0), 100 mM $MgCl_2$, and 50 mM NaCl] with 0.5 unit of DNA polymerase I (Klenow fragment, New England BioLabs). Unincorporated $[^{32}P]$dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled DNA substrate was collected. Approximately 2 nM 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 room temperature for 20 min in the presence of various concentrations of compounds. The reactions were terminated by adding SDS (0.5% final concentration), followed by 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 phosphorimager and ImageQuant software (Molecular Dynamics). For simplicity, cleavage sites were numbered as previously described in the 161-bp fragment.[53]

Gel-Based Assay Measuring the Inhibition of Recombinant TDP1. A 5′-$[^{32}P]$-labeled single-stranded DNA oligonucleotide containing a 3′-phosphotyrosine (N14Y) was generated as described by Dexheimer et al.[21] The DNA substrate was then incubated with 5 pM recombinant TDP1 in the absence or presence of inhibitor for 15 min at room temperature in reaction buffer [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]. Reactions were terminated by an 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 16% denaturing PAGE, and gels were exposed after drying to a PhosphorImager screen (GE Healthcare). Gel images were scanned using a Typhoon 8600 (GE Healthcare), and densitometric analyses were performed using the ImageQuant software (GE Healthcare).

ASSOCIATED CONTENT

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

AUTHOR INFORMATION

Corresponding Author
*Phone: 765-494-1465. Fax: 765-494-6790. E-mail: cushman@purdue.edu.

Notes
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.

ACKNOWLEDGMENTS

ABBREVIATIONS USED

APCI-MS, atmospheric-pressure chemical ionization mass spectrometry; CPT, camptothecin; DMSO-d_6, dimethyl-d_6 sulfoxide; EI/CI-MS, electron impact/chemical ionization mass spectrometry; ESI-MS, electrospray ionization mass spectrometry; HRMS, high resolution mass spectrometry; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry; PMB-Cl, p-methoxybenzyl chloride; TDP1, tyrosyl-DNA phosphodiesterase I; Top1, human topoisomerase type IB

REFERENCES

(1) Pommier, Y. Topoisomerase I Inhibitors: Camptothecins and Beyond. *Nature Rev. Cancer* **2006**, *6*, 789−802.

(2) Wang, J. C. DNA Topoisomerases. *Annu. Rev. Biochem.* **1996**, *65*, 635−692.

(3) Nguyen, T. X.; Morrell, A.; Conda-Sheridan, M.; Marchand, C.; Agama, K.; Bermingam, A.; Stephen, A. G.; Chergui, A.; Naumova, A.; Fisher, R.; O'Keefe, B. R.; 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.

(4) Pommier, Y.; Leo, E.; Zhang, H. L.; Marchand, C. DNA Topoisomerases and Their Poisoning by Anticancer and Antibacterial Drugs. *Chem. Biol.* **2010**, *17*, 421−433.

(5) Yang, S. W.; Burgin, A. B.; Huizenga, B. N.; Robertson, C. A.; Yao, K. C.; Nash, H. A. A Eukaryotic Enzyme that Can Disjoin Dead-End Covalent Complexes between DNA and Type I Topoisomerases. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 11534−11539.

(6) Interthal, H.; Pouliott, J. J.; Champoux, J. J. The Tyrosyl-DNA phosphodiesterase Tdp1 Is a Member of the Phospholipase D Superfamily. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 12009−12014.

(7) Pouliot, J. J.; Robertson, C. A.; Nash, H. A. Pathways for Repair of Topoisomerase I Covalent Complexes in *Saccharomyces cerevisiae*. *Genes to Cells* **2001**, *6*, 677−687.

(8) Debethune, L.; Kohlhagen, G.; Grandas, A.; Pommier, Y. Processing of Nucleopeptides Mimicking the Topoisomerase I−DNA Covalent Complex by Tyrosyl-DNA phosphodiesterase. *Nucleic Acids Res.* **2002**, *30*, 1198−1204.

(9) Dexheimer, T. S.; Antony, S.; Marchand, C.; Pommier, Y. Tyrosyl-DNA Phosphodiesterase as a Target for Anticancer Therapy. *Anti-Cancer Agents Med. Chem.* **2008**, *8*, 381−389.

(10) Thomas, C. J.; Rahier, N. J.; Hecht, S. M. Camptothecin: Current Perspectives. *Bioorg. Med. Chem.* **2004**, *12*, 1585−1604.

(11) Husain, I.; Mohler, J. L.; Seigler, H. F.; Besterman, J. M. Elevation of Topoisomerase-I Messenger-RNA, Protein, and Catalytic Activity in Human Tumors—Demonstration of Tumor-Type Specificity and Implications for Cancer Chemotherapy. *Cancer Res.* **1994**, *54*, 539−546.

(12) Pfister, T. D.; Reinhold, W. C.; Agama, K.; Gupta, S.; Khin, S. A.; Kinders, R. J.; Parchment, R. E.; Tomaszewski, J. E.; Doroshow, J. H.; Pommier, Y. Topoisomerase I Levels in the NCI-60 Cancer Cell Line Panel Determined by Validated ELISA and Microarray Analysis and Correlation with Indenoisoquinoline Sensitivity. *Mol. Cancer Ther.* **2009**, *8*, 1878−1884.

(13) Takashima, H.; Boerkoel, C. F.; John, J.; Saifi, G. M.; Salih, M. A. M.; Armstrong, D.; Mao, Y. X.; Quiocho, F. A.; Roa, B. B.; Nakagawa, M.; Stockton, D. W.; Lupski, J. R. Mutation of TDP1, Encoding a Topoisomerase I-Dependent DNA Damage Repair Enzyme, in Spinocerebellar Ataxia with Axonal Neuropathy. *Nature Genet.* **2002**, *32*, 267−272.

(14) Murai, J.; Huang, S. Y. N.; Das, B. B.; Dexheimer, T. S.; Takeda, S.; Pommier, Y. Tyrosyl-DNA Phosphodiesterase 1 (TDP1) Repairs DNA Damage Induced by Topoisomerases I and II and Base Alkylation in Vertebrate Cells. *J. Biol. Chem.* **2012**, *287*, 12848–12857.

(15) Marchand, C.; Lea, W. A.; Jadhav, A.; Dexheimer, T. S.; Austin, C. P.; 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.

(16) Zhou, T.; Lee, J. W.; Tatavarthi, H.; Lupski, J. R.; Valerie, K.; Povirk, L. F. Deficiency in 3′-Phosphoglycolate Processing in Human Cells with a Hereditary Mutation in Tyrosyl-DNA Phosphodiesterase (TDP1). *Nucleic Acids Res.* **2005**, *33*, 289–297.

(17) Barthelmes, H. U.; Habermeyer, M.; Christensen, M. O.; Mielke, C.; Interthal, H.; Pouliot, J. J.; Boege, F.; Marko, D. TDP1 Overexpression in Human Cells Counteracts DNA Damage Mediated by Topoisomerases I and II. *J. Biol. Chem.* **2004**, *279*, 55618–55625.

(18) Davies, D. R.; Interthal, H.; Champoux, J. J.; Hol, W. G. J. Insights into Substrate Binding and Catalytic Mechanism of Human Tyrosyl-DNA Phosphodiesterase (Tdp1) from Vanadate and Tungstate-Inhibited Structures. *J. Mol. Biol.* **2002**, *324*, 917–932.

(19) Antony, S.; Marchand, C.; Stephen, A. G.; Thibaut, L.; Agama, K. K.; Fisher, R. J.; Pommier, Y. Novel High-Throughput Electrochemiluminescent Assay for Identification of Human Tyrosyl-DNA Phosphodiesterase (Tdp1) Inhibitors and Characterization of Furamidine (NSC 305831) as an Inhibitor of Tdp1. *Nucleic Acids Res.* **2007**, *35*, 4474–4484.

(20) Liao, Z. Y.; Thibaut, L.; Jobson, A.; Pommier, Y. Inhibition of Human Tyrosyl-DNA Phosphodiesterase by Aminoglycoside Antibiotics and Ribosome Inhibitors. *Mol. Pharmacol.* **2006**, *70*, 366–372.

(21) Dexheimer, T. S.; Gediya, L. K.; Stephen, A. G.; Weidlich, I.; Antony, S.; Marchand, C.; Interthal, H.; Nicklaus, M.; Fisher, R. J.; Njar, V. C.; Pommier, Y. 4-Pregnen-21-ol-3,20-dione-21-(4-bromobenzenesulfonate) (NSC 88915) and Related Novel Steroid Derivatives as Tyrosyl-DNA Phosphodiesterase (Tdp1) Inhibitors. *J. Med. Chem.* **2009**, *52*, 7122–7131.

(22) Huang, S.-y. N.; Pommier, Y.; Marchand, C. Tyrosyl-DNA Phosphodiesterase 1 (Tdp1) Inhibitors. *Expert Opin. Ther. Pat.* **2011**, *21*, 1285–1292.

(23) Takagi, M.; Ueda, J.-y.; Hwang, J.-H.; Hashimoto, J.; Izumikawa, M.; Murakami, H.; Sekido, Y.; Shin-ya, K. Tyrosyl-DNA Phosphodiesterase 1 Inhibitor from an Anamorphic Fungus. *J. Nat. Prod.* **2012**, *75*, 764–767.

(24) Weidlich, I. E.; Dexheimer, T.; Marchand, C.; Antony, S.; Pommier, Y.; Nicklaus, M. C. Inhibitors of Human Tyrosyl-DNA Phospodiesterase (hTdp1) Developed by Virtual Screening Using Ligand-Based Pharmacophores. *Bioorg. Med. Chem.* **2010**, *18*, 182–189.

(25) Weidlich, I. E.; Dexheimer, T.; Marchand, C.; Antony, S.; Pommier, Y.; Nicklaus, M. C. Virtual Screening Using Ligand-Based Pharmacophores for Inhibitors of Human Tyrosyl-DNA Phosphodiesterase (hTdp1). *Bioorg. Med. Chem.* **2010**, *18*, 2347–2355.

(26) Sirivolu, V. R.; Vernekar, S. K. V.; Marchand, C.; Naumova, A.; Chergui, A.; Renaud, A.; Stephen, A. G.; Chen, F.; Sham, Y. Y.; Pommier, Y.; Wang, Z. 5-Arylidenethioxothiazolidinones as Inhibitors of Tyrosyl-DNA Phosphodiesterase I. *J. Med. Chem.* **2012**, *55*, 8671–8684.

(27) Takemura, H.; Rao, V. A.; Sordet, O.; Furuta, T.; Miao, Z.-H.; Meng, L.; Zhang, H.; Pommier, Y. Defective Mre11-Dependent Activation of Chk2 by Ataxia Telangiectasia Mutated in Colorectal Carcinoma Cells in Response to Replication-Dependent DNA Double-Strand Breaks. *J. Biol. Chem.* **2006**, *281*, 30814–30823.

(28) Dasika, G. K.; Lin, S. C. J.; Zhao, S.; Sung, P.; Tomkinson, A.; Lee, E. DNA Damage-Induced Cell Cycle Checkpoints and DNA Strand Break Repair in Development and Tumorigenesis. *Oncogene* **1999**, *18*, 7883–7899.

(29) Reinhold, W. C.; Sunshine, M.; Liu, H.; Varma, S.; Kohn, K. W.; Morris, J.; Doroshow, J.; Pommier, Y. CellMiner: A Web-Based Suite of Genomic and Pharmacologic Tools to Explore Transcript and Drug Patterns in the NCI-60 Cell Line Set. *Cancer Res.* **2012**, *72*, 3499–3511.

(30) Pommier, Y.; Cushman, M. The Indenoisoquinoline Non-camptothecin Topoisomerase I Inhibitors: Update and Perspectives. *Mol. Cancer Ther.* **2009**, *8*, 1008–1014.

(31) Antony, S.; Agama, K. K.; Miao, Z. H.; Takagi, K.; Wright, M. H.; Robles, A. I.; 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.

(32) Doroshow, J. H. ; Ji, J. J.; Chen, A.; Allen, D.; Zhang, Y.; Lawrence, S. M.; Pfister, T. D.; Wang, L.; E. Redon, C. E.; Bonner, W.; Speranza, G.; Weil, M. K.; Eiseman, J.; Holleran, J. L.; Kinders, R. L.; Beumer, J. H.; Parchment, R. E.; Pommier, Y.; Tomaszewski, J. E.; Kummar, S. Proof of Mechanism (POM) in the First-in-Human Trial of Two Novel Indenoisoquinoline, Non-camptothecin Topoisomerase I (TOP1) Inhibitors. In 48th Annual Meeting of the American Society of Clinical Oncology (ASCO), Chicago, IL, June 1–5, 2012; American Society of Clinical Oncology: 2318 Mill Road, Ste 800, Alexandra, VA 22314, 2012; Vol. 30.

(33) Cinelli, M. A.; Reddy, P. V. N.; Lv, P. C.; Liang, J. H.; Chen, L.; Agama, K.; Pommier, Y.; van Breemen, R. B.; 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.

(34) Morrell, A.; Placzek, M.; Parmley, S.; Grella, B.; Antony, S.; Pommier, Y.; Cushman, M. Optimization of the Indenone Ring of Indenoisoquinoline Topoisomerase I Inhibitors. *J. Med. Chem.* **2007**, *50*, 4388–4404.

(35) Morrell, A.; Placzek, M.; Parmley, S.; Antony, S.; Dexheimer, T. S.; Pommier, Y.; Cushman, M. Nitrated Indenoisoquinolines as Topoisomerase I Inhibitors: A Systematic Study and Optimization. *J. Med. Chem.* **2007**, *50*, 4419–4430.

(36) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. A Systematic Study of Nitrated Indenoisoquinolines Reveals a Potent Topoisomerase I Inhibitor. *J. Med. Chem.* **2006**, *49*, 7740–7753.

(37) Conda-Sheridan, M.; Reddy, P. V. N.; Morrell, A.; Cobb, B. T.; Marchand, C.; Agama, K.; Chergui, A.; Renaud, A.; Stephen, A. G.; Bindu, L. K.; Pommier, Y.; Cushman, M. Synthesis and Biological Evaluation of Indenoisoquinolines That Inhibit Both Tyrosyl-DNA Phosphodiesterase I (Tdp1) and Topoisomerase I (Top1). *J. Med. Chem.* **2013**, *56*, 182–200.

(38) Morrell, A.; Placzek, M. S.; Steffen, J. D.; Antony, S.; Agama, K.; Pommier, Y.; Cushman, M. Investigation of the Lactam Side Chain Length Necessary for Optimal Indenoisoquinoline Topoisomerase I Inhibition and Cytotoxicity in Human Cancer Cell Cultures. *J. Med. Chem.* **2007**, *50*, 2040–2048.

(39) Lebrun, S.; Couture, A.; Deniau, E.; Grandclaudon, P. Suzuki–Miyaura Cross-Coupling and Ring-Closing Metathesis: A Strategic Combination to the Synthesis of Indeno[1,2-*c*]isoquinolin-5,11-diones. *Tetrahedron Lett.* **2011**, *52*, 1481–1484.

(40) Cushman, M.; Cheng, L. Total Synthesis of Nitidine Chloride. *J. Org. Chem.* **1978**, *43*, 286–288.

(41) Cushman, M.; Cheng, L. Stereoselective Oxidation by Thionyl Chloride Leading to the Indeno[1,2-*c*]isoquinoline System. *J. Org. Chem.* **1978**, *43*, 3781–3783.

(42) Felix, A. M.; Heimer, E. P.; Lambros, T. J.; Tzougraki, C.; Meienhofer, J. Rapid Removal of Protecting Groups from Peptides by Catalytic Transfer Hydrogenation with 1,4-Cyclohexadiene. *J. Org. Chem.* **1978**, *43*, 4194–4196.

(43) Dexheimer, T. S.; Pommier, Y. DNA Cleavage Assay for the Identification of Topoisomerase I Inhibitors. *Nature Protoc.* **2008**, *3*, 1736–1750.

(44) Kiselev, E.; DeGuire, S.; Morrell, A.; Agama, K.; Dexheimer, T. S.; Pommier, Y.; Cushman, M. 7-Azaindenoisoquinolines as

Topoisomerase I Inhibitors and Potential Anticancer Agents. *J. Med. Chem.* **2011**, *54*, 6106−6116.

(45) Antony, S.; Kohlhagen, G.; Agama, K.; Jayaraman, M.; Cao, S.; Durrani, F. A.; Rustum, Y. M.; 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.

(46) Lv, P. C.; 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.

(47) Bihel, F.; Quelever, G.; Lelouard, H.; Petit, A. S.; da Costa, C. A.; Pourquie, O.; Checler, F.; Thellend, A.; Pierre, P.; Kraus, J. L. Synthesis of New 3-Alkoxy-7-amino-4-chloro-isocoumarin Derivatives as New Beta-Amyloid Peptide Production Inhibitors and Their Activities on Various Classes of Protease. *Bioorg. Med. Chem.* **2003**, *11*, 3141−3152.

(48) Whitmore, W. F.; Cooney, R. C. Preparation of Homophthalic Cyclic Hydrazide and 4-Aminohomophthalyl Cyclic Hydrazide. *J. Am. Chem. Soc.* **1944**, *66*, 1237−1240.

(49) Guthrie, D. A.; Frank, A. W.; Purves, C. B. Studies in the Polyoxyphenol Series. XI. Synthesis of Papaverine and Papaveraldine by the Pomeranz−Fritsch Method. *Can. J. Chem.* **1955**, *33*, 729−742.

(50) Duclos, R. I.; Tung, J. S.; Rapoport, H. A High-Yield Modification of the Pschorr Phenanthrine Synthesis. *J. Org. Chem.* **1984**, *49*, 5243−5246.

(51) Patel, B. A.; Ashby, C. R.; Hardej, D.; Talele, T. T. The Synthesis and SAR Study of Phenylalanine-Serived (Z)-5-Arylmethylidene Rhodanines as Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) Compounds. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5523−5527.

(52) Patel, B. A.; Krishnan, R.; Khadtare, N.; Gurukumar, K. R.; Basu, A.; Arora, P.; Bhatt, A.; Patel, M. R.; Dana, D.; Kumar, S.; Kaushik-Basu, N.; Talele, T. T. Design and Synthesis of L- and D-Phenylalanine Derived Rhodanines with Novel C5-Arylidenes as Inhibitors of HCV NS5B Polymerase. *Bioorg. Med. Chem.* **2013**, *21*, 3262−3271.

(53) Pourquier, P.; Ueng, L.-M.; Fertala, J.; Wang, D.; Park, H.-J.; Essigmann, J. M.; Bjornsti, M.-A.; Pommier, Y. Induction of Reversible Complexes Between Eukaryotic DNA Topoisomerase I and DNA-containing Oxidative Base Damages. 7,8-Dihydro-8-oxoguanine and 5-Hydroxycytosine. *J. Biol. Chem.* **1999**, *274*, 8516−8523.