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Design, Synthesis, and Evaluation of Dibenzo[*c*,*h*][1,6]naphthyridines as Topoisomerase I Inhibitors and Potential Anticancer Agents

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Indenoisoquinoline topoisomerase I (Top1) inhibitors are a novel class of anticancer agents. Modifications of the indenoisoquinoline A, B, and D rings have been extensively studied in order to optimize Top1 inhibitory activity and cytotoxicity. To improve understanding of the forces that stabilize drug-Top1-DNA ternary complexes, the five-membered cyclopentadienone C-ring of the indenoisoquinoline system was replaced by six-membered nitrogen heterocyclic rings, resulting in dibenzo[*c*,*h*][1,6]naphthyridines that were synthesized by a novel route and tested for Top1 inhibition. This resulted in several compounds that have unique DNA cleavage site selectivities and potent antitumor activities in a number of cancer cell lines.

Introduction

Cells of all living organisms possess topoisomerases to resolve the topological problems associated with DNA supercoiling during various cellular processes (e.g., replication, transcription, repair).[1] There are two major families of topoisomerases: type I and type II. Topoisomerase type I (Top1[a]) relaxes both positively and negatively supercoiled DNA via reversible single-strand nicks.[2] Top1 forms a covalent link with the 3′-oxygen atom of DNA.[3] The free 5′-end is then allowed to rotate about the intact strand, thus relieving tension. Once supercoils are removed, the broken DNA strand is religated and the Top1 released.[4] The importance of Top1 for DNA replication and cell division has made it an attractive drug target for anticancer therapy.[5,6] Camptothecin (CPT, **1**),[5] a natural product isolated from the Chinese tree *Camptotheca acuminata*, was the first small molecule to be identified as a Top1 inhibitor (Figure 1). Later, topotecan and irinotecan, two clinically relevant analogues of **1**, were developed, emphasizing the significance of Top1 as a drug target. The indenoisoquinoline NSC 314622 (**2**) was originally isolated as a byproduct in the total synthesis of nitidine chloride **3**, another naturally occurring Top1 inhibitor.[7,8] Interest in indenoisoquinolines was fueled by the fact that, despite their similarity to **1** in cytotoxic profile and ability to inhibit Top1, they lack the metabolically unstable lactone ring present in camptothecins.[9,10] Molecules like **1**−**3** convert Top1 into a poison by stabilizing the covalent Top1-DNA cleavage complex and preventing the religation step, thereby enhancing the formation of persistent DNA breaks that eventually result in cell death.[11]

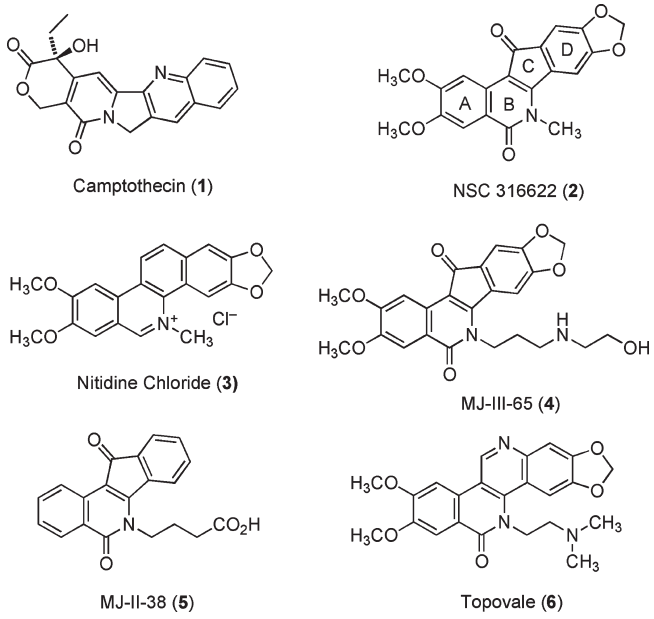

Figure 1. Representative Top1 inhibitors.

Further lead optimization and assessment of synthetic indenoisoquinoline analogues led to the discovery of a series of potent Top1 inhibitors.[12−14] To date, the lead optimization of indenoisoquinolines has mainly concentrated on varying the substituents on the A- and D-rings.[15,16] Also, a great deal of effort has been dedicated to finding the optimal replacement for the methyl group on the lactam B-ring of **2**.[17−19] In particular, the addition of an aminopropyl side chain to the lactam nitrogen was found to be advantageous for improving Top1 inhibitory activity of the indenoisoquinoline class of compounds. This resulted in the identification of potent Top1 inhibitor MJ-III-65 (**4**),[13] as well as MJ-II-38 (**5**), the

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[a] Abbreviations: CAN, ceric ammonium nitrate; DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone; DMSO-d_6, dimethyl-d_6 sulfoxide; MGM, mean-graph midpoint; NaHMDS, sodium hexamethyldisilazide; rmsd, root-mean-square deviation; TBDMS, *tert*-butyldimethylsilyl; Top1, Topoisomerase type I; SAR, structure−activity relationships.

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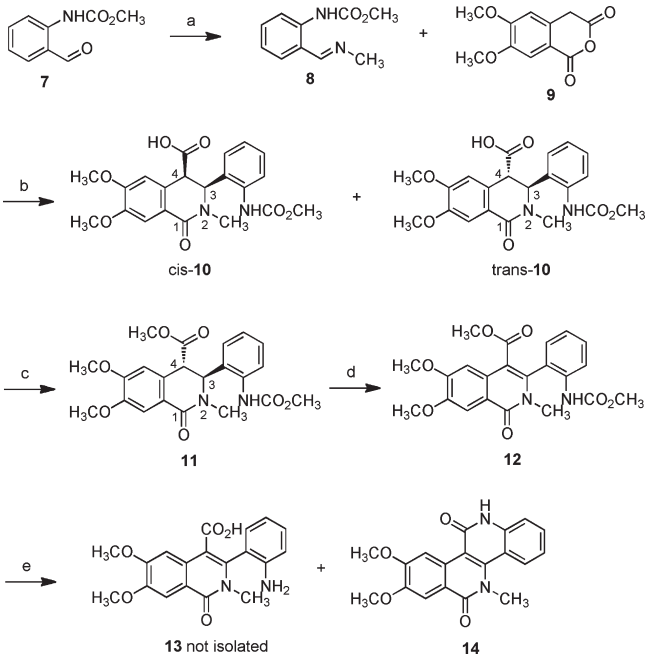
latter of which was used for cocrystallization with Top1-DNA cleavage complex to establish the molecular mechanism of Top1 inhibition by indenoisoquinolines.[20] As a part of an ongoing study of indenoisoquinoline structure−activity relationships (SAR), the exploration of the role and importance of the C-ring's size, geometry and constitution has been undertaken. For that purpose, the five-membered ring of the indenone fragment of the indenoisoquinoline was expanded to a six-membered pyridone ring. The designed dibenzonaphthyridinediones contain the isoquinolinone moiety of the indenoisoquinolines. The position of the added nitrogen atom was chosen to resemble the polycyclic core of the previously studied synthetic Top1 inhibitor topovale (**6**).[21] The tetracyclic system of the dibenzonaphthyridinediones closely resembles systems **3** and **6**. The overall character and position of the substituents on the dibenzonaphthyridinediones were chosen to be related to those of previously published indenoisoquinolines for ease of comparison.

Chemistry

Only two protocols for the preparation of dibenzo[*c*,*h*]-[1,6]naphthyridine-6,11(5*H*,12*H*)-diones have been reported so far.[22,23] Unfortunately, neither of the published syntheses is suitable for the preparation of the desired compounds. Therefore, a novel synthetic pathway was established in order to prepare several naphthyridinediones with various chains attached to the isoquinolinone lactam. The synthesis of *N*-methyl derivative **14** is outlined in Scheme 1. The *N*-protected *o*-aminobenzaldehyde **7**, prepared in two steps from commercially available *o*-aminobenzylalcohol,[24] was converted to imine **8** by treatment with a methanolic solution of methylamine. The less soluble cis-**10** was isolated by filtration of the reaction mixture derived from Schiff base **8** and 4,5-dimethoxyhomophthalic anhydride (**9**).[25] The trans isomer **10** was obtained by evaporation of the filtrate and recrystallization of the crude product. The lower yield of cis-**10** relative to its trans analogue can be attributed to the presence of the bulky carbamate in the ortho position. This also explains the nearly complete isomerization within 24 h of cis to trans configuration that was observed in an NMR sample of cis-**10** dissolved in dimethyl-d_6 sulfoxide (DMSO-d_6). The mixture of both cis- and trans-**10** was esterified to produce the more stable trans ester **11**. The chemical shifts and coupling constants of H-3 and H-4 were used to establish the cis/trans relationships for acid **10** and ester **11**.[7] In the case of cis-**10**, H-3 and H-4 appear as doublets with coupling constants of 6.6 Hz, whereas in the ^{1}H NMR spectrum of trans-**10**, they appear as singlets, consistent with pseudodiaxial substituents at C-3 and C-4 in trans-**10**, and a pseudoaxial phenyl substituent and pseudoequatorial carboxylic acid in cis-**10**. In both diastereomers, the C-3 phenyl substituent is pseudoaxial due to a steric A-strain interaction with the N-methyl group.[26−28] The ^{1}H NMR assignments of the relative configurations of Schiff base−homophthalic anhydride condensation products were previously corroborated through their use in the syntheses of a variety of natural products, including nitidine chloride, chelidonine, corynoline, and corydalic acid methyl ester.[7,29−31] In addition, the stereochemical assignment of cis and trans isomers based on the coupling constants of the vicinal methine protons have been more recently supported through the crystallographic studies of condensation products of homophthalic anhydride with imines.[32,33] The enolate formed after treatment of **11** with sodium hexamethyldisilazide (NaHMDS) was quenched with phenylselenyl chloride. Oxidation of the selenide with hydrogen peroxide resulted in dehydrogenated product **12**. A similar strategy was previously devised for conversion of trans-isoquinolonic acids and esters into indenoisoquinolines.[28] To our surprise, the saponification of ester **12** in the presence of lithium hydroxide, followed by acidification with acetic acid, provided the desired product **14** rather than the carboxylic acid **13**.

Scheme 1. Synthesis of Dibenzo[*c*,*h*][1,6]naphthyridinedione **14**[a]

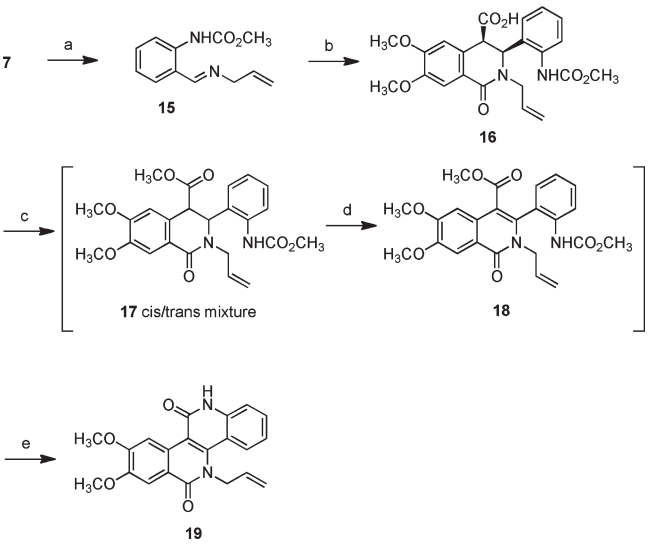


[a] Reagents and conditions: (a) CH_3NH_2, $MgSO_4$, CH_3OH, room temperature, 6 h (99%); (b) $CHCl_3$, room temperature, 2 h (cis-**10** 35%, trans-**10** 60%); (c) $SOCl_2$, CH_3OH, 0 °C, 6 h (96%); (d) (1) NaHMDS, THF, then PhSeCl, −78 °C to room temperature, 6 h, (2) H_2O_2, CH_3CO_2H, 0 °C to room temperature, 6 h (62%); (e) KOH, water−ethylene glycol (1:1), reflux, 4 d (83%).

In order to prepare a variety of analogues similar to previously prepared libraries of indenoisoquinolines for comparison purposes, a method that allows introduction of hydroxy- and aminoalkyl chains in place of the lactam methyl group of the original lead compound **2** was required. The allyl chain was envisioned as a stable enough potential precursor for further modifications and functionalizations once the polycyclic core of the dibenzonaphthyridinedione is complete. Synthesis of the allyl analogue of **14** was accomplished in a similar fashion to that described above (Scheme 2). Imine **15** was isolated from the reaction of aldehyde **7** with allyl amine. The following condensation of **15** with anhydride **9** yieled *cis*-isoquinolonic acid **16**. The esterification of cis-**16** was accompanied by only partial isomerization of the cis isomer and resulted in a cis/trans mixture **17**. Similar to the case of isoquinolonic acid **10**, the coupling constant between H-3 and H-4 (J_{3-4} = 5.9 Hz) in the ^{1}H NMR spectrum was used to assign the cis stereochemistry of **16**. Dehydrogenation followed by saponification and cyclization yielded naphthyridinedione **19**.

The allyl group proved to be stable enough to allow completion of the synthesis of **19**, but unfortunately, further derivatization of the allyl group was difficult to perform, mostly due to the extremely low solubility of the dibenzonaphthyridine **19**. This called for further adjustments of the synthetic strategy.

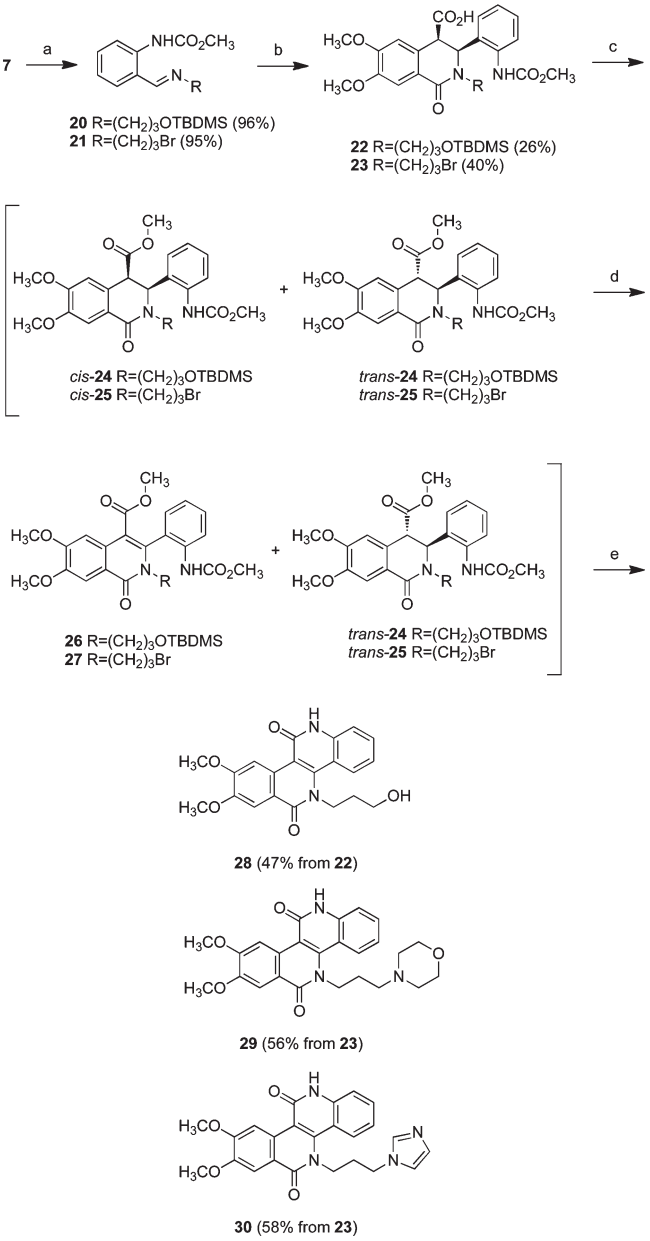
Scheme 2. Synthesis of Allyl Derivative **19**[a]



[a] Reagents and conditions: (a) $H_2C{=}CHCH_2NH_2$, $MgSO_4$, $CHCl_3$, room temperature, overnight (99%); (b) **9**, $CHCl_3$, room temperature, 18 h (26%); (c) $SOCl_2$, CH_3OH, 0 °C to room temperature, 6 h; (d) (1) NaHMDS, THF, then PhSeCl, −78 °C to room temperature, overnight, (2) H_2O_2, CH_3CO_2H, 0 °C to room temperature, overnight; (e) KOH, water/ethylene glycol (1:3), reflux, 12 h (43% over three steps).

Syntheses of hydroxypropyl- and two different aminopropyldibenzonaphthyridinediones are shown in Scheme 3. Starting from the *O*-TBDMS-protected Schiff base **20**, the hydroxypropyl analogue **28** was prepared. Similarly, bromide **21** became the launching point toward aminopropyl derivatives **29** and **30**. Condensation of **20** and **21** with anhydride **9** produced isoquinolonic acids **22** and **23** (Scheme 3). The coupling constants of 5.9 and 6.0 Hz between vicinal methine protons were observed in the ^{1}H NMR spectra of **22** and **23**, respectively, indicating cis stereochemistry of the isolated acids. Introduction of bromide and TBDMS-protected alcohol functionalities made it difficult to use the NaHMDS/phenylselenyl chloride/hydrogen peroxide sequence of steps and reagents for the dehydrogenation of the dihydroisoquinolone moieties of **22** and **23**. It was shown previously that dehydrogenation of the dihydroisoquinoline fragment could be performed easily on cis-substrates, whereas trans-analogues remain unresponsive to the treatment with oxidants like DDQ, CAN, and SeO_2.[7,8] This is due to the fact that in the cis diastereomers, the C-4 proton is pseudoaxial, resulting in overlap of the C−H bond with the neighboring π-system of the aromatic ring, thus facilitating deprotonation.[28] It is clear that, for the strategy to succeed, the retention of cis stereochemistry is required during esterification of **22** and **23**. The near-complete retention of cis configuration was achieved by means of esterification with trimethylsilyldiazomethane at low temperatures. The presence of small amounts of the trans isomers of **24** and **25** was confirmed by NMR spectroscopy of the crude products. In order to avoid further loss or isomerization of the cis-**24** and cis-**25** during column chromatography, the unpurified mixtures of cis- and trans-esters were used in the dehydrogenation step with DDQ. NMR analysis of the products revealed the presence of unchanged trans-**24** and trans-**25**, along with the desired compounds **26** and **27**. Treatment of **26** with potassium hydroxide at high temperature, followed by acidification with acetic acid, yielded the cyclized and unprotected hydroxypropyldibenzonaphthyridine **28**. Reaction of

Scheme 3. Synthesis of Hydroxy- and Aminopropyl Derivatives **28**−**30**[a]



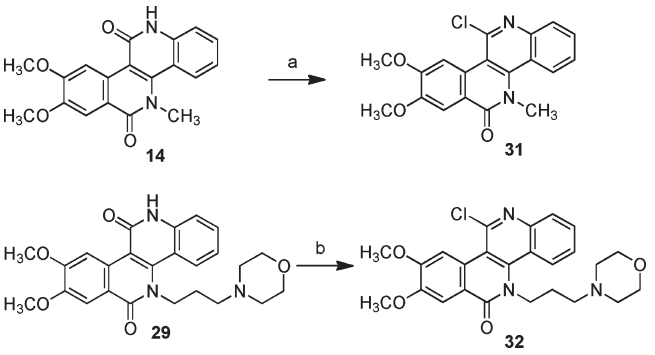
[a] Reagents and conditions: (a) RNH_2, $MgSO_4$, $CHCl_3$, room temperature, overnight; (b) **9**, $CHCl_3$, room temperature, 18 h; (c) $TMSCHN_2$, CH_3OH/THF (1:3), −10 to 0 °C, 30−45 min; (d) DDQ (2.2 equiv), 1,4-dioxane, reflux, 3−4 h; (e) KOH, water/ethylene glycol (1:3), reflux for 24 h for **28**, or morpholine, DMF, reflux 18 h for **29**, or imidazole, DMF, reflux 18−20 h for **30**.

27 with morpholine or imidazole in hot DMF provided **29** or **30**, respectively. In all cases, the final dibenzonaphthyridines were isolated as less soluble solids and the more soluble byproduct remained in solution.

The core polycyclic system of the dibenzo[*c*,*h*][1,6]naphthyridines has been synthesized before. In the present work, the naphthyridinedione system has been manipulated to provide a variety of substitution patterns. Naphthyridinediones **14** and **29** were reacted with phosphorus(V)oxychloride to yield chloronaphthyridinones **31** and **32** (Scheme 4).

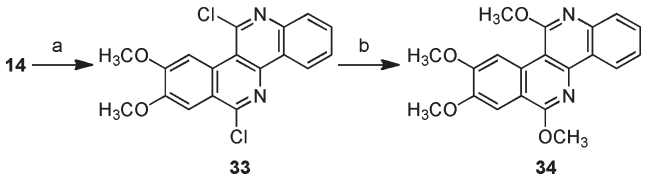
Heating a solution of **14** in $POCl_3$ for 6 h yielded dichloride **33** as the major product (Scheme 5). Both chlorines were

Scheme 4. Synthesis of Chloronaphthyridinones[a]



[a] Reagents and conditions: (a) $POCl_3$, DMF, room temperature to 70 °C, 7 h (61%); (b) $POCl_3$, PCl_5, room temperature, 1 h, reflux, 1.5 h (66%).

Scheme 5. Synthesis of Naphthyridines[a]



[a] Reagents and conditions: (a) $POCl_3$, PCl_5, reflux, 6 h (96%); (b) CH_3ONa, CH_3OH, reflux, 10 h (71%).

subsequently substituted with methoxy groups by the reaction with sodium methoxide providing tetramethoxydibenzonaphthyridine **34**.

Biological Results and Discussion

All of the target compounds were tested for induction of DNA damage in Top1-mediated DNA cleavage assays. For this purpose the ^{32}P 3′-end labeled 161 DNA fragment was incubated with Top1 and four 10-fold dilutions starting from 100 μM of a tested compound. The DNA fragments were separated on the denaturing gel. On the basis of the visual inspection of the number and intensities of the bands corresponding to those fragments, the Top1 inhibitory activities were assigned. The results of this assay are designated relative to the Top1 inhibitory activity of compounds **1** and **2**, and expressed in semiquantitative fashion: 0, no detectable activity; +, weak activity; ++, similar activity to compound **2**; +++, greater activity than **2**; ++++, equipotent to **1**. Naphthyridinediones **14**, **19**, and **28** expressed no detectable activity as Top1 inhibitors. Introduction of an imidazole or a morpholine at the end of the propyl chain in **29** and **30** made the naphthyridinediones Top1 inhibitors, but only at the lower + level. The loss of Top1 inhibitory activity with expansion of the five-membered indenone ring of the indenoisoquinoline might result from the decreased overall solubility, especially for **14**, **19**, and **28**, rather than differences in intercalation between DNA base pairs at the Top1 cleavage site. With **29** and **30**, incorporation of amines increased water solubility and affinity toward DNA due to attraction of their positively charged protonated amines with the phosphates of the DNA backbone. The comparison of the naphthyridinedione **30** to indenoisoquinoline **35**[34] revealed that, despite having similar substituents on the isoquinolinone moiety, the activity of the naphthyridinedione is significantly lower (Figure 2).

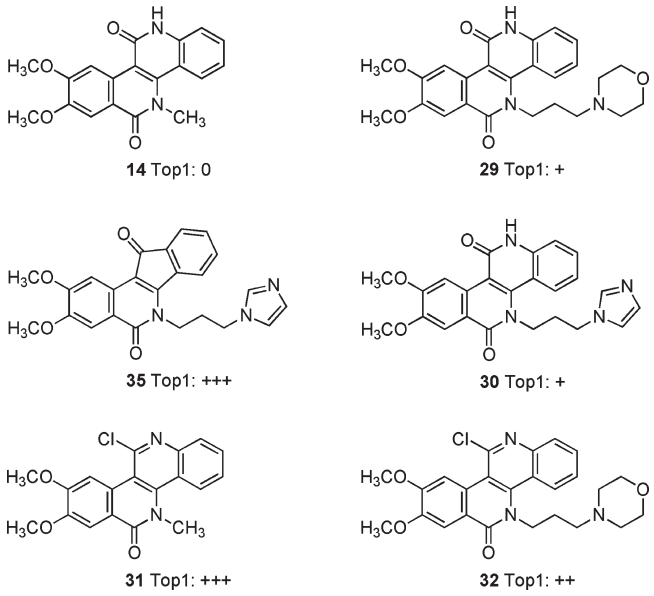


Figure 2. Relative Top1 inhibitory potencies of the compounds are presented as follows: 0, no detectable activity; +, weak activity; ++, similar activity as compound **2**; +++ and ++++, greater activity than compound **2**; ++++, similar activity as 1 μM **1**.

Interestingly, after conversion of the quinolinone fragment to a chloroquinoline, the observed Top1 inhibitory activity increased from 0 for **14** to +++ for **31**, and from + for **29** to ++ for **32**. The solubilities of **31** and **32** in solvents like dimethyl sulfoxide, methanol, and chloroform also increased substantially relative to their precursors **14** and **29**. Further aromatization of the isoquinolinone moiety led to complete loss of activity for dichloride **33** and tetramethoxynaphthyridine **34** despite similarity of their polycyclic systems to nitidine chloride (**3**). In the case of the indenoisoquinolines, Top1 inhibitory activity could generally be improved by replacement of an N-methyl group by a chain of 2−4 carbon atoms with a polar group attached at its end.[17−19] It is particularly noteworthy that this general trend was not observed with the chlorodibenzonaphthyridines **31** and **32**. In fact, introduction of the morpholinopropyl fragment in **32** in place of the N-methyl group in **31** led to somewhat weaker Top1 inhibitory activity.

The Top1-mediated DNA fragmentation patterns produced by camptothecin, indenoisoquinolines **2**, **4**, and synthesized compounds **19** and **28**−**34** are presented in Figure 3. Inspection of the cleavage band intensities for compound **31** shows that, unlike indenoisoquinolines, cleavage at base pairs 44 and 62 is weak. However, the band for cleavage at the base pair 97 is more intense. This shows that the DNA cleavage site selectivity of chloronaphthyridinone **31** bears greater similarity to camptothecins than to indenoisoquinolines. In the case of compound **32**, it is difficult to come to a similar conclusion, as only bands for cleavages at the 97 and 119 sites are intense enough to be significant. The behavior of **32** is different from the camptothecins, the indenoisoquinolines, and the chlorodibenzonaphthyridinone **31**. Therefore, the dibenzonaphthyridines offer the opportunity to target the genome differently from either the camptothecins or the indenoisoquinolines.

Despite the fact that a number of structures of different classes of Top1 inhibitors within drug-Top1-DNA ternary complex have been obtained by X-ray crystallography,[20] the binding orientations of **3** and dibenzonaphthyridines like **6**

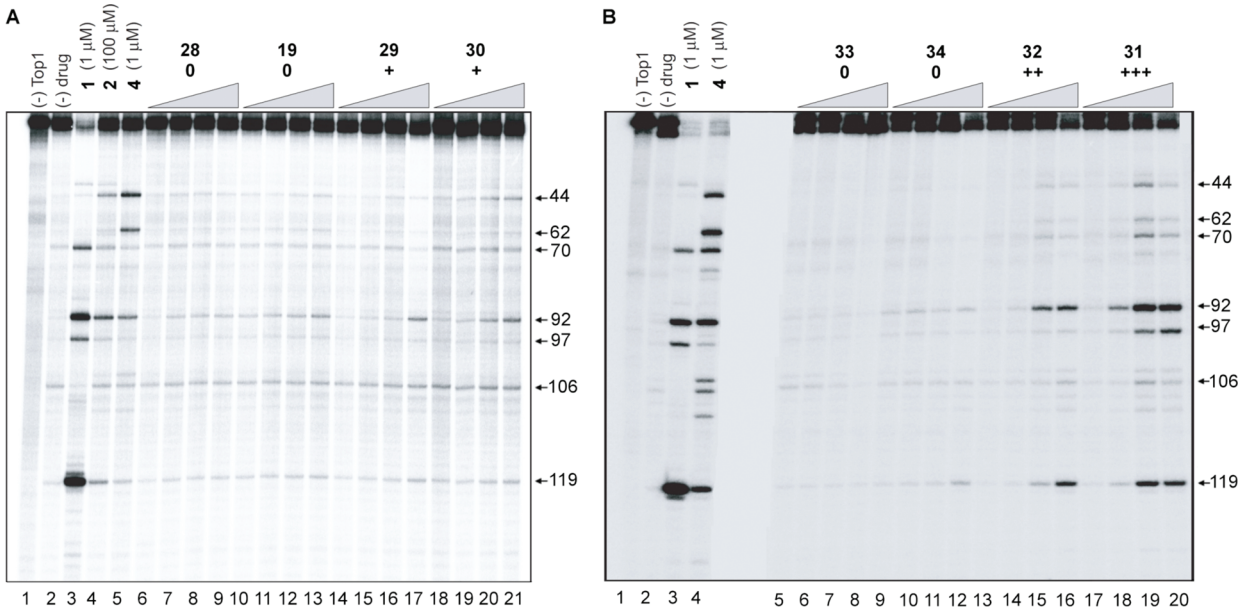


Figure 3. (A) Lane 1: DNA alone. Lane 2: Top1 alone. Lane 3: Top1 + **1** (1 μM). Lane 4: Top1 + **2** (100 μM). Lane 5: Top1 + **4** (1 μM). Lanes 6−21 (for compounds **19**, **28**−**30**): Top1 + indicated compound at 0.1 μM, 1 μM, 10 μM, 100 μM. (B) Lane 1: DNA alone. Lane 2: Top1 alone. Lane 3: Top1 + **1** (1 μM). Lane 4: Top1 + **4** (1 μM). Lanes 5−20 (for compounds **31**−**34**): Top1 + indicated compound at 0.1 μM, 1 μM, 10 μM, 100 μM. Numbers on right and arrows show the cleavage site positions. The activity of the compounds to produce Top1-mediated DNA cleavage was expressed semiquantitatively as follows: +, weak activity; ++ and +++, moderate activity; ++++, similar activity as 1 μM **1**.

have not been determined. Therefore, hypothetical binding models of naphthyridine-Top1-DNA ternary complexes were constructed in order to better understand the reasons underlying the change of activity in transitioning from indenoisoquinolines to naphthyridinedione and to chloronaphthyridinones. Binding models were constructed by means of docking and molecular mechanics tools. The geometry-optimized structures of dibenzonaphthyridines **14** and **31** and indenoisoquinoline **5** were docked using GOLD[35] in place of the ligand of the reported crystal structure of the **5**-Top1-DNA ternary complex (PDB ID: 1SC7[20]), and the geometries of the docked structures were optimized using the MMFF94s force field in *Sybyl 8.1*.[36] The docking protocol was capable of reproducing the original position of **5** within ternary complex with the 1.15 Å root-mean-square deviation (rmsd) of the heavy atoms of the indenoisoquinoline polycyclic core. The main goal of this molecular modeling was to calculate the preferred binding orientation of the dibenzonaphthyridine polycyclic core. Both **14** and **31** are oriented with the longer axis of the ligand oriented along the longer axis of the base pair. In the case of **14**, the isoquinolinone moiety faces the minor groove of the DNA and the distance of 2.6 Å between isoquinolinone oxygen and a nitrogen of the Arg364 side chain is consistent with a hydrogen bond (Figure 4). The most favorable orientation calculated for **31** is turned almost 180° around the axis perpendicular to the plane of the molecule relative to **14**. The chloroquinoline moiety of **31** faces the minor groove with a 3.2 Å distance between quinoline nitrogen and Arg364. Both molecules appear slightly bent out-of-plane. This bend results in one the benzene rings of **14** being closer to the thymine of the AT base pair, possibly causing unfavorable steric interactions. On the contrary, the bend of **31** nearly perfectly mirrors the out-of-plane distortion of the AT base pair, which may contribute to the better fit and higher Top1 inhibitory activity of this type of inhibitor.

Also, according to calculated binding orientation of these dibenzonaphthyridines, the methoxy groups of **14** would be located on the side of the intact DNA strand causing some additional unfavorable steric interaction with the DNA backbone (Figure 4). On the other hand, the methoxy groups of **31** would be placed toward the less restrictive scissile strand. This might explain the difference in activity between **14** and **31**. Further similarities between chlorodibenzonaphthyridinones and **1** are evident from comparison of the experimentally determined binding orientation for **1** and the calculated binding mode of **31**, which indicates that in both cases the quinoline fragment is located on the side of the intact DNA strand with the quinoline nitrogen facing Arg364 in the minor groove (Figure 5). In the case of the hypothetically determined binding mode of **31**, the quinoline nitrogen is placed somewhat closer to the Arg364 compared to **1**, forcing the polycyclic core of the dibenzonaphthyridinone to move deeper between flanking base pairs within the ternary complex.

In order to determine the antipoliferative activity, each compound was tested against 55 different human cancer cell lines in the National Cancer Institute screen.[37,38] The cells were incubated with the tested compounds at 100, 10, 1, 0.1, and 0.01 μM concentrations for 48 h before treatment with sulforhodamine B dye. Optical densities were recorded, and their ratios relative to that of the control were plotted as percentage growth against the $\log_{10}$ of the tested compound concentrations. The concentration that corresponds to 50% growth inhibition (GI_{50}) is calculated by interpolation between the points located above and below the 50% percentage growth. The chlorodibenzonaphthyridinone **32** bearing a morpholinopropyl chain on the lactam nitrogen was cytotoxic against human cancer cells at low micromolar concentrations. The cytotoxicities of **32** in selected cell lines are presented in Table 1, along with the mean-graph midpoint value of 5.6 μM. Cytotoxicity data recorded for the parent indenoisoquinoline **2** were added to Table 1 for comparison purposes. In general, cytotoxicity GI_{50} values were in the low micromolar range, although **32** was particularly cytotoxic in the leukemia K-562 and RPMI-8226 cell lines, the ovarian IGROV1 cancer

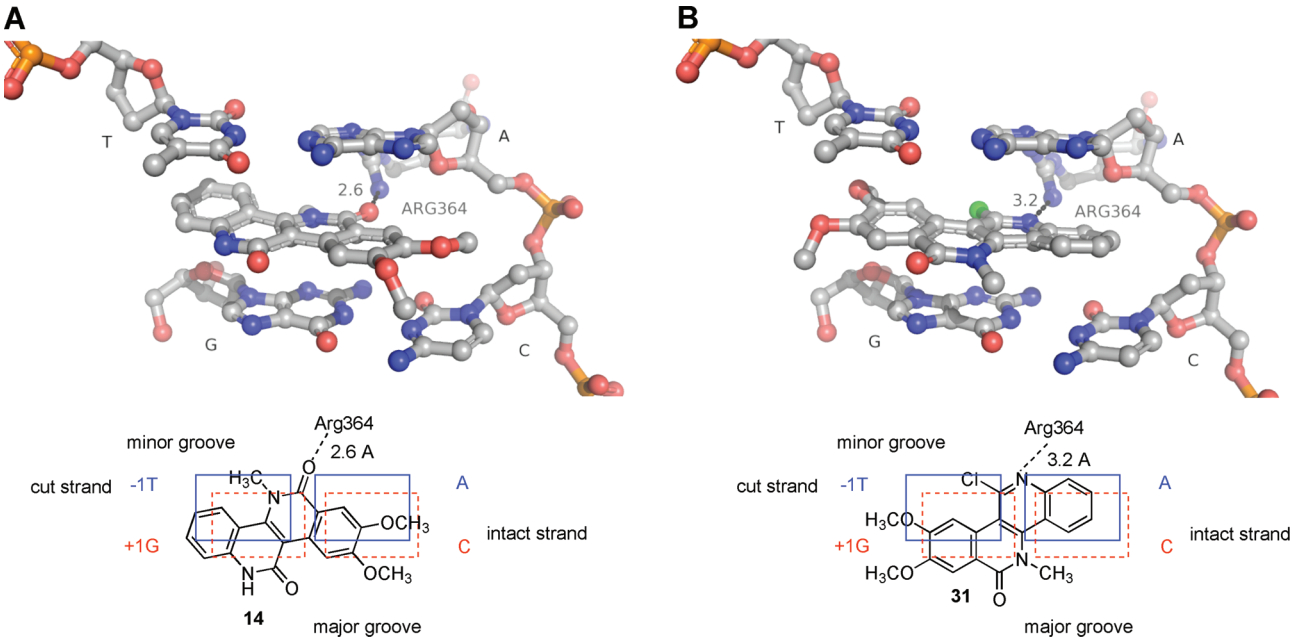


Figure 4. (A) Hypothetical binding mode of **14** within ternary complex. (B) Hypothetical binding mode of **31** within ternary complex.

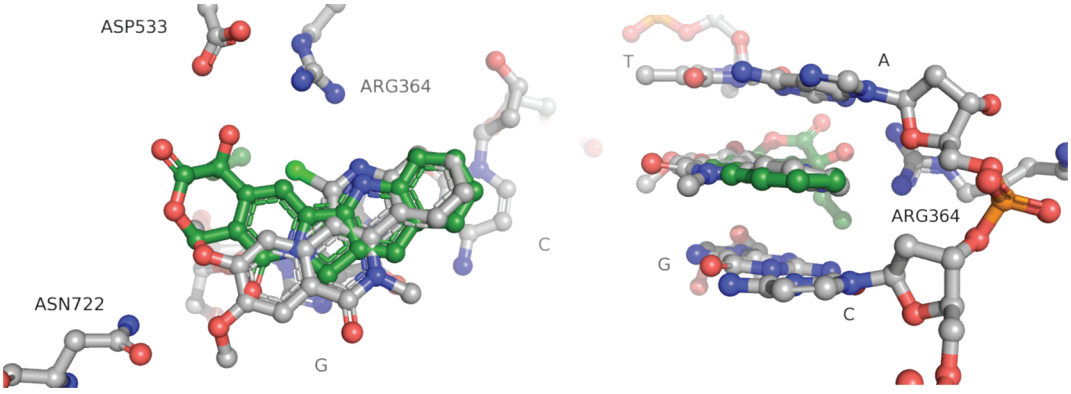


Figure 5. Comparison of the hypothetical binding orientation of **31** (gray) and the crystallographically obtained orientation of **1** (green). Left: view from the side of the AT base pair (not shown). Right: view from the side of the intact DNA strand.

cell line, the renal UO-31 cancer cell line, and the breast MCF7 cancer cell line.

In conclusion, a novel method for preparation of dibenzo[*c,h*][1,6]naphthyridinediones and chlorodibenzo[*c,h*][1,6]naphthyridinones has been developed. Two different pathways were successfully explored. The synthesis allowed introduction of a variety of chemically sensitive functional groups into the naphthyridinedione structure. The new chloronaphthyridinones represent promising lead compounds with Top1 inhibitory activities and low micromolar to submicromolar cytotoxicity GI_{50} values. Further replacement of the chloride of the quinoline moiety can serve as a route for expansion of this class of Top1 inhibitors. The tetramethoxy dibenzonaphthyridinedione **34** serves as an example of such modification. All target compounds were evaluated in a Top1-mediated DNA cleavage assay, and unique patterns of DNA cleavage were observed for **31** and **32** relative to the indenoisoquinolines, camptothecins, and each other. The hypothetical binding modes of dibenzonaphthyridinediones and chloronaphthyridinones were constructed by means of molecular docking and molecular mechanics energy minimization. Molecular modeling indicates that the lack of activity among naphthyridinediones could be attributed to a number of unfavorable steric interactions between the ligand and the cleavage complex. The calculated binding orientation of naphthyridinediones like **14** suggests that the conformationally flexible methoxy groups would face the intact DNA strand within ternary complex resulting in steric repulsion from the phosphodiester backbone and flanking base pairs. Replacement of the quinolinone moiety of **14** with chloroquinoline in **31** drastically changed the calculated binding mode of the tetracyclic core and eliminated such unfavorable interactions, which may explain the greater Top1 inhibitory activity of **31** vs **14**. The lactam substituent effects on Top1 inhibitory activity observed for **31** and **32** are different from those generally seen with the indenoisoquinolines, in which aminoalkyl substituents confer greater potency vs a methyl substituent.

Experimental Section

General. Melting points were determined using capillary tubes with a Mel-Temp apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded using an ARX300 300 MHz and DRX500 500 MHz Bruker NMR spectrometers. IR spectra were recorded using a

Table 1. Antiproliferative Activity of Dibenzonaphthyridine **32** and Indenoisoquinoline **2**

cancer cell line	**32** GI_{50} (μM)[a]	**2** GI_{50} (μM)[a]
	Leukemia	
K-562	0.2	6.0
RPMI-8226	0.1	> 100
	Lung	
HOP-62	4.7	2.8
	Colon	
HCT-116	4.16	11.5
COLO 205	0.33	> 100
	CNS	
SF-539	12.3	1.7
	Melanoma	
UACC-62	6.8	0.6
	Ovarian	
OVCAR-3	6.3	22.4
IGROV1	0.03	0.01
	Renal	
SN12C	13.2	25.7
UO-31	0.02	1.7
	Prostate	
DU-145	10.5	4.8
	Breast	
MCF7	0.4	1.9
MGM[b]	4.3	8.5

[a] The cytotoxicity GI_{50} values listed are the concentrations corresponding to 50% growth inhibition, and are the result of single determinations. [b] Mean-graph midpoint for growth inhibition of all human cancer cell lines successfully tested.

Perkin-Elmer 1600 series FTIR spectrometer. Purity of all tested compounds was ≥95%, as established by combustion analysis. Combustion microanalyses were performed at the Purdue University Microanalysis Laboratory, and the reported values were within 0.4% of the calculated values. Analytical thin-layer chromatography was carried out on Baker-flex silica gel IB2-F plates, and compounds were visualized with short-wavelength UV light. Silica gel flash chromatography was performed using 230−400 mesh silica gel.

Methyl 2-[(Methylimino)methyl]phenylcarbamate (8). A solution of methylamine in methanol (2 N, 3.6 mL, 7.2 mmol) and magnesium sulfate (3 g, 25 mmol) was added to **7** (1 g, 5.6 mmol), and the mixture was stirred for 6 h. Then, the precipitate was filtered off and washed with chloroform (3 × 20 mL). The combined filtrate was concentrated on a rotary evaporator to afford pale-yellow oil (1.08 g, 99%). IR (film) 1733, 1640 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 12.18 (s, 1 H), 8.41 (d, J = 8.4 Hz, 1 H), 8.28 (d, J = 1.0 Hz, 1 H), 7.41−7.33 (m, 1 H), 7.30−7.24 (m, 1 H), 7.08−6.99 (m, 1 H), 3.77 (s, 3 H), 3.50 (d, J = 1.3 Hz, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.57, 154.76, 140.29, 132.99, 131.32, 121.52, 120.46, 118.05, 52.12, 47.96; EIHRMS m/z M^+ calcd. for $C_{10}H_{12}N_2O_2$, 192.0899; found, 192.0897.

***cis*-3-[2-(Methoxycarbonylamino)phenyl]-4-carboxy-3,4-dihydro-6,7-dimethoxy-2-methyl-1(2*H*)-isoquinolone (10).** 4,5-Dimethoxyhomophthalic anhydride (**9**, 1.16 g, 5.2 mmol) was added to a solution of **8** (1 g, 5.2 mmol) in chloroform (10 mL) and the mixture was stirred for 2 h at room temperature. The precipitate was filtered, washed with chloroform (2 × 10 mL), and dried to yield acid cis **10** (750 mg, 35%): mp 212−213 °C (dec). IR (KBr) 1737, 1626, 1599, 1578 cm^{-1}; ^1H NMR (300 MHz, methanol-d_4) δ 7.80 (s, 1 H), 7.51 (s, 1 H), 7.29 (m, 2 H), 7.11 (t, J = 7.0 Hz, 1 H), 6.83 (m, 2 H), 5.33 (d, J = 6.60 Hz, 1 H), 4.45 (d, J = 6.60 Hz, 1 H), 3.80 (s, 3 H), 3.74 (s, 3 H), 3.65 (s, 3 H), 2.80 (s, 3 H); ESIMS m/z (rel intensity) 851 (35), 459 (13), 436 (100), 415 (6), 215 (15); ESIHRMS m/z MNa^+ calcd. for $C_{21}H_{22}N_2O_7$, 437.1325; found, 437.1333.

The combined filtrates were evaporated to dryness and the remaining solid was recrystallized from benzene to provide trans **10** (1.3 g, 60%): mp 148−150 °C (dec). IR (KBr) 1730, 1716, 1640 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.91 (s, 1 H), 9.18 (s, 1 H), 7.46 (s, 1 H), 7.36 (s, 1 H), 7.29 (d, J = 7.7 Hz, 1 H), 7.23 (t, J = 7.5 Hz, 1 H), 7.05 (t, J = 7.5 Hz, 1 H), 6.71 (s, 1 H), 6.63 (d, J = 7.8 Hz, 1 H), 5.58 (s, 1 H), 3.81 (s, 3 H), 3.80 (s, 1 H), 3.71 (s, 3 H), 3.69 (s, 3 H), 2.88 (s, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.38, 163.02, 155.44, 151.52, 148.23, 135.30, 134.59, 128.34, 128.18, 126.55, 126.25, 125.40, 121.11, 112.12, 109.24, 58.18, 55.63, 55.45, 51.98, 47.78, 33.82.

***trans*-3-[2-(Methoxycarbonylamino)phenyl]-3,4-dihydro-6,7-dimethoxy-4-methoxycarbonyl-2-methyl-1(2*H*)-isoquinolone (11).** Thionyl chloride (10 mL) was added slowly to a suspension of cis **10** (0.75 g, 1.8 mmol) and trans **10** (1.35 g, 3.3 mmol) in methanol (100 mL) at 0 °C. The resulting mixture was stirred for 6 h at 0 °C. After completion of the reaction (TLC), the reaction mixture was poured slowly into a mixture of ice (200 g) and a saturated solution of sodium bicarbonate (100 mL). The resulting mixture was extracted with chloroform (3 × 150 mL), and the combined extracts were dried with sodium sulfate, filtered though a thin pad of silica gel, and evaporated to dryness to obtain **11** as amorphous glassy solid (2.1 g, 96%). IR (film) 1733, 1637 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.67 (s, 1 H), 7.52 (d, J = 7.7 Hz, 1 H), 7.25 (t, J = 7.0 Hz, 1 H), 7.03 (t, J = 7.5 Hz, 1 H), 6.83 (d, J = 7.8 Hz, 1 H), 6.73 (s, 1 H), 6.54 (s, 1 H), 5.45 (s, 1 H), 3.95 (s, 3 H), 3.89 (s, 1 H), 3.82 (s, 3 H), 3.72 (s, 3 H), 3.63 (s, 1 H), 3.03 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.06, 164.17, 155.49, 152.15, 149.18, 134.39, 132.53, 129.10, 126.67, 126.53, 125.93, 125.14, 121.60, 111.86, 110.17, 59.32, 56.29, 56.20, 53.27, 53.06, 48.66, 34.57; ESIMS m/z (rel intensity) 429 (MH^+, 100), 451 (56); ESIHRMS m/z MH^+ calcd. for $C_{22}H_{24}N_2O_7$, 429,1662; found, 429.1665.

Methyl 6,7-Dimethoxy-3-(2-(methoxycarbonylamino)phenyl)-2-methyl-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (12). NaHMDS (1 M solution in THF−heptanes, 1.8 mL, 1.8 mmol) was slowly added to a solution of **11** (320 mg, 0.75 mmol) in dry THF (20 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1 h, and then a solution of phenylselenyl chloride (216 g, 1.13 mmol) in dry THF (5 mL) was added dropwise and the mixture was stirred at −78 °C for 2 h. The reaction mixture was allowed to warm to room temperature and stirred at this temperature for 3 h. The reaction mixture was quenched by slow addition of 1 N HCl (5 mL) at 0 °C, diluted with water (50 mL), and extracted with chloroform (4 × 50 mL). The combined extracts were washed with water and brine, dried with sodium sulfate, and evaporated under reduced pressure. The residue was dissolved in THF (10 mL). Acetic acid (1 mL) and hydrogen peroxide (30%, 5 mL) were added sequentially at 0 °C. The reaction mixture was warmed to room temperature and stirred for 6 h. Saturated aqueous sodium bicarbonate (5 mL) was added to the mixture at 0 °C. Chloroform (3 × 10 mL) was used to extract the product. The combined extracts were washed with water and brine, dried with sodium sulfate, and evaporated to afford the product (198 mg, 62%): mp 165−170 °C. IR (film) 1730, 1718, 1638 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.05 (d, J = 6.2 Hz, 1 H), 7.86 (s, 1 H), 7.49 (t, J = 7.9 Hz, 1 H), 7.22−7.16 (m, 2 H), 7.16−7.11 (m, 1 H), 6.53 (s, 1 H), 4.04 (s, 3 H), 3.99 (s, 3 H), 3.72 (s, 3 H), 3.45 (s, 3 H), 3.24 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.39, 162.03, 154.22, 154.07, 150.14, 139.65, 136.41, 130.97, 129.29, 128.59, 124.49, 119.35, 112.69, 108.14, 104.80, 56.52, 56.37, 52.75, 52.26, 33.18; ESIMS m/z (rel intensity) 449 (18), 427 (MH^+, 100), 395 (78); ESIHRMS m/z MH^+ calcd. for $C_{22}H_{22}N_2O_7$, 427,1505; found, 427.1507.

8,9-Dimethoxy-5-methyldibenzo[*c*,*h*][1,6]naphthyridine-6,11-(5*H*,12*H*)-dione (14). The methyl ester **12** (160 mg, 0.38 mmol) was added to a stirred solution of KOH (1.04 g, 18.5 mmol) in

water−ethylene glycol mixture (15:15 mL), and the resulting mixture was heated to reflux for 4 d. After the mixture was cooled to room temperature, it was diluted with water (20 mL), acidified with acetic acid (2.5 mL), and extracted with chloroform (3 × 100 mL); the combined extracts were washed with water (50 mL) and brine (50 mL), dried with sodium sulfate, and evaporated to obtain a white powder (105 mg, 83%): mp > 350 °C. IR (KBr) 1710, 1670, 1651 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.86 (s, 1 H), 9.41 (s, 1 H), 8.18 (d, J = 8.30 Hz, 1 H), 7.72 (s, 1 H), 7.55 (t, J = 7.20 Hz, 1 H), 7.44 (d, J = 8.30 Hz, 1 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 3.86 (s, 3 H); positive ESIMS m/z (rel intensity) 374 (60), 337 (MH^+, 19), 318 (100); negative ESIMS m/z (rel intensity) 335 ([M − H^+], 100). Anal. Calcd for $C_{19}H_{16}N_2O_4$: C, 67.85; H, 4.79; N, 8.33. Found: C, 67.52; H, 4.56; N, 7.97.

Methyl 2-[(Allylimino)methyl]phenylcarbamate (15). Methyl 2-formylphenylcarbamate (**7**, 1 g, 5.6 mmol) and magnesium sulfate (3 g, 25 mmol) were added to a solution of allylamine (1.5 g, 26 mmol) in chloroform (10 mL) and mixture was stirred overnight. Then, the mixture was filtered and the residue was washed with chloroform (2 × 10 mL). The combined filtrates were washed with water (3 × 10 mL) brine (10 mL), dried with sodium sulfate, and concentrated on a rotary evaporator to afford **15** as yellow oil (1.2 g, 99%). IR (film) 1732, 1635 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 12.25 (s, 1 H), 8.43 (d, J = 8.4 Hz, 1 H), 8.28 (s, 1 H), 7.40−7.34 (m, 1 H), 7.27 (dd, J = 7.7, 1.4 Hz, 1 H), 7.02 (td, J = 7.5, 0.9 Hz, 1 H), 6.05 (ddt, J = 17.1, 10.5, 5.3 Hz, 1 H), 5.25 (ddd, J = 17.2, 3.3, 1.6 Hz, 1 H), 5.20−5.13 (m, 1 H), 4.26−4.17 (m, 2 H), 3.76 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.94, 154.73, 140.38, 135.38, 133.25, 131.54, 121.49, 120.32, 118.05, 115.99, 62.88, 52.10; ESIMS m/z (rel intensity) 219 (MH^+, 100); EIHRMS m/z M^+ calcd. for $C_{12}H_{14}N_2O_2$, 218.1055; found, 218.1053.

***cis*-2-Allyl-6,7-dimethoxy-3-(2-(methoxycarbonylamino)phenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (16).** 4,5-Dimethoxyhomophthalic anhydride (**9**, 444 mg, 2 mmol) was added to a solution of **15** (700 mg, 2 mmol) in chloroform (5 mL), and the mixture was stirred at room temperature for 18 h. The precipitate was collected and washed with chloroform (2 × 20 mL) to obtain a white solid (300 mg, 26%): mp 219−220 °C (dec). IR (KBr) 1746, 1618 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 12.80 (s, 1 H), 8.79 (s, 1 H), 7.52 (s, 1 H), 7.37 (d, J = 7.9 Hz, 1 H), 7.30−7.20 (m, 1 H), 7.12−7.01 (m, 2 H), 6.87 (s, 1 H), 5.72−5.50 (m, 1 H), 5.39 (d, J = 5.9 Hz, 1 H), 4.99 (dd, J = 10.3, 1.3 Hz, 1 H), 4.91 (dd, J = 17.2, 1.5 Hz, 1 H), 4.46−4.36 (m, 2 H), 3.83 (s, 3 H), 3.77 (s, 3 H), 3.63 (s, 3 H), 3.29 (dd, J = 15.4, 6.6 Hz, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.54, 164.02, 155.37, 151.91, 148.35, 136.46, 133.58, 131.43, 128.66, 128.30, 128.26, 125.43, 121.80, 117.18, 110.54, 110.25, 55.95, 55.84, 54.54, 52.18, 48.37, 47.13; ESIMS m/z (rel intensity) 479 (MK^+, 100), 441 (MH^+, 26); ESIHRMS m/z MH^+ calcd. for $C_{23}H_{24}N_2O_7$, 441.1662; found, 441.1657.

5-Allyl-8,9-dimethoxydibenzo[*c*,*h*][1,6]naphthyridine-6,11(5*H*,-12*H*)-dione (19). Thionyl chloride (10 mL) was added slowly to a suspension of **16** (990 mg, 2.24 mmol) in methanol (50 mL) at 0 °C. The resulting mixture was stirred for 6 h at room temperature. After completion of the reaction, the mixture was poured slowly into ice (200 g) and neutralized with a saturated aqueous sodium bicarbonate solution (100 mL). The organic products were extracted with chloroform (3 × 50 mL), and the combined extracts were dried with sodium sulfate and concentrated. The solid residue (**17**, cis/trans mixture) was redissolved in dry THF (30 mL). NaHMDS (1 M solution in THF−heptanes, 5 mL, 5 mmol) was slowly added to the resulting solution. The reaction mixture was stirred at −78 °C for 2 h, and then a solution of phenylselenyl chloride (728 mg, 3.1 mmol) in dry THF (10 mL) was added dropwise and the mixture was stirred at −78 °C for an additional 2 h. The reaction mixture was allowed to warm to room temperature and stirred at this temperature overnight. The reaction mixture was quenched by slow addition of 1 N HCl (55 mL) at 0 °C, diluted with water (50 mL), and extracted with chloroform (4 × 50 mL). The combined extracts were washed with water and brine, dried with sodium sulfate, and evaporated under reduced pressure. The residue was dissolved in THF (50 mL). Acetic acid (5 mL) and hydrogen peroxide (30%, 30 mL) were added sequentially at 0 °C. The reaction mixture was warmed to room temperature and stirred overnight. Saturated sodium bicarbonate (15 mL) was added to the mixture at 0 °C. The precipitate was collected by filtration, then washed with water and hot methanol, providing **19** as a white solid mass (118 mg, 15%). The combined filtrates were extracted with chloroform (3 × 10 mL). The combined extracts were washed with water and brine, dried with sodium sulfate, and evaporated to dryness. The residue was added to a mixture of potassium hydroxide (560 mg, 10 mmol), water (10 mL), and ethylene glycol (30 mL). The resulting solution was heated to reflux for 12 h. After cooling down to ambient temperature, acetic acid was used to neutralize the solution. The precipitate was collected by filtration and washed with water and hot methanol, providing the rest of **19** (225 mg, 28%, total yield 343 mg, 43%): mp 298−300 °C. IR (KBr) 1691, 1602, 1500 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 11.86 (s, 1 H), 9.38 (s, 1 H), 8.14 (d, J = 8.4 Hz, 1 H), 7.69 (s, 1 H), 7.53 (t, J = 7.6 Hz, 1 H), 7.43 (d, J = 8.2 Hz, 1 H), 7.18 (t, J = 7.7 Hz, 1 H), 6.33−6.14 (m, 1 H), 5.31 (d, J = 10.7 Hz, 1 H), 5.17 (d, J = 17.5 Hz, 1 H), 4.87 (s, 2 H), 3.91 (s, 3 H), 3.90 (s, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.69, 161.09, 153.31, 149.53, 144.72, 137.67, 135.41, 130.73, 129.05, 126.25, 121.17, 118.98, 116.65, 116.25, 113.17, 108.21, 107.71, 107.41, 55.93, 55.79, 53.72; ESIMS m/z (rel intensity) 475 (MNa^+, 100), 453 (MH^+, 18). Anal. Calcd for $C_{21}H_{18}N_2O_4$: C, 69.60; H, 5.01; N, 7.73. Found: C, 69.22; H, 4.88; N, 7.64.

Methyl 2-[(3-Bromopropylimino)methyl]phenylcarbamate (21). Methyl 2-formylphenylcarbamate (**7**, 2.5 g, 14 mmol) and magnesium sulfate (5 g, 42 mmol) were added to a solution of 3-bromopropylamine hydrochloride (3.68 g, 16.8 mmol) and triethylamine in chloroform (30 mL), and the mixture was stirred for 24 h. The mixture was filtered, and the residue was washed with chloroform (2 × 30 mL). The combined filtrates were washed with water (2 × 30 mL) and brine (30 mL), dried with sodium sulfate, and concentrated to afford **21** as a yellow oil (3.95 g, 94.5%). IR (film) 1730, 1638 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.38 (d, J = 8.4 Hz, 1 H), 8.30 (s, 1 H), 7.35 (t, J = 7.7 Hz, 2 H), 7.26 (d, J = 7.7 Hz, 1 H), 7.00 (t, J = 7.4 Hz, 1 H), 3.74 (s, 3 H), 3.69 (t, J = 6.3 Hz, 2 H), 3.50 (t, J = 6.6 Hz, 2 H), 2.20 (pent, J = 6.3 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.74, 154.30, 140.07, 133.00, 131.32, 121.25, 119.79, 117.69, 58.17, 52.87, 33.16, 31.15; ESIMS m/z (rel intensity) 299/301 (MH^+, 100/99); EIHRMS m/z M^+ calcd. for $C_{12}H_{15}BrN_2O_2$, 298.0317; found, 298.0320.

***cis*-2-[3-(*tert*-Butyldimethylsilyloxy)propyl]-6,7-dimethoxy-3-(2-(methoxycarbonylamino)phenyl)-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (22).** Methyl 2-formylphenylcarbamate (**7**, 1.79 g, 10 mmol) and magnesium sulfate (3 g, 25 mmol) were added to a solution of 3-(*tert*-butyldimethylsilyloxy)propan-1-amine (1.89 g, 10 mmol) in chloroform (10 mL) and the mixture was stirred overnight. Then, the mixture was filtered, and the residue was washed with chloroform (2 × 10 mL). The combined filtrate was concentrated on a rotary evaporator to afford crude **20** as yellow oil (3.37 g, 96%) that was used without additional purification. ^1H NMR (300 MHz, $CDCl_3$) δ 8.38 (d, J = 8.4 Hz, 1 H), 8.30 (s, 1 H), 7.35 (t, J = 7.7 Hz, 2 H), 7.26 (d, J = 7.7 Hz, 1 H), 7.00 (t, J = 7.4 Hz, 1 H), 3.71−3.69 (m, 5 H), 3.60 (m, 2 H), 1.83 (pent, J = 6.3 Hz, 2 H), 0.84 (s, 9 H), −0.01 (s, 6 H); ESIMS m/z (rel intensity) 299/301 (MH^+, 100/99). Anhydride **9** (444 mg, 2 mmol) was added to a solution of **20** (700 mg, 2 mmol) in chloroform (5 mL), and the mixture was stirred at room temperature for 18 h. The precipitate was collected and washed with chloroform (2 × 20 mL) to obtain a white solid (300 mg, 26%): mp 252−258 °C (dec). IR (KBr) 3357, 1733 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.91 (s, 1 H), 7.50 (s, 1 H), 7.41 (d, J = 8.3 Hz,

1 H), 7.28−7.19 (m, 1 H), 7.01 (d, J = 6.2 Hz, 2 H), 6.86 (s, 1 H), 5.46 (d, J = 5.9 Hz, 1 H), 4.39 (d, J = 5.9 Hz, 1 H), 3.85−3.72 (m, 7 H), 3.64 (s, 3 H), 3.52−3.39 (m, 2 H), 2.78 (ddd, J = 13.7, 9.1, 5.0 Hz, 1 H), 1.54 (ddd, J = 21.7, 13.6, 7.4 Hz, 2 H), 0.78 (s, 9 H), −0.07 (s, 6 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.17, 163.66, 155.01, 151.40, 147.92, 136.25, 128.22, 127.86, 124.82, 121.78, 110.13, 109.91, 60.88, 55.58, 55.49, 54.52, 51.81, 48.07, 42.37, 30.23, 25.79, 17.88, −5.42, −5.45; ESIMS m/z (rel intensity) 573 (MH^+, 64), 441 (100); ESIHRMS m/z MH^+ calcd. for $C_{29}H_{40}N_2O_8Si$, 573.2632; found, 573.2636.

***cis*-2-(3-Bromopropyl)-6,7-dimethoxy-3-[2-(methoxycarbonylamino)phenyl]-1-oxo-1,2,3,4-tetrahydroisoquinoline-4-carboxylic Acid (23).** 4,5-Dimethoxyhomophthalic anhydride (**9**, 222 mg, 1 mmol) was added to a solution of **21** (300 mg, 10 mmol) in chloroform (5 mL), and the mixture was stirred at room temperature for 16 h. The precipitate was collected and washed with chloroform (2 × 20 mL) to obtain a white solid (208 mg, 40%): mp 264−266 °C (dec). IR (KBr) 3391, 1726 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.81 (s, 1 H), 8.84 (s, 1 H), 7.52 (s, 1 H), 7.40 (d, J = 8.1 Hz, 1 H), 7.30−7.20 (m, 1 H), 7.02 (t, J = 7.3 Hz, 1 H), 6.96 (dd, J = 7.9, 1.3 Hz, 1 H), 6.86 (s, 1 H), 5.50 (d, J = 6.0 Hz, 1 H), 4.51 (d, J = 6.0 Hz, 1 H), 3.91−3.79 (m, 4 H), 3.76 (d, J = 14.9 Hz, 3 H), 3.66 (d, J = 12.4 Hz, 3 H), 3.45−3.37 (m, 2 H), 2.84 (ddd, J = 13.6, 8.2, 5.6 Hz, 1 H), 1.96 (ddt, J = 21.7, 14.2, 7.0 Hz, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.03, 163.68, 155.04, 151.52, 147.93, 136.30, 128.36, 127.63, 127.56, 125.03, 121.49, 110.05, 109.99, 55.58, 55.50, 54.67, 51.89, 47.82, 44.09, 32.09, 30.63; ESIMS m/z (rel intensity) 299/301 (MH^+, 100/99) 543/545 (MNa^+, 68/63), 441 (100).

5-(3-Hydroxypropyl)-8,9-dimethoxydibenzo[*c*,*h*][1,6]naphthyridine-6,11(5*H*,12*H*)-dione (28). Trimethylsilyldiazomethane (0.12 mL, 2.0 M in diethyl ether, 0.25 mmol) was added dropwise to a suspension of **22** (110 mg, 0.19 mmol) in methanol (1 mL) and THF (3 mL) at −10 to 0 °C, and the mixture was stirred at −10 °C for 30−45 min after addition. The solvent was removed under reduced pressure at 20−25 °C. The residue (**24**, cis/trans mixture) was dissolved in anhydrous 1,4-dioxane (5 mL), and DDQ (100 mg, 0.44 mmol) was added to the solution. The reaction mixture was heated at reflux for 3−4 h. 1,4-Dioxane was evaporated under reduced pressure and chloroform (30 mL) was added to the residue. The mixture was washed with sodium bicarbonate (5%, 2 × 10 mL), water (15 mL), dried with sodium sulfate, and filtered through a thin layer of silica gel, eluting with chloroform. The combined filtrates were evaporated under reduced pressure. The amorphous solid containing **26** was added to a stirred solution of KOH (180 mg, 18.5 mmol) in water−ethylene glycol mixture (1 + 3 mL) at room temperature, and the mixture was heated at reflux on oil bath for 24 h. After the mixture was cooled to room temperature, it was diluted with water (5 mL) and acidified with acetic acid (0.5 mL). The white precipitate was collected by filtration to obtain **28** as a white powder (24 mg, 47%): mp 313 °C. IR (KBr) 1650, 1604 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.85 (s, 1 H), 9.36 (s, 1 H), 8.02 (d, J = 8.3 Hz, 1 H), 7.71 (s, 1 H), 7.54 (t, J = 7.6 Hz, 1 H), 7.44 (d, J = 8.1 Hz, 1 H), 7.23 (t, J = 7.6 Hz, 1 H), 4.51 (t, J = 6.8 Hz, 2 H), 4.43 (t, J = 4.8 Hz, 1 H), 3.92 (s, 3 H), 3.91 (s, 3 H), 3.22 (dd, J = 10.8, 5.5 Hz, 2 H), 2.05−1.81 (m, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.82, 160.88, 152.96, 149.17, 144.41, 137.37, 130.28, 128.54, 126.19, 121.07, 118.95, 115.89, 113.14, 107.83, 107.40, 57.90, 55.63, 55.50, 48.77, 31.35; positive ESIMS m/z (rel intensity) 381 (MH^+, 100). Anal. Calcd for $C_{21}H_{20}N_2O_5$: C, 66.31; H, 5.30; N, 7.36. Found: C, 66.03; H, 5.16; N, 7.26.

8,9-Dimethoxy-5-(3-morpholinopropyl)dibenzo[*c*,*h*][1,6]naphthyridine-6,11(5*H*,12*H*)-dione (29). Trimethylsilyldiazomethane (0.12 mL, 2.0 M in diethyl ether, 0.25 mmol) was added dropwise to a suspension of **23** (100 mg, 0.19 mmol) in methanol (1 mL) and THF (3 mL) at −10 to 0 °C. The mixture was kept in this temperature range for 30 min after addition. The reaction mixture became clear after stirring at 0 °C for 30−45 min. The solvent was removed under reduced pressure at 20−25 °C to yield solid residue **25** that melts at 183−184 °C. Without additional purification, the residue was dissolved in anhydrous 1,4-dioxane (5 mL) and DDQ (100 mg, 0.44 mmol) was added to the solution. The reaction mixture was heated at reflux for 3−4 h. 1,4-Dioxane was evaporated under reduced pressure, and chloroform (30 mL) was added to the residue. The resulting mixture was washed with sodium bicarbonate (5%, 2 × 10 mL), water (15 mL), dried with sodium sulfate, and filtered through a thin layer of silica gel, eluting with chloroform. The combined filtrates were evaporated under reduced pressure. The residue containing **27** was redissolved in dry DMF (5 mL). Morpholine (200 mg, 2.3 mmol) was added to the solution, and the mixture was heated to reflux for 18 h. The mixture was then cooled to room temperature (precipitate started forming prior to cooling). The precipitate was collected by filtration and washed with methanol and diethyl ether on a filter, and the product was dried to yield pure **29** (48 mg, 56%): mp 250−252 °C. IR (KBr) 1649, 1603 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.83 (s, 1 H), 9.35 (s, 1 H), 7.94 (d, J = 8.3 Hz, 1 H), 7.71 (d, J = 2.5 Hz, 1 H), 7.62−7.51 (m, 1 H), 7.44 (dd, J = 8.2, 1.1 Hz, 1 H), 7.29−7.14 (m, 1 H), 4.57 (t, J = 6.1 Hz, 2 H), 3.91 (s, 3 H), 3.91 (s, 3 H), 3.13 (s, 4 H), 1.94 (s, 4 H), 1.89−1.82 (m, 2 H), 1.79 (d, J = 5.6 Hz, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.66, 160.94, 152.99, 149.12, 145.03, 137.34, 130.16, 128.54, 126.05, 120.89, 118.80, 115.78, 113.64, 107.73, 107.42, 107.09, 65.78, 55.61, 55.50, 53.85, 52.65, 48.80; positive ESIMS m/z (rel intensity) 450 (MH^+, 92); negative ESIMS m/z (rel intensity) 448 ([M − H$^+$], 100). Anal. Calcd for $C_{25}H_{27}N_3O_5$: C, 66.80; H, 6.05; N, 9.35. Found: C, 66.49; H, 5.73; N, 9.22.

5-(3-(1*H*-Imidazol-1-yl)propyl)-8,9-dimethoxydibenzo[*c*,*h*][1,6]-naphthyridine-6,11(5*H*,12*H*)dione (30). Trimethylsilyldiazomethane (0.12 mL, 2.0 M in diethyl ether, 0.25 mmol) was added dropwise to a suspension of **23** (100 mg, 0.19 mmol) in methanol (1 mL) and THF (3 mL) at −10 to 0 °C. The mixture was kept at this temperature for 30 min after addition. The reaction mixture became clear after stirring at 0 °C for 30−45 min. The solvent was removed under reduced pressure at 20−25 °C to yield solid residue **25** that melts at 183−184 °C. Without additional purification, the residue was dissolved in anhydrous 1,4-dioxane (5 mL) and DDQ (100 mg, 0.44 mmol) was added to the solution. The reaction mixture was heated at reflux for 3−4 h. 1,4-Dioxane was evaporated under reduced pressure and chloroform (30 mL) was added to the residue. The mixture was washed with aqueous sodium bicarbonate (5%, 2 × 10 mL), water (15 mL), dried with sodium sulfate, and filtered through a thin layer of silica gel, eluting with chloroform. The combined filtrates were evaporated under reduced pressure. The residue containing **27** was redissolved in dry DMF (5 mL). Imidazole (160 mg, 2.3 mmol) was added to the solution, and the mixture was heated to reflux for 18−20 h. The mixture was then cooled to room temperature (precipitate started forming prior to cooling). The precipitate was collected by filtration and washed with methanol and diethyl ether on a filter, and the product was dried to yield pure **30** (47 mg, 58%): mp 288−290 °C. IR (KBr) 1659, 1636 1601 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.86 (s, 1 H), 9.36 (s, 1 H), 7.77 (d, J = 8.3 Hz, 1 H), 7.71 (s, 1 H), 7.56−7.48 (m, 2 H), 7.42 (d, J = 8.2 Hz, 1 H), 7.13 (t, J = 7.7 Hz, 1 H), 7.08 (s, 1 H), 6.81 (s, 1 H), 4.40−4.28 (m, 2 H), 3.99−3.91 (m, 5 H), 3.91 (s, 3 H), 2.40−2.23 (m, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.77, 160.78, 153.03, 149.23, 143.94, 137.34, 137.14, 130.29, 128.59, 128.46, 125.56, 121.15, 119.16, 118.82, 115.92, 112.77, 107.86, 107.55, 107.39, 55.63, 55.50, 48.67, 43.54, 29.61; positive ESIMS m/z (rel intensity) 431 (MH^+, 100); negative ESIMS m/z (rel intensity) 429 ([M − H$^+$], 100); ESIHRMS m/z MH^+ calcd. for $C_{24}H_{22}N_4O_4$, 431.1719; found, 431.1120. Anal. Calcd for $C_{24}H_{22}N_4O_4 \cdot 0.7H_2O$: C, 65.06; H, 5.32; N, 12.65. Found: C, 64.98; H, 5.40; N, 12.37.

11-Chloro-8,9-dimethoxy-5-methyldibenzo[*c*,*h*][1,6]naphthyridin-6(5*H*)-one (31). Dry DMF (1 mL) was added slowly to a mixture of **14** (84 mg, 0.25 mmol) and phosphoryl chloride (10 mL,

108 mmol) at 0 °C. The mixture was allowed to warm up to room temperature. The mixture was heated to 70 °C and kept at 65−70 °C for 2 h. After disappearance of starting material (TLC), the mixture was cooled to 0 °C, poured into the ice (50 g), and neutralized with concentrated ammonium hydroxide. The precipitate was collected by filtration and purified by preparative TLC to get an off-white solid (54 mg, 61%): mp 242−243 °C. IR (KBr) 1650, 1605 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1 H), 8.20 (d, J = 8.6 Hz, 1 H), 8.01 (d, J = 8.3 Hz, 1 H), 7.94 (s, 1 H), 7.71 (t, J = 7.6 Hz, 1 H), 7.54 (t, J = 7.7 Hz, 1 H), 4.07 (s, 3 H), 4.06 (s, 3 H), 4.03 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.12, 152.95, 150.30, 146.61, 145.57, 145.40, 130.36, 128.69, 126.51, 125.75, 124.93, 119.96, 118.71, 111.14, 108.76, 107.97, 56.48, 40.73; positive ESIMS m/z (rel intensity) 355/357 (MH^+, 100/33). Anal. Calcd for $C_{19}H_{15}ClN_2O_3 \cdot 0.5H_2O$: C, 62.73; H, 4.43; N, 7.70. Found: C, 62.74; H, 4.75; N, 7.38.

11-Chloro-8,9-dimethoxy-5-(3-morpholinopropyl)dibenzo[*c*,*h*]-[1,6]-naphthyridin-6(5*H*)-one (32). Precursor **29** (22.5 mg, 0.05 mmol) was mixed with phosphoryl chloride (3 mL, 32 mmol) and phosphorus pentachloride (100 mg, 0.48 mmol) was added slowly at room temperature. Starting material dissolved upon PCl_5 addition, forming a clear yellow solution. The reaction mixture was stirred for 1 h and then heated at reflux for 1.5 h. After disappearance of starting material, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The brown oily residue was dissolved completely in ice-cold water (3 mL), and concentrated ammonium hydroxide solution was added dropwise to neutral pH. The cloudy aqueous solution was extracted with ethyl acetate (3 × 30 mL). The combined extracts were washed with concentrated ammonium hydrochloride solution, dried with sodium sulfate, and evaporated to dryness under reduced pressure to yield **32** as an amorphous solid (15 mg, 66%): mp 185−190 °C (dec). IR (KBr) 1651, 1606 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.52 (s, 1 H), 8.17 (d, J = 8.5 Hz, 1 H), 7.95 (d, J = 7.6 Hz, 1 H), 7.82 (t, J = 7.6 Hz, 1 H), 7.77 (s, 1 H), 7.71 (t, J = 7.4 Hz, 1 H), 4.56−4.28 (m, 2 H), 3.97 (s, 3 H), 3.96−3.88 (m, 5 H), 3.76 (t, J = 12.0 Hz, 2 H), 3.42 (d, J = 11.9 Hz, 2 H), 3.18 (s, 2 H), 3.05 (d, J = 11.1 Hz, 2 H), 2.42 (s, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.90, 152.44, 149.88, 145.75, 144.76, 144.57, 130.45, 127.92, 126.50, 125.47, 124.66, 119.34, 117.80, 110.25, 108.06, 107.89, 63.23, 55.91, 55.78, 53.12, 50.89, 48.68, 22.93; positive ESIMS m/z (rel intensity) 468/470 (MH^+, 100/32), 381/383 (97/30). Anal. Calcd for $C_{25}H_{28}Cl_3N_3O_4 \cdot 1.2H_2O$: C, 53.38; H, 5.45; N, 7.47. Found: C, 53.36; H, 5.45; N, 7.10.

6,11-Dichloro-8,9-dimethoxydibenzo[*c*,*h*][1,6]naphthyridine (33). Compound **14** (700 mg, 2.04 mmol) and phosphorus pentachloride (868 mg, 4 mmol) were dissolved in phosphoryl chloride (25 mL, 270 mmol) at room temperature. The resulting solution was stirred for 2 h, and then heated at reflux for 6 h. The reaction mixture was cooled to room temperature, concentrated to about 5−6 mL, and quenched by pouring slowly into ice (50 g). The mixture was neutralized by adding a concentrated solution of ammonium hydroxide. The precipitate was separated by filtration and washed several times with small portions (5 mL) of ice-cold water providing light-gray powder (690 mg, 96%): mp 245−246 °C. IR (KBr) 1655, 1616, 1570, 1559, 1517 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.23 (s, 1 H), 9.03 (d, J = 7.8 Hz, 1 H), 8.06 (d, J = 8.1 Hz, 1 H), 7.82 (t, J = 7.2 Hz, 1 H), 7.79−7.69 (m, 2 H), 4.15 (s, 3 H), 4.10 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.73, 153.76, 150.54, 146.72, 146.51, 144.52, 131.02, 129.64, 128.02, 127.96, 124.95, 122.10, 114.65, 106.93, 106.83, 56.67, 56.39; positive ESIMS m/z (rel intensity) 359/360/361 (MH^+, 100/22/65). Anal. Calcd for $C_{18}H_{12}Cl_2N_2O_2 \cdot 0.6H_2O$: C, 58.43; H, 3.60; N, 7.57. Found: C, 58.32; H, 3.43; N, 7.53.

6,8,9,11-Tetramethoxydibenzo[*c*,*h*][1,6]naphthyridine (34). Sodium methoxide (63 mg, 1.17 mmol) and **33** (70 mg, 0.2 mmol) were mixed in methanol (10 mL). The mixture was heated to reflux for 10 h. The reaction mixture was cooled to room temperature and ice-cold water (10 mL) was added. The precipitated white amorphous solid (50 mg, 71%) was collected by filtration: mp 173−175 °C. IR (KBr) 1650, 1610, 1590, 1515 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 8.80 (d, J = 7.2 Hz, 1 H), 8.57 (s, 1 H), 7.78 (d, J = 7.8 Hz, 1 H), 7.70 (t, J = 7.3 Hz, 1 H), 7.59−7.45 (m, 2 H), 4.24 (s, 3 H), 4.19 (s, 3 H), 3.95 (s, 3 H), 3.91 (s, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.78, 159.51, 153.11, 149.48, 146.29, 143.82, 129.96, 129.49, 126.60, 124.42, 124.34, 123.50, 114.22, 107.61, 105.68, 103.90, 55.82, 55.72, 54.24, 53.95; positive ESIMS m/z (rel intensity) 351 (MH^+, 100). Anal. Calcd for $C_{20}H_{18}N_2O_4 \cdot 0.6H_2O$: C, 66.51; H, 5.36; N, 7.76. Found: C, 66.41; H, 5.08; N, 7.54.

Topoisomerase I-Mediated DNA Cleavage Reactions. Human recombinant Top1 was purified from Baculovirus as described previously.[39] The 161 bp fragment from pBluescript SK(-) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonucleases PvuII and *Hin*dIII (New England Biolabs, Beverly, MA) supplied in NE buffer 2 (50 μL reactions) for 1 h at 37 °C, and separated by electrophoresis in a 1% agarose gel made in 1 × TBE buffer. The 161 bp fragment was eluted from the gel slice using the QIAEX II kit (QIAGEN Inc., Valencia, CA). Approximately 200 ng of the fragment was 3′-end labeled at the *Hin*dIII site by fill-in reaction with [α-^{32}P]-dGTP and 0.5 mM dATP, dCTP, and dTTP, 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). Unincorporated ^{32}P-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3′-end-labeled 161 bp fragment was collected. Aliquots (approximately 50 000 dpm/reaction) were incubated with topoisomerase I at 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration). The samples (10 μL) were mixed with 30 μL of loading buffer (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, pH 8.0). Aliquots were separated in denaturing gels (16% polyacrylamine, 7 M urea). Gels were dried and visualized by using a Phosphoimager and *ImageQuant* software (Molecular Dynamics, Sunnyvale, CA).

Molecular Modeling. Structures of **14** and **31** were built and geometry optimized with *Sybyl 8.1* using the MMFF94s force field and MMFF94 charges.[36] The X-ray crystal structure of the **5**-Top1-DNA ternary complex was obtained from the Protein Data Bank (PDB ID: 1SC7). Hydrogens were added to all atoms and MMFF94 charges were assigned. The positions of hydrogen atoms were optimized with the MMFF94s force field. The original ligand **5** was removed from the structure of the ternary complex,[20] and 100 docking runs were performed for both **14** and **31** using the docking genetic algorithm and Goldscore fitness function within *GOLD 3.2*.[35] The best solutions were merged with the Top1-DNA cleavage complex. In order to refine the position of the naphthyridine ligands, geometry optimizations of the ligands within newly obtained ternary complexes were performed by 100 iterations with steepest descent minimization followed by 200 iterations with conjugate gradient using the MMFF94s force field and MMFF94 charges within *Sybyl 8.1*.

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