Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Discovery of Potent Indenoisoquinoline Topoisomerase I Poisons Lacking the 3-Nitro Toxicophore

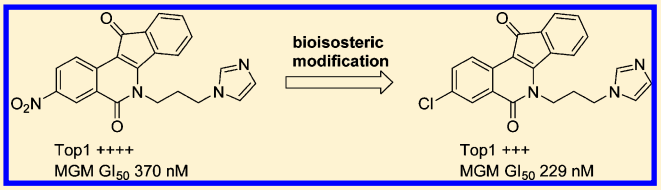
Daniel E. Beck,[†] Monica Abdelmalak,[‡] Wei Lv,[†] P. V. Narasimha Reddy,[†] Gabrielle S. Tender,[‡] Elizaveta O'Neill,[†] Keli Agama,[‡] Christophe Marchand,[‡] Yves Pommier,[‡] and Mark Cushman*[,†]

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

[‡]Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, NCI-Frederick, Frederick, Maryland 21702, United States

Supporting Information

ABSTRACT: 3-Nitroindenoisoquinoline human topoisomerase IB (Top1) poisons have potent antiproliferative effects on cancer cells. The undesirable nitro toxicophore could hypothetically be replaced by other functional groups that would retain the desired biological activities and minimize potential safety risks. Eleven series of indenoisoquinolines bearing 3-nitro bioisosteres were synthesized. The molecules were evaluated in the Top1-mediated DNA cleavage assay and in the National Cancer Institute's 60 cell line cytotoxicity assay. The data reveal that fluorine and chlorine may substitute for the 3-nitro group with minimal loss of Top1 poisoning activity. The new information gained from these efforts can be used to design novel indenoisoquinolines with improved safety.



INTRODUCTION

The camptothecin (**1**) derivatives topotecan (**2**) and irinotecan (**3**) are cancer chemotherapeutic drugs that inhibit DNA religation in the ternary drug−DNA−topoisomerase IB (Top1) cleavage complex, and they are therefore referred to as Top1 "poisons", as opposed to Top1 "suppressors", which inhibit the initial enzyme-catalyzed DNA cleavage reaction. Both topotecan and irinotecan were approved by the FDA for the treatment of solid tumors in 1996. Although these drugs are able to limit the growth of solid tumors, neither is curative and both have significant drawbacks to their use which include dose-limiting bone marrow suppression and the requirement of prolonged infusion times. The latter drawback is caused by the rapid diffusion of camptothecins out of their target site. In addition, the camptothecin core structure is susceptible to lactone hydrolysis in human blood plasma, and its ring-opened carboxylate form readily binds to blood plasma proteins.[1] New camptothecins are currently in clinical development, and several PEGylated and liposomal camptothecins have been granted orphan drug status by the FDA.[2] Another strategy for overcoming the challenges associated with camptothecins is to develop structurally dissimilar Top1 poisons.

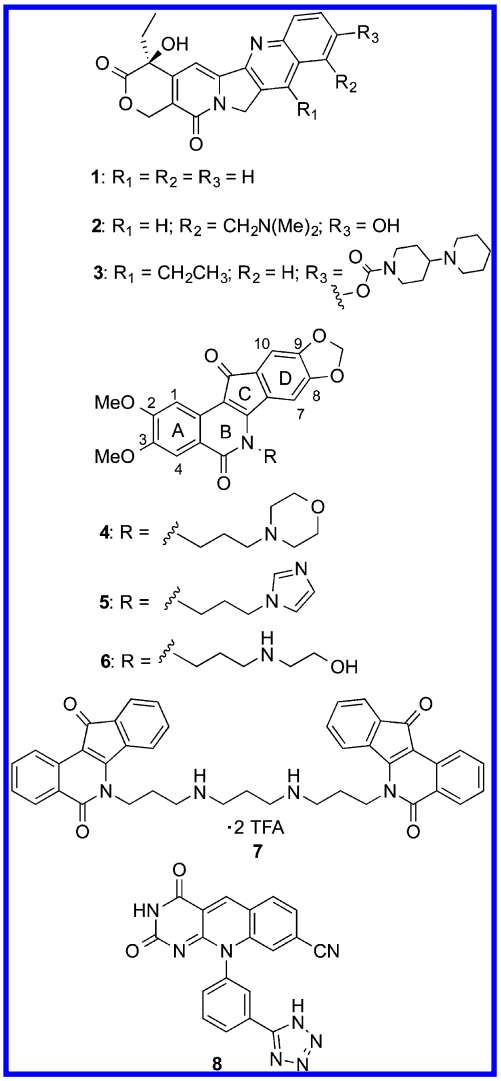
Top1 poisons based on the indenoisoquinoline core scaffold could offer solutions to the problems posed by the current Top1 poison arsenal. Our group designed and synthesized two indenoisoquinolines, indotecan (**4**, LMP400)[3] and indimitecan (**5**, LMP776),[3] which are being studied in phase I clinical trials at the National Institutes of Health.[4,5] These agents are well tolerated, and preliminary evidence of efficacy has been found.[6] Their side chain analogue MJ-III-65 (**6**, LMP744)[7] is under consideration for human clinical study.[8,9] The indenoisoquinolines overcome the inherent chemical instability of the camptothecins, and blood plasma protein binding has not been a significant problem in their development. Additional advantages of the indenoisoquinolines over camptothecins include the fact that some are not substrates for drug efflux pumps, their ability to overcome Top1 mutations that confer resistance to camptothecins, and their longer residence times in the binding site.[1,10,11]

Camptothecins and indenoisoquinolines stabilize the catalytic intermediate in the Top-1-mediated DNA relaxation process known as the cleavage complex, wherein Top1 and its DNA substrate are covalently bound. Under normal conditions, this intermediate exists only transiently as the enzyme removes excessive supercoiling from DNA. Top1 poisons intercalate between the DNA base pairs at the cleavage site and inhibit reversal of the complex. Collision of advancing DNA replication forks with Top1 cleavage sites produces double-strand breaks in the DNA which, if not properly dealt with, can lead the cell to enter apoptosis.[1,12,13]

Numerous 3-nitroindenoisoquinolines have been identified that display Top1 poisoning activities that are greater than the camptothecins, along with mean graph midpoint (MGM) GI_{50} values in the double-digit nanomolar range.[14−18] 3-Position nitration also contributes to inhibitory activity against tyrosyl DNA phosphodiesterase 1 (TDP1), an enzyme involved in the repair of DNA damage caused by Top1 poisons.[19] Although an aromatic nitro group may be acceptable if the drug is selectively delivered to cancer cells, systemic therapy with a nontargeted

Received: February 21, 2015

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX



molecule would expose healthy cells to the nitro toxicophore.[20] Metabolic reduction of aryl nitro groups converts them to potentially toxic aryl nitroso and hydroxylamine species that can covalently modify the DNA and proteins of healthy cells.[21] The NCI-60 screening service recently instituted a policy wherein submission of molecules containing "problematic" functionalities, including nitro groups, is discouraged.[22] For these reasons, indenoisoquinolines were sought that lack this potential metabolic and toxicological liability but maintain similar pharmacological activities.

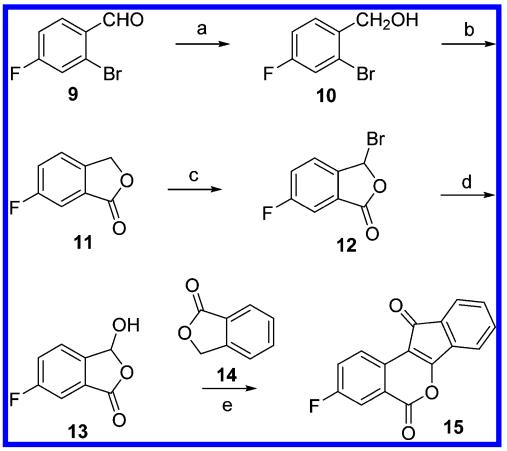
This report details the design, synthesis, and biological evaluation of bioisosteric indenoisoquinolines that display potent Top1 poisoning and growth inhibitory activities. In nearly all cases, the analogues were outperformed by their 3-nitro counterparts in one or both of these regards. One of the new Top1 poisons exhibits inhibitory activity against the DNA repair enzymes TDP1 and tyrosyl DNA phosphodiesterase 2 (TDP2, an enzyme that participates in the repair of DNA damage caused by topoisomerase II poisons).[23] These triple enzyme inhibitory activities increase the attractiveness of indenoisoquinolines for continued development as cancer chemotherapeutic agents. At present, there are few reported TDP1 and TDP2 inhibitors.[19,24−33] We discovered bis-(indenoisoquinoline) **7**, which is a potent dual Top1 poison and TDP1 inhibitor.[32] A separate group recently disclosed a series of deazaflavin TDP2 inhibitors.[30] Although some of the deazaflavins display submicromolar IC_{50} values against TDP2 (e.g., **8**), the series is plagued by cell membrane permeability issues.[30]

The pharmacological data for our compounds were rationalized using molecular mechanics-based molecular modeling and quantum mechanics calculations. Molecular mechanics calculations indicate hydrogen bonding and van der Waals forces stabilize the bioisosteric compounds in the Top1−DNA cleavage complex. Quantum mechanics calculations provide support for the hypothesis that electron-withdrawing groups in the 3-position favor stabilization of the drugs between DNA base pairs in the binding site.[15]

CHEMISTRY

Benzaldehyde **9** was reduced with $NaBH_4$ to provide the bromobenzylic alcohol **10** (Scheme 1). Rosenmund−von

Scheme 1[a]



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

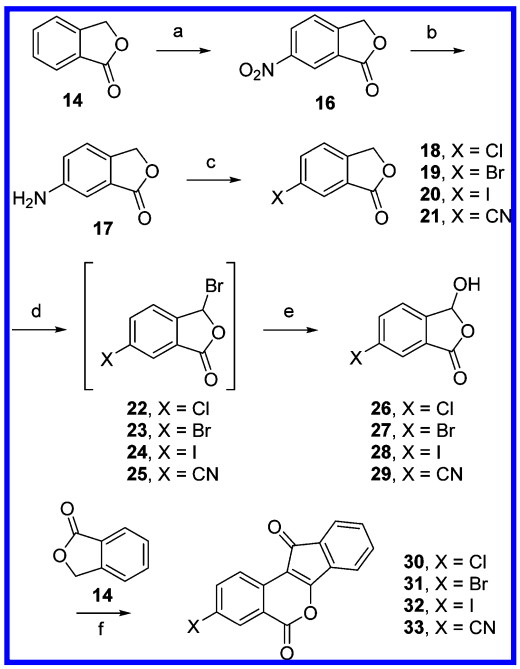
Braun reaction with CuCN and in situ hydrolysis and lactonization of the intermediate yielded compound **11**. Phthalide **11** was subjected to radical bromination, and the obtained 3-bromophthalide intermediate **12** was hydrolyzed to produce 3-hydroxyphthalide **13**. Compound **13** was condensed with phthalide (**14**) in refluxing methanol−EtOAc with NaOMe and then dehydratively cyclized in refluxing Ac_2O to afford 3-fluoroindenobenzopyran **15**.

Indenobenzopyrans **30**−**33** were synthesized by following nearly identical synthetic routes, whose only point of difference was in step c (Scheme 2). 6-Aminophthalide (**17**) was prepared in two steps from phthalide (**14**) following known procedures.[34,35] Sandmeyer chemistry was employed to introduce halogens or a nitrile group. Radical bromination and hydrolysis of each 3-bromophthalide **18**−**21** gave 3-hydroxyphthalides **26**−**29**, which were used to make indenobenzopyrans **30**, **31**,[36] **32**, and **33**.[19]

A fortuitous byproduct of the synthetic route to indenobenzopyran **43** permitted a comparison of the effect of substituting either the 2- or 3-positions with a trifluoromethyl group (Scheme 3). Phthalic acid **34** was converted to its anhydride **35** in refluxing AcCl. Anhydride **35** was reduced with $NaBH_4$ in THF to give a 1:1 mixture of 5- and 6-(trifluoromethyl)phthalides **36** and **37**, which were readily

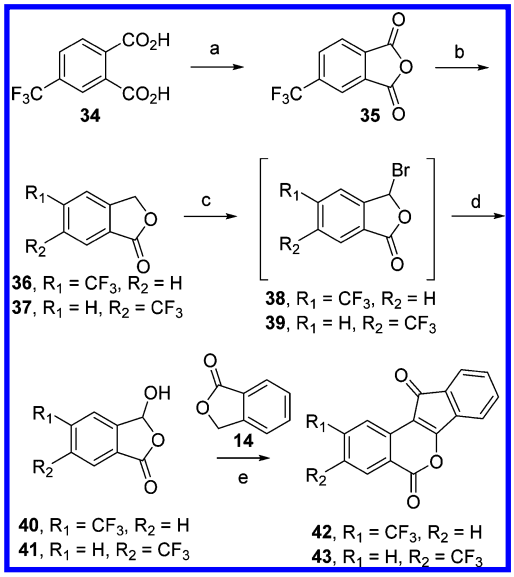
DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2[a]



[a]Reagents and conditions: (a) KNO_3, H_2SO_4, 0 °C to room temp; (b) H_2, Pd/C, EtOAc; (c) (i) $NaNO_2$, 37% HCl, 0 °C, (ii) CuCl, 37% HCl, 0 °C to reflux (**18**), or (i) $NaNO_2$, 48% HBr, 0 °C, (ii) CuBr, 48% HBr, 0–80 °C (**19**), or (i) $NaNO_2$, 37% HCl, 0 °C, (ii) KI, 0 °C to room temp (**20**), or (i) $NaNO_2$, 37% HCl, 0 °C, (ii) NaCN, CuCN, 0 °C to room temp (**21**); (d) NBS, AIBN, CCl_4, reflux; (e) KOH, H_2O, reflux; (f) (i) NaOMe, MeOH, EtOAc, reflux, (ii) Ac_2O, reflux.

Scheme 3[a]

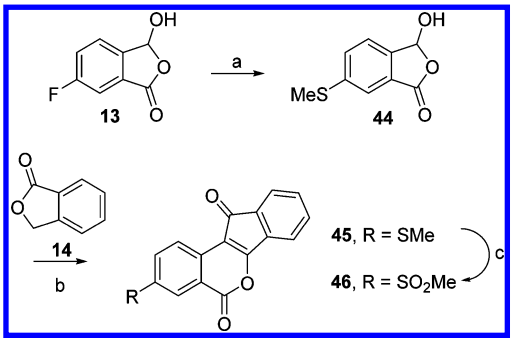


[a]Reagents and conditions: (a) AcCl, reflux; (b) (i) $NaBH_4$, THF, 0 °C, (ii) HCl; (c) NBS, AIBN, CCl_4, reflux; (d) KOH, H_2O, reflux; (e) (i) NaOMe, MeOH, EtOAc, reflux, (ii) Ac_2O, reflux.

separated by silica gel column chromatography. The identity of each isomer was confirmed by comparing ^1H NMR spectral information to that reported in the literature.[37] Phthalides **36** and **37** were separately subjected to radical bromination and subsequent hydrolysis to provide 3-hydroxyphthalides **40** and **41**. Indenobenzopyrans **42** and **43** were obtained by the condensation of 3-hydroxyphthalide **40** or **41** with phthalide (**14**), and dehydrative cyclization of the unisolated intermediate was carried out as before in refluxing Ac_2O.

3-Hydroxyphthalide **13** underwent nucleophilic aromatic substitution with NaSMe to deliver product **44** (Scheme 4). 3-

Scheme 4[a]



[a]Reagents and conditions: (a) NaSMe, DMF, 120 °C; (b) (i) NaOMe, MeOH, EtOAc, reflux, (ii) Ac_2O, reflux; (c) *m*-CPBA, $CHCl_3$, room temp.

Hydroxyphthalide **44** was condensed with phthalide (**14**) and dehydratively cyclized as described before to provide lactone **45**. Oxidation of lactone **45** with *m*-CPBA provided indenobenzopyran **46**.

(*S*)-3-Amino-1,2-propanediol (**48**) was condensed with indenobenzopyrans **15**, **30**, **31**,[36] **32**, **33**,[19] **42**, **43**, **45**, **46**, and **47**[36] to produce indenoisoquinolines **49**–**58** (Scheme 5). 1-(3-Aminopropyl)imidazole (**59**) was condensed with indenobenzopyrans **15**, **30**, **32**, **42**, **43**, **45**, and **46** to yield indenoisoquinolines **60**–**66**.

3-Morpholinopropylamine (**67**) was condensed with indenobenzopyrans **15**, **30**, and **32** to produce indenoisoquinolines **68**–**70** (Scheme 6).

Anhydride **71** was reduced with $NaBH_4$ in PhMe-DMF, and the reduction product was cyclized in refluxing 5 M HCl to provide phthalide **72** (Scheme 7). Phthalide **72** was subjected to radical bromination, and hydrolysis of the product **73** produced 3-hydroxyphthalide **74**. 3-Hydroxyphthalide **74** and phthalide (**14**) were condensed under basic conditions, and the intermediate was cyclized in situ in refluxing Ac_2O to yield lactone **75**.

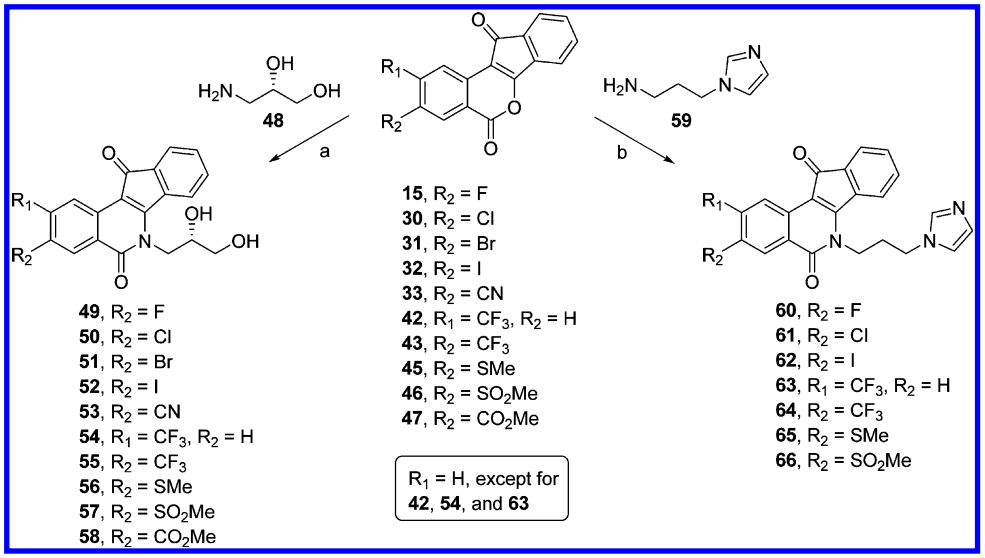
In similar fashion to its carbocyclic analogues, lactone **75** was condensed with primary amines **67**, *N*,*N*-dimethyl-1,3-diaminopropane (**77**), or **59** to yield indenoisoquinolines **76**, **78**, and **79**, respectively (Scheme 8).

RATIONALE, BIOLOGICAL RESULTS, AND DISCUSSION

The objective of this study was to discover a suitable bioisosteric replacement for the 3-nitro group on the indenoisoquinoline system that would maintain or improve Top1 poisoning activity and growth inhibitory potency. Accordingly, the newly synthesized compounds were subjected to the Top1-mediated DNA cleavage assay to assess Top1 poisoning activity and the NCI-60 human tumor cell line screen to evaluate growth inhibitory activity. The first assay scores the performances of Top1 poisons according to a rubric that is based on the activity of 1 μM camptothecin. Test agents are incubated at 0.1, 1, 10, and 100 μM concentrations with a 3′-[^{32}P]-labeled double-stranded DNA fragment and Top1

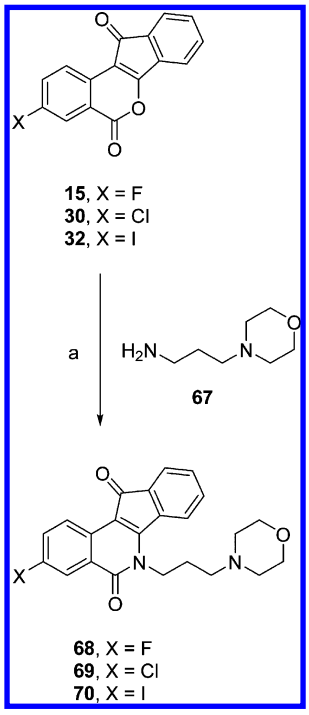
DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 5[a]



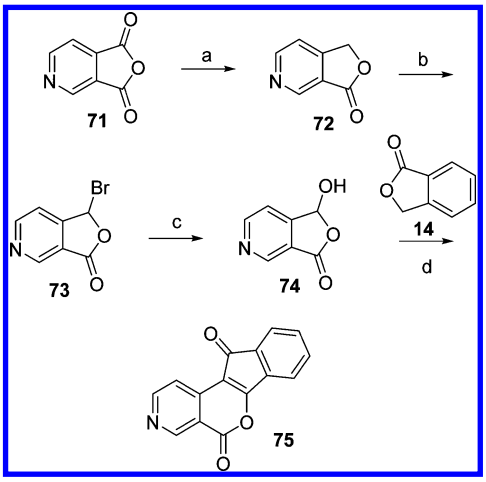
[a]Reagents and conditions: (a) MeOH−$CHCl_3$, reflux; (b) $CHCl_3$, reflux.

Scheme 6[a]



[a]Reagents and conditions: (a) $CHCl_3$, reflux.

Scheme 7[a]



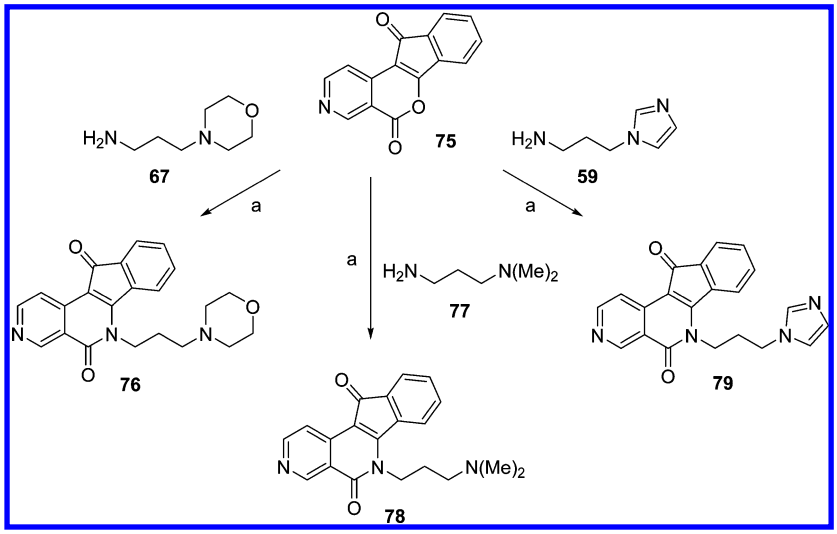
[a]Reagents and conditions: (a) (i) $NaBH_4$, PhMe, DMF, −20 to 35 °C, (ii) 5 M HCl, reflux; (b) NBS, AIBN, CCl_4, reflux; (c) H_2O, reflux; (d) (i) NaOMe, MeOH, EtOAc, reflux, (ii) Ac_2O, reflux.

enzyme. Top1 poisons bind to and trap Top1−DNA cleavage complexes. Separation of the resulting cleaved DNA via denaturing gel electrophoresis reveals a DNA cleavage pattern. The number and intensity of the bands observed in test agent lanes is visually compared to that seen with 1 μM CPT. The relative activity is estimated and is used to assign a semiquantitative score which ranges from 0 (no activity) to ++++ (activity equal to that of 1 μM CPT; see Table 2 caption for a complete description of the scoring rubric). A representative gel is shown in Figure 1.

The representative Top1 assay gel reveals additional information beyond Top1 poisoning activity. First, the intensities of the DNA cleavage bands in test agent lanes do not increase in a simple dose-dependent manner. The intensities appear to subside at high drug concentration after increasing steadily at lower concentrations (e.g., compound **69**, bands at positions 92 and 119). One possible cause is that high drug concentrations produce a significant population of DNA with intercalated test agent, which is a poorer enzyme substrate than nonintercalated DNA. Intercalation of drug molecules into DNA suppresses Top1 nonspecifically, and this mechanism is distinct from Top1 poisoning.[38] Second, the DNA cleavage patterns induced by test agents indicate preferences for different flanking base pairs at the binding site, or cleavage site specificities, versus the control compounds. For example, the dark band at position 62 for compound **6**, which has a unique hydroxyethylaminopropyl side chain, is not observed for **1** or the test agents. Different cleavage site specificities may translate into unique antitumor activity profiles for Top1 poisons.[8,10]

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 8[a]



[a]Reagents and conditions: (a) $CHCl_3$, reflux.

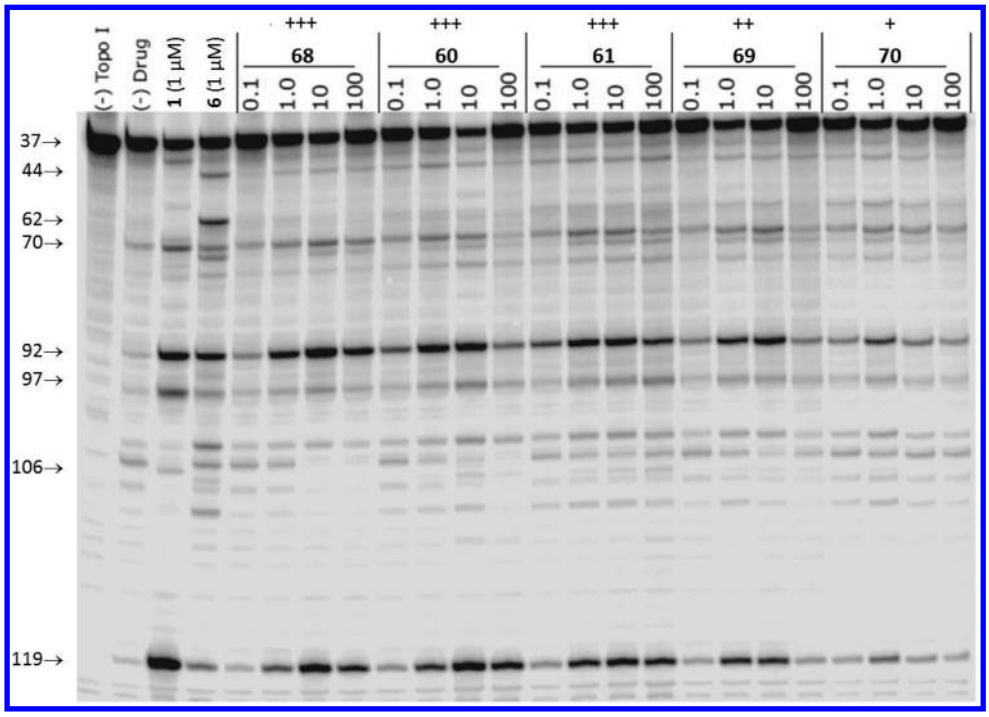


Figure 1. Top1-mediated DNA cleavage assay gel: lane 1, DNA alone; lane 2, DNA and Top1; lane 3, DNA, Top1, and 1 μM **1**; lane 4, DNA, Top1, and 1 μM **6**; remaining lanes, DNA, Top1, and indicated concentration (μM) of test compound. The numbers and arrows at the left indicate cleavage site positions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

Recent investigations have focused on mapping out the structural features of indenoisoquinolines that contribute to potent TDP1 inhibition.[19,31−33] TDP1 is a DNA-repair enzyme that is specifically involved in the recognition and repair of DNA lesions induced by Top1 poisons. Co-treatment with a TDP1 inhibitor and Top1 poison may achieve a selective cytotoxic effect on cancer cells versus normal cells because cancer cells often have a reduced capacity for DNA repair.[39] A representative TDP1 inhibition assay gel is shown in Figure 2. TDP2 is a distinct DNA repair enzyme that is involved in the removal of stalled topoisomerase II (Top2) cleavage complexes. A representative TDP2 inhibition assay gel is shown in Figure 3. Similar to the idea with dual Top1 and TDP1 activities, dual Top2 and TDP2 activities may produce a synergistic effect that selectively targets cancer cells. TDP1 and TDP2 inhibitors would be useful not just for DNA-damaging cancer chemotherapy but also in radiation and antiviral therapies, where DNA breakage is induced to create a therapeutic effect.[23] Only one compound prepared in the present investigation, **78**, showed activity against either TDP1 or TDP2. The activity of **78** was scored as "+" (IC_{50} 37−111 μM) against both TDP1 and TDP2. Its activity was not surprising, given that sterically undemanding aminopropyl lactam side chains are usually necessary for indenoisoquinolines to inhibit TDP1.[19]

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

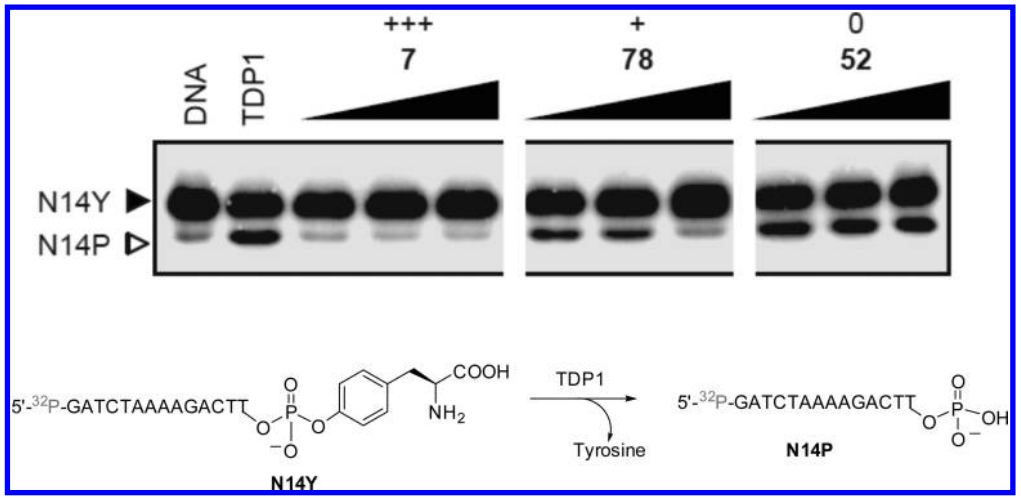


Figure 2. TDP1 inhibition assay gel. The concentrations of test compounds were 12.3, 37, and 111 μM (left to right). N14Y is 5′-end labeled DNA oligonucleotide with a 3′-phosphate group, and N14P is 5′-end labeled DNA oligonucleotide (see Experimental Section). Gel-based assays are commonly acquired twice for each compound. The positive control used for the TDP1 assay was **7**. The "+"-based scoring system rubric is as follows: 0, IC_{50} > 111 μM; +, IC_{50} 37−111 μM; ++, IC_{50} 12−37 μM; +++, IC_{50} > 1−12 μM; ++++, $IC_{50} \leq 1$ μM. The image was cropped to remove irrelevant compounds that also appeared on this gel.

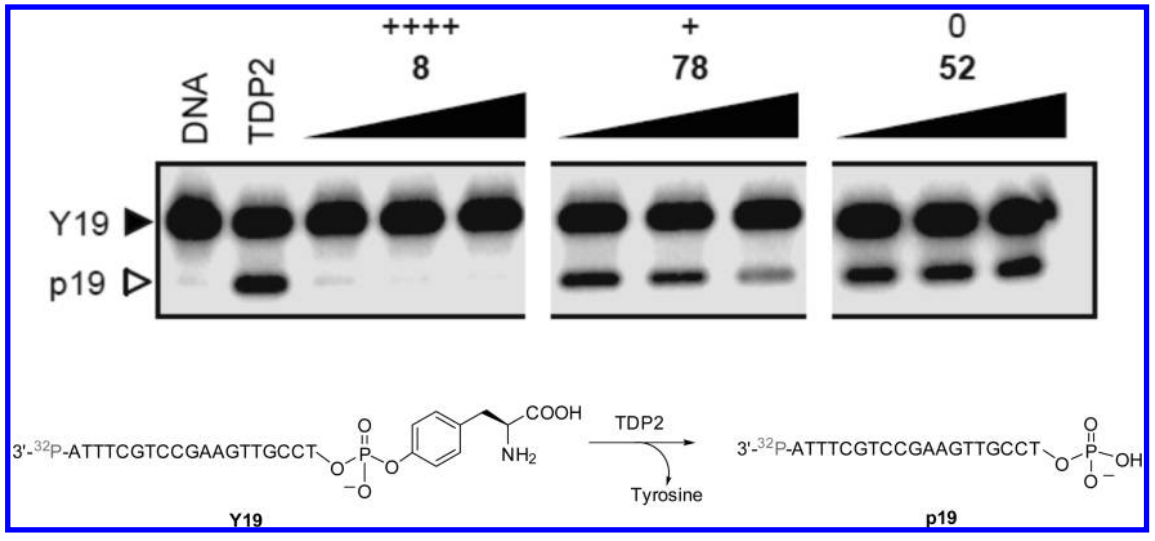


Figure 3. TDP2 inhibition assay gel. The concentrations of test compounds were 12.3, 37, and 111 μM (left to right). The TDP2 substrate Y19 corresponds to a 3′-end labeled DNA oligonucleotide with a 5′ phosphotyrosyl, and the TDP2 product p19 corresponds to a 3′-end labeled DNA oligonucleotide with a 5′ phosphate group (see Experimental Section). Gel-based assays are commonly acquired twice for each compound. The positive control used for the TDP2 assay was **8**. The "+"-based scoring system rubric is the same as that for the TDP1 assay (see Figure 2 caption). The image was cropped to remove irrelevant compounds that also appeared on this gel.

The NCI-60 human tumor cell line screen[40] provides information on the cytotoxic effect of a test agent across 60 cell lines originating from human breast, colon, central nervous system (CNS), melanoma, and other tissues. Compounds are first screened at 10 μM, and an averaged growth percent across all of the tested cell lines is reported as a mean growth percent. If the mean growth percent is sufficiently low (normally below ca. 60%), the compound is promoted to a five-dose assay. The five testing concentrations range from 10^{-8} to 10^{-4} μM, and the resulting dose−response curves are used to calculate the concentration required for 50% growth inhibition relative to control, or GI_{50}, for each cell line. In cases where a GI_{50} is calculated to fall outside of the testing range, it is replaced with either the minimum (10^{-8} μM) or maximum (10^{-4} μM) concentration. The mean GI_{50} calculated this way for all cell lines tested is called a mean graph midpoint (MGM) GI_{50}.

Molecular modeling studies performed by Morrell et al. suggest two explanations for the marked enhancement in biological activity observed with nitrated indenoisoquinolines.[15] The first possibility is that a 3-nitro is appropriately positioned to engage the side chain carboxamide of the nearby residue Asn722 in a direct hydrogen bond. Replacement substituents CN, SO_2Me, CO_2Me, and 3-aza were investigated here because they too could function as hydrogen bond acceptors. A second possibility is that the electronegative 3-nitro group creates favorable polar interactions between the ligand and surrounding DNA bases. All of the atoms and groups selected for study exert an electron-withdrawing effect. A comparison of the electronegativities of several of these chemical groups, obtained from ab initio calculations performed by de Proft et al.,[41] is displayed in Table 1.

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 1. Electronegativities of Bioisosteres from the Present Study[41]

group	electronegativity (eV)[a]
NO_2	7.84
F	10.01
Cl	7.65
CN	8.63
CF_3	6.30
CO_2Me	5.48
SMe	4.99

[a]Calculated at the CISD level.

Biological testing data are organized in Table 2. Compounds **80**−**88** were previously studied[17,19,36,42] and are included in the table for easy referencing. Among the bioisosteric compounds, **60**, **61**, **68**, and **86** display the best Top1 poisoning activities. The Top1-mediated DNA cleavage induced by these four compounds is between 75 and 95% that of 1 μM CPT (i.e., ++ +). MGM GI_{50} values were calculated to be 0.692 μM for **60**, 0.229 μM for **61**, and 2.75 μM for **68**. Compound **86** was not advanced to five-dose testing for determination of MGM GI_{50} because it failed to significantly reduce the mean growth of the same cancer cell lines at 10 μM. Compounds **60**, **61**, **68**, and **86** are substituted with the halogens F, Cl, and Br. It was not immediately clear why "smaller" halogens were better replacements for nitro than other atoms and functional groups. For this reason, several model systems were studied using molecular mechanics and quantum mechanics calculations. It is not unprecedented that F should act as a bioisostere of nitro.[43] In the course of optimizing adrenoceptor antagonists, a *p*-nitrophenyl ring was replaced by a 3,4-difluorophenyl one with retention or improvement of K_i. This exchange provided additional value by side-stepping toxicity concerns about the nitro group.[44]

Indenoisoquinoline lactam side chains are known to exert a significant influence on Top1 poisoning and growth inhibitory activities.[45,46] Unexpectedly, dihydroxy side chain analogues **49**

Table 2. Antiproliferative and Top1 Poisoning Activities of Indenoisoquinolines Substituted with 3-Nitro Bioisosteres

				cytotoxicity (GI_{50}, μM)								
	compd	Top1 cleavage[a]	mean growth percent[b]	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MCF7	MGM[c]
	2	++++	ND	0.01	0.03	0.01	0.01	0.22	0.02	0.01	0.01	0.0405 ± 0.0187
3-NO_2	**80**	++++	ND	0.089	0.025	0.157	0.098	0.309	0.241	0.040	0.016	0.156 ± 0.061
	81	++++	ND	0.19	0.274	0.016	0.012	0.864	0.015	0.017	2.17	0.370 ± 0.28
	82	+++	ND	0.021	0.038	0.095	0.380		0.309	0.085		0.632 ± 0.029
3-F	**49**	++	61.14									NS[d]
	60	+++	−8.15	0.490	0.309	0.417	0.245	1.29	0.437	0.575	0.135	0.692
	68	+++	30.31	2.09	1.29	2.95	1.38	4.27	2.34	2.95	0.347	2.75
3-Cl	**50**	++	14.53	0.692	0.437	0.417	0.398	2.24	0.977	2.09	0.257	1.12
	61	+++	0.06	0.186	0.107	0.204	0.074	0.407	0.110	0.309	0.031	0.229
	69	++	44.73	2.14	1.62	2.40	3.31	6.03	5.01	4.07	0.372	4.17
3-Br	**51**	++	46.84									NS
	83	++	27.33	0.513	0.251	0.676	0.427	3.31	0.468	0.676	0.191	1.26
	86	+++	84.03									NS
3-I	**52**	++	39.73	NT[e]	0.631	3.02	2.14	5.27	4.27	3.02	0.331	3.16
	62	++	43.54	0.437	0.200	0.550	2.29	3.89	0.708	0.603	0.074	1.62
	70	+	96.00									NS
3-CN	**53**	+(+)	42.65									NS
	84	++	41.11	0.537	0.112	0.501	0.407	4.68	0.575	0.562	0.033	0.944 ± 0.015
	87	++	101.69									NS
2-CF_3	**54**	+	82.67									NS
	63	0/+	49.34									NS
3-CF_3	**55**	+	52.24									NS
	64	0/+	6.57	0.407	0.646	1.29	0.537	1.51	1.78	1.10	0.257	1.07
3-SMe	**56**	+	39.68	1.82	0.741	2.34	1.38	4.37	1.95	2.51	0.427	2.51
	65	+	42.65									NS
3-SO_2Me	**57**	++	96.74									NS
	66	++	59.38									NS
3-CO_2Me	**58**	+	NA[f]									NA
	85	++	62.13	0.589	0.398	0.490	100	100	0.525	0.692	0.316	5.37
	88	+	87.47									NS
3-aza	**76**	++	NA									NA
	78	++	−9.25	0.871	0.380	0.708	1.23	2.00	0.955	0.977	0.331	0.977
	79	+	47.53									NS

[a]Compound-induced DNA cleavage due to Top1 poisoning, with scores given according to the following system based on the activity of 1 μM camptothecin: 0, no activity; +, between 20 and 50% activity; ++, between 50 and 75% activity; +++, between 75 and 95% activity; ++++, equal activity. [b]Growth percent of cultured cells of ∼60 cancer cell lines treated with 10 μM compound relative to vehicle-treated control. [c]Mean graph midpoint of growth inhibition from 5 dose assay, ranging from 10^{-8}−10^{-4} M. [d]NS = not selected for 5 dose assay. [e]NT = cell line not tested. [f]NA = not accepted by the National Cancer Institute for screening in the NCI-60.

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

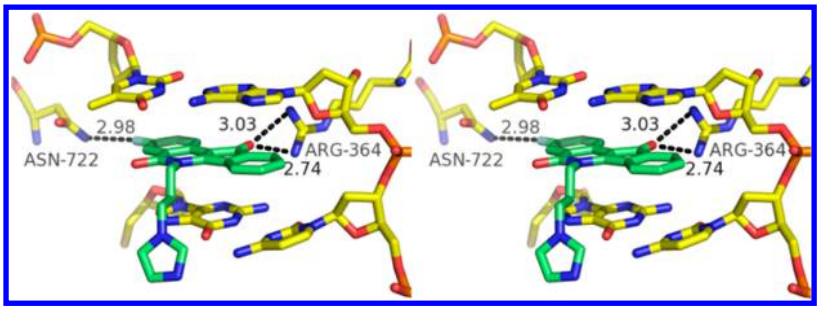


Figure 4. Energy-minimized hypothetical binding pose of **60** (green) within the X-ray crystal structure of a stalled Top1−DNA cleavage complex cocrystallized with indenoisoquinoline **89** (PDB code 1SC7). Potential interactions are indicated by dashed lines. The distances between heavy atoms appear next to the dashed lines. The stereoview is programmed for wall-eyed (relaxed) viewing.

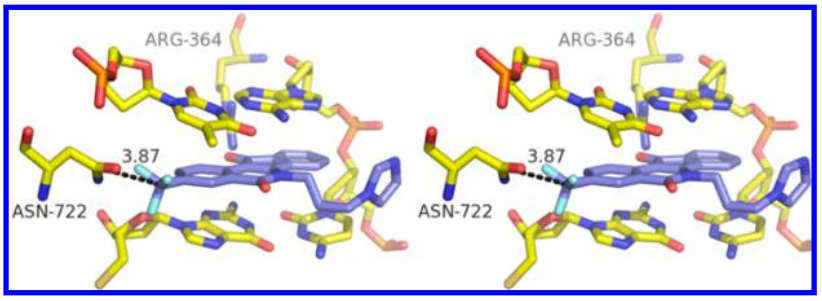
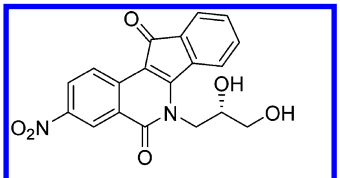


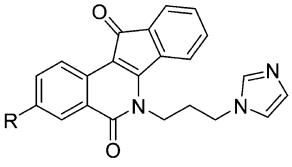
Figure 5. Energy-minimized hypothetical binding pose of **64** (blue) within the X-ray crystal structure of a stalled Top1−DNA cleavage complex cocrystallized with indenoisoquinoline **89** (PDB code 1SC7). Potential interactions are indicated by dashed lines. For clarity, only one potential interaction is shown. The distance between heavy atoms appears next to the dashed line. The stereoview is programmed for wall-eyed (relaxed) viewing.

and **50** displayed worse Top1 poisoning activity and cytotoxicity than imidazolylpropyl side chain analogues **60** and **61**. This trend held generally true for all of the other potential bioisosteres. The (*S*)-2,3-dihydroxypropyl lactam side chain was recently reported to impart equivalent or greater Top1 poisoning activity, and improved MGM GI_{50} values, compared to imidazolylpropyl and morpholinopropyl lactam side chains.[31] This trend is applicable to 2,3-dimethoxy-8,9-methylenedioxy- and 3-nitro-substituted indenoisoquinolines but clearly not for all aromatic ring substitution patterns.

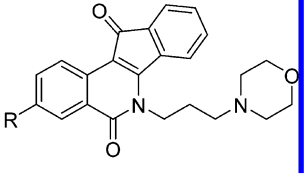
There are several reports in the literature of successful nitro aryl replacement with a pyridine ring.[43] It might therefore be expected that the 3-azaindenoisoquinolines would display Top1 poisoning and growth inhibitory activities comparable to 3-nitroindenoisoquinolines **80**−**82**. Two possibilities to explain why this "swap" has turned out to be successful in other studies include (1) the pyridine nitrogen, like nitro group oxygens, can function as a hydrogen bond acceptor, and (2) the electronegative nitrogen within the pyridine ring mimics the electron-withdrawing effect of nitro. On the contrary, the 3-azaindenoisoquinolines' Top1 poisoning scores were mediocre to poor (++ or +). A single compound, **78**, progressed to the five-dose growth inhibition assay, and its MGM GI_{50} was 0.977 μM. None of the other 3-aza compounds displayed submicromolar MGM GI_{50} values or Top1 poisoning scores above ++.



80



81, R = NO_2
83, R = Br
84, R = CN
85, R = CO_2Me



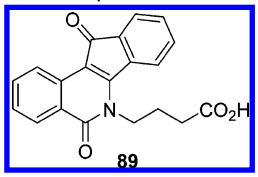
82, R = NO_2
86, R = Br
87, R = CN
88, R = CO_2Me

The other potential bioisosteres (i.e., I, CN, CF_3, SMe, SO_2Me, and CO_2Me) did not display Top1-mediated DNA cleavage assay scores above ++. Although all of the new compounds had some activity, the majority were clustered with

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

"+" and "++" Top1 assay scores. Only four of these compounds, **52**, **62**, **63**, and **84**, were advanced to the five-dose assay for MGM GI_{50} determination. However, none of these four displayed MGM GI_{50} values better than 0.944 μM.

In an effort to rationalize the observed biological activities on the basis of molecular mechanics, several ligands were docked into the PDB code 1SC7 X-ray crystal structure of the indenoisoquinoline **89**–Top1–DNA ternary complex.[47] The ligands, Top1–DNA cleavage complex, and resulting ligand–Top1–DNA ternary complexes were energy minimized as described in the Experimental Section. The graphics in Figures 4 and 5 were prepared in PyMOL.[48]



89

The binding mode of **60** may provide insight into its relatively potent Top1 poisoning activity (Figure 4). The 3-fluoro substituent is calculated to be within hydrogen bonding distance (2.98 Å) of the carboxamide nitrogen of Asn722. The angle defined by the fluorine of **60** and the carboxamide hydrogen and nitrogen of Asn722 would ideally be >150° for hydrogen bonding,[49] but the measured angle in the docking pose is 116°. The fluorine atom occupies a pocket defined by two deoxyribose sugars and Top1 residues Asn722 and Thr718 (not shown). Its close contacts (≤3.5 Å) with heavy atoms from each of these four structures favorably impact the van der Waals contribution to the ligand's binding energy.

Compound **61** was also studied by molecular mechanics molecular modeling. The binding mode of **61** (not shown), which is similar to that obtained for **60**, does not suggest a halogen bonding interaction between the chlorine and Asn722. In halogen bonding, chlorine, bromine, or iodine interacts as a Lewis acid with a Lewis basic atom such as oxygen or nitrogen.[50] The electron density surrounding the halogen atom is unevenly distributed, such that a small region of electropositivity exists at one face.[49] This type of bonding seems unlikely for **61**, given that the distance between the chlorine and the carboxamide oxygen of Asn722 is calculated to be 4.2 Å. The carbonyl does not align with the C–Cl bond on the ligand. Furthermore, its 3-bromo and 3-iodo analogues do not show the marked enhancement in activity relative to 3-fluoro observed with some halogen-bonding ligands.[49,51] Another possibility is that the 3-chloro substituent has appropriate shape complementarity for its surroundings. Significantly larger monatomic bioisosteres (i.e., bromine and iodine) and multiatom groups (e.g., CO_2Me and SO_2Me) were unable to confer similar Top1 poisoning and growth inhibitory activities.

Trifluoromethylated indenoisoquinoline **64** exhibits poor Top1 poisoning activity. In its molecular model (Figure 5), the CF_3 group packs closely with the Asn722 residue. The same attractive intermolecular interactions that could stabilize the binding of **60** and **61** seem plausible. As can be observed from close visual inspection of the ligands in Figures 4 and 5, **64** is rotated relative to **60** so as to maximize its interaction with Asn722, an effect which may consequently weaken other important interactions for ligand binding. The trifluoromethyl group is unusual among the bioisosteres selected for this study because of its tetrahedral geometry (the only other being SO_2Me). Its geometry may help to explain why the 2- and 3-CF_3 compounds exhibit very poor Top1 poisoning activities (0/+ or +).

Previous quantum mechanics studies suggest that the interactions between an indenoisoquinoline and its flanking base pairs within the cleavage complex are predictive of ligand binding orientation.[52] Electrostatic potential surfaces calculated using quantum mechanics showed that the electron withdrawing effect of 3-nitro pulls electron density away from the lactam carbonyl. This polar effect serves to minimize poor charge complementarity at the lactam carbonyl and to maximize the charge complementarity just outside of the A-ring 3-position with the flanking base pairs.[52] The theoretical binding energy, electron correlation, and dispersion forces that stabilize azaindenoisoquinolines in their binding site are sensitive to both incorporation and position of the nitrogen atom in the D-ring.[53] In the present study, it was predicted that the quantum mechanics-calculated binding energies of bioisosterically modified indenoisoquinolines would be similar to those for nitrated indenoisoquinolines.

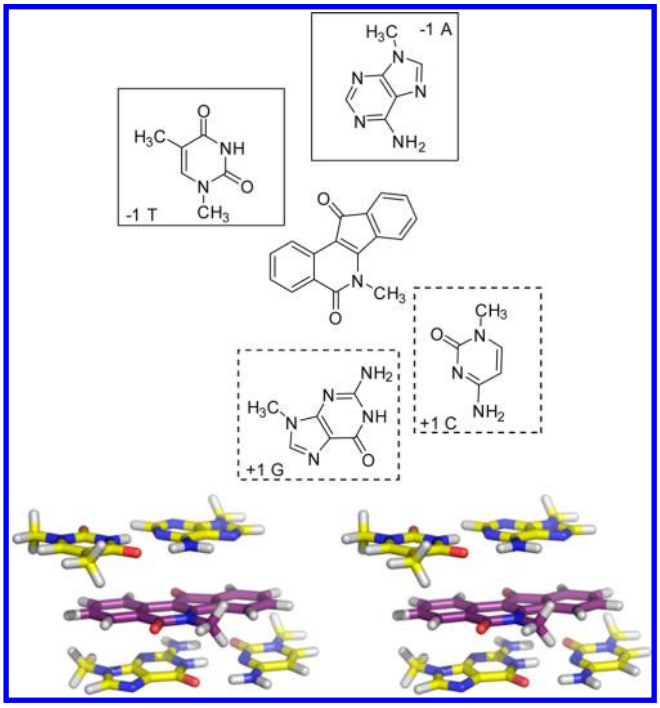
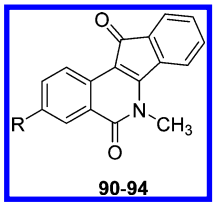


Figure 6. Two- and three-dimensional depictions of the model complex used for quantum mechanics calculations. The three-dimensional depiction is shown in stereoview and is programmed for wall-eyed (relaxed) viewing.

The binding energies of five model indenoisoquinolines **90**–**94** in a simplified version of a Top1–DNA cleavage complex (Figure 6) were calculated by quantum mechanics at the MP2/6-31G* level of theory. The quantum mechanics calculations were performed with a model Top1–DNA cleavage complex containing only the heterocyclic bases shown in Figure 6 and simplified indenoisoquinolines in which the lactam side chains were replaced by a methyl group. These approximations were made because (1) previous theoretical studies have demonstrated that the experimentally observed indenoisoquinoline binding orientation can be predicted using this simplified binding site model[52] and (2) the lactam side chain of the

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX–XXX

Table 3. Calculated Binding Energies of Hypothetical Indenoisoquinolines A–E in the Model Binding Site



90-94

ligand	R	E_{inter} (kcal/mol)[a]	electronegativity of R (eV)[b]
90	NO_2	−21.34	7.84
91	H	−15.87	
92	F	−17.36	10.01
93	Cl	−18.60	7.65
94	CF_3	−16.09	6.30

[a]Binding energies were calculated at the MP2/6-31G* level and include the basis set superposition error. [b]Calculated at CISD level by de Proft et al.[41]

indenoisoquinolines projects out of the simplified binding site model and into the DNA major groove.[47]

The quantum mechanics-derived binding energies (Table 3) display a correlation with Top1 assay scores. The two nitro bioisosteres that result in +++ Top1 assay scores, F and Cl (i.e., compounds **60** and **61**), also have the most favorable theoretical binding energies, at −17.36 and −18.60 kcal/mol, respectively. In line with Top1 assay scores, neither fluorinated **92** nor chlorinated **93** were calculated to bind as favorably as **90**, the nitrated analogue. The negative control ligand **91** is substituted with 3-H, and it has the least favorable calculated binding energy. Ligand **94** is substituted with a CF_3 group in the 3-position. Its calculated binding energy was slightly better than **91**'s, although considerably worse than that of **90**, **92**, or **93**.

The calculated binding energies of the ligands also show a correlation with group electronegativity. The substituents on ligands **90** and **93**, NO_2 and Cl respectively, have similar electronegativities, and their theoretical binding energies are the most favorable of all five systems studied. With its substantially greater electronegativity, fluorinated analogue **92** could be anticipated to have a more favorable theoretical binding energy than **90** and **93**, but it does not. The CF_3 group present on ligand **94** has the lowest electronegativity among the nitro bioisosteres, and this may factor into its unfavorable calculated binding energy and low Top1 assay scores. It is possible that both, or either, direct substituent-substituent interactions and polarized aromatic system interactions dominate the calculated theoretical binding energies.[54,55]

CONCLUSIONS

Twenty-three new 3-substituted indenoisoquinolines were synthesized in order to investigate the effect of bioisosteric replacements of the nitro group in the 3-nitroindenoisoquinoline Top1 poison family. Although many 3-nitroindenoisoquinolines display potent activity in the Top1-mediated DNA cleavage assay and growth inhibitory effect in the NCI-60 antiproliferative assay, it would be advantageous to identify bioisosteres that mitigate potential toxicity risks associated with aryl nitro groups. Fluorine and chlorine were identified as bioisosteres on the basis of Top1 poisoning activities and growth inhibitory potencies. Molecular mechanics modeling and quantum mechanics calculations indicate that hydrogen-bonding, van der Waals forces, and interactions between the flanking base pairs and the indenoisoquinoline could stabilize bioisosterically modified indenoisoquinolines within their binding site in the Top1–DNA cleavage complex.

The findings from this study are valuable because they can aid the design of novel Top1 poisons. 3,9-Difluorinated indolocarbazole glycosides have demonstrated efficacy in preclinical models and advanced to human clinical trials.[56,57] The 3-position substituents on the indenoisoquinoline and indolocarbazole scaffolds project toward Asn722.[47] This observation provides support for a universal structure–activity relationship for the Top1 poisons, and may therefore aid in the structure-based design and optimization of new and different Top1 poisons.[58–61]

EXPERIMENTAL SECTION

Reactions were monitored by silica gel analytical thin-layer chromatography, and 254 nm UV light was used for visualization. Melting points were determined using capillary tubes and are uncorrected. ^{1}H Nuclear magnetic resonance spectroscopy was performed using a 300 MHz spectrometer. Infrared spectra were obtained using an FTIR spectrometer. High-resolution mass spectra were recorded on a double-focusing sector mass spectrometer with magnetic and electrostatic mass analyzers. Compound purities were estimated by reversed phase C18 HPLC, with UV detection at 254 nm. The major peak area of each tested compound was ≥95% of the combined total peak area.

(2-Bromo-4-fluorophenyl)methanol (10). Aldehyde **9** (5.014 g, 24.70 mmol) was dissolved in MeOH (50 mL), and the solution was cooled to 0–5 °C. $NaBH_4$ (1.873 g, 49.51 mmol) was added all at once, and the mixture was stirred at 0–5 °C for 1 h, and then at room temperature for 24 h. Saturated $NaHCO_3$ solution (50 mL) was added to quench the reaction, and the mixture was concentrated in vacuo to remove MeOH. The resulting suspension was extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with brine (1 × 50 mL), dried over Na_2SO_4, and concentrated in vacuo. The obtained oil was purified by silica gel column chromatography, eluting with $CHCl_3$, to yield **10** (4.240 g, 84%) as a white solid: mp 63–66 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (dd, J = 8.6 Hz, J = 6.1 Hz, 1 H), 7.31 (dd, J = 8.2 Hz, J = 2.6 Hz, 1 H), 7.06 (td, J = 8.3 Hz, J = 2.6 Hz, 1 H), 4.72 (d, J = 6.4 Hz, 2 H), 2.02 (t, J = 6.3 Hz, 1 H).

6-Fluorophthalide (11). (2-Bromo-4-fluorophenyl)methanol (**10**, 6.720 g, 32.78 mmol) and CuCN (5.891 g, 65.78 mmol) were diluted in DMF (35 mL). The system was flushed with argon and then heated to reflux with stirring for 2 h 45 min. The mixture was cooled to 100 °C, H_2O (3.5 mL) was added, and the mixture was stirred with heating to reflux for 21 h. EtOAc (75 mL) was added to the reaction mixture once it cooled to room temperature, and the mixture was filtered through a plug of Celite, washing with EtOAc (25 mL). Brine (150 mL) was added, the mixture partitioned between the two phases, and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 × 70 mL). The combined organic layers were washed with brine (2 × 70 mL) and saturated LiCl solution (70 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with $CHCl_3$, to provide **11** (2.945 g, 59%) as an off-white solid: mp 100–105 °C (lit.[35] mp 100–102 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (dd, J = 7.2, 2.3 Hz, 1 H), 7.53–7.37 (m, 2 H), 5.32 (s, 2 H). CIMS m/z (rel intensity) 153 (MH^+, 100).

3-Bromo-6-fluorophthalide (12). 6-Fluorophthalide (**11**, 1.001 g, 6.580 mmol), NBS (1.204 g, 6.764 mmol), and AIBN (39 mg) were diluted in CCl_4 (25 mL) and heated to reflux with stirring under argon for 1 h 30 min. The mixture was cooled to room temperature, filtered, and the residue was washed with CCl_4 (10 mL). The filtrate was concentrated in vacuo to provide a yellow oil, which was purified by silica gel column chromatography, eluting with 80:20 hexanes–EtOAc, to provide **12** (1.230 g, 81%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (ddd, J = 8.4, 4.2, 0.7 Hz, 1 H), 7.56–7.44 (m, 2 H),

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX–XXX

7.40 (s, 1 H). CIMS m/z (rel intensity) 231/233 (MH^+, 6/6); 153 (MH^+ − Br, 100).

6-Fluoro-3-hydroxyphthalide (13). Potassium hydroxide (0.542 g, 9.66 mmol) and H_2O (15 mL) were added to 3-bromo-6-fluorophthalide (**12**, 1.077 g, 4.662 mmol). The mixture was heated to reflux with stirring for 1 h, cooled to room temperature, and EtOAc (20 mL) and $NaHSO_4$ (1.8 g) were added. The organic layer was removed, and the aqueous layer was extracted twice more with EtOAc (2 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo to yield **13** (0.617 g, 79%) as a white solid: mp 95−97 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (s, 1 H), 7.85−7.55 (m, 3 H), 6.66 (s, 1 H). CIMS m/z (rel intensity) 169 (MH^+, 13), 151 ($MH^+ - H_2O$, 100).

3-Fluoroindeno[1,2-*c*]isochromene-5,11-dione (15). 6-Fluoro-3-hydroxyphthalide (**13**, 0.582 g, 3.46 mmol) and phthalide (**14**, 0.479 g, 3.57 mmol) were suspended in EtOAc (10 mL), and a freshly prepared solution of NaOMe (0.349 g, 15.2 mmol of Na) in MeOH (10 mL) was added. The resulting solution was heated to reflux with stirring for 18 h. The resulting mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (3 mL), concentrated in vacuo, and azeotroped with PhMe (10 mL). Ac_2O (20 mL) was added to the residue, and the mixture was heated to reflux with stirring for 1 h. The mixture was cooled to room temperature, and hexanes (20 mL) were added. The mixture was filtered, and the residue was washed with hexanes (20 mL) and H_2O (10 mL) to provide **15** (196 mg, 31%) as an orange−red solid: mp 215−218 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (dd, J = 8.8, 5.2 Hz, 1 H), 7.95 (dd, J = 8.6, 2.7 Hz, 1 H), 7.65−7.37 (m, 5 H). EIMS m/z (rel intensity) 266 (M^+, 100). HREIMS calcd for $C_{16}H_8FO_3$ (M^+) 266.0374, found 266.0380.

6-Chlorophthalide (18). 3-Aminophthalide (**17**, 3.500 g, 23.49 mmol) was suspended in 37% HCl (25 mL) 0 °C. An ice-cold solution of $NaNO_2$ (1.713 g, 24.83 mmol) in H_2O (10 mL) was added dropwise, and the resulting solution was stirred at 0−5 °C for 10 min. An ice-cold suspension of CuCl (2.91 g, 29.4 mmol) in 37% HCl (10 mL) was added dropwise. The mixture was stirred at room temperature for 10 min, then at reflux for 3 h, and room temperature for 16 h. The suspension was extracted with EtOAc (3 × 80 mL), and the combined organic layers were washed with saturated $NaHCO_3$ solution (1 × 50 mL) and H_2O (1 × 50 mL). The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with $CHCl_3$, to yield **18** (2.852 g, 72%) as a white solid: mp 106−107 °C (lit.[62] mp 107−108 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 1.9 Hz, 1 H), 7.66 (dd, J = 8.2 Hz, J = 1.9 Hz, 1 H), 7.45 (dd, J = 8.2 Hz, J = 0.8 Hz, 1 H), 5.31 (s, 2 H). CIMS m/z (rel intensity) 169/171 (MH^+, 100/32).

6-Bromophthalide (19). 3-Aminophthalide (**17**, 2.98 g, 20.0 mmol) was suspended in 48% HBr (15 mL) and H_2O (15 mL) at 0 °C. A room temperature solution of $NaNO_2$ (1.45 g, 21.0 mmol) in H_2O (7 mL) was added dropwise, and the resulting solution was stirred at 0−5 °C for 20 min. A room temperature suspension of CuBr (3.58 g, 25.0 mmol) in 48% HBr (20 mL) was added dropwise. The mixture was stirred at room temperature for 20 min, at 80 °C for 2 h, and room temperature for 16 h. EtOAc (70 mL) was added, the suspension shaken vigorously, the aqueous layer removed, and the organic layer washed with 1 N HCl (2 × 25 mL), saturated $NaHCO_3$ solution (1 × 25 mL), and H_2O (2 × 25 mL), and concentrated. The residue was purified by silica gel column chromatography, eluting with $CHCl_3$, to yield **19** (2.71 g, 64%) as a white solid: mp 96−98 °C (lit.[62] mp 114−115 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 1.7 Hz, 1 H), 7.80 (dd, J = 8.1 Hz, J = 1.8 Hz, 1 H), 7.39 (dd, J = 8.1 Hz, J = 0.7 Hz, 1 H), 5.29 (s, 2 H). CIMS m/z (rel intensity) 213/215 (MH^+, 100/100).

6-Iodophthalide (20). 3-Aminophthalide (**17**, 5.414 g, 36.34 mmol) was suspended in 37% HCl (25 mL) at 0 °C. An ice-cold solution of $NaNO_2$ (3.027 g, 43.87 mmol) in H_2O (8 mL) was added dropwise, and the resulting solution was stirred at 0 °C for 25 min. A solution of KI (30.5 g, 184 mmol) in H_2O (30 mL) was added dropwise. The mixture was stirred at room temperature for 18 h. The aqueous suspension was extracted with EtOAc (3 × 100 mL), and the combined organic layers were washed with saturated aq $Na_2S_2O_3$ (2 × 100 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with $CHCl_3$, to provide **20** (5.973 g, 63%) as an off-white solid: mp 87−90 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (dd, J = 1.5 Hz, J = 0.7 Hz, 1 H), 7.99 (dd, J = 8.0 Hz, J = 1.5 Hz, 1 H), 7.29 (d, J = 0.8 Hz, 1 H), 5.28 (s, 2 H). CIMS m/z (rel intensity) 261 (MH^+, 100).

6-Cyanophthalide (21). 3-Aminophthalide (**17**, 1.100 g, 7.375 mmol) was suspended in 37% HCl (2 mL) and H_2O (12 mL) at 0 °C. An ice-cold solution of $NaNO_2$ (691 mg, 10.0 mmol) in H_2O (2 mL) was added dropwise, and the resulting mixture was added dropwise to a prepared solution of CuCN (804 mg, 9.00 mmol) and NaCN (1.240 g, 25.30 mmol) in H_2O (10 mL) stirring at 0 °C. The mixture was stirred with warming to room temperature for 3.5 h and then filtered through a plug of Celite (2 cm height), which was then washed with EtOAc (50 mL). The aqueous layer of the filtrate was extracted with EtOAc (50 mL), and the combined organic layers were washed with brine (25 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with a 50:50 EtOAc−hexanes →80:20 EtOAc−hexanes gradient, to provide **21** (498 mg, 42%) as a yellow solid: mp 185−190 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (s, 1 H), 7.97 (dd, J = 7.9, 1.5 Hz, 1 H), 7.67 (dq, J = 7.9, 0.9 Hz, 1 H), 5.41 (s, 2 H).

6-Chloro-3-hydroxyphthalide (26). 6-Chlorophthalide (**18**, 2.786 g, 16.58 mmol), NBS (2.962 g, 16.64 mmol), and benzoyl peroxide (33 mg) were suspended in CCl_4 (22 mL). The mixture was heated to reflux with stirring under an argon atmosphere for 1 h, cooled to room temperature, and filtered. The residue was washed with CCl_4 (2 × 20 mL), and the filtrate was concentrated in vacuo. H_2O (25 mL) and KOH (1.88 g, 33.5 mmol) were added to the obtained yellow oil, and the mixture was heated to reflux with stirring for 1 h. After cooling to room temperature, the mixture was acidified with $NaHSO_4$ (1.9 g) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to provide **26** (2.60 g, 85%) as a white solid: mp 111−116 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (d, J = 7.4 Hz, 1 H), 7.98−7.77 (m, 2 H), 7.77−7.59 (m, 1 H), 6.67 (d, J = 8.2 Hz, 1 H). CIMS m/z (rel intensity) 185/187 (MH^+, 15/4), 167/169 ($MH^+ - H_2O$, 100/47).

6-Bromo-3-hydroxyphthalide (27). 6-Bromophthalide (**19**, 2.69 g, 12.6 mmol), NBS (2.25 g, 12.6 mmol), and benzoyl peroxide (30 mg) were suspended in CCl_4 (15 mL). The mixture was heated to reflux with stirring under an argon atmosphere for 50 min, cooled to room temperature, and filtered. The residue was washed with CCl_4 (2 × 10 mL), and the filtrate was concentrated in vacuo. H_2O (25 mL), THF (10 mL), and KOH (1.409 g, 25.11 mmol) were added to the obtained yellow oil, and the mixture was heated to reflux with stirring for 1 h. After cooling to room temperature, the mixture was acidified with $NaHSO_4$ (1.9 g) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to provide **27** (2.78 g, 96%) as a white solid: mp 118−120 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (d, J = 7.0 Hz, 1 H), 8.06−7.92 (m, 2 H), 7.64 (dd, J = 7.9 Hz, J = 3.9 Hz, 1 H), 6.65 (d, J = 7.6 Hz, 1 H).

3-Hydroxy-6-iodophthalide (28). 6-Iodophthalide (**20**, 0.806 g, 3.10 mmol), NBS (0.553 g, 3.11 mmol), and benzoyl peroxide (23 mg) were suspended in CCl_4 (15 mL). The mixture was heated to reflux with stirring under an argon atmosphere for 2.5 h, cooled to room temperature, and filtered. The residue was washed with CCl_4 (2 × 20 mL), and the filtrate was concentrated in vacuo. H_2O (25 mL) and KOH (0.34 g, 6.1 mmol) were added to the obtained yellow oil, and the mixture was heated to reflux with stirring for 3.5 h. After cooling to room temperature, the mixture was acidified with $NaHSO_4$ (2.0 g) and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to provide **28** (0.954 g, 96%) as a white solid: mp 127−130 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (d, J = 7.0 Hz, 1 H), 8.19−8.09 (m, 2 H), 7.49 (d, J = 7.8 Hz, 1 H), 6.63 (d, J = 7.6 Hz, 1 H). CIMS m/z (rel intensity) 277 (MH^+, 8), 259 ($MH^+ - H_2O$), 100.

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

6-Cyano-3-hydroxyphthalide (29). 6-Cyanophthalide (**21**, 802 mg, 5.04 mmol), NBS (990 mg, 5.56 mmol), and AIBN (57 mg) were suspended in CCl_4 (50 mL). The mixture was heated to reflux with stirring under an argon atmosphere for 2 h, cooled to room temperature, and concentrated in vacuo. The residue was purified by flash silica gel column chromatography (10 cm × 3 cm, 80:20 hexanes−EtOAc 50:50 hexanes−EtOAc, 10 mL fractions). The product-containing fractions were pooled and concentrated in vacuo to yield a solid residue. H_2O (15 mL) was added to the solid, and the mixture was heated to reflux with stirring for 18 h. The product **29** (408 mg, 46%) precipitated from solution upon cooling to room temperature as a yellowish-white solid: mp 167−169 °C (lit.[19] mp 138−140 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 (s, 1 H), 8.39 (s, 1 H), 8.24 (dd, J = 7.9, 1.4 Hz, 1 H), 7.89 (d, J = 7.9 Hz, 1 H), 6.77 (s, 1 H).

3-Chloroindeno[1,2-*c*]isochromene-5,11-dione (30). 6-Chloro-3-hydroxyphthalide (**26**, 2.437 g, 13.24 mmol) and phthalide (**14**, 1.779 g, 13.28 mmol) were suspended in EtOAc (20 mL), and a freshly prepared solution of NaOMe (1.16 g, 50.4 mmol of Na) in MeOH (40 mL) was added. The resulting solution was heated to reflux with stirring under an argon atmosphere for 23 h. The resulting mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (4.5 mL), concentrated in vacuo, and azeotroped with PhMe (1 × 25 mL). Ac_2O (75 mL) was added to the residue, and the mixture was heated to reflux with stirring under an argon atmosphere for 21 h. After cooling to room temperature, the mixture was concentrated in vacuo, the residue taken up in $CHCl_3$ (100 mL), and the organic solution washed with H_2O (3 × 30 mL). The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was washed with 70:30 hexanes−EtOAc (3 × 30 mL) to provide **30** (2.009 g, 54%) as an orange solid: mp 244−246 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.24−8.14 (m, 2 H), 7.98 (dd, J = 8.5 Hz, J = 2.3 Hz, 1 H), 7.70−7.48 (m, 4 H). CIMS m/z (rel intensity) 283/285 (MH^+, 100/36). HRCIMS calcd for $C_{16}H_8ClO_3$ (MH^+) 283.0162, found 283.0153.

3-Bromoindeno[1,2-*c*]isochromene-5,11-dione (31). 6-Bromo-3-hydroxyphthalide (**27**, 2.52 g, 11.0 mmol) and phthalide (**14**, 1.47 g, 11.0 mmol) were suspended in EtOAc (20 mL), and a freshly prepared solution of NaOMe (0.91 g, 40 mmol of Na) in MeOH (40 mL) was added. The resulting solution was heated to reflux with stirring under an argon atmosphere for 72 h. The resulting mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (4 mL), concentrated in vacuo, and azeotroped with PhMe (1 × 25 mL). Ac_2O (50 mL) was added to the residue, and the mixture was heated to reflux with stirring under an argon atmosphere for 17 h. After cooling to room temperature, H_2O (30 mL) and $CHCl_3$ (50 mL) were added and the mixture was filtered to obtain some product. The filtrate was washed with H_2O (2 × 20 mL) and brine (1 × 20 mL), concentrated in vacuo, and azeotroped with PhMe (2 × 10 mL) to provide additional product. The combined crude products were washed with $CHCl_3$ (2 × 30 mL) and EtOAc (1 × 30 mL) to provide **31** (1.920 g, 53%) as an orange solid: mp 236−238 °C (lit.[36] mp 230−232 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (d, J = 1.6 Hz, 1 H), 8.13−8.08 (m, 2 H), 7.64−7.48 (m, 4 H). CIMS m/z (rel intensity) 327/329 (MH^+, 100/96), 248 (MH^+ − Br, 77).

3-Iodoindeno[1,2-*c*]isochromene-5,11-dione (32). 3-Hydroxy-6-iodophthalide (**28**, 0.465 g, 1.68 mmol) and phthalide (**14**, 0.232 g, 1.73 mmol) were suspended in EtOAc (10 mL), and a freshly prepared solution of NaOMe (0.19 g, 8.3 mmol of Na) in MeOH (10 mL) was added. The resulting solution was heated to reflux with stirring for 27 h. The resulting mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (1.5 mL), concentrated in vacuo, and azeotroped with PhMe (15 mL). Ac_2O (20 mL) was added to the residue, and the mixture was heated to reflux with stirring for 4 h. The mixture was concentrated in vacuo, the residue taken up in $CHCl_3$ (30 mL), and the organic layer washed with H_2O (2 × 15 mL). The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The crude product was washed with 80:20 hexanes−EtOAc (2 × 10 mL) to provide **32** (196 mg, 31%) as an orange−red solid: mp 199−201 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.43 (d, J = 1.8 Hz, 1 H), 8.24 (dd, J = 8.3 Hz, J = 1.9 Hz, 1 H), 7.97 (d, J = 8.3 Hz, 1 H), 7.64−7.48 (m, 4 H). CIMS m/z (rel intensity) 375 (MH^+, 100). HRCIMS calcd for $C_{16}H_8IO_3$ (MH^+) 374.9518, found 374.9512.

3-Cyanoindeno[1,2-*c*]isochromene-5,11-dione (33). 6-Cyano-3-hydroxyphthalide (**29**, 404 mg, 2.31 mmol) and phthalide (**14**, 318 mg, 2.37 mmol) were suspended in EtOAc (10 mL), and a freshly prepared solution of NaOMe (0.20 g, 8.7 mmol of Na) in MeOH (20 mL) was added. The resulting solution was heated to reflux with stirring for 17.5 h. The resulting mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (1 mL), concentrated in vacuo, and azeotroped with PhMe (2 × 10 mL). Ac_2O (20 mL) was added to the residue, and the mixture was heated to reflux with stirring for 18.5 h. The resulting mixture was azeotroped with PhMe (4 × 10 mL) to remove the solvents. The residue was purified by silica gel column chromatography, eluting with $CHCl_3$, to provide an oil, which was triturated with hexanes (10 mL) and filtered to collect a solid. **33** (225 mg, 36%) was obtained as a yellow-orange solid: mp 215−218 °C (lit.[19] mp 225−227 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (dd, J = 1.7, 0.6 Hz, 1 H), 8.49 (dd, J = 8.3, 0.7 Hz, 1 H), 8.00 (dd, J = 8.3, 1.8 Hz, 1 H), 7.66 (dt, J = 6.4, 1.2 Hz, 1 H), 7.61−7.45 (m, 3 H). CIMS m/z (rel intensity) 274 (MH^+, 100).

5-(Trifluoromethyl)isobenzofuran-1,3-dione (35). Diacid **34** (2.519 g, 10.759 mmol) was suspended in AcCl (15 mL), and the mixture was heated to reflux with stirring for 17 h. The mixture was concentrated in vacuo, and the product was purified by silica gel column chromatography, eluting with 4:1 EtOAc−hexanes, to yield a yellow oil. The yellow oil was dried under high vacuum to provide **35** (1.683 g, 72%) as a faint-yellow solid: mp 54−58 °C (lit.[63] mp 65−69 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (dt, J = 1.5, 0.7 Hz, 1 H), 8.41−8.23 (m, 2 H).

5-(Trifluoromethyl)phthalide (36) and 6-(Trifluoromethyl)-phthalide (37). Anhydride **35** (2.476 g, 11.46 mmol) was dissolved in THF (20 mL) with stirring, and the mixture was cooled to 0 °C. $NaBH_4$ (436 mg, 11.5 mmol) was added all at once, and the mixture was stirred at 0 °C for 1 h 25 min. The mixture was concentrated in vacuo, and a solution of H_2O (5 mL) and aqueous HCl (37%, 1 mL) was added to the residue. The aqueous solution was extracted with $CHCl_3$ (3 × 25 mL), and the combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried over Na_2SO_4, and concentrated in vacuo. The products were first purified on a silica gel column, eluting with 50:50 hexanes−EtOAc, to obtain a mixture of **36** and **37**, and then the two regioisomers were separated on a second silica gel column, eluting with 80:20 hexanes−EtOAc. Products **36** (405 mg, 17%) and **37** (408 mg, 18%) were each obtained as white solids: **36** mp 65−68 °C (lit.[37] mp 65−67 °C); **37** mp 88−89 °C (lit.[37] mp 88−90 °C). **36** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 7.9 Hz, 1 H), 7.86−7.78 (m, 2 H), 5.41 (s, 2 H). **37** ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 1 H), 7.95 (dd, J = 8.1 Hz, J = 0.8 Hz, 1 H), 7.67 (d, J = 8.0 Hz, 1 H), 5.41 (s, 2 H).

3-Hydroxy-5-(trifluoromethyl)phthalide (40). Phthalide **36** (1.101 g, 5.447 mmol), NBS (982 mg, 5.52 mmol), and AIBN (39 mg) were suspended in CCl_4 (25 mL) with stirring, and the mixture was heated to reflux for 2 h. The product mixture was then cooled and filtered to remove succinimide. The residue was washed with CCl_4 (10 mL), and the combined filtrate was concentrated in vacuo to provide an oil that was purified by silica gel column chromatography eluting with 80:20 hexanes−EtOAc. The pure product-containing fractions were concentrated in vacuo to provide a red oil. H_2O (20 mL) and KOH (630 mg, 11.2 mmol) were added to the oil, and the mixture was stirred and heated to reflux for 2 h 30 min. $NaHSO_4$ (1.1 g) was added to the obtained solution, and the product was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo to provide **40** (480 mg, 40%) as a yellow solid: mp 110−112 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1 H), 8.00 (dd, J = 8.1, 0.8 Hz, 1 H), 7.82 (d, J = 8.0 Hz, 1 H), 6.75 (s, 1 H), 3.81 (s, 1 H). CIMS m/z (rel intensity) 201 [$(M + H^+ - H_2O)^+$, 100], 219 (MH^+, 92).

3-Hydroxy-6-(trifluoromethyl)phthalide (41). Phthalide **37** (873 mg, 4.32 mmol), NBS (784 mg, 4.40 mmol), and AIBN (32 mg) were suspended in CCl_4 (25 mL) with stirring, and the mixture

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

was heated to reflux for 2 h 10 min. The product mixture was then cooled and filtered to remove succinimide. The residue was washed with CCl_4 (10 mL), and the combined filtrate was concentrated in vacuo to provide an oil which was purified by silica gel column chromatography, eluting with 80:20 hexanes−EtOAc. The pure product-containing fractions were concentrated in vacuo to provide a colorless oil. H_2O (20 mL) and KOH (538 mg, 9.59 mmol) were added to the oil, and the mixture was stirred and heated to reflux for 3 h 10 min. $NaHSO_4$ (1.1 g) was added to the obtained solution, and the product was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo to provide **41** (595 mg, 63%) as a white solid: mp 107−110 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, J = 8.0 Hz, 1 H), 7.95 (s, 1 H), 7.90 (d, J = 8.2 Hz, 1 H), 6.86 (s, 1 H), 5.08 (s, 1 H). CIMS m/z (rel intensity) 219 (MH^+, 100).

2-(Trifluoromethyl)indeno[1,2-*c*]isochromene-5,11-dione (42). 3-Hydroxy-5-(trifluoromethyl)phthalide (**40**, 480 mg, 2.20 mmol) and phthalide (**14**, 310 mg, 2.31 mmol) were suspended in EtOAc (5 mL), and a freshly prepared solution of NaOMe (0.21 g, 9.0 mmol of Na) in MeOH (10 mL) was added. The solution was heated to reflux with stirring for 20.5 h. The mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (1 mL), concentrated in vacuo, and azeotroped with PhMe (10 mL). Ac_2O (10 mL) was added to the residue, and the mixture was heated to reflux with stirring for 5 h. After cooling to room temperature, the mixture was azeotroped with PhMe (2 × 20 mL). The orange solid residue was taken up in $CHCl_3$ (50 mL) and washed with H_2O (2 × 25 mL). The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was washed with 50:50 $CHCl_3$−hexanes (15 mL) to yield **42** (220 mg, 32%) as an orange solid: mp 224−225 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (s, 1 H), 8.51 (d, J = 8.4 Hz, 1 H), 8.01 (dd, J = 8.5 Hz, J = 1.9 Hz, 1 H), 7.65 (dt, J = 6.6 Hz, J = 1.1 Hz, 1 H), 7.57−7.45 (m, 3 H). CIMS m/z (rel intensity) 317 (MH^+, 100). HRCIMS calcd for $C_{17}H_8F_3O_3$ (MH^+) 317.0420, found 317.0412.

3-(Trifluoromethyl)indeno[1,2-*c*]isochromene-5,11-dione (43). 3-Hydroxy-6-(trifluoromethyl)phthalide (**41**, 595 mg, 2.73 mmol) and phthalide (**14**, 377 mg, 2.81 mmol) were suspended in EtOAc (5 mL), and a freshly prepared solution of NaOMe (0.25 g, 11 mmol of Na) in MeOH (10 mL) was added. The solution was heated to reflux with stirring for 20.5 h. The mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (1 mL), concentrated in vacuo, and azeotroped with PhMe (10 mL). Ac_2O (10 mL) was added to the residue, and the mixture was heated to reflux with stirring for 5 h. After cooling to room temperature, the mixture was azeotroped with PhMe (2 × 10 mL). The orange solid residue was taken up in $CHCl_3$ (50 mL) and washed with H_2O (2 × 25 mL). The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was washed with 50:50 $CHCl_3$−hexanes (15 mL) to yield **43** (287 mg, 33%) as an orange solid: mp 200−202 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (s, 1 H), 8.42 (d, J = 8.4 Hz, 1 H), 7.73 (dd, J = 8.4 Hz, J = 1.4 Hz, 1 H), 7.65 (dt, J = 6.7 Hz, J = 1.1 Hz, 1 H), 7.58−7.42 (m, 3 H). CIMS m/z (rel intensity) 317 (MH^+, 100). HRCIMS calcd for $C_{17}H_8F_3O_3$ (MH^+) 317.0420, found 317.0433.

3-Hydroxy-6-(methylthio)phthalide (44). 6-Fluoro-3-hydroxyphthalide **12** (1.604 g, 9.541 mmol) was dissolved with stirring in DMF (11 mL), and solid NaSMe (1.358 mg, 19.38 mmol) was added. The mixture was heated between 115−120 °C for 2 h 30 min. The reaction mixture was cooled, and H_2O (20 mL) and 37% HCl (3 mL) were added. The product was extracted with Et_2O (6 × 30 mL). The combined Et_2O layers were washed with brine (2 × 30 mL), dried over Na_2SO_4, and concentrated in vacuo to provide a yellow−orange oil. The oil was purified by silica gel column chromatography, eluting with 4:1 EtOAc−hexanes, to yield **44** (1.299 g, 69%) as a peach solid: mp 95−100 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1 H), 7.65−7.47 (m, 2 H), 2.55 (s, 3 H). CIMS m/z (rel intensity) 197 (MH^+, 100).

3-(Methylthio)indeno[1,2-*c*]isochromene-5,11-dione (45). 3-Hydroxy-6-(methylthio)phthalide (**44**, 1.000 g, 5.096 mmol) and phthalide (**14**, 692 mg, 5.159 mmol) were suspended in EtOAc (15 mL), and a freshly prepared solution of NaOMe (0.50 g, 22 mmol of Na) in MeOH (30 mL) was added. The solution was heated to reflux with stirring under an argon atmosphere for 24.5 h. The mixture was cooled to room temperature, acidified to pH 1 with 37% HCl (2 mL), concentrated in vacuo, and azeotroped with PhMe (2 × 10 mL). Ac_2O (15 mL) was added to the residue, and the mixture was heated to reflux with stirring under an argon atmosphere for 1 h 10 min. The obtained suspension was azeotroped with PhMe (3 × 20 mL) to provide a red solid residue, which was suspended in $CHCl_3$ (125 mL) and washed with H_2O (2 × 25 mL). The organic solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was washed with $CHCl_3$−hexanes (50:50, 50 mL) and filtered. The filtrate was concentrated in vacuo until 10 mL of solvent remained, and the suspension was filtered. The collected solids were combined, yielding **45** (844 mg, 56%) as an orange solid: mp 220−222 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (dd, J = 8.4 Hz, J = 0.5 Hz, 1 H), 8.05 (d, J = 2.1 Hz, 1 H), 7.65 (dd, J = 8.4 Hz, J = 2.1 Hz, 1 H), 7.58 (td, J = 6.8 Hz, J = 0.9 Hz, 1 H), 7.51−7.36 (m, 3 H), 2.59 (s, 3 H). CIMS m/z (rel intensity) 295 (MH^+, 100). HRCIMS calcd for $C_{17}H_{11}O_3S$ (MH^+) 295.0423, found 295.0429.

3-(Methylsulfonyl)indeno[1,2-*c*]isochromene-5,11-dione (46). Lactone **45** (224 mg, 0.761 mmol) was dissolved with stirring in $CHCl_3$ (35 mL) at room temperature, and *m*-CPBA (labeled 57−86%, assumed 57%, 477 mg, 1.58 mmol) was added. The color of the solution changed from orange to yellow−orange almost instantaneously upon addition of *m*-CPBA. After stirring for 48.5 h, the reaction mixture color had changed back to orange. The suspension was filtered to yield **46** (131 mg, 53%) as an orange solid: mp 305 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (t, J = 1.2 Hz, 1 H), 8.40 (d, J = 1.3 Hz, 2 H), 7.70−7.54 (m, 4 H), 3.34 (s, 3 H). CIMS m/z (rel intensity) 327 (MH^+, 100). HRESIMS calcd for $C_{17}H_{10}O_5SNa$ (MNa^+) 349.0147, found 349.0130.

(*S*)-3-Fluoro-5,6-dihydro-6-(2,3-dihydroxypropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (49). Lactone **15** (86 mg, 0.32 mmol) was suspended in MeOH (15 mL) and $CHCl_3$ (10 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 75 mg, 0.82 mmol) was added with stirring. The mixture was heated to reflux for 15 h, cooled to room temperature, and filtered to provide a red−orange solid. The filtrate was concentrated in vacuo, suspended in $CHCl_3$ (5 mL), and filtered to provide additional product. The products were combined and washed with $CHCl_3$ (5 mL) and hexanes (10 mL) to yield **49** (88 mg, 81%) as a red-orange solid: mp 183−185 °C. IR (film) 3369, 1692, 1647, 1554, 1509 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (dd, J = 9.0, 5.4 Hz, 1 H), 8.04 (d, J = 7.5 Hz, 1 H), 7.88 (dd, J = 9.5, 2.8 Hz, 1 H), 7.74 (td, J = 8.8, 2.9 Hz, 1 H), 7.65−7.41 (m, 3 H), 5.14 (d, J = 5.1 Hz, 1 H), 4.99 (t, J = 5.6 Hz, 1 H), 4.65−4.41 (m, 2 H), 4.06−3.94 (m, 1 H), 3.59 (t, J = 5.2 Hz, 2 H). CIMS m/z (rel intensity) 340 (MH^+, 84). HRCIMS calcd for $C_{19}H_{15}FNO_4$ (MH^+) 340.0980, found 340.0977. HPLC purity, 95.01% (MeOH−H_2O, 90:10).

(*S*)-3-Chloro-5,6-dihydro-6-(2,3-dihydroxypropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (50). Lactone **30** (88 mg, 0.31 mmol) was suspended in MeOH (15 mL) and $CHCl_3$ (10 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 64 mg, 0.70 mmol) was added with stirring. The mixture was heated to reflux for 15 h, cooled to room temperature, and filtered to provide a red−orange solid. The product was washed with $CHCl_3$ (5 mL) and hexanes (10 mL) to yield **50** (62 mg, 56%) as a red−orange solid: mp 240−242 °C. IR (film) 3435, 1690, 1647, 1540, 1500 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.59 (d, J = 8.7 Hz, 1 H), 8.14 (d, J = 2.3 Hz, 1 H), 8.06 (d, J = 7.4 Hz, 1 H), 7.87 (dd, J = 8.7, 2.4 Hz, 1 H), 7.63−7.43 (m, 3 H), 5.13 (d, J = 5.0 Hz, 1 H), 4.98 (t, J = 5.6 Hz, 1 H), 4.62−4.46 (m, 2 H), 3.99 (s, 1 H), 3.58 (t, J = 5.3 Hz, 2 H). ESIMS m/z (rel intensity) 378/380 (MNa^+, 100/42). HRCIMS calcd for $C_{19}H_{14}ClNO_4Na$ (MNa^+) 378.0509, found 378.0504. HPLC purity, 95.09% (MeOH−H_2O, 85:15).

(*S*)-3-Bromo-5,6-dihydro-6-(2,3-dihydroxypropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (51). Lactone **31** (84 mg, 0.26 mmol) was suspended in MeOH (15 mL) and $CHCl_3$ (10 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 55 mg, 0.60 mmol) was added with stirring. The mixture was heated to reflux for 15 h, cooled to room temperature, and filtered to provide a red−orange solid. The

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

product was washed with $CHCl_3$ (5 mL) and hexanes (10 mL) to yield **51** (44 mg, 42%) as a red−orange solid: mp 224−226 °C. IR (film) 3436, 1664, 1643, 1540, 1315 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.49 (d, J = 8.7 Hz, 1 H), 8.26 (d, J = 2.2 Hz, 1 H), 8.14−7.89 (m, 2 H), 7.65−7.37 (m, 3 H), 5.13 (d, J = 5.0 Hz, 1 H), 4.99 (t, J = 5.5 Hz, 1 H), 4.68−4.39 (m, 2 H), 3.98 (d, J = 9.8 Hz, 1 H), 3.57 (t, J = 5.2 Hz, 2 H). CIMS m/z (rel intensity) 400/402 (MH^+, 94/100). HRCIMS calcd for $C_{19}H_{15}BrNO_4$ (MH^+) 400.0184/402.0166, found 400.0192/402.0173. HPLC purity, 95.18% (MeOH−H_2O, 85:15).

(*S*)-5,6-Dihydro-6-(2,3-dihydroxypropyl)-3-iodo-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (52). Lactone **32** (52 mg, 0.14 mmol) was dissolved in MeOH/$CHCl_3$ (15:10 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 32 mg, 0.35 mmol) was added. The mixture was heated at reflux under Ar overnight, cooled, and concentrated. The residue was suspended in $CHCl_3$−hexanes (10:5 mL), and the suspension was filtered to provide **52** (58 mg, 93%) as orange solid: mp 186−193 °C (dec). IR (film) 3488, 1706, 1624, 1533, 1304 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.49 (d, J = 1.9 Hz, 1 H), 8.36 (d, J = 8.5 Hz, 1 H), 8.13 (dd, J = 8.5, 1.9 Hz, 1 H), 8.07 (d, J = 7.6 Hz, 1 H), 7.62−7.44 (m, 3 H), 5.14 (d, J = 5.1 Hz, 1 H), 4.99 (t, J = 5.5 Hz, 1 H), 4.63−4.45 (m, 2 H), 4.06−3.93 (m, 1 H), 3.59 (t, J = 5.3 Hz, 2 H). ESIMS m/z (rel intensity) 470 (MNa^+, 52). HRESIMS calcd for $C_{19}H_{14}INO_4Na$ (MNa^+) 469.9865, found 469.9855. HPLC purity, 96.81% (MeOH−H_2O, 90:10).

(*S*)-3-Cyano-5,6-dihydro-6-(2,3-dihydroxypropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (53). Lactone **33** (67 mg, 0.25 mmol) was suspended in MeOH (10 mL) and $CHCl_3$ (15 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 56 mg, 0.61 mmol) was added with stirring. The mixture was heated to reflux for 19 h, cooled to room temperature, and concentrated until ∼1 mL of solvent remained. The mixture was filtered, and the solid residue was washed with hexanes (5 mL) to yield **53** (44 mg, 52%) as a red−orange solid: mp 314−315 °C. IR (film) 3583, 1734, 1671, 1608, 1534 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.66 (dd, J = 8.4, 0.7 Hz, 1 H), 8.54 (dd, J = 1.8, 0.6 Hz, 1 H), 8.22−8.05 (m, 2 H), 7.67−7.46 (m, 3 H), 5.15 (d, J = 5.0 Hz, 1 H), 5.01 (t, J = 5.6 Hz, 1 H), 4.54 (dd, J = 6.8, 3.6 Hz, 2 H), 3.99 (dt, J = 9.3, 5.0 Hz, 1 H), 3.66−3.52 (m, 2 H). CIMS m/z (rel intensity) 347 (MH^+, 100). HRESIMS calcd for $C_{20}H_{13}N_2O_4$ (M − H^+) 345.0875, found 345.0865. HPLC purity, 95.12% (MeOH−H_2O, 90:10).

(*S*)-2-(Trifluoromethyl)-5,6-dihydro-6-(2,3-dihydroxypropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (54). Lactone **42** (66 mg, 0.21 mmol) was suspended in MeOH (10 mL) and $CHCl_3$ (15 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 52 mg, 0.42 mmol) was added with stirring. The mixture was heated to reflux for 14 h, cooled to room temperature, and concentrated until ∼1 mL of solvent remained. Precipitation of product occurred upon standing for 30 min, and the mixture was filtered. The product was washed with hexanes (5 mL) to provide **54** (60 mg, 73%) as a bright-orange solid: mp 214−215 °C. IR (film) 3583, 3294, 1665, 1546, 1314, 1109 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.72 (d, J = 8.7 Hz, 1 H), 8.38 (s, 1 H), 8.13−8.07 (m, 2 H), 7.60−7.46 (m, 3 H), 5.13 (d, J = 5.0 Hz, 1 H), 5.00 (t, J = 5.5 Hz, 1 H), 4.62−4.46 (m, 2 H), 3.99 (dt, J = 9.3 Hz, J = 4.9 Hz, 1 H), 3.59 (t, J = 5.5 Hz, 2 H). CIMS m/z (rel intensity) 390 (MH^+, 100). HRCIMS calcd for $C_{20}H_{15}F_3NO_4$ (MH^+) 390.0948, found 390.0951. HPLC purity, 97.24% (MeOH−H_2O, 90:10).

(*S*)-3-(Trifluoromethyl)-5,6-dihydro-6-(2,3-dihydroxypropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (55). Lactone **43** (62 mg, 0.20 mmol) was suspended in MeOH (10 mL) and $CHCl_3$ (15 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 54 mg, 0.43 mmol) was added with stirring. The mixture was heated to reflux for 14 h, cooled to room temperature, and concentrated until ∼1 mL of solvent remained. Hexanes (2 mL) were added, and the suspension was filtered. The product was washed with hexanes (5 mL) to yield **55** (49 mg, 63%) as a red−orange solid: mp 228−230 °C. IR (film) 3401, 1668, 1559, 1501, 1312, 1128 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.87 (s, 1 H), 8.40 (d, J = 8.4 Hz, 1 H), 8.10 (d, J = 7.4 Hz, 1 H), 7.81 (dd, J = 8.5 Hz, J = 1.9 Hz, 1 H), 7.62−7.46 (m, 3 H), 5.15 (d, J = 5.0 Hz, 1 H), 5.00 (t, J = 5.6 Hz, 1 H), 4.59−4.47 (m, 2 H), 4.06−3.95 (m, 1 H), 3.59 (t, J = 5.4 Hz, 2 H). CIMS m/z (rel intensity) 390 (MH^+, 100). HRCIMS calcd for $C_{20}H_{15}F_3NO_4$ (MH^+) 390.0948, found 390.0952. HPLC purity, 96.12% (MeOH−H_2O, 90:10).

(*S*)-5,6-Dihydro-6-(2,3-dihydroxypropyl)-3-(methylthio)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (56). Lactone **45** (57 mg, 0.19 mmol) was suspended in MeOH (10 mL) and $CHCl_3$ (15 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 39 mg, 0.43 mmol) was added as a solution in MeOH (1 mL) with stirring. The mixture was heated to reflux with stirring under an argon atmosphere for 14.5 h, cooled to room temperature, and concentrated to a volume of ∼1 mL. Hexanes (0.5 mL) were added, the mixture was vigorously stirred, and the mixture was filtered. The solid residue that collected was washed with hexanes (1 mL) to yield **56** (51 mg, 72%) as a red−orange solid: mp 195 °C. IR (film) 3401, 1698, 1656, 1647, 1498, 1424 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.50 (d, J = 8.6 Hz, 1 H), 8.02 (d, J = 7.5 Hz, 1 H), 7.95 (d, J = 2.1 Hz, 1 H), 7.72 (dd, J = 8.6, 2.2 Hz, 1 H), 7.60−7.37 (m, 3 H), 5.13 (d, J = 5.0 Hz, 1 H), 4.97 (t, J = 5.6 Hz, 1 H), 4.65−4.41 (m, 2 H), 4.07−3.89 (m, 1 H), 3.57 (t, J = 5.3 Hz, 2 H), 2.57 (s, 3 H). CIMS m/z (rel intensity) 368 (MH^+, 100). HRESIMS calcd for $C_{20}H_{16}NO_4S$ (M − H^+) 366.0800, found 366.0794. HPLC purity, 96.39% (MeOH−H_2O, 90:10).

(*S*)-5,6-Dihydro-6-(2,3-dihydroxypropyl)-3-(methylsulfonyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (57). Lactone **46** (62 mg, 0.19 mmol) was suspended in MeOH (anhydrous, 10 mL) and $CHCl_3$ (15 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 36 mg, 0.40 mmol) was added with stirring. The mixture was heated to reflux for 16 h, cooled to room temperature, and concentrated in vacuo. 80:20 hexanes−EtOAc (10 mL) was added to the residue, and the suspension was filtered. The product was washed with MeOH (5 mL) to yield **57** (49 mg, 65%) as a red−orange solid: mp 185 °C (dec). IR (film) 3468, 1702, 1670, 1647, 1605, 1539 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.78 (d, J = 8.6 Hz, 1 H), 8.65 (d, J = 1.9 Hz, 1 H), 8.28 (dd, J = 8.6, 2.1 Hz, 1 H), 8.14 (d, J = 7.4 Hz, 1 H), 7.71−7.48 (m, 3 H), 5.16 (d, J = 5.0 Hz, 1 H), 5.02 (t, J = 5.5 Hz, 1 H), 4.65−4.51 (m, 2 H), 4.14−3.93 (m, 1 H), 3.61 (t, J = 5.3 Hz, 2 H), 3.30 (s, 3 H). APCIMS m/z (rel intensity) 400 (MH^+, 100). HRESIMS calcd for $C_{20}H_{17}NO_6SNa$ (MNa^+) 422.0674, found 422.0670. HPLC purity, 97.27% (MeOH, 100).

(*S*)-5,6-Dihydro-6-(2,3-dihydroxypropyl)-3-(methoxycarbonyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (58). Lactone **47** (42 mg, 0.14 mmol) was dissolved in MeOH/$CHCl_3$ (15:10 mL), and (*S*)-3-amino-1,2-propanediol (**48**, 31 mg, 0.34 mmol) was added. The mixture was heated at reflux under Ar overnight, cooled, and concentrated. The residue was suspended in $CHCl_3$/hexanes (10:5 mL), and the suspension was filtered to provide **58** (44 mg, 86%) as dark-orange solid: mp 174−175 °C (dec). IR (film) 3503, 1719, 1637, 1612, 1541, 1438, 1278 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.71 (d, J = 1.8 Hz, 1 H), 8.64 (d, J = 8.5 Hz, 1 H), 8.27 (dd, J = 8.5, 1.9 Hz, 1 H), 8.10 (d, J = 7.3 Hz, 1 H), 7.64−7.44 (m, 3 H), 5.15 (d, J = 5.0 Hz, 1 H), 5.01 (t, J = 5.5 Hz, 1 H), 4.65−4.44 (m, 2 H), 4.08−3.94 (m, 1 H), 3.90 (s, 3 H), 3.60 (t, J = 5.4 Hz, 2 H). CIMS m/z (rel intensity) 380 (MH^+, 78). HRCIMS calcd for $C_{21}H_{18}NO_6$ (MH^+) 380.1129, found 380.1134. HPLC purity, 95.02% (MeOH−H_2O, 85:15).

3-Fluoro-5,6-dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (60). Lactone **15** (78 mg, 0.29 mmol) was dissolved in $CHCl_3$ (25 mL) with stirring, and 1-(3-aminopropyl)imidazole (**59**, 73 mg, 0.58 mmol) was added. The mixture was heated to reflux for 26 h, cooled, and concentrated to ∼1 mL. Hexanes (5 mL) were added, and the suspension was filtered to provide **60** (74 mg, 68%) as a red−orange solid: mp 220−222 °C. IR (film) 1698, 1660, 1556, 1509, 1432 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.71 (dd, J = 9.0 Hz, J = 5.3 Hz, 1 H), 7.97 (dd, J = 9.2 Hz, J = 2.8 Hz, 1 H), 7.64 (s, 1 H), 7.59 (dd, J = 6.8 Hz, J = 1.6 Hz, 1 H), 7.47 (td, J = 8.5 Hz, J = 2.7 Hz, 1 H), 7.40−7.29 (m, 2 H), 7.23−7.17 (m, 1 H), 7.11−7.02 (m, 1 H), 6.66 (d, J = 7.2 Hz, 1 H), 4.58−4.47 (m, 2 H), 4.24 (t, J = 6.4 Hz, 2 H), 2.37 (p, J = 6.7 Hz, 2 H). APCIMS m/z 374 (MH^+, 100). HRESIMS calcd for $C_{22}H_{17}FN_3O_2$ (MH^+) 374.1305, found 374.1307. HPLC purity, 95.21% (1% TFA in MeOH−H_2O, 85:15).

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

3-Chloro-5,6-dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (61). Lactone **30** (82 mg, 0.29 mmol) was dissolved in $CHCl_3$ (25 mL) with stirring, and 1-(3-aminopropyl)imidazole (**59**, 73 mg, 0.58 mmol) was added. The mixture was heated to reflux for 20 h, cooled, and concentrated to ∼1 mL. Hexanes (5 mL) were added, and the suspension was filtered to provide **61** (87 mg, 77%) as a red solid: mp 215−217 °C. IR (film) 1698, 1664, 1541, 1500, 1317 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.62 (d, J = 8.7 Hz, 1 H), 8.28 (d, J = 2.3 Hz, 1 H), 7.80−7.52 (m, 3 H), 7.41−7.28 (m, 2 H), 7.20 (d, J = 1.1 Hz, 1 H), 7.15−7.00 (m, 1 H), 6.64 (d, J = 7.4 Hz, 1 H), 4.51 (t, J = 7.8 Hz, 2 H), 4.24 (t, J = 6.4 Hz, 2 H), 2.35 (p, J = 6.7 Hz, 2 H). ESIMS m/z 390/392 (MH^+, 100/37). HRESIMS calcd for $C_{22}H_{17}ClN_3O_2$ (MH^+) 390.1009, found 390.1009. HPLC purity, 95.04% (1% TFA in MeOH, 100).

5,6-Dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-3-iodo-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (62). Lactone **32** (72 mg, 0.19 mmol) was dissolved in $CHCl_3$ (25 mL) with stirring, and 1-(3-aminopropyl)imidazole (**59**, 51 mg, 0.41 mmol) was added. The mixture was heated to reflux for 17 h, cooled, and concentrated to ∼1 mL. Hexanes (5 mL) were added, and the suspension was filtered to provide **62** (92 mg, 100%) as a red−orange solid: mp 248−250 °C. IR (film) 3401, 1693, 1662, 1601, 1534, 1495, 1424 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, J = 1.9 Hz, 1 H), 8.42 (d, J = 8.5 Hz, 1 H), 7.99 (dd, J = 8.6 Hz, J = 1.9 Hz, 1 H), 7.90−7.76 (m, 1 H), 7.60 (d, J = 6.5 Hz, 1 H), 7.43−7.29 (m, 2 H, signal obscured by $CHCl_3$ in $CDCl_3$), 7.18−7.05 (m, 1 H), 6.75 (d, J = 7.2 Hz, 1 H), 4.64−4.44 (m, 2 H), 4.27 (t, J = 6.5 Hz, 2 H), 2.37 (s, 2 H). ESIMS m/z 482 (MH^+, 100). HRESIMS calcd for $C_{22}H_{17}IN_3O_2$ (MH^+) 482.0366, found 482.0364. HPLC purity, 100% (MeOH−H_2O, 85:15).

2-(Trifluoromethyl)-5,6-dihydro-6-(3-(1*H*-imidazol-1-yl)-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (63). Lactone **42** (74 mg, 0.23 mmol) was dissolved in $CHCl_3$ (25 mL) with stirring, and 1-(3-aminopropyl)imidazole (**59**, 59 mg, 0.47 mmol) was added. The mixture was heated to reflux for 17 h, cooled to room temperature, and hexanes (5 mL) were added. The suspension was stirred for 5 min and filtered to give **63** (97 mg, 100%) as a red−orange solid: mp 220−223 °C. IR (film) 1698, 1669, 1516, 1436, 1314, 1122 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.81 (d, J = 8.6 Hz, 1 H), 8.61 (s, 1 H), 7.91 (dd, J = 8.6 Hz, J = 2.0 Hz, 1 H), 7.68−7.61 (m, 2 H), 7.45−7.29 (m, 2 H), 7.22 (s, 1 H), 7.08 (s, 1 H), 6.69 (d, J = 7.4 Hz, 1 H), 4.56 (t, J = 8.2 Hz, 2 H), 4.26 (t, J = 6.3 Hz, 2 H), 2.38 (p, J = 6.6 Hz, 1 H). CIMS m/z (rel intensity) 424 (MH^+, 100). HRESIMS calcd for $C_{23}H_{17}F_3N_3O_2$ (MH^+) 424.1273, found 424.1278. HPLC purity, 95.27% (MeOH−H_2O, 90:10).

3-(Trifluoromethyl)-5,6-dihydro-6-(3-(1*H*-imidazol-1-yl)-propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (64). Lactone **43** (76 mg, 0.24 mmol) was dissolved in $CHCl_3$ (25 mL) with stirring, and 1-(3-aminopropyl)imidazole (**59**, 63 mg, 0.50 mmol) was added. The mixture was heated to reflux for 17 h, cooled to room temperature, and hexanes (5 mL) were added. The suspension was stirred for 5 min and filtered to yield **64** (91 mg, 90%) as a red−orange solid: mp 194−195 °C. IR (film) 1697, 1669, 1561, 1504, 1312, 1128 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.97 (s, 1 H), 8.43 (d, J = 8.4 Hz, 1 H), 7.74−7.56 (m, 3 H), 7.41−7.30 (m, 2 H), 7.21 (t, J = 1.1 Hz, 1 H), 7.07 (t, J = 1.3 Hz, 1 H), 6.68 (d, J = 7.4 Hz, 1 H), 4.64−4.46 (m, 2 H), 4.26 (t, J = 6.3 Hz, 2 H), 2.47−2.29 (m, 2 H). CIMS m/z (rel intensity) 424 (MH^+, 100). HRESIMS calcd for $C_{23}H_{17}F_3N_3O_2$ (MH^+) 424.1273, found 424.1266. HPLC purity, 98.31% (1% TFA in MeOH−H_2O, 90:10).

5,6-Dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-3-(methylthio)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (65). Lactone **45** (64 mg, 0.22 mmol) was dissolved in $CHCl_3$ (20 mL) with stirring, and a solution of 1-(3-aminopropyl)imidazole (**59**, 58 mg, 0.46 mmol) dissolved in $CHCl_3$ (1 mL) was added. The mixture was heated to reflux with stirring under an argon atmosphere for 14.5 h, cooled to room temperature, and concentrated to a volume of ∼1 mL. Hexanes (0.5 mL) were added, the mixture was vigorously stirred, and the mixture was filtered. The solid residue that collected was washed with hexanes (1 mL) to yield **65** (71 mg, 81%) as a blood-red solid: mp 234 °C (dec). IR (film) 1693, 1641, 1573, 1497, 1423 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.49 (d, J = 8.5 Hz, 1 H), 7.97 (d, J = 2.1 Hz, 1 H), 7.82 (s, 1 H), 7.74 (dd, J = 8.6, 2.2 Hz, 1 H), 7.62−7.37 (m, 3 H), 7.34 (s, 1 H), 7.22 (d, J = 7.0 Hz, 1 H), 7.01 (s, 1 H), 4.47 (t, J = 7.6 Hz, 2 H), 4.24 (t, J = 6.7 Hz, 2 H), 2.59 (s, 3 H), 2.35−2.15 (m, 2 H). EIMS m/z (rel intensity) 401 (M^+, 100). HRESIMS calcd for $C_{23}H_{19}N_3O_2S$ (MH^+) 402.1276, found 402.1262. HPLC purity, 95.37% (1% TFA in MeOH−H_2O, 85:15).

5,6-Dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-3-(methylsulfonyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (66). Lactone **46** (45 mg, 0.14 mmol) was dissolved in $CHCl_3$ (20 mL) with stirring, and 1-(3-aminopropyl)imidazole (**58**, 36 mg, 0.29 mmol) was added. The mixture was heated to reflux for 15.5 h, cooled to room temperature, and concentrated in vacuo. Then 80:20 hexanes−EtOAc (10 mL) was added to the residue, and the suspension was filtered. The product was washed with MeOH (5 mL) to provide **66** (34 mg, 57%) as a red−orange solid: mp 142 °C (dec). IR (film) 1698, 1668, 1606, 1505, 1304, 1148 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.73 (d, J = 8.6 Hz, 1 H), 8.65 (d, J = 2.0 Hz, 1 H), 8.28 (dd, J = 8.5, 2.1 Hz, 1 H), 7.75 (s, 1 H), 7.66−7.43 (m, 3 H), 7.33−7.22 (m, 2 H), 6.97 (s, 1 H), 4.49 (t, J = 7.7 Hz, 2 H), 4.24 (t, J = 6.7 Hz, 2 H), 3.31 (s, 3 H), 2.34−2.14 (m, 2 H). EIMS m/z 433 (rel intensity) (M^+, 37), 95 (100). HRESIMS calcd for $C_{23}H_{19}N_3O_4SNa$ (MNa^+) 456.0994, found 456.0972. HPLC purity, 95.27% (MeOH−H_2O, 90:10).

3-Fluoro-5,6-dihydro-6-(3-morpholinopropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (68). Lactone **15** (88 mg, 0.33 mmol) was dissolved in $CHCl_3$ (25 mL) with stirring, and 3-morpholinopropylamine (**67**, 95 mg, 0.66 mmol) was added. The mixture was heated to reflux for 21.5 h, cooled, and concentrated to ∼1 mL. Hexanes (5 mL) were added, and the suspension was filtered to provide **68** (108 mg, 83%) as a brick-red solid: mp 200−202 °C. IR (film) 1693, 1655, 1511, 1456, 1112, 909 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.73 (dd, J = 8.9 Hz, J = 5.3 Hz, 1 H), 7.97 (dd, J = 9.3 Hz, J = 2.8 Hz, 1 H), 7.74 (d, J = 7.4 Hz, 1 H), 7.63 (dd, J = 6.8 Hz, J = 1.7 Hz, 1 H), 7.51−7.36 (m, 3 H), 4.67−4.55 (m, 2 H), 3.73 (t, J = 4.7 Hz, 4 H), 2.60 (t, J = 6.3 Hz, 2 H), 2.53 (s, 4 H), 2.08 (p, J = 6.5 Hz, 2 H). ESIMS m/z 393 (MH^+, 100). HRESIMS calcd for $C_{23}H_{22}FN_2O_3$ (MH^+) 393.1614, found 393.1612. HPLC purity, 95.05% (1% TFA in MeOH−H_2O, 90:10).

3-Chloro-5,6-dihydro-6-(3-morpholinopropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (69). Lactone **30** (79 mg, 0.28 mmol) was dissolved in $CHCl_3$ (25 mL) with stirring, and 3-morpholinopropylamine (**67**, 82 mg, 0.57 mmol) was added. The mixture was heated to reflux for 22 h, cooled, and concentrated to ∼1 mL. Hexanes (5 mL) were added, and the suspension was filtered to provide **69** (111 mg, 97%) as a red-orange solid: mp 199−200 °C. IR (film) 1698, 1654, 1500, 1427, 1113 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.58 (d, J = 8.7 Hz, 1 H), 8.16 (d, J = 2.3 Hz, 1 H), 7.97−7.84 (m, 2 H), 7.70−7.48 (m, 3 H), 4.56 (t, J = 7.5 Hz, 2 H), 3.49 (t, J = 4.3 Hz, 4 H), 2.42−2.24 (m, 4 H), 2.03−1.87 (m, 2 H, obscured by solvent peak). APCIMS m/z 409/411 (MH^+, 100/35). HRESIMS calcd for $C_{23}H_{22}ClN_2O_3$ (MH^+) 409.1319, found 409.1318. HPLC purity, 96.24% (1% TFA in MeOH−H_2O, 85:15).

5,6-Dihydro-3-iodo-6-(3-morpholinopropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (70). Lactone **32** (69 mg, 0.18 mmol) was dissolved in $CHCl_3$ (25 mL) with stirring, and 3-morpholinopropylamine (**67**, 53 mg, 0.37 mmol) was added. The mixture was heated to reflux for 22 h, cooled, and concentrated to ∼1 mL. Hexanes (5 mL) were added, and the suspension was filtered to provide **70** (78 mg, 87%) as a red−orange solid: mp 231−232 °C. IR (film) 1698, 1654, 1493, 1424, 1110 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, J = 1.7 Hz, 1 H), 8.41 (d, J = 8.4 Hz, 1 H), 7.96 (dd, J = 8.5 Hz, J = 1.7 Hz, 1 H), 7.74 (d, J = 6.4 Hz, 1 H), 7.62 (dd, J = 6.7 Hz, J = 1.8 Hz, 1 H), 7.51−7.36 (m, 2 H), 4.66−4.54 (m, 2 H), 3.75 (t, J = 4.4 Hz, 4 H), 2.71−2.44 (m, 6 H), 2.24−1.98 (m, 2 H). APCIMS m/z 501 (MH^+, 100). HRESIMS calcd for $C_{23}H_{22}IN_2O_3$ (MH^+) 501.0675, found 501.0671. HPLC purity, 96.88% (1% TFA in MeOH, 100).

6-Azaphthalide (72). The method of Orlek and co-workers[64] was followed to yield **72** (1.5 g, 33%) as an off-white solid: mp 128−130 °C (lit.[64] mp 150−153 °C). IR (KBr) 1764, 1572, 1432, 1005, 756

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

cm^{-1}. ^{1}H NMR ($CDC1_3$, 300 MHz) δ 9.18 (s, 1 H), 8.86 (d, J = 5.2 Hz, 1 H), 7.50 (d, J = 5.2 Hz, 1 H), 5.35 (s, 2 H). ESIMS m/z (rel intensity) 136 (MH^+, 100).

6-Aza-3-bromophthalide (73). Furo[3,4-*c*]pyridin-3(1*H*)-one (**72**, 1.0 g, 7.4 mmol) was heated at reflux with NBS (1.4 g, 8.1 mmol) and AIBN (20 mg) in CCl_4 (40 mL) for 2 h. The reaction mixture was cooled to room temperature, precipitated solids were filtered off, and the filtrate was concentrated. The residue was purified by silica gel column chromatography, eluting with 3:1 EtOAc–hexanes, to afford **73** (0.90 g, 57%) as a white solid: mp 96–97 °C. IR (KBr) 1745, 1587, 668 cm^{-1}. ^{1}H NMR ($CDC1_3$, 300 MHz) δ 9.22 (s, 1 H), 8.99 (d, J = 5.2 Hz, 1 H), 7.60 (d, J = 5.2 Hz, 1 H), 7.36 (s, 1 H).

6-Aza-3-hydroxyphthalide (74). Compound **73** (0.8 g, 4 mmol) was heated at reflux in H_2O (40 mL) for 2 h. The obtained mixture was concentrated to dryness to afford compound **74** (0.5 g, 88%) as a brown syrup. IR (KBr) 3256, 1734, 1081, 768 cm^{-1}. ^{1}H NMR ($CDC1_3$, 300 MHz) δ 10.62 (s, 0.1 H), 9.16 (s, 1 H), 8.98 (s, 1 H), 7.87 (d, J = 4.4 Hz, 1 H), 6.76 (s, 0.9 H). ESIMS m/z (rel intensity) 152 (MH^+, 100).

3-Azaindeno[1,2-*c*]isochromene-5,11-dione (75). Hydroxyphthalide **74** (0.5 g, 3 mmol) and phthalide (**14**, 0.413 g, 3.08 mmol) were diluted in EtOAc (15 mL). Sodium metal (0.354 g, 15.4 mmol) was dissolved in MeOH (30 mL), and the solution was added to reaction mixture. The solution was heated at reflux for 24 h, cooled to room temperature, acidified with concd HCl (~4 mL), and concentrated. The obtained solid was diluted with Ac_2O (20 mL), and the mixture was heated at reflux for 6 h. The solution was concentrated, diluted with $CHCl_3$ (100 mL), and washed with saturated $NaHCO_3$ (3 × 50 mL). The organic layer was washed with brine (75 mL), dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography, eluting with 9:1 $CHCl_3$-hexanes to yield **75** (0.090 g, 11%) as an orange solid: mp 203–205 °C. IR (KBr) 1745, 1706, 1389, 998, 693 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.43 (s, 1 H), 8.89 (d, J = 5.0 Hz, 1 H), 8.15 (d, J = 5.2 Hz, 1 H), 7.66 (m, 1 H), 7.55 (m, 3 H). CIMS (m/z relative intensity) 250 (MH^+, 100). HRESIMS calcd for $C_{15}H_8NO_3$ 250.0504 (MH^+), found 250.0495.

3-Aza-5,6-dihydro-6-(3-morpholinopropyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (76). 3-Morpholinopropylamine (**67**, 0.043 g, 0.30 mmol) was added to a solution of lactone **75** (0.050 g, 0.20 mmol) in $CHCl_3$ (30 mL). The solution was allowed to stir at reflux temperature for 15 h, diluted with $CHCl_3$ (45 mL), and washed with H_2O (3 × 25 mL) and brine (25 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to provide a crude solid. The solid was purified by silica gel column chromatography, eluting with 1% MeOH in $CHCl_3$, to provide **76** (0.058 mg, 82%) as an orange–red solid: mp 210–211 °C. IR (KBr) 1756, 1665, 1189, 756 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.50 (s, 1 H), 8.77 (d, J = 5.5 Hz, 1 H), 8.43 (d, J = 5.5 Hz, 1 H), 7.82 (d, J = 6.1 Hz, 1 H), 7.71 (d, J = 6.1 Hz, 1 H), 7.53 (m, 2 H), 4.66 (t, J = 7.6 Hz, 2 H), 3.77 (br s, 4 H), 2.60 (m, 6 H), 2.12 (m, 2 H). ESIMS (m/z relative intensity) 376 (MH^+, 100). HRESIMS calcd for $C_{22}H_{22}N_3O_3$ 376.1661 (MH^+), found 376.1662. HPLC purity, 95.31% (1% TFA in MeOH–H_2O, 90:10).

3-Aza-5,6-dihydro-6-(3-(dimethylamino)propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (78). *N*,*N*-Dimethyl-1,3-diaminopropane (77, 0.030 g, 0.30 mmol) was added to a solution of lactone **75** (0.050 g, 0.20 mmol) in $CHCl_3$ (30 mL). The solution was allowed to stir at reflux temperature for 15 h, diluted with $CHCl_3$ (60 mL), and washed with H_2O (3 × 30 mL) and brine (30 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to provide a crude solid. The solid was purified by silica gel column chromatography, eluting with 4% MeOH in $CHCl_3$, to afford **78** (0.049 g, 72%) as an orange–red solid: mp 168–170 °C. IR (KBr) 1710, 1675, 1567, 765 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.50 (s, 1 H), 8.75 (dd, J = 5.5 Hz, 1 H), 8.40 (d, J = 5.7 Hz, 1 H), 7.79 (m, 1 H), 7.68 (m, 1 H), 7.50 (m, 2 H), 4.61 (m, 2 H), 2.53 (t, J = 6.45 Hz, 2 H), 2.29 (s, 6 H), 2.05 (m, 2 H). CIMS (m/z relative intensity) 334 (MH^+, 100). HRESIMS calcd for $C_{20}H_{20}N_3O_2$ 334.1556 (MH^+), found 334.1557. HPLC purity, 99.46% (1% TFA in MeOH–H_2O, 90:10).

3-Aza-5,6-dihydro-6-(3-(1*H*-imidazol-1-yl)propyl)-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (79). 1-(3-Aminopropyl)-imidazole (**59**, 0.037 g, 0.30 mmol) was added to a solution of lactone **75** (0.050 g, 0.20 mmol) in $CHCl_3$ (30 mL). The solution was allowed to stir at reflux temperature for 15 h, diluted with $CHCl_3$ (50 mL), and washed with H_2O (3 × 20 mL) and brine (20 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to provide a crude solid. The solid was purified by silica gel column chromatography, eluting with 97:3 $CHCl_3$–MeOH, to provide **79** (0.030 mg, 71%) as an orange–red solid: mp 251–252 °C. IR (KBr) 1723, 1678, 767, 685 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.52 (s, 1 H), 8.79 (d, J = 5.6 Hz, 1 H), 8.42 (d, J = 5.5 Hz, 1 H), 7.68 (m, 2 H), 7.46 (t, J = 7.0 Hz, 1 H), 7.36 (t, J = 7.0 Hz, 1 H), 7.21 (s, 1 H), 7.07 (s, 1 H), 6.71 (d, J = 7.4 Hz, 1 H), 4.56 (t, J = 7.8 Hz, 2 H), 4.28 (t, J = 6.2 Hz, 2 H), 2.37 (m, 2 H). ESIMS (m/z relative intensity) 357 (MH^+, 100). HRESIMS calcd for $C_{21}H_{17}N_4O_2$ 357.1352 (MH^+), found 357.1351. HPLC purity, 95.74% (1% TFA in MeOH–H_2O, 90:10).

Topoisomerase I-Mediated DNA Cleavage Reactions. A 3′-[^{32}P]-labeled 117-bp DNA oligonucleotide was prepared as previously described. The oligonucleotide contains previously identified Top1 cleavage sites in 161-bp pBluescript SK(−) phagemid DNA. Approximately 2 nM radiolabeled DNA substrate was incubated with recombinant Top1 in 20 μL of reaction buffer [10 mM Tris-HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.2 mM DTT, and 15 μg/mL BSA] at 25 °C for 20 min in the presence of various concentrations of test compounds. The reactions were terminated by adding SDS (0.5% final concentration), followed by the addition of two volumes of loading dye (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). Aliquots of each reaction mixture were subjected to 20% denaturing PAGE. Gels were dried and visualized by using a phosphoimager and ImageQuant software (Molecular Dynamics). For simplicity, cleavage sites were numbered as previously described in the 161-bp fragment.[65]

Recombinant TDP1 Assay. A 5′-[^{32}P]-labeled single-stranded DNA oligonucleotide containing a 3′-phosphotyrosine (N14Y)[28] was incubated at 1 nM with 10 pM recombinant TDP1 in the absence or presence of inhibitor for 15 min at room temperature in the LMP1 assay buffer containing 50 mM Tris HCl, pH 7.5, 80 mM KCl, 2 mM EDTA, 1 mM DTT, 40 μg/mL BSA, and 0.01% Tween-20.[32] Reactions were terminated by the addition of 1 volume of gel loading buffer [99.5% (v/v) formamide, 5 mM EDTA, 0.01% (w/v) xylene cyanol, and 0.01% (w/v) bromophenol blue]. Samples were subjected to a 16% denaturing PAGE with multiple loadings at 12 min intervals. Gels were dried and exposed to a PhosphorImager screen (GE Healthcare). Gel images were scanned using a Typhoon 8600 (GE Healthcare), and densitometry analyses were performed using ImageQuant software (GE Healthcare).

Recombinant TDP2 Assay. TDP2 reactions were carried out as described previously[66] with the following modifications. The 19-mer single-stranded oligonucleotide DNA substrate containing a 5′ phosphotyrosine (Y19, α^{32}P-cordycepin-3′-labeled) was incubated at 1 nM with 25 pM recombinant human TDP2 in the absence or presence of inhibitor for 15 min at room temperature in the LMP2 assay buffer containing 50 mM Tris-HCl, pH 7.5, 80 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 40 μg/mL BSA, and 0.01% Tween 20. Reactions were terminated and treated similarly to recombinant TDP1 reactions (see above).

Molecular Mechanics Calculations. The 1SC7 X-ray crystal structure file was prepared for molecular modeling by correcting several atom types, removing all of the water molecules, and adding hydrogens. The hydrogens were energy minimized, while the remaining atoms were frozen in aggregate, in SYBYL.[67] The details of this minimization, and all of the other minimizations, were set as follows: Powell method, MMFF94s force field,[68] MMFF94 charges, and 0.05 kcal/mol·Å energy gradient convergence. Selected indenoisoquinolines were constructed in SYBYL and energy minimized. The ligands were docked into the prepared crystal structure using GOLD 3.2 software.[69] The centroid of the binding site was defined by the

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX–XXX

crystallized ligand. Ten GOLD algorithm runs were executed per ligand. Default parameters were used. The top ten docking poses per ligand were inspected visually following the docking runs. The docking poses that had a favorable GOLD score and similar binding mode to the crystallized ligand were selected for further analysis. Ligand atom types were inspected and corrected as necessary. Lone pairs added by GOLD were deleted prior to a subsequent energy minimization of the docked complexes. Minimization was executed by allowing only the ligand to move while freezing the surrounding crystal structure as an aggregate.

Quantum Mechanics Calculations. The A−T and C−G base pairs and indenoisoquinoline **91** were individually subjected to geometry optimization and frequency calculation at the HF/6-31G** level in Gaussian 09.[70] The original 1SC7 X-ray crystal structure complex was then replaced with the geometry optimized parts. The hydrogen in the 3-position of indenoisoquinoline **91** was replaced with a nitro group (**90**), fluorine (**92**), chlorine (**93**), or trifluoromethyl (**94**), respectively. The same geometry optimizations were performed on these molecules before placing them into complexes with the DNA base pairs. A schematic representation of the model "indenoisoquinoline−Top1−DNA ternary complex" used in this study is shown in Figure 6.

After the complexes were assembled, single-point energy calculations were performed at the MP2/6-31G* level in Gaussian 09. The basis set superposition error (BSSE) for each complex was calculated using the counterpoise correction method within the Gaussian 09 package, specifying two fragments: (1) the two flanking DNA base pairs and (2) the indenoisoquinoline. The base pair−indenoisoquinoline interaction energy was calculated as $E_{int} = E_{complex} - E_{ligand} - E_{bp} +$ BSSE, where $E_{complex}$, E_{ligand}, and E_{bp} are the corresponding MP2/6-31G* calculated energies of the complex, the ligand, and the DNA base pairs at their normal distance in the absence of an intercalator.

■ ASSOCIATED CONTENT

Supporting Information

SMILES molecular formula strings (CSV). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00303.

■ AUTHOR INFORMATION

Corresponding Author

* Phone: 765-494-1465. Fax: 765-494-6970. 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

This work was made possible by the National Institutes of Health (NIH) through support with Research Grants U01CA089566 and P30CACA023168. This research was also supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. In vitro cytotoxicity testing was performed by the Developmental Therapeutics Program at the National Cancer Institute, under contract NO1-CO-56000. D.E.B. thanks Stephanie Pitman, Rubayat Khan, Huaping Mo, and Karl Wood for technical assistance.

■ ABBREVIATIONS USED

APCIMS, atmospheric-pressure chemical ionization mass spectrometry; CIMS, chemical ionization mass spectrometry; CPT, camptothecin; DTT, dithiothreitol; EIMS, electron ionization mass spectrometry; ESIMS, electrospray ionization mass spectrometry; FDA, U.S. Food and Drug Administration; TDP1, tyrosyl-DNA phosphodiesterase 1; TDP2, tyrosyl-DNA phosphodiesterase 2; Top1, human topoisomerase IB

■ REFERENCES

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

(2) *Search Orphan Drug Designations and Approvals*; U.S. Food and Drug Administration: Silver Spring, MD; http://www.accessdata.fda.gov/scripts/opdlisting/oopd/index.cfm (accessed March 20, 2015).

(3) Nagarajan, M.; Morrell, A.; Ioanoviciu, A.; Antony, S.; Kohlhagen, G.; Agama, K.; Hollingshead, M.; Pommier, Y.; Cushman, M. Synthesis and Evaluation of Indenoisoquinoline Topoisomerase I Inhibitors Substituted with Nitrogen Heterocycles. *J. Med. Chem.* **2006**, *49*, 6283−6289.

(4) A Phase I Study of Indenoisoquinolines LMP400 and LMP776 in Adults with Relapsed Solid Tumors and Lymphomas. *ClinicalTrials.gov*; U.S. National Institutes of Health: Bethesda, MD; http://clinicaltrials.gov/ct2/show/NCT01051635 (accessed November 17, 2013).

(5) Indenoisoquinoline LMP400 for Advanced Solid Tumors and Lymphomas. *ClinicalTrials.gov*; U.S. National Institutes of Health: Bethesda, MD; http://clinicaltrials.gov/ct2/show/NCT01794104 (accessed September 2, 2014).

(6) Ji, J. J.; Kummar, S.; Chen, A.; Zhang, Y.; Kinders, R. J.; Parchment, R. E.; Bonner, W.; Pommier, Y.; Tomaszewski, J. E.; Doroshow, J. H. Pharmacodynamic Response in Phase I Study of Indenoisoquinolines LMP400 and LMP776 in Adults with Relapsed Solid Tumors and Lymphomas. 46th Annual Meeting of the American Society of Clinical Oncology , Chicago, June 4−8, 2010.

(7) Cushman, M.; Jayaraman, M.; Vroman, J. A.; Fukunaga, A. K.; Fox, B. M.; Kohlhagen, G.; Strumberg, D.; Pommier, Y. Synthesis of New Indeno[1,2-*c*]isoquinolines: Cytotoxic Non-camptothecin Topoisomerase I Inhibitors. *J. Med. Chem.* **2000**, *43*, 3688−3698.

(8) Antony, S.; Jayaraman, M.; Laco, G.; Kohlhagen, G.; Kohn, K. W.; Cushman, M.; Pommier, Y. Differential Induction of Topoisomerase I−DNA Cleavage Complexes by the Indenoisoquinoline MJ-III-65 (NSC 706744) and Camptothecin: Base Sequence Analysis and Activity against Camptothecin-Resistant Topoisomerases I. *Cancer Res.* **2003**, *63*, 7428−7435.

(9) Antony, S.; Kohlhagen, G.; Agama, K.; Jayaraman, M.; Cao, S. 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.

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

(11) 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.

(12) Pommier, Y. DNA Topoisomerase I Inhibitors: Chemistry, Biology, and Interfacial Inhibition. *Chem. Rev.* **2009**, *109*, 2894−2902.

(13) Pommier, Y.; Marchand, C. Interfacial Inhibitors: Targeting Macromolecular Complexes. *Nature Rev. Drug Discovery* **2012**, *11*, 25−36.

(14) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of Benz[*d*]indeno[1,2-*b*]pyran-5,11-diones: Versatile Intermediates for the Design and Synthesis of Topoisomerase I Inhibitors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1846−1849.

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

(15) 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.
(16) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of Nitrated Indenoisoquinolines as Topoisomerase I Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3659−3663.
(17) 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.
(18) 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.
(19) 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.
(20) Kazius, J.; McGuire, R.; Bursi, R. Derivation and Validation of Toxicophores for Mutagenicity Prediction. *J. Med. Chem.* **2005**, *48*, 312−320.
(21) Smith, G. F. Designing Drugs to Avoid Toxicity. *Prog. Med. Chem.* **2011**, *50*, 1−47.
(22) *Selection Guidelines for Small Molecule Structures for Screening*; NCI Developmental Therapeutics Program: Bethesda, MD, 2013; http://dtp.nci.nih.gov/docs/misc/common_files/guidelines.html.
(23) Pommier, Y.; Huang, S. Y. N.; Gao, R.; Das, B. B.; Murai, J.; Marchand, C. Tyrosyl−DNA-phosphodiesterases (TDP1 and TDP2). *DNA Repair* **2014**, *19*, 114−129.
(24) 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.
(25) 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.
(26) 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.
(27) 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.
(28) 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.
(29) 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. Q. 5-Arylidenethioxothiazolidinones as Inhibitors of Tyrosyl−DNA Phosphodiesterase I. *J. Med. Chem.* **2012**, *55*, 8671−8684.
(30) Raoof, A.; Depledge, P.; Hamilton, N. M.; Hamilton, N. S.; Hitchin, J. R.; Hopkins, G. V.; Jordan, A. M.; Maguire, L. A.; McGonagle, A. E.; Mould, D. P.; Rushbrooke, M.; Small, H. F.; Smith, K. M.; Thomson, G. J.; Turlais, F.; Waddell, I. D.; Waszkowycz, B.; Watson, A. J.; Ogilvie, D. J. Toxoflavins and Deazaflavins as the First Reported Selective Small Molecule Inhibitors of Tyrosyl−DNA Phosphodiesterase II. *J. Med. Chem.* **2013**, *56*, 6352−6370.
(31) Beck, D. E.; Agama, K.; Marchand, C.; Chergui, A.; Pommier, Y.; Cushman, M. Synthesis and Biological Evaluation of New Carbohydrate-Substituted Indenoisoquinoline Topoisomerase I Inhibitors and Improved Syntheses of the Experimental Anticancer Agents Indotecan (LMP400) and Indimitecan (LMP776). *J. Med. Chem.* **2014**, *57*, 1495−1512.
(32) 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.
(33) 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.
(34) Borsche, W.; Diacont, K.; Hanau, H. Regarding 5-Carboxyhomophthalic Acid, 6-Nitrophthalide and 6-Nitroisoindolin-1-one. *Chem. Ber.* **1934**, *67*, 675−686.
(35) Irvine, R. W.; Kinloch, S. A.; McCormick, A. S.; Russell, R. A.; Warrener, R. N. Anthracyclines XVII: The Synthesis of 2-Fluoro-4-demethoxydaunomycin and 3-Fluoro-4-demethoxydaunomycin. *Tetrahedron* **1988**, *44*, 4591−4604.
(36) Conda-Sheridan, M.; Park, E. J.; Beck, D. E.; Reddy, P. V. N.; Nguyen, T. X.; Hu, B. J.; Chen, L.; White, J. J.; van Breemen, R. B.; Pezzuto, J. M.; Cushman, M. Design, Synthesis, and Biological Evaluation of Indenoisoquinoline Rexinoids with Chemopreventive Potential. *J. Med. Chem.* **2013**, *56*, 2581−2605.
(37) Marzi, E.; Spitaleri, A.; Mongin, F.; Schlosser, M. Fluoro- or Trifluoromethyl-substituted Benzyl and Phenethyl Alcohols: Substrates for Metal-Mediated Site-Selective Functionalization. *Eur. J. Org. Chem.* **2002**, 2508−2517.
(38) Antony, S.; Agama, K. K.; Miao, Z. H.; Hollingshead, M.; Holbeck, S. L.; Wright, M. H.; Varticovski, L.; Nagarajan, M.; Morrell, A.; Cushman, M.; Pommier, Y. Bisindenoisoquinoline bis-1,3-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propylamino}-propane bis(trifluoroacetate) (NSC 727357), a DNA intercalator and topoisomerase inhibitor with antitumor activity. *Mol. Pharmacol.* **2006**, *70*, 1109−1120.
(39) 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.
(40) Shoemaker, R. H. The NCI60 Human Tumour Cell Line Anticancer Drug Screen. *Nature Rev. Cancer* **2006**, *6*, 813−823.
(41) De Proft, F.; Langenaeker, W.; Geerlings, P. Ab Initio Determination of Substituent Constants in a Density Functional Theory Formalism: Calculation of Intrinsic Group Electronegativity, Hardness, and Softness. *J. Phys. Chem.* **1993**, *97*, 1826−1831.
(42) Peterson, K. E.; Cinelli, M. A.; Morrell, A. E.; Mehta, A.; Dexheimer, T. S.; Agama, K.; Antony, S.; Pommier, Y.; Cushman, M. Alcohol-, Diol-, and Carbohydrate-Substituted Indenoisoquinolines as Topoisomerase I Inhibitors: Investigating the Relationships Involving Stereochemistry, Hydrogen Bonding, and Biological Activity. *J. Med. Chem.* **2011**, *54*, 4937−4953.
(43) Meanwell, N. A. Synopsis of Some Recent Tactical Application of Bioisosteres in Drug Design. *J. Med. Chem.* **2011**, *54*, 2529−2591.
(44) Nagarathnam, D.; Miao, S. W.; Lagu, B.; Chiu, G.; Fang, J.; Dhar, T. G. M.; Zhang, J.; Tyagarajan, S.; Marzabadi, M. R.; Zhang, F. Q.; Wong, W. C.; Sun, W. Y.; Tian, D.; Wetzel, J. M.; Forray, C.; Chang, R. S. L.; Broten, T. P.; Ransom, R. W.; Schorn, T. W.; Chen, T. B.; O'Malley, S.; Kling, P.; Schneck, K.; Bendesky, R.; Harrell, C. M.; Vyas, K. P.; Gluchowski, C. Design and Synthesis of Novel α_{1a} Adrenoceptor-Selective Antagonists. 1. Structure−Activity Relationship in Dihydropyrimidinones. *J. Med. Chem.* **1999**, *42*, 4764−4777.
(45) Kiselev, E.; Sooryakumar, D.; Agama, K.; Cushman, M.; Pommier, Y. Optimization of the Lactam Side Chain of 7-Azaindenoisoquinoline Topoisomerase I Inhibitors and Mechanism of Action Studies in Cancer Cells. *J. Med. Chem.* **2014**, *57*, 1289−1298.

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX

(46) 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.
(47) Staker, B. L.; Feese, M. D.; Cushman, M.; Pommier, Y.; Zembower, D.; Stewart, L.; Burgin, A. B. Structures of Three Classes of Anticancer Agents Bound to the Human Topoisomerase I−DNA Covalent Complex. *J. Med. Chem.* **2005**, *48*, 2336−2345.
(48) *The PyMOL Molecular Graphics System*, version 1.5.0.4; Schrödinger, LLC, 2013.
(49) Bissantz, C.; Kuhn, B.; Stahl, M. A Medicinal Chemist's Guide to Molecular Interactions. *J. Med. Chem.* **2010**, *53*, 5061−5084.
(50) Wilcken, R.; Zimmermann, M. O.; Lange, A.; Joerger, A. C.; Boeckler, F. M. Principles and Applications of Halogen Bonding in Medicinal Chemistry and Chemical Biology. *J. Med. Chem.* **2013**, *56*, 1363−1388.
(51) Hardegger, L. A.; Kuhn, B.; Spinnler, B.; Anselm, L.; Ecabert, R.; Stihle, M.; Gsell, B.; Thoma, R.; Diez, J.; Benz, J.; Plancher, J. M.; Hartmann, G.; Isshiki, Y.; Morikami, K.; Shimma, N.; Haap, W.; Banner, D. W.; Diederich, F. Halogen Bonding at the Active Sites of Human Cathepsin L and MEK1 Kinase: Efficient Interactions in Different Environments. *ChemMedChem* **2011**, *6*, 2048−2054.
(52) Xiao, X. S.; Antony, S.; Pommier, Y.; Cushman, M. On the Binding of Indeno[1,2-*c*]isoquinolines in the DNA−topoisomerase I Cleavage Complex. *J. Med. Chem.* **2005**, *48*, 3231−3238.
(53) Kiselev, E.; Agama, K.; Pommier, Y.; Cushman, M. Azaindenoisoquinolines as Topoisomerase I Inhibitors and Potential Anticancer Agents: A Systematic Study of Structure−Activity Relationships. *J. Med. Chem.* **2012**, *55*, 1682−1697.
(54) Hunter, C. A.; Sanders, J. K. M. The Nature of pi−pi Interactions. *J. Am. Chem. Soc.* **1990**, *112*, 5525−5534.
(55) Martinez, C. R.; Iverson, B. L. Rethinking the term "pi-stacking". *Chem. Sci.* **2012**, *3*, 2191−2201.
(56) Balasubramanian, B. N.; Laurent, D. R. S.; Saulnier, M. G.; Long, B. H.; Bachand, C.; Beaulieu, F.; Clarke, W.; Deshpande, M.; Eummer, J.; Fairchild, C. R.; Frennesson, D. B.; Kramer, R.; Lee, F. Y.; Mahler, M.; Martel, A.; Naidu, B. N.; Rose, W. C.; Russell, J.; Ruediger, E.; Solomon, C.; Stoffan, K. M.; Wong, H.; Zimmermann, K.; Vyas, D. M. Design and Synthesis of a Fluoroindolocarbazole Series as Selective Topoisomerase I Active Agents. Discovery of Water-Soluble 3,9-Difluoro-12,13-dihydro-13-[6-amino-*β*-D-glucopyranosyl]-5*H*,13*H*-benzo[*b*]-thienyl[2,3-*a*]pyrrolo[3,4-*c*]carbazole-5,7(6*H*)-dione (BMS-251873) with Curative Antitumor Activity against Prostate Carcinoma Xenograft Tumor Model. *J. Med. Chem.* **2004**, *47*, 1609−1612.
(57) Saulnier, M. G.; Balasubramanian, B. N.; Long, B. H.; Frennesson, D. B.; Ruediger, E.; Zimmermann, K.; Eummer, J. T., St; Laurent, D. R.; Stoffan, K. M.; Naidu, B. N.; Mahler, M.; Beaulieu, F.; Bachand, C.; Lee, F. Y.; Fairchild, C. R.; Stadnick, L. K.; Rose, W. C.; Solomon, C.; Wong, H.; Martel, A.; Wright, J. J.; Kramer, R.; Langley, D. R.; Vyas, D. M. Discovery of a Fluoroindolo[2,3-*a*]carbazole Clinical Candidate with Broad Spectrum Antitumor Activity in Preclinical Tumor Models Superior to the Marketed Oncology Drug, CPT-11. *J. Med. Chem.* **2005**, *48*, 2258−2261.
(58) Taliani, S.; Pugliesi, I.; Barresi, E.; Salerno, S.; Marchand, C.; Agama, K.; Simorini, F.; La Motta, C.; Marini, A. M.; Di Leva, F. S.; Marinelli, L.; Cosconati, S.; Novellino, E.; Pommier, Y.; Di Santo, R.; Da Settimo, F. Phenylpyrazolo[1,5-*a*]quinazolin-5(4*H*)-one: A Suitable Scaffold for the Development of Noncamptothecin Topoisomerase I (Top1) Inhibitors. *J. Med. Chem.* **2013**, *56*, 7458−7462.
(59) Dong, G. Q.; Sheng, C. Q.; Wang, S. Z.; Miao, Z. Y.; Yao, J. Z.; Zhang, W. N. Selection of Evodiamine as a Novel Topoisomerase I Inhibitor by Structure-Based Virtual Screening and Hit Optimization of Evodiamine Derivatives as Antitumor Agents. *J. Med. Chem.* **2010**, *53*, 7521−7531.
(60) Ioanoviciu, A.; Antony, S.; Pommier, Y.; Staker, B. L.; Stewart, L.; Cushman, M. Synthesis and Mechanism of Action Studies of a Series of Norindenoisoquinoline Topoisomerase I Poisons Reveal an Inhibitor with a Flipped Orientation in the Ternary DNA−enzyme−inhibitor Complex as Determined by X-ray Crystallographic Analysis. *J. Med. Chem.* **2005**, *48*, 4803−4814.
(61) Cinelli, M. A.; Morrell, A.; Dexheimer, T. S.; Scher, E. S.; Pommier, Y.; Cushman, M. Design, Synthesis, and Biological Evaluation of 14-substituted Aromathecins as Topoisomerase I Inhibitors. *J. Med. Chem.* **2008**, *51*, 4609−4619.
(62) Pokhodylo, N. T.; Matiychuk, V. S.; Obushak, M. D. Synthesis of Isothiocoumarin Derivatives. *Chem. Heterocycl. Compd.* **2010**, *46*, 140−145.
(63) Nandhikonda, P.; Heagy, M. D. Dual Fluorescent *N*-Aryl-2,3-naphthalimides: Applications in Ratiometric DNA Detection and White Organic Light-Emitting Devices. *Org. Lett.* **2010**, *12*, 4796−4799.
(64) Orlek, B. S.; Blaney, F. E.; Brown, F.; Clark, M. S. G.; Hadley, M. S.; Hatcher, J.; Riley, G. J.; Rosenberg, H. E.; Wadsworth, H. J.; Wyman, P. Comparison of Azabicyclic Esters and Oxadiazoles as Ligands for the Muscarinic Receptor. *J. Med. Chem.* **1991**, *34*, 2726−2735.
(65) Dexheimer, T. S.; Pommier, Y. DNA Cleavage Assay for the Identification of Topoisomerase I Inhibitors. *Nature. Protoc.* **2008**, *3*, 1736−1750.
(66) Gao, R.; Huang, S. Y. N.; Marchand, C.; Pommier, Y. Biochemical Characterization of Human Tyrosyl−DNA Phosphodiesterase 2 (TDP2/TTRAP): A Mg^{2+}/Mn^{2+}-dependent Phosphodiesterase Specific for the Repair of Topoisomerase Cleavage Complexes. *J. Biol. Chem.* **2012**, *287*, 30842−30852.
(67) *SYBYL*; Tripos, Inc.: St. Louis, MO.
(68) Halgren, T. A. Merck Molecular Force Field.1. Basis, Form, Scope, Parameterization, and Performance of MMFF94. *J. Comput. Chem.* **1996**, *17*, 490−519.
(69) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved Protein−Ligand Docking Using GOLD. *Proteins: Struct., Funct., Genet.* **2003**, *52*, 609−623.
(70) Frisch, M. J. T., G, W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, M. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, revision D.01; Gaussian, Inc., Wallingford, CT, 2009.

DOI: 10.1021/acs.jmedchem.5b00303
J. Med. Chem. XXXX, XXX, XXX−XXX