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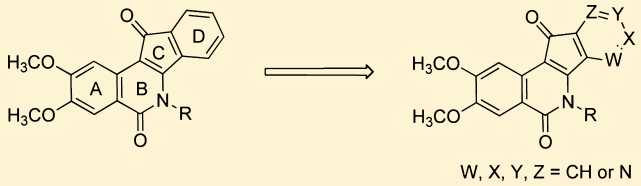
Azaindenoisoquinolines as Topoisomerase I Inhibitors and Potential Anticancer Agents: A Systematic Study of Structure–Activity Relationships

Evgeny Kiselev,[†] Keli Agama,[‡] Yves Pommier,[‡] and Mark Cushman*[,†]

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

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

ABSTRACT: A comprehensive study of a series of azaindenoisoquinoline topoisomerase I (Top1) inhibitors is reported. The synthetic pathways have been developed to prepare 7-, 8-, 9-, and 10-azaindenoisoquinolines. The present study shows that 7-azaindenoisoquinolines possess the greatest Top1 inhibitory activity and cytotoxicity. Additionally, the introduction of a methoxy group into the D-ring of 7-azaindenoisoquinolines improved their biological activities, leading to new lead molecules for further development. A series of QM calculations were performed on the model "sandwich" complexes of azaindenoisoquinolines with flanking DNA base pairs from the Drug–Top1–DNA ternary complex. The results of these calculations demonstrate how changes in two forces contributing to the $\pi-\pi$ stacking (dispersion and charge-transfer interactions) affect the binding of the drug to the Top1–DNA cleavage complex and thus modulate the drug's Top1 inhibitory activity.



INTRODUCTION

After the discovery of NSC 314622's (**1**, Figure 1) cytotoxic and topoisomerase I (Top1) inhibitory properties, the indenoisoquinolines emerged as potential anticancer agents. Following the demonstration of similarity between the cytotoxicity profiles of **1** and the plant alkaloid Top1 inhibitor camptothecin (**2**) by the National Cancer Institute's (NCI's) COMPARE analysis, it was shown that **1** is also capable of inducing single-strand DNA breaks in the presence of Top1 at micromolar concentrations.[1] Top1 plays its vital role in cell survival and replication by unwinding supercoiled DNA, thus allowing its processing. The major step in Top1-mediated DNA relaxation is reversible single strand cleavage of DNA by Top1 that results in the formation of a covalent Top1–DNA cleavage complex (Top1-DNAcc).[2,3] A series of elegant crystallographic studies of Top1-DNAcc in the presence of inhibitors **2** and MJ-III-65 (**3**) revealed that by binding between DNA base pairs at the site of cleavage, molecules like **2** and **3** cause separation of the ends of the cleaved strand, preventing DNA religation and release of active enzyme.[4,5] Both camptothecins and indenoisoquinolines have demonstrated similarity in their mode of action by showing the ability to intercalate their polyaromatic cores between DNA base pairs and to selectively form hydrogen bond interactions with Top1, thereby acting as interfacial inhibitors (Figure 2).[6]

In comparison with **2**, indenoisoquinolines offer greater chemical stability. The lactone hydrolysis of **2** at physiological pH results in loss of its biological activity.[7] The indenoisoquinoline Top1 inhibitors express DNA cleavage cite specificity different from that of **2**, in addition to slower reversibility and thus greater stability of drug–Top1–DNA ternary complexes. These advantages of indenoisoquinolines triggered their development as anticancer agents. The structure optimization of the lead compound **1** resulted in the identification and promotion of two members of the family, LMP400 (**4**) and LMP776 (**5**), into phase I clinical study at the NCI (Figure 1).[8−10]

Through the synthesis and evaluation of a large number of analogues of **1**, a series of important modifications that advantageously affect the inhibitory and cytotoxic properties of the indenoisoquinolines have been identified. It was shown that replacement of lactam methyl group with ω-aminoalkyl substituents having two to four carbon atoms (e.g., **4** and **5**) positively affects the biological activities of the drug.[8,11,12] Diverse substituents on the A- and D-rings of the core indenoisoquinoline molecules have been investigated leading to drugs with greatly improved cytotoxic properties.[11,13] The modifications of the indenone side of the indenoisoquinolines were almost exclusively confined to electron-donating alkoxy groups, and only a very limited number of electron withdrawing substituents at the ninth position of indenoisoquinoline were studied.[14,15] Recently, the syntheses of 7-azaindenoisoquinolines **6** and **7** have been reported.[16] The replacement of the benzene D-ring with a pyridine ring has afforded improved water solubility. The introduction of the pyridine motif into the indenoisoquinoline system was in accordance with our hypothesis that the increase in electron deficiency of the system would potentially provide an increase in charge-transfer interactions.

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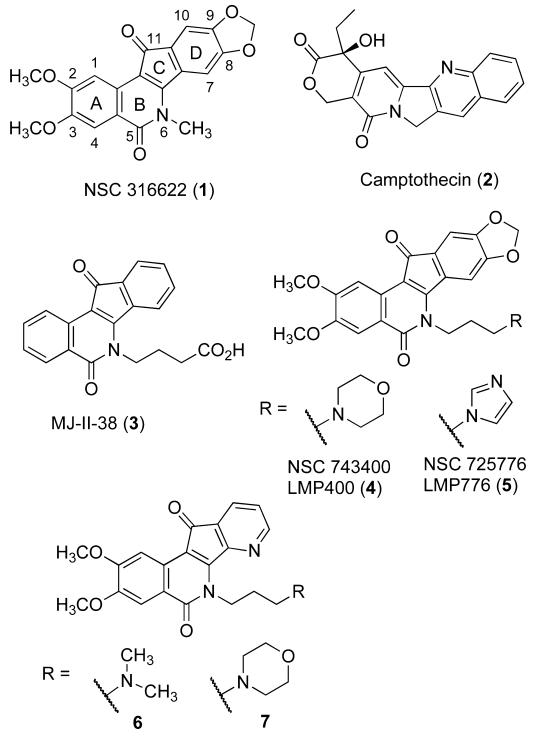


Figure 1. Representative Top1 inhibitors.

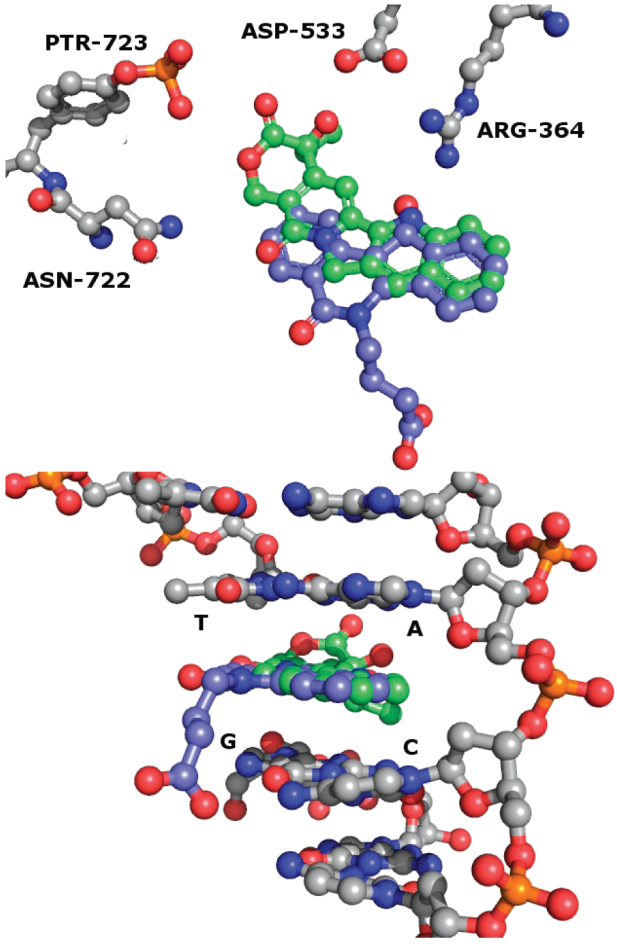


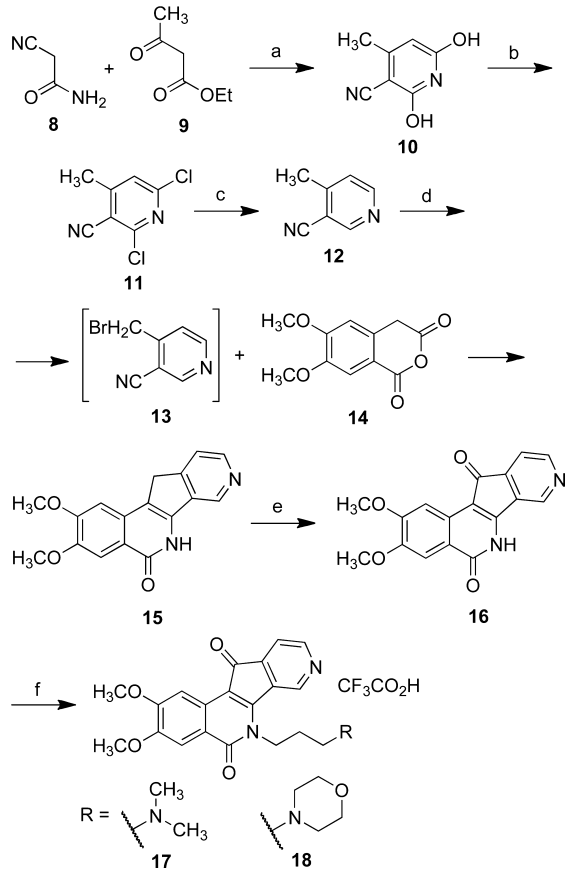
Figure 2. Superimposition of ternary complexes of **2** (green) and **3** (blue) with Top1-DNAcc.

A series of 8-, 9-, and 10-azaindenoisoquinolines have therefore been prepared in order to complete the systematic study of indenoisoquinolines with electron-deficient D-rings.

CHEMISTRY

The desired series of 8-, 9-, and 10-azaindenoisoquinolines was prepared from the corresponding cyanomethylpyridines (Schemes 1−3), similar to the synthesis of 7-azaindenoisoquinolines from 2-cyano-3-methylpyridine.[16,17] Synthesis of 8-azaindenoisoquinolines was started with preparation of the key starting material, 4-methylnicotinonitrile (**12**, Scheme 1).[18]

Scheme 1[a]



[a]Reagents and conditions: (a) KOH, methanol, reflux, 12 h (75%); (b) $POCl_3$, sealed tube, 150−180 °C, 8 h (67%); (c) H_2, $PdCl_2$, CH_3CO_2Na, methanol, 23 °C, 14 h (93%); (d) (1) NBS, AIBN, CCl_4, reflux, 3.5 h, (2) **14**, triethylamine, acetonitrile, reflux 14 h (13%); (e) SeO_2, 1,4-dioxane, reflux, 4 h (49%); (f) (1) DIAD, triphenylphosphine, 3-dimethylamino-1-propanol (for **17**) or 4-(3-hydroxypropyl)-morpholine (for **18**), THF, 23 °C, 3 h, (2) TFA, diethyl ether, chloroform, 23 °C (**17** 34%, **18** 35%).

Cyanoacetamide (**8**) was condensed with ethyl acetoacetate (**9**) in the presence of potassium hydroxide to afford dihydroxypyridine **10**. Conversion of **10** to dichloride **11** by heating with excess of phosphorus oxychloride in a sealed reaction vessel followed by hydrogenation in the presence of palladium dichloride yielded **12**. Further synthesis proceeded as previously described for 7-azaindenoisoquinolines.[16] In short, intermediate bromide **13** obtained by bromination of **12** with *N*-bromosuccinimide (NBS) was reacted with 4,5-dimethoxyhomophthalic anhydride (**14**)[19] to produce **15** (Scheme 1).

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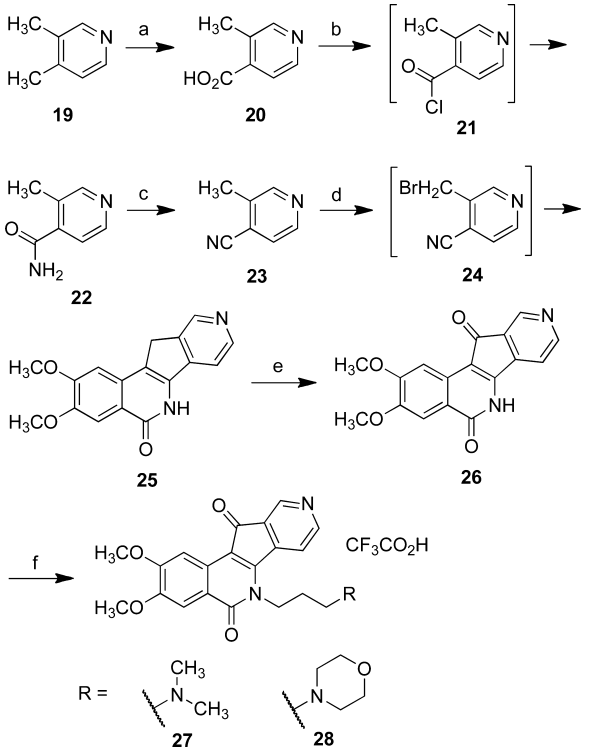
Oxidation of **15** with selenium dioxide afforded the key intermediate dioxoindenoisoquinoline **16**. The synthesis of 8-azaindenoisoquinoline analogues was completed with alkylation of **16** by means of Mitsunobu reaction to prepare the dimethylaminopropyl and morpholinopropyl analogues **17** and **18**, respectively.

The 3-methyl-4-cyanopyridine (**23**) necessary for the preparation of 9-azaindenoisoquinolines was obtained from 3,4-lutidine (**19**, Scheme 2). Oxidation of **19** with selenium dioxide in hot diphenyl ether yielded acid **20**.[20] Treatment of **20** with thionyl chloride gave crude acyl chloride **21** that was added in small portions to a cold concentrated ammonium hydroxide solution in order to obtain amide **22**. Dehydration of **22** in phosphorus oxychloride produced the key starting material **23**. The crude bromide **24** derived from **23** was condensed with **14** to prepare **25**. Oxidation of **25** to **26** with selenium dioxide, followed by derivatization through Mitsunobu reaction, yielded the final products **27** and **28** (Scheme 2).

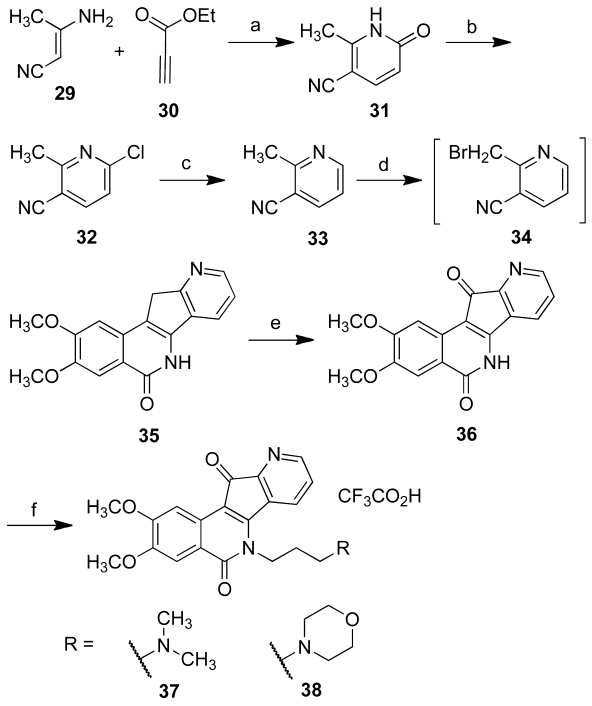
Scheme 2[a]



[a]Reagents and conditions: (a) SeO_2, Ph_2O, 180 °C, 1 h (47%); (b) (1) $SOCl_2$, reflux, 3 h, (2) conc aq NH_3, 0−5 °C (60%); (c) $POCl_3$, reflux, 24 h (90%); (d) (1) NBS, AIBN, CCl_4, reflux, 2 h, (2) **14**, triethylamine, acetonitrile, reflux 14 h (14%); (e) SeO_2, 1,4-dioxane, reflux, 4 h (94%); (f) (1) DIAD, triphenylphosphine, 3-dimethylamino-1-propanol (for **27**) or 4-(3-hydroxypropyl)morpholine (for **28**), THF, 23 °C, 3 h, (2) TFA, diethyl ether, chloroform, 23 °C (**27** 62%, **28** 10%).

The synthesis of 10-azaindenoisoquinolines began with the preparation of 3-cyano-2-methylpyridine (**33**, Scheme 3). Nitrile **33** was obtained from 3-aminocrotonitrile (**29**) and ethyl propiolate (**30**) in three steps. Condensation of **29** and **30** afforded pyridone **31**, which was further converted into chloropyridine **32** by treatment with phosphorus oxychloride.[21] Compound **32** was reduced to **33** with ammonium formate in the presence of palladium on charcoal. Benzylic bromination of **33** and condensation of the intermediate bromide with **14** yielded compound **35**, which contains the 10-azaindenoisoquinoline polycyclic core. Compound **36**, the product of selenium dioxide oxidation of **35**, was introduced into Mitsunobu reaction to derive **37** and **38**.

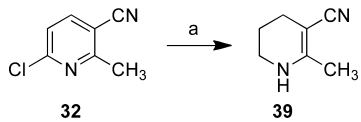
Scheme 3[a]



[a]Reagents and conditions: (a) DMF, reflux, 3 days (31%); (b) $POCl_3$, reflux, 6 h (54%); (c) HCO_2NH_4, Pd/C, methanol, 23 °C, 12 h (81%); (d) (1) NBS, AIBN, 1,2-dichloroethane, reflux, 9 h, (2) **14**, triethylamine, acetonitrile, reflux 2 days (31%); (e) SeO_2, 1,4-dioxane, reflux, 3 days (90%); (f) (1) DIAD, triphenylphosphine, 3-dimethylamino-1-propanol (for **37**) or 4-(3-hydroxypropyl)morpholine (for **38**), THF, 23 °C, 12 h, (2) TFA, diethyl ether, chloroform, 23 °C (**37** 22%, **38** 7%).

It is interesting to note that reduction of **32** to **33** required different reaction conditions than were employed to convert **11** to **12**. Instead of catalytic hydrogenation of **32**, reduction with ammonium formate in the presence of palladium on carbon was required (Scheme 3). When conditions required for the reduction of **11** were applied to compound **32**, the only isolated product was tetrahydropyridine **39** (Scheme 4).[22] In contrast, attempted reduction of compound **11** with ammonium formate in the presence of palladium on activated carbon resulted in isolation of monochloride **40** in high yield, but pyridine **12** was not detected (Scheme 5). A similar reported reduction produced **40** in less than 10% yield in a mixture with **12** as a major component.[23] The conversion of **11** to **40** reported here might provide a pathway for preparation of similarly substituted pyridines.

Scheme 4[a]



[a]Reagents and conditions: (a) H_2, $PdCl_2$, CH_3CO_2Na, methanol, 23 °C (84%).

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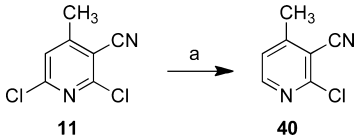
Scheme 5[a]



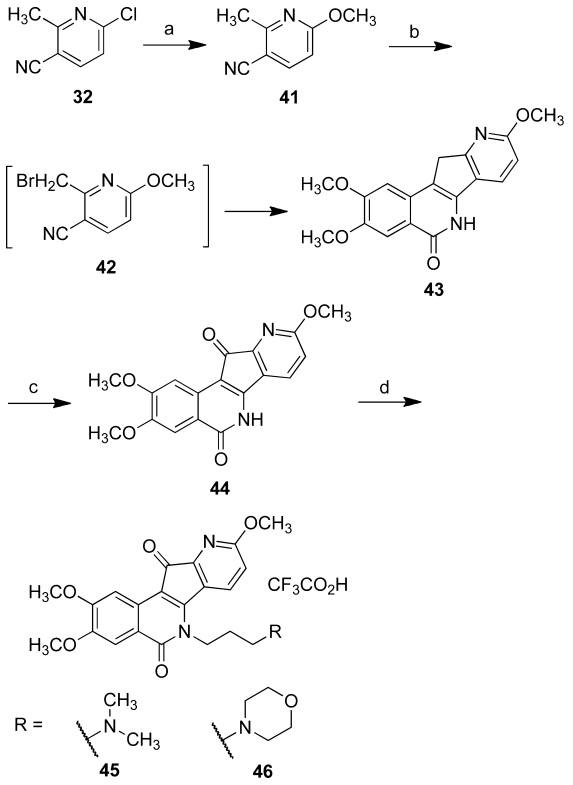
11 40

[a]Reagents and conditions: (a) HCO_2NH_4, Pd/C, methanol, 23 °C, 3 days (87%).

It was found in earlier SAR studies of indenoisoquinolines that the introduction of alkoxy groups in the ninth position generally benefits the Top1 inhibitory activities and cytotoxic properties of the target compounds. Having the chloropyridine **32** in hand provided the opportunity to investigate the effect of the methoxy group on the activity of 10-azaindenoisoquinolines.

Luckily, the arrangement of substituents in the pyridine **32** is such that the displacement of chlorine with a methoxy group would produce **41**, which could be converted to the 9-methoxy derivatives of 10-azaindenoisoquinolines (Scheme 6). 3-Cyano-6-methoxy-2-methylpyridine (**41**) was subjected to radical bromination with NBS followed by condensation with **14** (Scheme 6). The isolated azaindenoisoquinoline **43** was oxidized

Scheme 6[a]



32 41 42 43 44 45 46

[a]Reagents and conditions: (a) $NaOCH_3$, methanol, reflux, 1.5 h (87%); (b) (1) NBS, AIBN, 1,2-dichloroethane, reflux, 3.5 h, (2) **14**, triethylamine, acetonitrile, reflux 14 h (19%); (c) SeO_2, 1,4-dioxane, reflux, 24 h (89%); (d) DIAD, triphenylphosphine, 3-dimethylamino-1-propanol (for **45**) or 4-(3-hydroxypropyl)morpholine (for **46**), THF, 23 °C, 3 days, (2) TFA, diethyl ether, chloroform, 23 °C (**45** 30%, **46** 31%).

with selenium dioxide, and the product **44** was reacted with 3-dimethylamino-1-propanol and 4-(3-hydroxypropyl)morpholine under Mitsunobu conditions to prepare **45** and **46**, respectively.

The evaluation of the Top1 inhibitory activity and cytotoxicity of the azaindenoisoquinolines showed that the introduction of a methoxy group into the ninth position of 10-azaindenoisoquinoline resulted in an increase in Top1 inhibitory activity.[14,15] But unfortunately, it did not improve cytotoxicity and 10-azaindenoisoquinolines **37**, **45**, and **46** remained largely nontoxic to cancer cell lines (Table 1). In contrast, the 7-azaindenoisoquinoline series has demonstrated the greatest cytotoxicity of all of the currently available azaindenoisoquinolines (Table 1).[16] In order to investigate the effect of the 9-methoxy substituent in the 7-azaindenoisoquinoline series, analogues **55** and **56** were synthesized (Scheme 7).

The key starting material required for the preparation of this series, 2-cyano-5-methoxy-3-methylpyridine (**51**), was prepared in four steps from commercially available 2-amino-3-picoline (**47**, Scheme 7). Bromination of **47** with NBS in acetonitrile in the presence of ammonium acetate yielded 2-amino-5-bromo-3-methylpyridine (**48**).[24] The amino group of **48** was replaced with bromide to provide **49**, followed by further displacement with cyanide to yield pyridine **50**. For this transformation sodium nitrite was added to the solution of aminopyridine **48** and bromine in concentrated hydrobromic acid at −15 °C.[25−27] Treatment of **49** with 1 equiv of copper(I) cyanide in dry DMF led to the selective substitution of only the bromide in the second position of the pyridine, yielding **50**.[28] The direct conversion of aminopyridine **48** to nitrile **50** via Sandmeyer-type reaction is prevented by the instability of the intermediate 2-pyridyldiazonium salt that is apparently capable of decomposing at relatively low temperatures prior to its transformation into nitrile **50** in the presence of copper(I) cyanide. Nucleophilic substitution of the second bromide with methoxide provided **51**.

A series of transformations starting from **51** to the targets **55** and **56** were performed in a way that is similar to that described for other azaindenoisoquinolines (Scheme 7). Bromination of **51** in the presence of radical initiator AIBN, followed by condensation with **14**, produced **53**. Oxidation of **53** to **54** and Mitsunobu reaction of **54** with 3-dimethylamino-1-propanol and 4-(3-hydroxypropyl)morpholine led to analogues **55** and **56**, respectively.

In order to further explore the potential of the 7-aza-9-methoxyindenoisoquinoline series as anticancer agents, compounds **63**−**66** were prepared (Scheme 8). These analogues contain a differently substituted indenoisoquinoline A-ring compared to compounds **55** and **56**. Pyridine **51** was utilized in the preparation of **63**−**66** (Scheme 8). Bromination of **51** followed by condensation with homophthalic and 5-nitro-homophthalic anhydrides[29] (**57** and **58**) yielded azaindenoisoquinolines **59** and **60**, respectively. Compounds **59** and **60** underwent oxidation to **61** and **62**, respectively, followed by Mitsunobu transformation to yield **63**−**66**.

BIOLOGICAL RESULTS AND DISCUSSION

All of the target compounds were tested for induction of DNA damage in Top1-mediated DNA cleavage assays.[30] For this purpose, a ^{32}P 3′-end-labeled 117-bp DNA fragment was incubated with human recombinant Top1 and increasing concentrations of the test compounds. The DNA fragments were separated on a denaturing gel (Figure 3). The Top1 inhibitory activity was assigned based on the visual inspection of the number and intensities of the DNA cleavage bands and expressed in semiquantitative fashion relative to the Top1 inhibitory activities of compounds **1** and **2**: 0, no detectable

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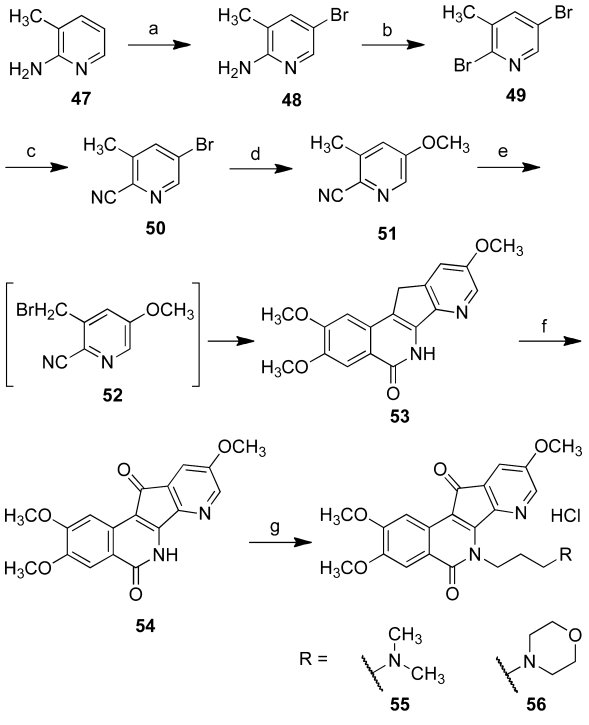
Table 1. Top1 Inhibitory and Antiproliferative Activity of Azaindenoisoquinolines

compd	Top1 cleavage[a]	cytotoxicity (GI_{50}, μM)[c] MGM[b]	lung, HOP-62	colon, HCT-116	CNS, SF-539	melanoma, UACC-62	ovarian, OVCAR-3	renal, SN12C	prostate, DU-145	breast, MCF7
1	++	8.5	2.8	11.5	1.7	0.56	22	26	4.8	1.9
2	++++	0.040	0.010	0.030	0.010	0.010	0.22	0.020	0.010	0.013
6	+++	4.5	3.4	1.6	4.1	13	3.6	3.2	1.7	0.44
7	++	0.30	0.30	0.22	0.29	0.10	0.37	0.52	0.31	0.052
17	++	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]
18	++	16	6.5	2.5	40	48	24	16	24	4.1
27	+	6.5	6.5	0.62	12	>100	7.4	8.5	2.9	3.9
28	++	9.5	5.0	0.39	>100	85	12	5.9	7.6	1.9
37	++	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]
38	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]
45	+++	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]
46	+++	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]
55	+++	1.8	0.92	1.5	1.1	3.9	2.9	3.6	0.88	0.13
56	++	0.48	0.24	0.33	0.27	0.22	0.31	0.34	0.34	0.10
63	+++	0.40	0.30	0.34	0.57	0.54	0.94	0.26	0.30	0.21
64	++	3.0	4.1	2.5	3.5	1.7	6.3	2.6	3.6	0.60
65	+++	0.11	0.054	0.074	0.078	0.052	0.14	0.057	0.051	0.024
66	++++	0.085	0.051	0.050	0.035	0.040	0.11	0.043	0.040	0.020

[a]The relative Top1 inhibitory potencies of the compounds are presented as follows: 0: no detectable activity; +: weak activity; ++: similar activity as compound **1**; +++ and ++++: greater activity than compound **1**; ++++: similar activity as 1 μM **2**. [b]Mean graph midpoint (MGM) for growth inhibition of all human cancer cell lines successfully tested. [c]The cytotoxicity GI_{50} values listed are the concentrations corresponding to 50% growth inhibition and are the result of single determinations. [d]GI_{50} values were not determined because the low activities revealed in the initial single-concentration testing at 10 μM did not warrant the multiple-concentration testing required for determination of GI_{50} values. [e]Not tested.

Scheme 7[a]



[a]Reagents and conditions: (a) NBS, $CH_3CO_2NH_4$, acetonitrile, 0–23 °C, 25 min (65%); (b) Br_2, $NaNO_2$, aq HBr, −15 °C, then 23 °C, 3 h (94%); (c) CuCN, DMF, reflux, 2 h (74%); (d) $NaOCH_3$, methanol, reflux, 12 h (79%); (e) (1) NBS, AIBN, 1,2-dichloroethane, reflux, 24 h, (2) **14**, triethylamine, acetonitrile, reflux 24 h (21%); (f) SeO_2, 1,4-dioxane, reflux, 24 h (92%); (g) (1) DIAD, triphenylphosphine, 3-dimethylamino-1-propanol (for **55**) or 4-(3-hydroxypropyl)-morpholine (for **56**), THF, 23 °C, 2 days, (2) HCl, methanol, chloroform, 23 °C (**55** 52%, **56** 59%).

activity; +, weak activity; ++, similar activity to compound **1**; +++, greater activity than **1**; ++++, equipotent to **2** (Table 1).

The antiproliferative activity of each compound was determined in the National Cancer Institute (NCI) screen.[31,32] Cells of approximately 60 different human cancer cell lines were incubated for 48 h with five 10-fold dilutions of the test compounds starting from 100 μM and then treated with sulforhodamine B dye. The ratios of recorded optical densities relative to those of the control were plotted as a function of the common logarithm of the tested compound concentrations. The interpolation between the points located above and below the 50% percentage growth provided 50% growth inhibition (GI_{50}) values. The GI_{50} and the mean graph midpoint (MGM) values of the prepared indenoisoquinolines in selected cell lines are presented in Table 1. The MGM is based on a calculation of the average GI_{50} for all of the cell lines tested in which GI_{50} values above and below the tested range (10^{-4}–10^{-8} M) are taken as the maximum (10^{-4} M) and minimum (10^{-8} M) drug concentrations used in the screening test. The Top1 inhibitory and cytotoxicity (MGM and GI_{50}) data for lead compound **1**,[1,33] compound **2**, and previously reported 7-azaindenoisoquinolines **6** and **7**[16] are included in Table 1 for comparison purposes.

The results of the biological evaluations presented in Table 1 clearly show that 7-azaindenoisoquinolines **6** and **7** are more active than their corresponding isomers, 8-, 9-, and 10-azaindenoisoquinolines, **17**, **18**, **27**, **28**, and **37**. The Top1 inhibitory activities of **6** and **7** are at the +++/++ level, and their cytotoxicities are in the low micromolar concentration range. Top1 inhibition is observed at only the ++/+ level for 8-, 9-, and 10-azaindenoisoquinolines. The MGM of compounds **18**, **27**, and **28** increased 2–50 times relative to the MGM of **6** and **7**. The 8-azaindenoisoquinoline **17** and 10-azaindenoisoquinoline **37** were not cytotoxic enough for GI_{50} and MGM values to be

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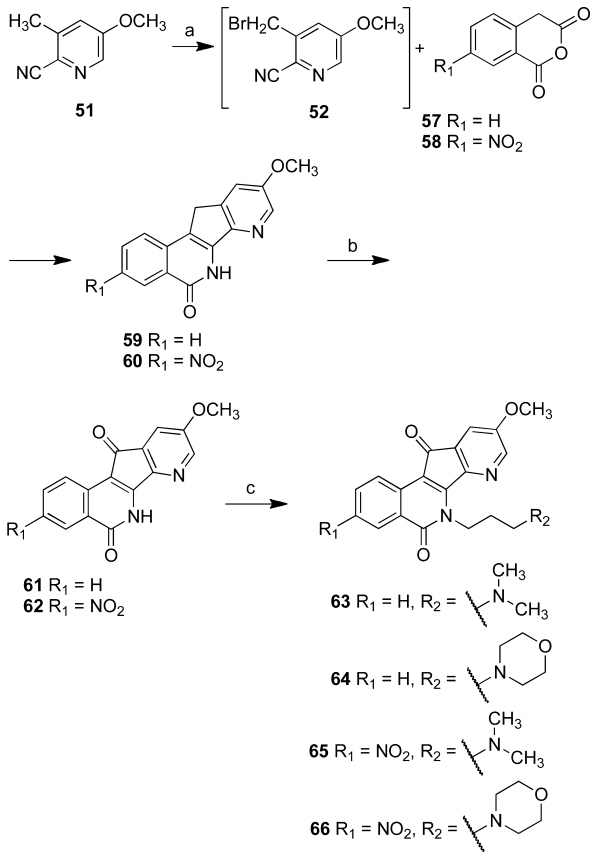
Scheme 8[a]



[a]Reagents and conditions: (a) (1) NBS, AIBN, 1,2-dichloroethane, reflux, 24 h, (2) homophthalic anhydride (**57**, for **59**) or 5-nitrohomophthalic anhydride (**58**, for **60**), triethylamine, acetonitrile, reflux 24 h (**59** 46%, **60** 26%); (b) SeO_2, 1,4-dioxane, reflux, 24 h (**61** 76%, **62** 86%); (c) DIAD, triphenylphosphine, 3-dimethylamino-1-propanol (for **63** and **65**) or 4-(3-hydroxypropyl)morpholine (for **64** and **66**), THF, 23 °C, 2 days (**63** 38%, **64** 40%, **65** 61%, **66** 47%).

determined in the NCI testing concentration range. These results indicate that the introduction of a nitrogen atom in positions other than 7 of the indenoisoquinoline polycyclic system results in compounds with diminished activity.

The synthetic protocol developed for the preparation of the 10-azaindenoisoquinolines **37** and **38** was modified, allowing for an addition of the methoxy group to position 9, which resulted in the preparation of compounds **45** and **46** (Schemes 3 and 6). It was previously noted that introduction of a methoxy group into position 9 of the indenoisoquinolines positively affects the inhibitory and cytotoxic activities of the target compounds.[14,15] 10-Aza-9-methoxyindenoisoquinolines **45** and **46** (Scheme 6) were ranked +++ in the Top1-mediated DNA cleavage assay, showing an improvement in comparison to the corresponding analogue **37** that lacks a 9-methoxy group (Top1: ++, Table 1). Unfortunately, the 10-azaindenoisoquinolines were largely noncytotoxic in the tested cancer cell lines.

The 7-aza-9-methoxyindenoisoquinoline series was prepared (Scheme 7) in view of the positive Top1 inhibition results observed on the introduction of a 9-methoxy group in the 10-aza series. In the case of 7-azaindenoisoquinolines, there was no apparent increase in the Top1 inhibitory activity of compounds **55** and **56** over **6** and **7**, respectively. However, in the case of 7-aza-9-methoxyindenoisoquinoline **55**, a nearly 3-fold improvement in cytotoxicity was observed relative to 7-azaindenoisoquinoline **6** (Table 1).

Having achieved the desired effect, 7-aza-9-methoxyindenoisoquinolines **63**–**66** were prepared that bear different substituents on the A-ring (Scheme 8). An improvement in cytotoxicity was observed for the dimethylaminopropyl analogues **63** and **65**, which had MGM values of 0.40 and 0.11 μM. No loss of Top1 inhibitory activity was observed for **63** and **65** in comparison with the closely related compounds **6** and **55**. In the case of compound **66**, there was a surprising improvement in Top1 inhibitory activity, up to ++++, making it equipotent with **2**. The MGM value obtained for compound **66** was 85 nM, making it the most active compound to date in the entire azaindenoisoquinoline series.

The cleavage band intensities displayed in Figure 3 indicate that the azaindenoisoquinolines offer different DNA cleavage site specificity in comparison to **2**. Most notably, the bands at positions 44, 62, and 106 become more prominent whereas bands at positions 32 and 97 are weaker than in the case of **2**. The cleavage site specificity of the azaindenoisoquinoline series is consistent with that previously observed for **1**, **4**, and **5**.[1,10] By trapping Top1-DNA cleavage complexes having different DNA cleavage sites than the camptothecins, the indenoisoquinolines and azaindenoisoquinolines target the genome differently than the camptothecins. This suggests that the cancer treatment profiles of the indenoisoquinolines might be different from the camptothecins.

In an attempt to understand the effect of introduction of nitrogen into various positions of the indenoisoquinoline D-ring on the π–π stacking interactions, a series of hypothetical azaindenoisoquinoline structures were modeled and subjected to quantum mechanical single point energy calculations. It was shown earlier that π–π stacking interactions of indenoisoquinolines with the flanking base pairs are responsible for their orientation in the ternary complex and their DNA cleavage site specificities. The results of these calculations indicate that π–π stacking interactions are the major complex stabilizing force.[34−36]

The model for these calculations was derived from the X-ray crystal structure of the **3**–Top1–DNA ternary complex (PDB entry 1SC7).[5] The deoxyribose rings of the flanking base pairs and the lactam side chain of **3** were replaced with methyl groups because they are considered to be nonparticipating groups in π–π stacking interactions.[35] The geometry optimizations and frequency calculations at the HF/6-31G** level were performed on the indenoisoquinoline molecule, as well as A–T and G–C base pairs, using the Gaussian 09 software package.[37] The original complex was then replaced with the geometry optimized parts (Figure 4 and Figure 5, model **A**). The CH groups in positions 7–10 of the D-ring of the inhibitor were replaced with nitrogen to produce 7-, 8-, 9-, and 10-azaindenoisoquinoline models (Figure 5, models **B**–**F**). Geometry optimizations were performed on these molecules before fitting them into their corresponding complexes with the DNA base pairs.

After the complexes were assembled, MP2/6-31G* single point energy calculations were performed. The basis set superposition error (BSSE) for each complex was calculated using the counterpoise correction method within the Gaussian 09 package, specifying two fragments: the first being the two flanking DNA base pairs and the second being the intercalating indenoisoquinoline inhibitor. The π–π staking interaction

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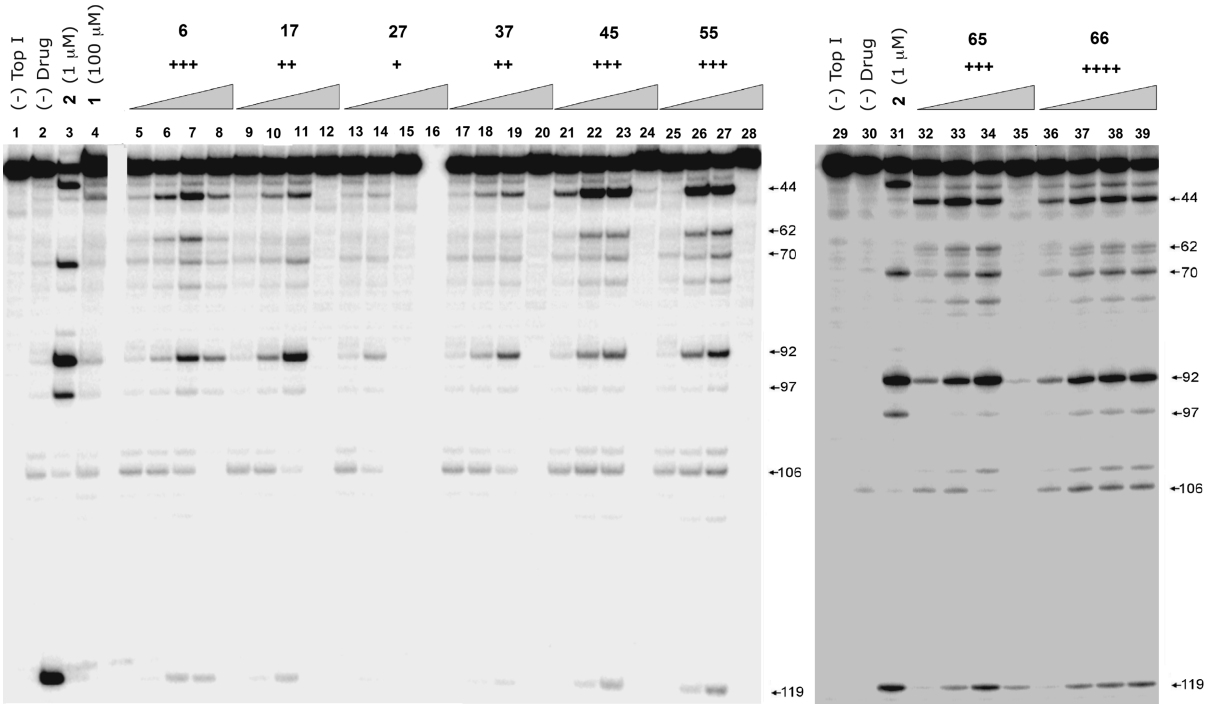

Figure 3. Top1-mediated DNA cleavage induced by azaindenoisoquinolines. Lanes 1, 29: DNA alone. Lanes 2, 30: Top1 alone. Lanes 3, 31: Top1 + **2** (1 μM). Lane 4: Top1 + **1** (100 μM). Lanes 5–8: Top1 + **6** at 0.1, 1, 10, 100 μM. Lanes 9–12: Top1 + **17** at 0.1, 1, 10, 100 μM. Lanes 13–16: Top1 + **27** at 0.1, 1, 10, 100 μM. Lanes 17–20: Top1 + **37** at 0.1, 1, 10, 100 μM. Lanes 21–24: Top1 + **45** at 0.1, 1, 10, 100 μM. Lanes 25–28: Top1 + **55** at 0.1, 1, 10, 100 μM. Lanes 32–35: Top1 + **65** at 0.1, 1, 10, 100 μM. Lanes 36–39: Top1 + **66** at 0.1, 1, 10, 100 μM. Numbers on right and arrows show the cleavage site positions.

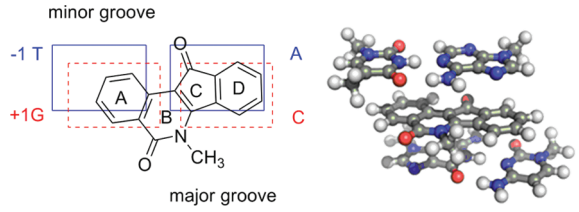

Figure 4. Model of the complex of the inhibitor with DNA base pairs.

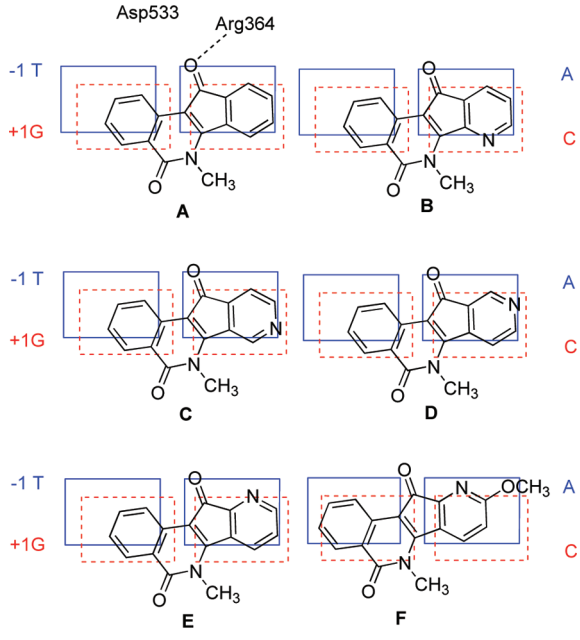

Figure 5. Modeled indenoisoquinoline–DNA complexes.

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 complex, ligand, and DNA base pairs at their normal distance in the absence of intercalator (Table 2). The polarizable continuum model (PCM) was used at the MP2/6-31G* level of theory to estimate the effect of solvation on stabilization of the complex ($E_{int,aq}$, Table 2). The dispersion energy E_{corr} was calculated as $E_{corr} = E_{int}(MP2) - E_{int}(HF)$. The energy associated with charge transfer was obtained as result of natural bond orbital (NBO) analysis in Gaussian 09.[38] The NBO analysis was performed at the HF/6-31G** level of theory and presented as E_{CT} in Table 2. The calculated transfer of 0.01e can be equated to 1 kcal/mol of complex stabilization energy.[39]

Although E_{corr} and E_{CT} could not be summed directly to obtain the whole or at least a part of E_{int}, they could be used to evaluate the effect of various modifications on particular forces responsible for stabilization of drug–Top1–DNA ternary complexes. For example, comparison of E_{corr} for "classical" indenoisoquinoline (model **A**) and 7-azaindenoisoquinoline (model **B**) shows that introduction of nitrogen into the seventh position did not decrease the dispersion energy contribution to the stabilization resulting from π–π stacking. Analysis of the E_{CT} column on the other hand suggests that as expected the charge-transfer stabilization energy is greater by 1.08 kcal/mol for 7-azindenoisoquinoline represented by model **B**, in comparison to indenoisoquinoline in model **A**. Table 2 indicates that the dispersion interaction is decreased significantly for models **C**–**E** but not **B** (relative to **A**). This decrease in dispersion energy contributes to the decrease of Top1 inhibitory activity and

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Table 2. MP2 Calculated Energies for Models A–F

model	E_{int} (au)	E_{int} [a] (kcal/mol)	$E_{int,aq}$ (kcal/mol)	E_{corr} [b] (kcal/mol)	E_{CT} [c] (kcal/mol)
A	−0.01108	−6.95	−15.17	−26.03	−11.50
B	−0.01273	−7.99	−15.57	−25.79	−12.58
C	−0.01452	−9.11	−16.20	−15.11	−13.57
D	−0.01663	−10.43	−17.01	−14.73	−11.99
E	−0.01459	−9.16	−18.59	−14.27	−10.05
F	−0.01305	−8.19	−17.03	−40.04	−10.91

[a] E_{int} is derived from MP2/6-31G* single point energy calculations. [b] $E_{corr} = E_{int(MP2/6\text{-}31G^*)} - E_{int(HF/6\text{-}31G^*)}$. [c] The magnitude of the charge transfer as estimated by NBO analysis at the HF/6-31G** level.

cytotoxicity. Even in the case of 8-azaindenosioquinolines (model **C**), despite the increase of the absolute magnitude of E_{CT} to almost 13.6 kcal/mol, the decrease in dispersion interactions was significant enough to decrease Top1 inhibitory activity. Comparison of the experimental results of Top1-mediated DNA cleavage for compounds **37** and **45** and calculated E_{CT} and E_{corr} values for models **E** and **F** demonstrates the importance of the methoxy group in position 9 of the indenoisoquinolines (Tables 1 and 2). This methoxy substitution drastically increases dispersion (E_{corr}) interaction energy while maintaining charge-transfer (E_{CT}) interactions. The increase in the dispersion interaction of 9-methoxy analogues could be attributed to the electron donating properties of the methoxy group and increase in electron delocalization in comparison to the analogues lacking this group. The increase of the Top1 inhibitory activity that corresponds to the addition of a methoxy group is observed in the case of 10-aza- and 10-aza-9-methoxyindenoisoquinolines, **37** and **45**, respectively. These findings were extrapolated to 7-azaindenoisoquinolines. The resulting 7-aza-9-methoxyindenoisoquinolines **55**, **56**, and **63–66** demonstrated improvement in their biological properties relative to those lacking the methoxy group in position 9.

It was previously hypothesized that a nitro group in the third position of the indenoisoquinoline system is capable of forming a direct hydrogen bond with Asn722.[14] Overlaying the structure of **66** with **3** in its ternary complex (PDB entry 1SC7)[5] revealed that this interaction would be possible in addition to the contact with Arg364 formed by the carbonyl oxygen of the indenone moiety (Figure 6). Addition of an extra hydrogen bond to the network of contacts of the drug with the Top1-DNAcc might explain the increase of Top1 inhibitory and antiproliferative potency of **66** in comparison to other azaindenoisoquinolines such as **7** and **56**.

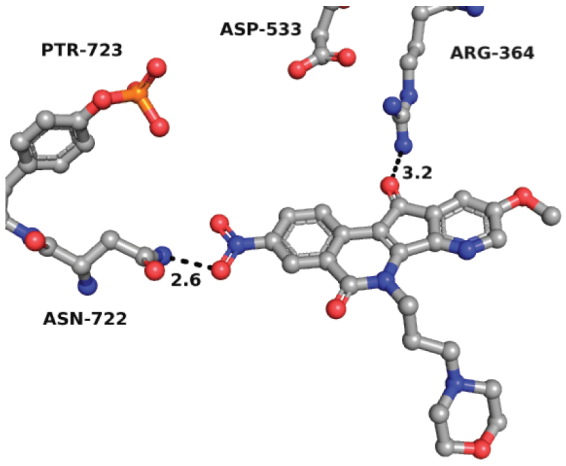

Figure 6. Hypothetical binding mode of **66** in complex with Top1-DNAcc. Hydrogen bonds (dashed lines) are represented as distances between corresponding heavy atoms.

In conclusion, a series of 7-, 8-, 9-, and 10-azaindenoisoquinolines have been prepared. The established synthetic protocols allow the preparation of these compounds from easily affordable starting materials. Azaindenoisoquinolines have been evaluated for their ability to stabilize the Top1-DNAcc and thus inhibit Top1. The ab initio calculations show that the values of the components of the $\pi-\pi$ stacking interaction energy, i.e., dispersion forces (E_{corr}) and charge-transfer interactions (E_{CT}), significantly depend on the position of the nitrogen atom. The 7-azaindenoisoquinoline system allows for an increase of charge transfer interaction without compromising the level of dispersion interactions. Previous studies have also revealed that incorporation of nitrogen into position 7 of indenoisoquinolines allows for improvement of water solubility without compromising the Top1 inhibitory activities and cytotoxicities of the resulting 7-azaindenoisoquinolines.[16] Further development of the 7-azaindenoisoquinolines presented in this study led to the discovery of 7-aza-9-methoxy-3-nitroindenoisoquinoline **66**, which demonstrated high activity in the Top1-mediated DNA cleavage assay, ++++, and in cytotoxicity screening at the NCI, with an MGM of 85 nM. Further aminopropyl chain modifications and optimization[8,13] could be done in order to further improve the Top1 inhibitory and cytotoxic performance of compounds such as **65** and **66**.

EXPERIMENTAL SECTION

General. Melting points were determined using capillary tubes with a Mel-Temp apparatus and are uncorrected. The nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded using ARX300 300 MHz and DRX500 500 MHz Bruker NMR spectrometers. IR spectra were recorded using a Perkin-Elmer 1600 series FTIR spectrometer. Purities of all tested compounds were ≥95%, as established by combustion and/or estimated HPLC analysis. Combustion microanalyses were performed at the Purdue University Microanalysis Laboratory or Galbraith Laboratories Inc., and the reported values were within 0.4% of the calculated values. HPLC analyses were performed on a Waters 1525 binary HPLC pump/Waters 2487 dual λ absorbance detector system. For purities estimated by HPLC, the major peak accounted for ≥95% of the combined total peak area when monitored by a UV detector at 254 nm. Analytical thin-layer chromatography was carried out on Baker-flex silica gel IB2-F plates, and compounds were visualized with UV light at 254 nm. Silica gel flash chromatography was performed using 230–400 mesh silica gel.

3-Cyano-2,6-dihydroxy-4-methylpyridine (10).[18] Cyanoacetamide (**8**, 34 g, 0.40 mol) and ethyl acetoacetate (**9**, 52 g, 0.40 mol) were dissolved in methanol (250 mL) at room temperature. A solution of potassium hydroxide (28 g, 0.42 mol) in methanol (200 mL) was slowly added, and the resulting mixture was heated to reflux for 12 h. The reaction mixture was cooled to room temperature, and the white amorphous precipitate was filtered and washed with methanol (2 × 50 mL). The solid product was redissolved in hot water. The solution was carefully acidified, and the off-white precipitate was allowed to form. The precipitate was filtered and washed with water and methanol to yield **10** (46 g, 75%): mp >300 °C (dec) [lit.[18] mp 315–320 °C (dec)]. ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.58 (s, 1 H), 2.22 (s, 3 H).

3-Cyano-2,6-dichloro-4-methylpyridine (11).[18] 3-Cyano-2,6-dihydroxy-4-methylpyridine (**10**, 10 g, 0.07 mol) and phosphorus oxychloride (25 mL, 0.27 mol) were sealed in a heavy-walled tube, and the mixture was heated to 150–180 °C in an oil bath for 8 h. The resulting mixture was allowed to cool to room temperature and carefully quenched by pouring it into ice (200 g). The light brown precipitate was filtered, washed with water, and dried to yield **11** (8.3 g, 67%): mp 114–118 °C (lit.[18] mp 109–110 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.77 (s, 1 H), 1.49 (s, 3 H).

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3-Cyano-4-methylpyridine (12).[18] Palladium dichloride (50 mg, 0.3 mmol) was added to a degassed solution of **11** (5.0 g, 27 mmol) and sodium acetate (4.5 g, 55 mmol) in methanol (100 mL). The resulting mixture was stirred under hydrogen (1 atm) for 14 h at room temperature. The precipitate was filtered and washed with methanol (3 × 20 mL). The combined filtrates were evaporated under reduced pressure, and chloroform (50 mL) was added to the residue. The chloroform solution was filtered through a thin pad of silica gel, washing with additional portions of chloroform. The filtrate was evaporated to dryness to provide **12** (2.9 g, 93%) as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.77 (s, 1 H), 8.63 (s, J = 6.0 Hz, 1 H), 7.3 (d, J = 6.0 Hz, 1 H), 2.56 (s, 3 H). 3-Cyano-4-methylpyridine (**12**) was used further without additional purification.

8-Aza-5,6-dihydro-2,3-dimethoxy-5-oxo-11*H*-indeno[1,2-*c*]-isoquinoline (15). 3-Cyano-4-methylpyridine (**12**, 1.5 g, 13 mmol), NBS (3.3 g, 19 mmol), and AIBN (100 mg, 0.6 mmol) were diluted with carbon tetrachloride (60 mL), and the mixture was heated at reflux for 3.5 h. The reaction mixture was concentrated to one-half its original volume, filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was diluted with acetonitrile (60 mL). 4,5-Dimethoxyhomophthalic anhydride (**14**, 5.6 g, 25 mmol) was added, followed by triethylamine (3.5 mL, 25 mmol), and the solution was heated at reflux for 14 h. The solution was allowed to cool to room temperature and the precipitate was filtered and washed with acetonitrile (2 × 15 mL) to provide a gray solid (500 mg, 13%): mp 270−272 °C. IR (KBr) 1633, 1611, 1593 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.31 (s, 1 H), 9.08 (s, 1 H), 8.48 (d, J = 6.0 Hz, 1 H), 7.61 (m, 2 H), 7.13 (s, 1 H) 3.93 (s, 5 H), 3.86 (s, 3 H). Positive ESIMS m/z (rel intensity): 295 (MH^+, 100).

8-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (16). 8-Aza-5,6-dihydro-2,3-dimethoxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (**15**, 250 mg, 0.85 mmol) and SeO_2 (190 mg, 1.7 mmol) were diluted with 1,4-dioxane (20 mL), and the mixture was heated at reflux for 4 h. The reaction mixture was filtered while hot, and the precipitate was washed with hot dioxane (3 × 10 mL). The combined filtrates were evaporated to dryness under reduced pressure. The solid residue was purified by flash column chromatography (silica gel), eluting with 5% methanol in chloroform, to obtain **16** (130 mg, 49%): mp 300−302 °C. IR (KBr) 1708, 1648, 1611, 1579 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.96 (s, 1 H), 8.56 (d, J = 6.2 and 1.4 Hz, 1 H), 7.86−7.81 (m, 2 H), 7.59 (s, 1 H), 7.40 (t, J = 6.8 Hz, 1 H). Positive ESIMS m/z (rel intensity): 309 (MH^+, 100).

8-Aza-5,6-dihydro-6-(3-dimethylaminopropyl)-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (17). 8-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline (**16**, 92 mg, 0.30 mmol), 3-dimethylamino-1-propanol (0.1 mL, 0.9 mmol), and PPh_3 (240 mg, 0.92 mmol) were diluted with THF (15 mL). Diisopropyl azodicarboxylate (0.18 mL, 0.92 mmol) was added to the THF solution, and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was then evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel), eluting with 10% methanol in chloroform, followed by preparative TLC (silica gel), eluting with 5% methanol in chloroform, to provide an orange solid. The solid was redissolved in chloroform (5 mL), and trifluoroacetic acid (2 M in diethyl ether, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its trifluoroacetate salt (53 mg, 34%): mp 230−232 °C (dec). IR (KBr) 1690, 1612 cm^{-1}. ^{1}H NMR (300 MHz, CD_3OD) δ 8.68−8.60 (m, 2 H), 7.73 (d, J = 5.5 Hz, 1 H), 7.69 (s, 1 H), 7.26 (s, 1 H), 4.57 (t, J = 6.1 Hz, 2 H), 3.76 (s, 3 H), 3.70 (s, 3 H), 3.24−3.08 (m, 2 H), 2.69 (s, 6 H), 2.24−2.06 (m, 2 H). Positive ion ESIMS m/z (rel intensity): 394 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{22}H_{23}N_3O_4$, 394.1767; found, 394.1769. HPLC purity: 97.44% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 80:20].

8-Aza-5,6-dihydro-6-(3-(4-morpholino)propyl)-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (18). 8-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (**16**, 100 mg, 0.32 mmol), 4-(3-hydroxypropyl)morpholine (94.3 mg, 0.65 mmol), and PPh_3 (170 mg, 0.65 mmol) were diluted with THF (10 mL). Diisopropyl azodicarboxylate (0.13 mL, 0.65 mmol) was added to the THF solution, and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was then evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel), eluting with 10% methanol in chloroform, followed by preparative TLC (silica gel), eluting with 5% methanol in chloroform, to provide an orange solid. The solid was redissolved in chloroform (5 mL), and trifluoroacetic acid (2 M in diethyl ether, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its trifluoroacetate salt (62 mg, 35%): mp 213−214 °C. IR (KBr) 1778, 1753, 1679, 1614 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.15 (s, 1 H), 8.83−8.58 (m, 2 H), 7.69 (s, 1 H), 7.44 (d, J = 4.5 Hz, 1 H), 7.31 (s, 1 H), 4.69 (t, J = 5.8 Hz, 2 H), 4.02 (d, J = 12.3 Hz, 2 H), 3.92 (s, 3 H), 3.90 (s, 3 H), 3.67 (t, J = 11.5 Hz, 2 H), 3.54 (d, J = 12.1 Hz, 2 H), 3.40 (s, 2 H), 3.16 (s, 2 H), 2.32 (s, 2 H). Positive ion ESIMS m/z (rel intensity): 436 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{24}H_{25}N_3O_5$, 436.1872; found, 436.1769. HPLC purity: 97.05% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 90:10]; 97.36% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 70:30].

3-Methyl-4-nicotinic Acid (20).[20] A solution of 3,4-lutidine (**19**, 30 g, 0.28 mol) in diphenyl ether (150 mL) was heated to 150−170 °C, and selenium dioxide (62 g, 0.56 mol) was carefully added to the hot solution in small portions in the course of 1 h. The resulting mixture was heated to 180 °C for 1 h. The reaction mixture was filtered while hot, and the collected precipitate was washed with boiling water (3 × 300 mL). The combined filtrates were extracted with chloroform (3 × 300 mL). The aqueous phase was evaporated to dryness and the remaining product was recrystallized from ethanol (450 mL) to obtain pure acid **20** (18 g, 47%): mp 220−222 °C (lit.[40] mp 232 °C). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.59 (s, 1 H), 8.04 (s, 1 H), 8.47 (d, J = 4.8, 1 H), 7.69 (d, J = 4.8, 1 H), 2.48 (s, 3 H).

3-Methyl-4-nicotinamide (22). A solution of 3-methyl-4-nicotinic acid (**20**, 5.0 g, 37 mmol) in thionyl chloride (20 mL, 0.28 mol) was heated at reflux for 3 h. The thionyl chloride was evaporated. The solid acid chloride **21** was added in small portions to a concentrated ammonium hydroxide solution (300 mL) while cooling the reaction mixture to 0−5 °C. The reaction mixture was saturated with potassium carbonate, and the solution was extracted with chloroform (2 × 150 mL) and ethyl acetate (2 × 150 mL). The aqueous phase was evaporated to dryness, and the resulting solid was extracted with hot ethyl acetate (3 × 150 mL). The combined extracts were evaporated to dryness to yield crude **22** (3.0 g, 60%): mp 140−142 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (s, 1 H), 8.44 (d, J = 4.9 Hz, 1 H), 7.94 (s, 1 H), 7.65 (s, 1 H), 7.29 (d, J = 4.9 Hz, 1 H), 2.32 (s, 3 H).

3-Methyl-4-cyanopyridine (23). Phosphorus oxychloride (100 mL, 1.1 mol) was slowly added to the crude amide **22** (15 g, 0.11 mol) while cooling the mixture in an ice bath. The resulting solution was heated at reflux for 24 h. The reaction mixture was cooled to room temperature, and the excess phosphorus oxychloride was removed under reduced pressure. Crushed ice (150 g) was slowly added to the oily residue, and the solution was neutralized with saturated ammonium hydroxide. The crude product was extracted with chloroform (3 × 100 mL). The combined extracts were filtered through a layer of silica gel, washing with extra portions of chloroform. The filtrates were evaporated to dryness to yield **23** as colorless crystals (12 g, 90%): mp 45−47 °C (lit.[41] mp 50 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (s, 1 H), 8.59 (d, J = 5.0 Hz, 1 H), 7.45 (d, J = 5.0 Hz, 1 H), 2.54 (s, 3 H).

9-Aza-5,6-dihydro-2,3-dimethoxy-5-oxo-11*H*-indeno[1,2-*c*]-isoquinoline (25). 3-Methylisonicotinonitrile (**23**, 590 mg, 5.0 mmol), NBS (1.2 g, 7.0 mmol), and AIBN (50 mg, 0.3 mmol) were diluted with carbon tetrachloride (20 mL), and the mixture was heated at reflux for 2 h. The reaction mixture was concentrated to one-half its original volume, filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was diluted with acetonitrile (25 mL), and **14** (2.2 g, 10 mmol) was added, followed by triethylamine (5 mL, 36 mmol), and the solution was heated at reflux for 14 h.

The solution was allowed to cool to room temperature, and the precipitate was filtered and washed with acetonitrile (50 mL) to provide a light-brown solid (200 mg, 14%): mp 306−308 °C. IR (KBr) 1639, 1613, 1592 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1 H), 8.73 (s, 1 H), 8.54 (d, J = 6.0 Hz, 1 H), 7.87 (d, J = 6.0 Hz, 1 H), 7.65 (s, 1 H), 7.23 (s, 1 H), 3.96 (s, 3 H), 3.88 (s, 3 H), 3.37 (s, 2 H). Positive ESIMS m/z (rel intensity): 295 (MH^+, 100).

9-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (26). 9-Aza-5,6-dihydro-2,3-dimethoxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (**25**, 100 mg, 0.34 mmol) and SeO_2 (75 mg, 0.68 mmol) were diluted with 1,4-dioxane (10 mL), and the mixture was heated at reflux for 4 h. The reaction mixture was filtered while hot, and the precipitate was washed with hot dioxane (3 × 10 mL). The combined filtrates were evaporated to dryness under reduced pressure. The solid residue was purified by flash column chromatography (silica gel), eluting with 5% methanol in chloroform, to obtain **26** (98 mg, 94%): mp 312−314 °C. IR (KBr) 1710, 1638, 1608 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 12.96 (s, 1 H), 8.56 (d, J = 6.2 and 1.4 Hz, 1 H), 7.86−7.81 (m, 2 H), 7.59 (s, 1 H), 7.40 (t, J = 6.8 Hz, 1 H). Positive ESIMS m/z (rel intensity): 309 (MH^+, 100). Negative ion ESIMS m/z (rel intensity): 307 [$(M - H^+)^-$, 100].

9-Aza-5,6-dihydro-6-(3-dimethylaminopropyl)-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (27). 9-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline (**26**, 92 mg, 0.3 mmol), 3-dimethylamino-1-propanol (0.1 mL, 0.9 mmol), and PPh_3 (240 mg, 0.92 mmol) were diluted with THF (15 mL). Diisopropyl azodicarboxylate (0.18 mL, 0.92 mmol) was added to the THF solution, and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was then evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel), eluting with 10% methanol in chloroform, to provide a dark-orange solid. The solid was redissolved in chloroform (10 mL), and trifluoroacetic acid (2 M in diethyl ether, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its trifluoroacetate salt (94 mg, 62%): mp 220 °C (dec). IR (KBr) 1772, 1688, 1633, 1612 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ 8.61 (d, J = 5.2 Hz, 1 H), 8.44 (s, 1 H), 7.54 (d, J = 7.0 Hz, 2 H), 7.16 (s, 1 H), 4.62 (t, J = 6.0 Hz, 2 H), 3.84 (s, 3 H), 3.81 (s, 3 H), 3.40−3.29 (m, 2 H), 2.89 (s, 6 H), 2.38−2.24 (m, 2 H). Positive ion ESIMS m/z (rel intensity): 394 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{22}H_{23}N_3O_4$, 394.1767; found, 394.1770. HPLC purity: 96.18% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 70:30]; 97.23% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 80:20].

9-Aza-5,6-dihydro-6-(3-(4-morpholino)propyl)-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (28). 9-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline (**26**, 85 mg, 0.28 mmol), 4-(3-hydroxypropyl)morpholine (120 mg, 0.84 mmol), and PPh_3 (230 mg, 0.84 mmol) were diluted with THF (10 mL). Diisopropyl azodicarboxylate (0.17 mL, 0.84 mmol) was added to the THF solution, and the resulting mixture was stirred at room temperature for 3 h. The reaction mixture was then evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel), eluting with 10% methanol in chloroform, to provide a dark-orange solid. The solid was redissolved in chloroform (2 mL), and trifluoroacetic acid (2 M in diethyl ether, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its trifluoroacetate salt (16 mg, 10%): mp 222−224 °C (dec). IR (KBr) 1772, 1712, 1677, 1635, 1612 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ 8.73 (s, 1 H), 8.62 (s, 1 H), 8.55 (d, J = 8.3 Hz, 1 H), 8.24 (d, J = 8.0 Hz, 1 H), 7.80 (t, J = 7.6 Hz, 2 H), 7.59 (t, J = 7.7 Hz, 1 H), 4.75 (d, J = 6.4 Hz, 2 H), 3.99 (d, J = 11.5 Hz, 2 H), 3.68 (t, J = 12.7 Hz, 2 H), 3.50 (d, J = 12.2 Hz, 2 H), 3.44−3.35 (m, 2 H), 3.13−2.98 (m, 2 H), 2.36 (td, J = 11.7, 5.7 Hz, 2 H). Positive ion ESIMS m/z (rel intensity): 436 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{20}H_{19}N_3O_2$, 436.1872; found, 436.1870. HPLC purity: 98.14% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 70:30]; 96.84% [C-18 reverse phase, MeOH (1% CF_3COOH), 100].

2-Methyl-6-oxo-1,6-dihydropyridine-3-carbonitrile (31).[21] 3-Aminocrotonitrile (**29**, 2.9 g, 36 mmol) and ethyl propiolate (**30**, 3.0 mL, 36 mmol) were dissolved in dry DMF (17 mL). The reaction mixture was stirred for 1 h at room temperature, and the mixture was heated at reflux for 3 days. The precipitate formed after cooling to room temperature was collected, washed with methanol (5 mL), ether (10 mL), and dried to yield **31** (1.5 g, 31%): mp >300 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 12.43 (s, 1 H), 7.58 (d, J = 9.6 Hz, 1 H), 6.23 (d, J = 9.6 Hz, 1 H), 2.37 (s, 3 H).

6-Chloro-2-methylnicotinonitrile (32).[21] A mixture of **31** (1.5 g, 11 mmol) and phosphorus oxychloride (9 mL, 0.1 mol) was heated at reflux for 6 h. The reaction mixture was cooled to room temperature, and the excess phosphorus oxychloride was removed under reduced pressure. Ice cold water (50 mL) was added to the residue. The brown precipitate was collected and washed with ice cold water (3 × 25 mL), ether (2 × 20 mL), and dried to provide **32** as a light-brown solid (0.9 g, 54%): mp 104−105 °C (lit.[21] mp 106−108 °C). ^1H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, J = 8.1 Hz, 1 H), 7.29 (d, J = 8.1 Hz, 1 H), 2.77 (s, 3 H).

2-Methylnicotinonitrile (33). 6-Chloro-2-methylnicotinonitrile (**32**, 10 g, 66 mmol) and ammonium formate (41 g, 0.65 mol) were dissolved in methanol (250 mL), and palladium (5% on activated carbon, 3.5 g, 2.5 mol %) was added. The mixture was stirred at room temperature for 12 h, filtered through Celite, and washed with methanol (3 × 50 mL). The combined filtrates were evaporated, and the yellow oily residue was subjected to flash column chromatography on silica gel, eluting with chloroform to provide **33** as an off-white solid (6.3 g, 81%): mp 55 °C (lit.[42] mp 56−58 °C). ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (dd, J = 4.9, 1.6 Hz, 1 H), 7.02 (dd, J = 7.8, 1.7 Hz, 1 H), 6.37 (dd, J = 7.8, 5.0 Hz, 1 H), 1.88 (s, 3 H). Positive ion ESIMS m/z (rel intensity): 119 (MH^+, 100).

10-Aza-5,6-dihydro-2,3-dimethoxy-5-oxo-11*H*-indeno[1,2-*c*]-isoquinoline (35). 2-Methylnicotinonitrile (**33**, 3.3 g, 34 mmol), NBS (5.5 g, 33 mmol), and AIBN (600 mg, 4 mmol) were diluted with 1,2-dichloroethane (60 mL), and the mixture was heated at reflux for 9 h. The reaction mixture was concentrated to the half its original volume, filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was redissolved in acetonitrile (70 mL). **14** (11 g, 48 mmol) was added, followed by triethylamine (7 mL, 50 mmol), and the solution was heated at reflux for 2 days. The hot solution was filtered, and the precipitate was washed with boiling acetonitrile (2 × 25 mL) to provide a gray solid (2.8 g, 31%): mp 270−272 °C. IR (KBr) 1635, 1610, 1528, 1503 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 12.26 (s, 1 H), 8.40 (d, J = 4.9 Hz, 1 H), 8.19 (d, J = 7.7 Hz, 1 H), 7.63 (s, 1 H), 7.34 (dd, J = 7.6, 5.1 Hz, 1 H), 7.19 (s, 1 H), 3.95 (s, 3 H), 3.91 (s, 2 H), 3.86 (s, 3 H). Positive ESIMS m/z (rel intensity): 295 (M^+, 100).

10-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (36). 10-Aza-5,6-dihydro-2,3-dimethoxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (**35**, 1.5 g, 5.1 mmol) and SeO_2 (1.13 g, 10.2 mmol) were diluted with 1,4-dioxane (50 mL), and the mixture was heated at reflux for 3 days. The reaction mixture was filtered while hot, and the precipitate was extracted in a Soxhlet extractor with a chloroform−methanol mixture (4:1). The extracts were evaporated to dryness to get **36** (1.4 g, 90%): mp >300 °C. IR (KBr) 1708, 1659, 1600, 1574 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 8.49 (d, J = 4.9 Hz, 1 H), 8.07 (d, J = 7.6 Hz, 1 H), 7.83 (s, 1 H), 7.56 (s, 1 H), 7.42 (dd, J = 7.4, 5.2 Hz, 1 H), 3.93 (s, 3 H), 3.87 (s, 3 H). Negative ion ESIMS m/z (rel intensity): 307 [$(M - H^+)^-$, 100].

10-Aza-5,6-dihydro-6-(3-dimethylaminopropyl)-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (37). 10-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (**36**, 92 mg, 0.3 mmol), 3-dimethylamino-1-propanol (0.1 mL, 0.9 mmol), and PPh_3 (240 mg, 0.92 mmol) were diluted with THF (15 mL). Diisopropyl azodicarboxylate (0.18 mL, 0.92 mmol) was added to the THF solution, and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was then evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel), eluting with 10% methanol in chloroform, followed by preparative TLC (silica gel),

eluting with 5% methanol in chloroform, to provide an orange solid. The solid was redissolved in chloroform (5 mL), and trifluoroacetic acid (2 M in diethyl ether, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its trifluoroacetate salt (34 mg, 22%): mp 212−214 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.60 (s, 1 H), 8.50 (dd, J = 5.1, 1.3 Hz, 1 H), 7.83 (dd, J = 7.5, 1.3 Hz, 1 H), 7.73 (s, 1 H), 7.41 (dd, J = 7.5, 5.1 Hz, 1 H), 7.32 (s, 1 H), 4.67 (t, J = 6.1 Hz, 2 H), 3.93 (s, 3 H), 3.90 (s, 3 H), 3.34−3.25 (m, 2 H), 2.81 (d, J = 4.7 Hz, 6 H), 2.38−2.28 (m, 2 H). Positive ion ESIMS m/z (rel intensity): 394 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{22}H_{23}N_3O_4$, 394.1767; found, 394.1769. HPLC purity: 98.32% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 80:20].

10-Aza-5,6-dihydro-6-[3-(4-morpholino)propyl]-2,3-dimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (38). 10-Aza-5,6-dihydro-2,3-dimethoxy-5,11-dioxo-11*H*-indeno-[1,2-*c*]isoquinoline (**36**, 100 mg, 0.32 mmol), 4-(3-hydroxypropyl)-morpholine (94 mg, 0.65 mmol), and PPh_3 (170 mg, 0.65 mmol) were diluted with THF (10 mL). Diisopropyl azodicarboxylate (130 mg, 0.65 mmol) was added to the THF solution, and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was then evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel), eluting with 10% methanol in chloroform, followed by preparative TLC (silica gel), eluting with 5% methanol in chloroform, to provide an orange solid. The solid was redissolved in chloroform (5 mL), and trifluoroacetic acid (2 M in diethyl ether, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its trifluoroacetate salt (12 mg, 7%): mp 208−210 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.05 (s, 1 H), 8.51 (d, J = 4.0 Hz, 1 H), 7.88 (d, J = 7.4 Hz, 1 H), 7.81 (s, 1 H), 7.44 (dd, J = 7.4, 5.1 Hz, 1 H), 7.40 (s, 1 H), 4.71 (t, J = 5.8 Hz, 2 H), 3.96 (t, J = 10.8 Hz, 8 H), 3.81 (dd, J = 20.1, 8.5 Hz, 2 H), 3.50 (d, J = 12.1 Hz, 2 H), 3.11 (dd, J = 21.2, 9.3 Hz, 4 H), 2.41−2.33 (m, 2 H). Positive ion ESIMS m/z (rel intensity): 436 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{24}H_{25}N_3O_5$, 436.1872; found, 436.1769. HPLC purity: 98.28% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 90:10]; 97.14% [C-18 reverse phase, MeOH(1% CF_3COOH)/H_2O, 70:30].

2-Methyl-1,4,5,6-tetrahydropyridine-3-carbonitrile (39). A solution of 6-chloro-2-methylnicotinonitrile (**32**, 6.0 g, 39 mmol) and potassium acetate (7.8 g, 80 mmol) in methanol (80 mL) was degassed. Palladium dichloride (350 mg, 2.0 mmol) was added to the solution, and the reaction vessel was filled with hydrogen (1 atm.). The mixture was stirred at room temperature until no more hydrogen was consumed. The solvent was removed under reduced pressure and the oily residue was subjected to flash column chromatography on silica gel, eluting with chloroform, to provide **39** as light-brown solid (4.0 g, 84%): mp 49−52 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.43 (s, 1 H), 3.18 (td, J = 6.2, 3.0 Hz, 2 H), 2.20 (t, J = 6.3 Hz, 2 H), 1.97 (s, 3 H), 1.80−1.64 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.22, 123.85, 71.19, 41.22, 23.27, 20.75, 19.85.

3-Cyano-2-chloro-4-methylpyridine (40). 3-Cyano-2,6-dichloro-4-methylpyridine (**11**, 35.1 g, 0.19 mol) and ammonium formate (235 g, 3.73 mol) were added to a suspension of palladium (5% on activated carbon, 4.0 g, 0.8 mol %) in methanol (600 mL), and the mixture was stirred at room temperature for 3 days. The mixture was filtered through a Celite bed. The filtrate was evaporated to dryness, and water (100 mL) and chloroform (100 mL) were added. The organic layer was separated, and the aqueous layer was extracted with chloroform (3 × 50 mL). The combined extracts were washed with water (50 mL), brine (100 mL), dried with sodium sulfate, and filtered through a thin pad of silica gel, washing with chloroform. The combined filtrates were evaporated to yield a light-brown solid (25.3 g, 87%): mp 104−106 °C (lit.[23] mp 105−108 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, J = 5.1 Hz, 1 H), 7.21 (d, J = 5.1 Hz, 1 H), 2.57 (s, 3 H).

6-Methoxy-2-methylnicotinonitrile (41). Sodium methoxide (20 g, 0.4 mol) was added to a solution of 6-chloro-2-methylnicotinonitrile (**32**, 10 g, 66 mmol) in methanol (150 mL), and the mixture was heated at reflux for 1.5 h and cooled to room temperature. The precipitate was removed by filtration, and the filtrate was concentrated to dryness. The crude solid was redissolved in chloroform, and the resulting solution was filtered through a layer of silica gel, washing with extra portions of chloroform. The combined filtrates were evaporated to dryness to yield **41** (8.3 g, 87%): mp 81−82 °C (lit.[21] mp 80−80.5 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, J = 8.5 Hz, 1H), 6.59 (d, J = 8.5 Hz, 1H), 3.94 (s, 3H), 2.62 (s, 3H).

10-Aza-5,6-dihydro-2,3,9-trimethoxy-5-oxo-11*H*-indeno-[1,2-*c*]isoquinoline (43). 6-Methoxy-2-methylnicotinonitrile (**41**, 2.2 g, 15 mmol), NBS (2.9 g, 16 mmol), and AIBN (100 mg, 0.6 mmol) were diluted with 1,2-dichloroethane (50 mL), and the mixture was heated at reflux for 3.5 h. The reaction mixture was concentrated to one-half its original volume, filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was diluted with acetonitrile (60 mL), and **14** (5.3 g, 24 mmol) was added, followed by triethylamine (3.5 mL, 25 mmol), and the solution was heated at reflux for 14 h. The solution was allowed to cool to room temperature and the obtained precipitate was filtered and washed with acetonitrile (2 × 15 mL) to provide an off-white solid (0.9 g, 19%): mp 284−286 °C. IR (KBr) 1648, 1614 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 (s, 1 H), 8.14 (d, J = 8.4 Hz, 1 H), 7.61 (s, 1 H), 7.10 (s, 1 H), 6.81 (d, J = 8.5 Hz, 1 H), 3.94 (s, 3 H), 3.91 (s, 3 H), 3.86 (s, 3H), 3.85 (s, 2 H). EIMS m/z: 324 (M^+). CIMS m/z (rel intensity): 325 (MH^+, 100).

10-Aza-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (44). 10-Aza-5,6-dihydro-2,3,9-trimethoxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (**43**, 0.7 g, 2.2 mmol) and SeO_2 (0.48 mg, 4.3 mmol) were diluted with 1,4-dioxane (50 mL), and the mixture was heated at reflux for 24 h. The reaction mixture was filtered while hot, and the precipitate was washed with hot dioxane (3 × 100 mL). The combined filtrates were evaporated to dryness under reduced pressure to obtain **44** (0.65 g, 89%): mp >350 °C. IR (KBr) 1713, 1645, 1624, 1612, 1592 cm^{-1}. NMR data have not been obtained because of poor solubility of the sample. Negative ion ESIMS m/z (rel intensity): 337 [$(M - H^+)^-$, 100].

10-Aza-5,6-dihydro-6-(3-dimethylaminopropyl)-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (45). 10-Aza-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**44**, 110 mg, 0.32 mmol) was added to a stirred solution of PPh_3 (170 mg, 0.65 mmol) and diisopropyl azodicarboxylate (0.13 mL, 0.65 mmol) in tetrahydrofuran (10 mL). The mixture was stirred at room temperature until the solid material completely disappeared to form a dark-red solution. 3-Dimethylamino-1-propanol (67 mg, 0.65 mmol) was added dropwise to the resulting solution over the course of 30 min, and the reaction mixture was stirred at room temperature for 3 days. The resulting mixture was evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel), eluting with 3% methanol in chloroform, to provide a red solid. The solid was redissolved in chloroform (10 mL), and trifluoroacetic acid (2 M in diethyl ether, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its trifluoroacetate salt (52 mg, 30%): mp 250−252 °C (dec). IR (KBr) 1692, 1621, 1605 1562 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD) δ 7.28 (d, J = 8.1 Hz, 1 H), 7.16 (s, 1 H), 6.95 (s, 1 H), 6.54 (d, J = 8.1 Hz, 1 H), 4.57 (t, J = 5.8 Hz, 2 H), 3.94 (s, 3 H), 3.83 (s, 3 H), 3.82 (s, 3 H), 3.58−3.48 (m, 2 H), 3.07 (s, 6 H), 2.46−2.32 (m, 2 H). Positive ion ESIMS m/z (rel intensity): 424 (MH^+, 100). Anal. Calcd for $C_{25}H_{26}F_3N_3O_7$: C, 55.87; H, 4.88; N, 7.82. Found: C, 55.45; H, 4.62; N, 7.75.

10-Aza-5,6-dihydro-6-(3-(4-morpholino)propyl)-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Trifluoroacetate (46). 10-Aza-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**44**, 110 mg, 0.32 mmol) was added to a stirred solution of PPh_3 (170 mg, 0.65 mmol) and diisopropyl azodicarboxylate (0.13 mL, 0.65 mmol) in tetrahydrofuran (10 mL). The mixture was stirred at room temperature until the solid material completely disappeared to form a dark-red solution. 4-(3-Hydroxypropyl)morpholine (94 mg, 0.65 mmol) was added dropwise to the resulting solution over the course of 30 min, and the reaction mixture was stirred at room temperature for 3 days. The resulting

mixture was evaporated to dryness under reduced pressure. The residue was purified by flash column chromatography (silica gel), eluting with 3% methanol in chloroform, to provide a red solid. The solid was redissolved in chloroform (10 mL), and hydrochloric acid (2 M in methanol, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its trifluoroacetate salt (61 mg, 31%): mp 237−238 °C (dec). IR (KBr) 1709, 1618, 1605, 1561 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 10.81 (s, 1 H), 7.79 (d, J = 8.2 Hz, 1 H), 7.70 (s, 1 H), 7.33 (s, 1 H), 6.86 (d, J = 8.2 Hz, 1 H), 4.68 (s, 2 H), 4.00 (s, 2 H), 3.94 (s, 3 H), 3.92 (s, 3 H), 3.90 (s, 3 H), 3.78 (t, J = 11.8 Hz, 2 H), 3.62−3.40 (m, 4 H), 3.18−3.04 (m, 2 H), 2.35 (s, 2 H). Positive ion ESIMS m/z (rel intensity): 466 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{25}H_{27}N_3O_6$, 466.1978; found, 466.1980. HPLC purity: 96.75% [C-18 reverse phase, MeOH (1% CF_3COOH)/H_2O, 70:30]; 95.27% [C-18 reverse phase, MeOH (1% CF_3COOH)].

5-Bromo-3-methylpyridin-2-amine (48).[24] *N*-Bromosuccinimide (170 g, 0.95 mol) was added to a solution of **47** (99 g, 0.92 mol) and ammonium acetate (7 g, 10 mol %) in acetonitrile (500 mL). The temperature of the reaction mixture during addition was controlled with an ice bath. After the full amount of NBS was added, the ice bath was removed and the reaction mixture was stirred at room temperature for 25 min and acetonitrile was removed under reduced pressure. A mixture of ethyl acetate (1 L) and water (1 L) was added to the solid residue. The resulting mixture was stirred, and the organic layer was separated. The water layer was extracted with ethyl acetate (3 × 500 mL). The combined extracts were washed with water (300 mL), saturated sodium bicarbonate solution (500 L), dried with sodium sulfate, and evaporated to dryness to give a dark brown solid. The crude product was redissolved in chloroform (300 mL), and the solution was filtered through a thin pad of silica gel, eluting with chloroform. The combined filtrates were evaporated under reduced pressure to yield **48** as a light-brown solid (113 g, 65%): mp 89−90 °C (lit.[43] mp 91−93 °C). ^1H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, J = 2.3 Hz, 1 H), 7.36 (d, J = 2.3 Hz, 1 H), 4.50 (s, 2 H), 2.09 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.10, 146.29, 140.03, 118.79, 108.58, 77.72, 77.30, 76.88, 17.27.

2,5-Dibromo-3-methylpyridine (49).[27] 5-Bromo-3-methylpyridin-2-amine (**48**, 69 g, 0.37 mol) was suspended in hydrobromic acid (200 mL, 48% in water), and the mixture was cooled to −15 °C. Bromine (95 g, 0.59 mol) was added dropwise to the mixture followed by addition of sodium nitrite (69 g, 1 mol) in water (100 mL). Temperature of the reaction mixture was kept below −15 °C during addition. After addition, the cooling bath was removed and the reaction mixture was stirred for 3 h. The reaction mixture was cooled to −15 °C and quenched with potassium hydroxide (112 g, 2 mol) in water (500 mL). The cooling bath was removed, and the mixture was stirred for 1.5 h. The products were extracted with ethyl acetate (3 × 300 mL). The combined extracts were washed with water (2 × 200 mL), saturated aqueous sodium bicarbonate (200 mL), dried with sodium sulfate, and evaporated to dryness. The oily residue was redissolved in chloroform (100 mL), and the solution was filtered through a pad of silica gel, washing with chloroform. The combined filtrates were evaporated to provide **49** as light-yellow solid (87 g, 94%): mp 38−40 °C (lit.[44] mp 41−42 °C). ^1H NMR (300 MHz, $CDCl_3$) δ 8.22 (d, J = 2.4 Hz, 1 H), 7.61 (d, J = 2.4 Hz, 1 H), 2.33 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.29, 143.05, 141.15, 137.15, 119.68, 22.06.

5-Bromo-3-methylpicolinonitrile (50). Copper(I) cyanide (21 g, 0.24 mol) was added to a solution of 2,5-dibromo-3-methylpyridine (**49**, 60 g, 0.24 mol) in dry DMF (200 mL), and the mixture was heated at reflux for 2 h. After the mixture was cooled to room temperature, water (1500 mL) was added to the mixture and the products were extracted with ethyl acetate (3 × 300 mL). The combined extracts were washed with water (3 × 300 mL), brine (300 mL), dried with sodium sulfate, and evaporated to dryness. The brown oily residue was subjected to flash column chromatography (silica gel), eluting with chloroform, to yield a white solid (35 g, 74%): mp 86−88 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, J = 1.8 Hz, 1 H), 7.84 (d, J = 1.5 Hz, 1 H), 2.53 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.75, 140.68, 139.95, 132.25, 124.61, 115.90, 18.59. EIMS m/z 196/198 (M^+). CIMS 197/199 (MH^+). The ^1H NMR spectrum is consistent with previously published data.[45]

5-Methoxy-3-methylpicolinonitrile (51). 5-Bromo-3-methylpicolinonitrile (**50**, 35 g, 0.18 mol) was added to a solution of sodium methoxide (18 g, 0.54 mol) in methanol (200 mL), and the mixture was heated at reflux for 12 h. The solution was cooled to room temperature and concentrated to one-third of its volume. The concentrated solution was diluted with water (150 mL), and the products were extracted with chloroform (3 × 50 mL). The combined extracts were washed with water (2 × 50 mL), brine (50 mL), dried with sodium sulfate, and filtered through a pad of silica gel, washing with chloroform, to produce **51** as an off-white solid (21 g, 79%): mp 80−81 °C. IR (film) 2225, 1645, 1589 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 2.6 Hz, 1 H), 7.03 (d, J = 2.4 Hz, 1 H), 3.83 (s, 3 H), 2.43 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.65, 139.99, 137.68, 125.05, 120.52, 116.76, 55.84, 18.71. EIMS m/z (rel intensity): 148 (M^+, 100). CIMS m/z (rel intensity): 149 (MH^+, 100).

7-Aza-5,6-dihydro-2,3,9-trimethoxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (53). 5-Methoxy-3-methylpicolinonitrile (**51**, 5.0 g, 34 mmol), NBS (6.6 g, 37 mmol), and AIBN (500 mg, 3 mmol) were diluted with 1,2-dichloroethane (50 mL), and the mixture was heated at reflux for 24 h. The reaction mixture was concentrated to one-half its original volume, filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was redissolved in acetonitrile (100 mL). 4,5-Dimethoxyhomophthalic anhydride (**14**, 11.2 g, 50 mmol) was added, followed by triethylamine (8 mL, 58 mmol), and the solution was heated at reflux for 24 h. The hot solution was filtered, and the precipitate was washed with boiling acetonitrile (2 × 25 mL) to provide a gray solid (2.4 g, 21%): mp >260 °C. IR (KBr) 1635, 1608 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 11.94 (s, 1 H), 8.22 (d, J = 2.6 Hz, 1 H), 7.63 (s, 2 H), 7.17 (s, 1 H), 3.95 (s, 3 H), 3.89 (s, 3 H), 3.87 (s, 3 H), 3.85 (s, 2 H). Positive ion ESIMS m/z (rel intensity): 265 (MH^+, 100).

7-Aza-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (54). 10-Aza-5,6-dihydro-2,3,9-trimethoxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (**53**, 2.08 g, 6.4 mmol) and SeO_2 (1.42 g, 12.8 mmol) were diluted with 1,4-dioxane (100 mL), and the mixture was heated at reflux for 24 h. The reaction mixture was filtered while hot, and the precipitate was washed with hot dioxane (2 × 500 mL). The combined filtrates were evaporated to dryness under reduced pressure to afford **54** (2.0 g, 92%): mp >300 °C. IR (KBr) 1705, 1662, 1615, 1602, 1563 cm^{-1}. NMR data have not been obtained because of poor solubility of the sample. Negative ion ESIMS m/z (rel intensity): 339 [$(M - H^+)^-$, 100]. The product was introduced into the next step without additional purification.

7-Aza-5,6-dihydro-6-(3-dimethylaminopropyl)-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (55). 7-Aza-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**54**, 338 mg, 1 mmol) was added to a stirred solution of PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) in tetrahydrofuran (10 mL). The mixture was stirred for 4 h at room temperature. 3-Dimethylamino-1-propanol (200 mg, 1.9 mmol) was added dropwise to the resulting solution over the course of 30 min, and the reaction mixture was stirred at room temperature for 12 h. PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) were added to the reaction mixture. The mixture was stirred for 6 h, and 3-dimethylamino-1-propanol (200 mg, 1.9 mmol) was added, forming a dark red solution. The solution was stirred at room temperature for 24 h and evaporated to dryness under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with a gradient of 1−5% methanol in chloroform, to provide a red solid. The solid was redissolved in chloroform (10 mL), and hydrochloric acid (1 M in methanol, 1 mL) was added. The precipitate was collected by filtration and washed with ether (2 × 2 mL) to yield the product in the form of its hydrochloride salt (238 mg, 52%): mp 245 °C (dec). IR (KBr) 3445, 1699, 1651, 1611 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ 9.90 (s, 1 H), 8.21 (s, 1 H), 7.81 (s, 1 H), 7.49 (s, 1 H), 7.43 (s, 1 H), 4.78 (s, 2 H), 3.94 (s, 3 H), 3.91 (s, 3 H), 3.86 (s, 3 H), 3.15 (s, 2 H), 2.76 (s, 3 H), 2.74 (s, 3 H), 2.14 (s, 2 H). Positive ion ESIMS m/z (rel intensity): 424 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{23}H_{25}N_3O_5$, 424.1822;

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found, 424.1869. HPLC purity: 98.61% [C-18 reverse phase, MeOH]; 97.99% [C-18 reverse phase, MeOH/H_2O, 85:15].

7-Aza-5,6-dihydro-6-[3-(4-morpholino)propyl]-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline Hydrochloride (56). 7-Aza-5,6-dihydro-2,3,9-trimethoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**54**, 338 mg, 1 mmol) was added to a stirred solution of PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) in tetrahydrofuran (10 mL). The mixture was stirred for 4 h at room temperature. 4-(3-Hydroxypropyl)morpholine (280 mg, 1.9 mmol) was added dropwise to the resulting solution over the course of 30 min, and the reaction mixture was stirred at room temperature for 12 h. PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) were added to the reaction mixture. The mixture was stirred for 6 h, and 4-(3-hydroxypropyl)morpholine (280 mg, 1.9 mmol) was added, forming a dark red solution. The solution was stirred at room temperature for 24 h and evaporated to dryness under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with a gradient of 1−5% methanol in chloroform, to provide a red solid. The solid was redissolved in chloroform (10 mL), and hydrochloric acid (1 M in methanol, 1 mL) was added. The precipitate was collected by filtration and washed with chloroform (20 mL) and diethyl ether (10 mL) to yield the product in the form of its hydrochloride salt (276 mg, 59%): mp 260−261 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.18 (d, J = 2.6 Hz, 1 H), 7.76 (s, 1 H), 7.45 (s, 1 H), 7.38 (d, J = 2.6 Hz, 1 H), 4.76 (s, 2 H), 3.93 (s, 3 H), 3.90 (s, 3 H), 3.84 (s, 3 H), 3.75 (t, J = 11.6 Hz, 4 H), 3.20 (s, 2 H), 3.06 (s, 4 H), 2.20 (s, 2 H). Positive ion ESIMS m/z (rel intensity): 466 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{23}H_{25}N_3O_5$, 466.1978; found, 466.1974. HPLC purity: 95.45% [C-18 reverse phase, MeOH]; 96.67% [C-18 reverse phase, MeOH/H_2O, 85:15].

7-Aza-5,6-dihydro-9-methoxy-5-oxo-11*H*-indeno[1,2-*c*]-isoquinoline (59). 5-Methoxy-3-methylpicolinonitrile (**51**, 5.0 g, 34 mmol), NBS (6.6 g, 37 mmol), and AIBN (500 mg, 3 mmol) were diluted with 1,2-dichloroethane (50 mL), and the mixture was heated at reflux for 24 h. The reaction mixture was concentrated to one-half its original volume, filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was redissolved in acetonitrile (100 mL). Homophthalic anhydride (**57**, 9 g, 55 mmol) was added, followed by triethylamine (8 mL, 58 mmol), and the solution was heated at reflux for 24 h. The hot solution was filtered, and the precipitate was washed with boiling acetonitrile (2 × 30 mL) to provide a gray solid (4.1 g, 46%): mp 232−233 °C. IR (KBr) 1666, 1621, 1607 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.11 (s, 1 H), 8.29−8.19 (m, 2 H), 7.75 (d, J = 4.1 Hz, 2 H), 7.68 (s, 1 H), 7.48 (dd, J = 8.1, 4.3 Hz, 1 H), 3.89 (s, 5 H). Positive ion ESIMS m/z (rel intensity): 310 (MH^+, 100).

7-Aza-5,6-dihydro-3-nitro-9-methoxy-5-oxo-11*H*-indeno-[1,2-*c*]isoquinoline (60). 5-Methoxy-3-methylpicolinonitrile (**51**, 5.0 g, 34 mmol), NBS (6.6 g, 37 mmol), and AIBN (500 mg, 3 mmol) were diluted with 1,2-dichloroethane (50 mL), and the mixture was heated at reflux for 24 h. The reaction mixture was concentrated to one-half its original volume, filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was redissolved in acetonitrile (100 mL). 5-Nitrohomophthalic anhydride (**58**, 11 g, 53 mmol) was added, followed by triethylamine (8 mL, 58 mmol), and the solution was heated at reflux for 24 h. The hot solution was filtered, and the precipitate was washed with boiling acetonitrile (2 × 30 mL) to provide a gray solid (2.7 g, 26%): mp >260 °C. IR (KBr) 1690, 1616, 1559 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.89 (d, J = 2.5 Hz, 1 H), 8.47 (dd, J = 8.8, 2.5 Hz, 1 H), 8.30 (d, J = 2.6 Hz, 1 H), 7.91 (d, J = 8.8 Hz, 1 H), 7.72 (d, J = 2.5 Hz, 1 H), 3.94 (s, 2 H), 3.91 (s, 3 H). Positive ion ESIMS m/z (rel intensity): 310 (MH^+, 100).

7-Aza-5,6-dihydro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*] isoquinoline (61). 7-Aza-5,6-dihydro-9-methoxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (**59**, 2.64 g, 10 mmol) and SeO_2 (2.22 g, 20 mmol) were diluted with 1,4-dioxane (120 mL), and the mixture was heated at reflux for 24 h. The reaction mixture was filtered while hot, and the precipitate was washed with hot dioxane (3 × 300 mL). The combined filtrates were evaporated to dryness under reduced pressure to afford **61** (2.10 g, 76%): mp >350 °C. IR (KBr) 1717, 1689, 1618, 1574 cm^{-1}. NMR and MS data have not been obtained because of poor solubility of the sample. The product was introduced into the next step without additional purification.

7-Aza-5,6-dihydro-3-nitro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (62). 7-Aza-5,6-dihydro-3-nitro-9-methoxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (**60**, 2.2 g, 7 mmol) and SeO_2 (1.6 g, 14 mmol) were diluted with 1,4-dioxane (100 mL), and the mixture was heated at reflux for 24 h. The reaction mixture was filtered while hot, and the precipitate was washed with hot dioxane (2 × 500 mL). The combined filtrates were evaporated to dryness under reduced pressure to yield **62** (1.94 g, 86%): mp >300 °C. IR (KBr) 1693, 1618, 1571 cm^{-1}. NMR data have not been obtained because ofpoor solubility of the sample. Negative ion ESIMS m/z (rel intensity): 322 [$(M - H^+)^-$, 100]. The product was introduced into the next step without additional purification.

7-Aza-5,6-dihydro-6-(3-dimethylaminopropyl)-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (63). 7-Aza-5,6-dihydro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**61**, 278 mg, 1 mmol) was added to a stirred solution of PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) in tetrahydrofuran (20 mL). The mixture was stirred at room temperature for 4 h. 3-Dimethylamino-1-propanol (200 mg, 1.9 mmol) was added dropwise to the resulting solution over the course of 15 min, and the reaction mixture was stirred at room temperature for 12 h. PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) were added to the reaction mixture. The mixture was stirred for 6 h, and 3-dimethylamino-1-propanol (200 mg, 1.9 mmol) was added, forming an orange solution. The solution was stirred at room temperature for 24 h and evaporated to dryness under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with a gradient of 1% to 5% methanol in chloroform, to provide an orange solid (138 mg, 38%): mp 190−192 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (d, J = 8.1 Hz, 1 H), 8.16 (d, J = 8.1 Hz, 1 H), 7.93 (d, J = 2.7 Hz, 1 H), 7.59−7.49 (m, 1 H), 7.35−7.27 (m, 1 H), 7.09 (d, J = 2.7 Hz, 1 H), 4.82−4.69 (m, 2 H), 3.81 (s, 3 H), 2.40 (t, J = 7.1 Hz, 2 H), 2.18 (s, 6 H), 1.89 (dt, J = 14.6, 7.5 Hz, 2 H). Positive ion ESIMS m/z (rel intensity): 364 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{21}H_{21}N_3O_3$, 364.1661; found, 364.1663. HPLC purity: 98.39% [C-18 reverse phase, MeOH]; 98.46% [C-18 reverse phase, MeOH/H_2O, 85:15].

7-Aza-5,6-dihydro-6-(3-(4-morpholino)propyl)-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (64). 7-Aza-5,6-dihydro-9-methoxy-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**61**, 278 mg, 1 mmol) was added to a stirred solution of PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) in tetrahydrofuran (20 mL). The mixture was stirred at room temperature for 4 h. 4-(3-Hydroxypropyl)morpholine (280 mg, 1.9 mmol) was added dropwise to the resulting solution over the course of 15 min, and the reaction mixture was stirred at room temperature for 12 h. PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) were added to the reaction mixture. The mixture was stirred for 6 h, and 4-(3-hydroxypropyl)morpholine (280 mg, 1.9 mmol) was added, forming an orange solution. The solution was stirred at room temperature for 24 h and evaporated to dryness under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with a gradient of 1−5% methanol in chloroform, to provide an orange solid (163 mg, 40%): mp 218−224 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (d, J = 8.2 Hz, 1 H), 8.25 (d, J = 2.5 Hz, 1 H), 8.20 (d, J = 7.8 Hz, 1 H), 7.82 (t, J = 7.7 Hz, 1 H), 7.61−7.45 (m, 2 H), 4.82 (s, 2 H), 3.92 (d, J = 12.6 Hz, 5 H), 3.74 (t, J = 11.9 Hz, 2 H), 3.39 (d, J = 12.2 Hz, 2 H), 3.21 (s, 2 H), 3.03 (d, J = 11.8 Hz, 2 H), 2.22 (s, 2 H). Positive ion ESIMS m/z (rel intensity): 406 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{21}H_{21}N_3O_3$, 406.1767; found, 406.1773. HPLC purity: 97.30% [C-18 reverse phase, MeOH]; 98.60% [C-18 reverse phase, MeOH/H_2O, 85:15].

7-Aza-5,6-dihydro-6-(3-dimethylaminopropyl)-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (65). 7-Aza-5,6-dihydro-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline (**62**, 323 mg, 1 mmol) was added to a stirred solution of PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg,

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1.9 mmol) in tetrahydrofuran (20 mL). The mixture was stirred at room temperature for 4 h. 3-Dimethylamino-1-propanol (200 mg, 1.9 mmol) was added dropwise to the resulting solution over the course of 15 min, and the reaction mixture was stirred at room temperature for 12 h. PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) were added to the reaction mixture. The mixture was stirred for 6 h, and 3-dimethylamino-1-propanol (200 mg, 1.9 mmol) was added, forming an orange solution. The solution was stirred at room temperature for 24 h and evaporated to dryness under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with a gradient of 1−5% methanol in chloroform, to provide a red solid (250 mg, 61%): mp 224−226 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.17 (d, J = 2.2 Hz, 1 H), 8.71 (d, J = 8.8 Hz, 1 H), 8.45 (dd, J = 8.9, 2.4 Hz, 1 H), 8.21 (d, J = 2.8 Hz, 1 H), 7.41 (d, J = 2.8 Hz, 1 H), 5.12−4.88 (m, 2 H), 3.98 (d, J = 4.0 Hz, 3 H), 2.48 (t, J = 7.0 Hz, 2 H), 2.22 (d, J = 3.9 Hz, 6 H), 1.99 (dt, J = 14.3, 7.1 Hz, 2 H). Positive ion ESIMS m/z (rel intensity): 409 (MH^+, 100). HRMS-ESI m/z: MH^+ calcd for $C_{21}H_{20}N_4O_5$, 409.1512; found, 409.1510. HPLC purity: 100% [C-18 reverse phase, MeOH]; 99.03% [C-18 reverse phase, MeOH/H_2O, 85:15].

7-Aza-5,6-dihydro-6-(3-(4-morpholino)propyl)-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (66). 7-Aza-5,6-dihydro-9-methoxy-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]-isoquinoline (**62**, 323 mg, 1 mmol) was added to a stirred solution of PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) in tetrahydrofuran (20 mL). The mixture was stirred at room temperature for 4 h. 4-(3-Hydroxypropyl)morpholine (280 mg, 1.9 mmol) was added dropwise to the resulting solution over the course of 15 min, and the reaction mixture was stirred at room temperature for 12 h. PPh_3 (510 mg, 1.9 mmol) and diisopropyl azodicarboxylate (390 mg, 1.9 mmol) were added to the reaction mixture. The mixture was stirred for 6 h, and 4-(3-hydroxypropyl)morpholine (280 mg, 1.9 mmol) was added, forming an orange solution. The solution was stirred at room temperature for 24 h and evaporated to dryness under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with a gradient of 1−5% methanol in chloroform, to provide a red solid (212 mg, 47%): mp 243−245 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.81 (s, 1 H), 8.55 (s, 2 H), 8.32 (d, J = 2.5 Hz, 1 H), 7.59 (d, J = 2.5 Hz, 1 H), 4.84 (s, 2 H), 3.96 (s, 3 H), 3.79 (s, 4 H), 3.21 (m, 6 H), 2.22 (s, 2 H). Positive ion ESIMS m/z (rel intensity): 451 (MH^+, 100). HPLC purity: 95.42% [C-18 reverse phase, MeOH]; 95.93% [C-18 reverse phase, MeOH/H_2O, 85:15].

Topoisomerase I Mediated DNA Cleavage Reactions. Human recombinant Top1 was purified from baculovirus as previously described.[46] DNA cleavage reactions were prepared as previously reported with the exception of the DNA substrate.[30] Briefly, a 117-bp DNA oligonucleotide (Integrated DNA Technologies) encompassing the previously identified Top1 cleavage sites in the 161-bp fragment from pBluescript SK(−) phagemid DNA was employed. This 117-bp oligonucleotide contains a single 5′-cytosine overhang, which was 3′-end-labeled by fill-in reaction with [α-^{32}P]dGTP in React 2 buffer (50 mM Tris-HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM NaCl) with 0.5 unit of DNA polymerase I (Klenow fragment, New England BioLabs). Unincorporated [^{32}P]dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled DNA substrate was collected. Approximately 2 nM radiolabeled DNA substrate was incubated with recombinant Top1 in 20 μL of reaction buffer [10 mM Tris-HCl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 15 μg/mL BSA] at 25 °C for 20 min in the presence of various concentrations of compounds. The reactions were terminated by adding SDS (0.5% final concentration) followed by the addition of two volumes of loading dye (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromphenol blue). Aliquots of each reaction mixture were subjected to 20% denaturing PAGE. Gels were dried and visualized by using a phosphoimager and ImageQuant software (Molecular Dynamics). For simplicity, cleavage sites were numbered as previously described in the 161-bp fragment.[46]

Quantum Mechanics Calculations. All calculations were performed in Gaussian 09. The structures of model indenoisoquinoline, azaindenoisoquinolines, and A−T and G−C base pairs from models **A**−**F** were optimized at the HF/6-31G** level of theory. The single point energy calculations were performed at the MP2/6-31G* and HF/6-31G* levels of theory. The NBO analysis was performed at the HF/6-31G** level of theory.

AUTHOR INFORMATION

Corresponding Author

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

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AIBN, azobisisobutyronitrile; DIAD, diisopropyl azodicarboxylate; DMF, *N*,*N*-dimethylformamide; DMSO-d_6, dimethyl-d_6 sulfoxide; NBS, *N*-bromosuccinimide; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Top1, topoisomerase type I; Top1-DNAcc, topoisomerase type I−DNA cleavage complex

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