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Nitro and amino substitution within the A-ring of 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*][1,6]-naphthyridin-6-ones: influence on topoisomerase I-targeting activity and cytotoxicity

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Abstract—Recently, 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-one, **1**, was identified as a TOP1-targeting agent with pronounced antitumor activity. In the present study, the effect on activity of substituting a single nitro or amino group in the A-ring in lieu of the methylenedioxy moiety of **1** was evaluated. The presence of either a nitro or amino substituent at the 4-position had a pronounced adverse affect on both TOP1-targeting activity and cytotoxicity. To a lesser extent, derivatives with a nitro or amino substituent at the 1-position were also less active than **1**. Replacement of the methylenedioxy moiety of **1** with either a nitro or amino substituent at either the 2- and 3-position did result in analogues with potent TOP1-targeting activity and cytotoxicity.

1. Introduction

Topoisomerases are enzymes that regulate the topology of DNA and are critical to replication and transcription. There are two major subtypes, topoisomerase I (TOP1) and topoisomerase II (TOP2) based upon differences in their initial mechanisms wherein either a single or double-stranded DNA break is implicated.[1–4] Topoisomerase-targeting agents that stabilize the cleavable complex formed between the enzyme and DNA have proved to be effective in the treatment of cancer. Molecules capable of stabilizing the topoisomerase–DNA cleavable complex in effect convert these enzymes into cellular poisons.

Camptothecin was the first agent identified as a TOP1-targeting agent.[5] Structure–activity relationships of camptothecin analogues have been intensely studied.[6,7] Among the more potent analogues are A-ring functionalized derivatives including 10,11-methylenedioxy-camptothecin, 9-nitrocamptothecin (9-NC) and 9- and 10-aminocamptothecin (9-AC and 10-AC, respectively). 9-NC[8–11] and 9-AC[12–19] are presently undergoing clinical trials. All camptothecin-based drugs have incorporated within their structures a δ-lactone, which is susceptible to hydrolysis. Hydrolysis of this lactone results in the formation of an inactive derivative that possesses high affinity for human serum albumin.[20–22] The metabolic instability of this lactone and the observation that both topotecan and irinotecan are substrates for efflux transporters associated with resistance, have prompted further studies on the development of novel TOP1-targeting agents.[23–26]

Several novel noncamptothecin TOP1-targeting agents have been identified. These include derivatives of bi- and

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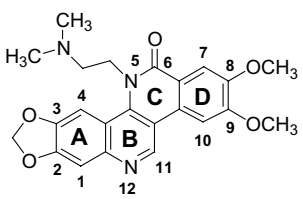
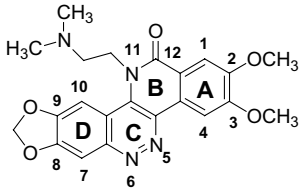


1



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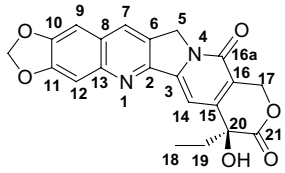
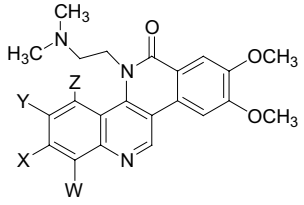


10,11-Methylenedioxy CPT

Figure 1. Structure and numbering of 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c*,*h*][1,6]-naphthyridin-6-one **1**, 11*H*-2,3-dimethoxy-11-(2-*N*,*N*-dimethylaminoethyl)-8,9-methylenedioxy-isoquino[4,3-*c*]cinnolin-12-one **2**, and 10,11-methylenedioxycamptothecin.



3a W = NO_2, X = H, Y = H, Z = H
3b W = H, X = NO_2, Y = H, Z = H
3c W = H, X = H, Y = NO_2, Z = H
3d W = H, X = H, Y = H, Z = NO_2

4a W = NH_2, X = H, Y = H, Z = H
4b W = H, X = NH_2, Y = H, Z = H
4c W = H, X = H, Y = NH_2, Z = H
4d W = H, X = H, Y = H, Z = NH_2

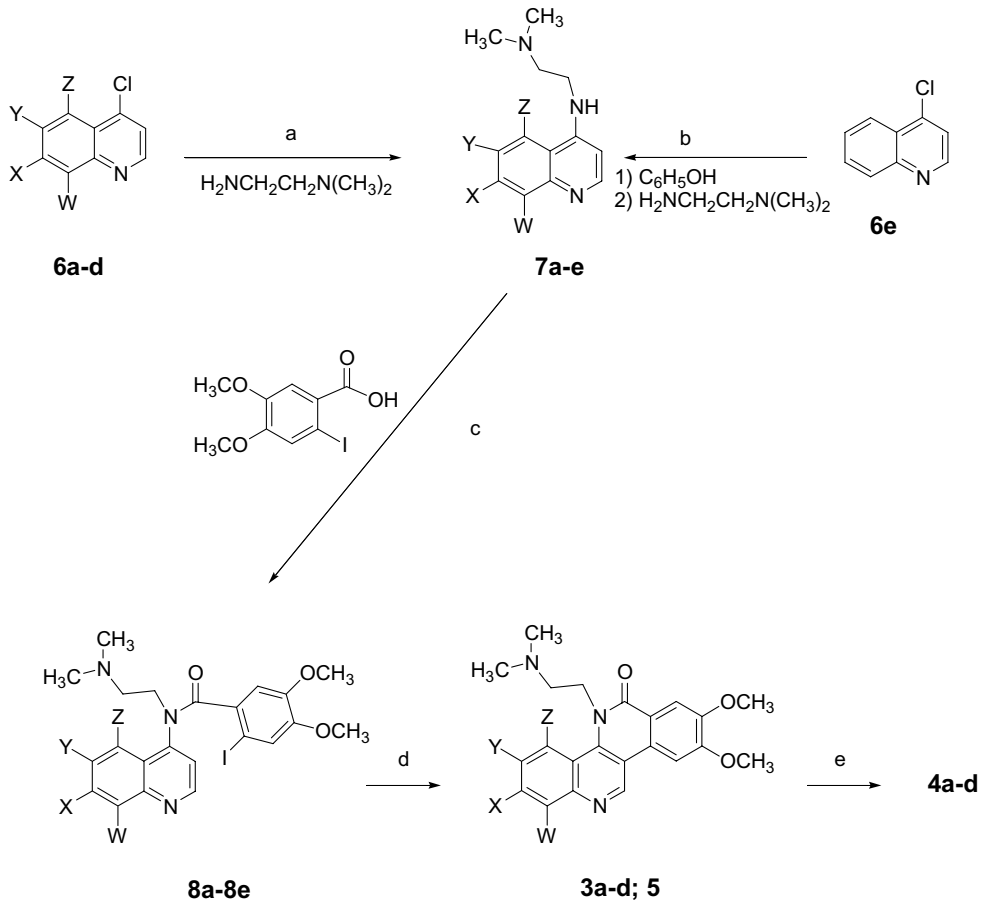
5 W = H, X = H, Y = H, Z = H

Figure 2. 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones synthesized and evaluated as TOP1-targeting agents and for cytotoxic activity.

terbenzimidazoles,[27–29] benz[*a*]anthracenes,[30] benzo[*c*]-phenanthridine and protoberberine alkaloids,[31,32] indolocarbazoles,[33] the fungal metabolites bulgarein[34] and saintopin,[35] and several indenoisoquinolines[36,37] and benzophenazines.[38] Studies in our laboratory have demonstrated that 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-one, **1**, and 11*H*-isoquino[4,3-*c*]cinnolin-12-ones, such as **2** (Fig. 1) can exhibit potent TOP1-targeting activity and pronounced cytotoxicity.[39,40] Potent antitumor activity was observed for **1** administered orally and parenterally to athymic nude mice bearing the human tumor xenograft, MDA-MB-435.

Both **1** and **2** were investigated as TOP1-targeting agents based upon SAR data observed for variously substituted benzo[*i*]phenanthridines and dibenzo[*c*,*h*]cinnolines. Enhanced TOP1-targeting activity and cytotoxicity within a series of substituted dibenzo[*c*,*h*]cinnolines are associated with (1) the presence of methoxy substituents at both the 2- and 3-position of the A-ring, (2) a 8,9-methylenedioxy moiety in the D-ring, and (3) a nitrogen heteroatom adjacent to the benzo-ring that incorporates the methylenedioxy substituent.[40–42] A similar spatial arrangement of substituents relative to the nitrogen heteroatom of benzo[*i*]phenanthridines also appears to be associated with potent TOP1-targeting activity.[43]

A recent molecular modeling study has investigated the ternary complex formed between TOP1 enzyme, DNA and either dibenzo[*c*,*h*][1,6]naphthyridin-6-ones (e.g., **1**) or isoquino[4,3-*c*]cinnolin-12-ones (e.g., **2**).[44] This work suggests that the binding mode used by these compounds may be similar to the binding mode observed in the X-ray crystal structure[45] of topotecan/DNA/TOP1. Compound **1** has a quinoline ring system in common with the camptothecins, and the methylenedioxy substituent present within the quinoline substructure occupies the site corresponding to the methylenedioxy group in 10,11-methylenedioxy CPT (see Fig. 1). That this substitution should confer a substantial increase in potency to both camptothecins and dibenzo[*c*,*h*]-[1,6]naphthyridin-6-ones supports the premise that the quinoline ring of both families plays a similar role in binding. As both 9-amino- and 9-nitrocamptothecin both retain potent TOP1-targeting activity and cytotoxicity,[8–19] it was speculated that similarly substituted A-ring derivatives of 8,9-dimethoxydibenzo[*c*,*h*][1,6]-naphthyridin-6-ones would exhibit potent activity. As it is known that aryl nitro groups in a low dielectric environment can act as hydrogen bond acceptors,[46] it was hypothesized that analogues wherein a nitro substituent in ring-A replaced the methylenedioxy group of **1** would retain similar TOP1-targeting activity. The synthetic approach employed for the preparation of 5-substituted 5*H*-8,9-dimethoxy-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-ones provides the opportunity to assess the influence of a nitro substituent at each available site on the A-ring. The present study was undertaken to determine if one or more such nitro



Scheme 1. Methods used for the synthesis of **3a**–**d**, **4a**–**d**, and **5**. Reagents and conditions: (a) Reflux; (b) **6e** was added to refluxing phenol; then cooled to 100 °C prior to addition of amine; (c) $(COCl)_2$, then **7a**–**e**, TEA, CH_2Cl_2; (d) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Ag_2CO_3 in DMF at reflux; (e) $SnCl_2$, Sn, HCl, EtOH for **4a**–**c**; Ra–Ni, $H_2NNH_2{\cdot}H_2O$ for **4d**.

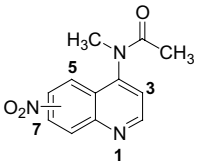
derivatives could retain similar TOP1-targeting activity as **1**. In this study we synthesized 1-, 2-, 3-, and 4-nitro-5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*]-[1,6]naphthyridin-6-ones, **3a**–**d**, as well as their amino derivatives, **4a**–**d**, to assess their relative TOP1-targeting activity and cytotoxicity (Fig. 2). The biological activities of these nitro and amino derivatives were compared to **1** as well as the unsubstituted A-ring derivative, 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*][1,6] naphthyridin-6-one, **5**.

2. Chemistry

The methods used for the preparation of 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-dibenzo[*c*,*h*][1,6]naphthyridin-6-one **5** and its four isomeric A-ring nitro derivatives **3a**–**d** are outlined in Scheme 1. Compound **6e** was prepared by treatment of the 4-quinolone with $POCl_3$.[47] Nitration of **6e** using a mixture of sulfuric acid and fuming nitric acid gives a separable mixture of the 8- and 5-nitro isomers, **6a** and **6d**, respectively.[48] As previously described, 4-hydroxy-7-nitroquinoline was synthesized, and was chlorinated using phosphorus oxychloride to give **6b**.[49] For the preparation of **6c**, 4-hydroxyquinoline was nitrated with nitric acid in sulfuric acid, furnishing 4-hydroxy-6-nitroquinoline, which was chlorinated using phosphorus oxychloride.[50]

In the case of **6a**–**d**, treatment of these 4-chloro nitroquinolines with *N*,*N*-dimethylethylenediamine did provide **7a**–**d**. In the case of **6e**, the chloroquinoline was initially refluxed in phenol for 2.5 h prior to treatment with *N*,*N*-dimethylethylenediamine to form **7e**.[51,52] Compounds **7a**–**e** were treated with the acid chloride of 3,4-dimethoxy-6-iodobenzoic acid to form the 2-iodo-4,5-dimethoxybenzamides **8a**–**e**.

Heck cyclization of the benzamides was performed using palladium acetate, tri(*o*-tolylphosphine) and silver carbonate in refluxing DMF, as previously described.[53] In these reactions, silver carbonate functions as a base, and also acts to sequester iodide.[54–56] Thus, after oxidative addition of aryl iodide to the palladium(0) complex, abstraction of iodide results in the formation of a tricoordinate, positively charged complex. Such a cationic complex tends to react fastest with an electron rich double bond (a poor π-acceptor and a good σ-donor).[57] Therefore, under these reaction conditions C–C bond formation occurs optimally between an aryl halide and an electron rich aromatic carbon.



Isomer	Potential at C3	Heck rxn yield
5-nitro	C3 = +6.21 ± 0.18	35%
6-nitro	C3 = +5.94 ± 0.16	42%
7-nitro	C3 = +7.47 ± 0.31	12%
8-nitro	C3 = +5.61 ± 0.37	36%

Figure 3. Electrostatic potential at C-3 for the various nitro compounds from B3LYP/6-31G* calculation.

The results from our Heck reactions are in accord with this mechanism. The reactions of benzamides **8a**, **8c**, and **8d** were completed within 1 h, and provided the desired 1-, 3-, and 4-nitrodibenzo[*c*,*h*][1,6]naphthyridin-6-ones **3a**, **3c**, and **3d** in 35–42% yield. On the other hand, reaction of **8b** was extremely sluggish, requiring 18 h to go to completion and providing only 12% of the 2-nitrodibenzo[*c*,*h*][1,6]naphthyridin-6-one **3b**. This disparity is due to the resonance effect of the nitro group, which based on its position within the quinoline ring induces a variable degree of deactivation toward the Heck reaction. The 7-nitro isomer has a paucity of electron density at the 3-position, which is unfavorable for the success for the Heck reaction. In contrast, none of the resonance structures of 6-nitroquinoline has a positive charge on the 3-position. Thus, a nitro group at the 6-position is much less electron withdrawing at C3, and should be not be detrimental to the Heck reaction. The resonance effect in the 5-position is not as strong because the nitro group is sterically forced by the amido group in the 4-position into a dihedral angle that places the group perpendicular to the plane of the quinoline ring. The disruption of orbital overlap reduces the electron-withdrawing ability of the nitro group in this position.

To lend further support to this explanation, single-point B3LYP/6-31G* computations were performed to quantify the electron-withdrawing effect of the nitro group at the relevant sites. We used a simplified construct, nitro-substituted 4-*N*-methylacetamido quinolines, to study the impact of the nitro group on the Heck reaction. The single-point B3LYP/6-31G*[58,59] computations on the various nitro substituted 4-*N*-methylacetamido quinolines were performed starting from AM1[60] geometries. The results from these calculations show a good correlation ($R^2 = 0.87$) between the electrostatic potential at C3 and % yield in the Heck reaction (Figs. 3 and 4). Electrostatic potential maps of the constructs are shown in Figure 5.

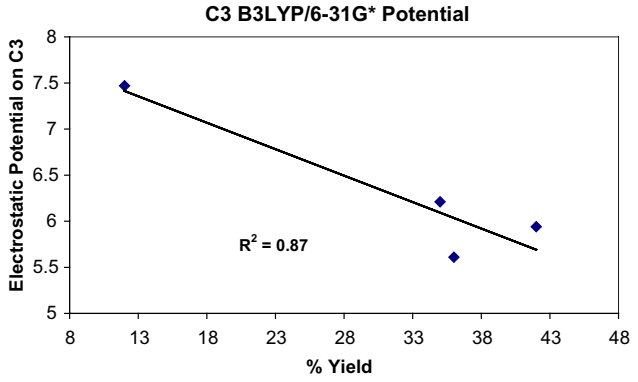


Figure 4. Plot of electrostatic potential on C3 (*y*-axis) versus % yield on the *x*-axis.

The amino compounds **3a**–**d** were obtained by reduction of the corresponding nitro compounds **4a**–**d**. For the synthesis of **3a**–**c**, best results were obtained using $SnCl_2$ and granular tin in a mixture of ethanol and concentrated HCl. For the conversion of **3d** to **4d**, complete conversion required the use of hydrazine hydrate and Raney nickel.

3. Pharmacology

The relative TOP1-targeting activity of **3a**–**d** and **4a**–**d** are listed in Table 1. The influence of various concentrations of **3a**, **3c**, and **4a** on DNA fragmentation in the presence of TOP1 and DNA is illustrated in Figure 6. The potency of these analogues as TOP1-targeting agents correlates with their cytotoxicity as observed in the human lymphoblastoma cell line, RPMI8402 and the mouse leukemia cell line, P388. Evidence that their cytotoxic activity is associated with their TOP1-targeting activity is also apparent by the resistance observed with CPT-K5 cell, the camptothecin-resistant variant of RPMI8402,[61] which possesses a mutant form of TOP1. In addition, these analogues, together with **1** and **2**, exhibited significantly decreased cytotoxic activity in a cell line (P388/CPT45) that does not express topoisomerase I relative to its parental cell line (P388).[62] These data are consistent with TOP1-targeting activity being the principal mechanism by which these agents exert their cytotoxic activity.

It is evident that there are major differences in activity among these various 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones. The positional isomer **3d**, which has its nitro substituent within a bay-region, does not exhibit significant activity as a TOP1-targeting agent and is much less cytotoxic than the other positional isomers. The absence of TOP1-targeting activity contributing to its cytotoxic activity is apparent by the absence of a difference in cytotoxicity as observed between either RPMI8402 and CPT-K5, or P388 and P388/CPT45 cell lines. Compound **3a**, with its nitro substituent adjacent to the nitrogen heteroatom at the 12-position, has less TOP1-targeting activity and is less cytotoxic than **3b**, **3c**, or the A-ring unsubstituted analogue, **5**. The 3-nitro derivative, **3c**, is the most potent of these four nitro dibenzo[*c*,*h*][1,6]naphthyridin-6-ones with regard to both TOP-1-targeting activity and cytotoxicity.

The results obtained for the amino derivatives parallels those obtained for their nitro analogues. Their relative potency as TOP1-targeting agents is **4c** > **4b** ≫ **4a** > **4d**. The differences in activity between amino and nitro substituents at the same site on the molecule were not as great as the differences observed between certain positional isomers within either series. Among the more potent TOP1-targeting positional isomers, the nitro

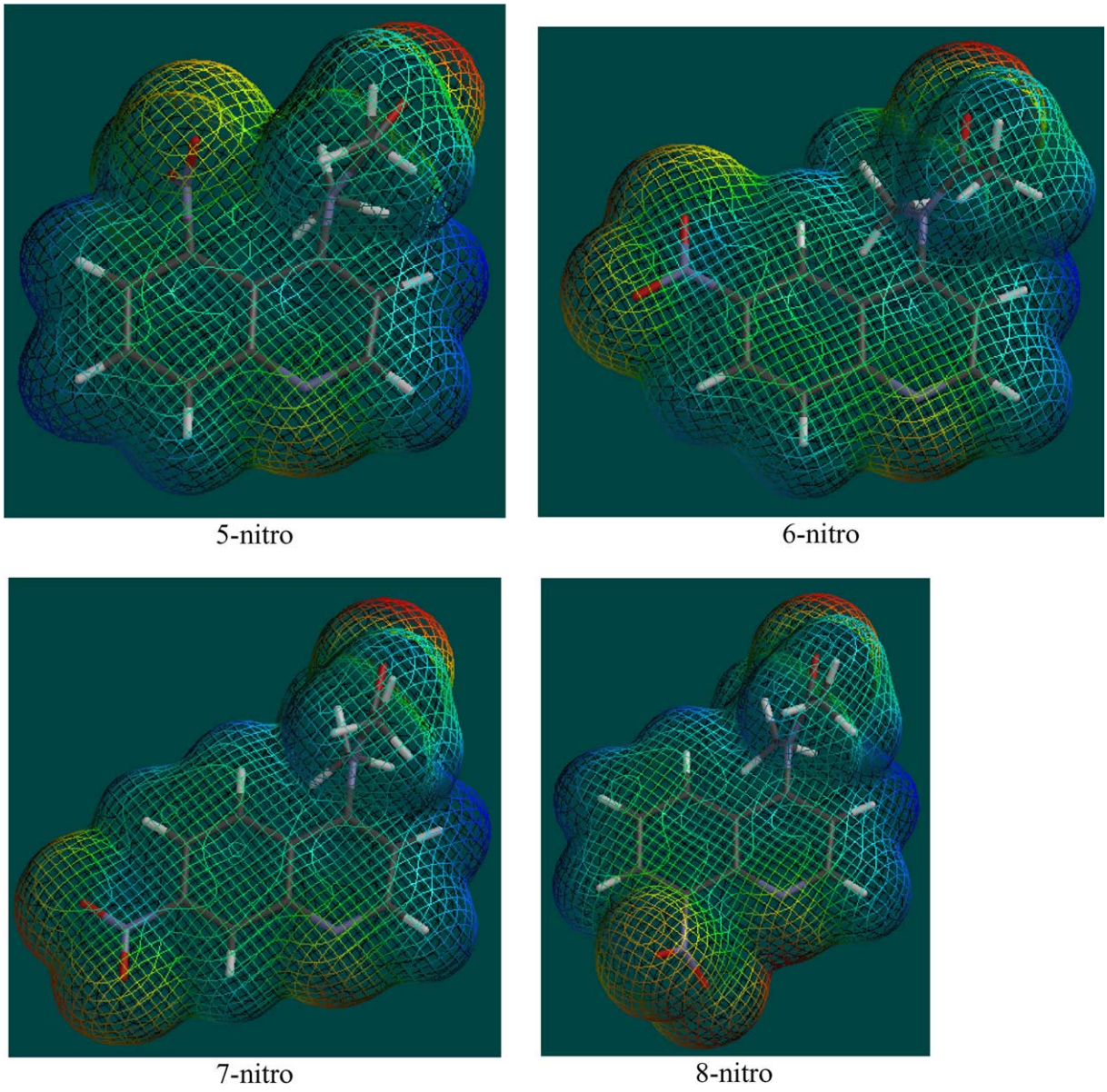


Figure 5. Electrostatic potential mapped to mesh contour of electron density.

analogues **3a**–**c** are more active as TOP1-targeting agents and, in general, exhibit greater cytotoxicity relative to their amino counterparts, **4a**–**c**, in RPMI8402 or P388 cells.

These data indicate that a nitro substituent at the 2- or 3-position permits significant retention of TOP1-targeting activity. This activity is enhanced relative to 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-dibenzo[*c*,*h*]-[1,6]naphthyridin-6-one, **5**, but is less than that observed for the 2,3-methylenedioxy analogue, **1**. Recently, it was demonstrated that 8- and 9-nitro-5-(2-dimethylaminoethyl)-2,3-methylenedioxy-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones have comparable activity to **1**, indicating that nitro substituents at these positions can be effective replacements for the dimethoxyl groups on the D-ring.[63] The results from this study indicate that the nitro analogue, **3c**, wherein a 3-nitro group replaces the 2,3-methylenedioxy substituent of **1**, has similar TOP1-targeting activity and cytotoxicity to that observed for topotecan.

4. Experimental

Melting points were determined with either a Thomas-Hoover Unimelt or Meltemp capillary melting point apparatus. Column chromatography refers to flash chromatography conducted on SiliTech 32–63 μm, (ICN Biomedicals, Eschwege, Ger.) using the solvent systems indicated. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^{1}H and 50 MHz ^{13}C) were recorded in the deuterated solvent indicated with chemical shifts reported in δ units downfield from tetramethylsilane

Table 1. TOP1-targeting activity and cytotoxicity of 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones

Compound	TOP1-mediated[a] DNA cleavage	Cytotoxicity IC_{50} (μM)[b]			
		RPMI 8402	CPT-K5	P388	P388/CPT45
1	0.5	0.002	0.90	0.001	0.23
2	0.3	0.001	0.60	0.002	0.36
3a	9	0.22	3.0	0.19	2.1
3b	6	0.075	3.35	0.03	0.34
3c	2	0.018	0.80	0.04	0.2
3d	>300	5.5	12	6.0	7.0
4a	100	0.65	2.0	0.35	0.21
4b	12	0.1	2.1	0.06	0.23
4c	6	0.04	1.4	0.02	0.33
4d	>300	4.0	8.0	3.0	3.5
5	10	0.1	1.7	0.07	0.30
CPT	0.2	0.005	61	0.009	>10
CPT-11	25	0.57	>100	2.0	>10
Topotecan	1	0.012	>50	0.035	>10

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration, that is, concentrations relative to Topotecan, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage of plasmid DNA in the presence of human TOP1.
[b] IC_{50} has been calculated after 4 days of continuous drug exposure.

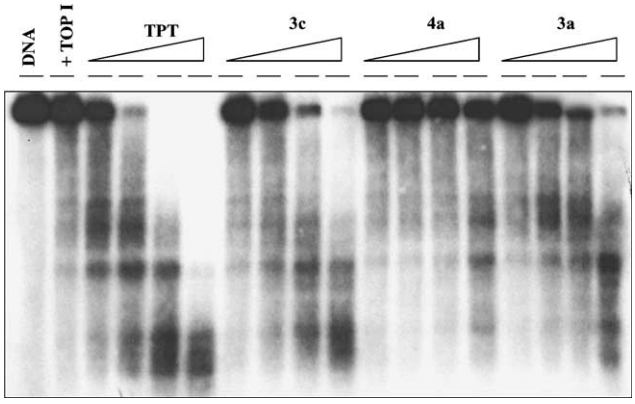


Figure 6. Stimulation of enzyme-mediated DNA cleavage by topotecan (TPT), **3a**, **3c**, and **4a** using human TOP1. The first lane is the DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.001 to 1.0 μM.

(TMS). Coupling constants are reported in hertz (Hz). Mass spectra were obtained from Washington University Resource for Biomedical and Bio-organic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO.

4.1. General procedure for the preparation of nitro 5-[2-(*N*,*N*-dimethylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-ones

4.1.1. 8,9-Dimethoxy-1-nitro-5-[2-(*N*,*N*-dimethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (3a). A mixture of **8a** (660 mg, 1.2 mmol), $Pd(OAc)_2$ (54 mg, 0.24 mmol), P(*o*-tolyl)$_3$ (147 mg, 0.48 mmol), and Ag_2CO_3 (660 mg, 2.4 mmol) in DMF (36 mL) was heated to reflux and stirred for 30 min. The reaction mixture was cooled, diluted with chloroform, filtered through Celite, and the solvent removed under vacuum. The crude residue was chromatographed in 98:2 chloroform/methanol, to provide 190 mg (36%) of the cyclized product as a pale-yellow solid; mp 257–258 °C; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6H), 3.01 (t, 2H, $J = 6.9$), 4.09 (s, 3H), 4.13 (s, 3H), 4.70 (t, 2H, $J = 6.9$), 7.67 (dd, 1H, $J = 9.0$, $J = 7.7$), 7.72 (s, 1H), 7.92 (s, 1H), 7.99 (dd, 1H, $J = 7.7$, $J = 1.1$), 8.80 (dd, 1H, $J = 9.0$, $J = 1.1$), 9.68 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.9, 49.4, 56.4, 56.5, 57.8, 102.2, 108.9, 113.0, 120.0, 120.2, 122.5, 124.5, 126.8, 128.4, 140.1, 140.6, 147.7, 149.5, 151.2, 154.6, 163.5; HRMS calcd for $C_{22}H_{22}O_5N_4H$: 423.1660; found: 423.1650.

4.1.2. 8,9-Dimethoxy-2-nitro-5-[2-(*N*,*N*-dimethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (3b). Prepared from **8b** (660 mg, 1.2 mmol); reaction time 6 h at which point $Pd(OAc)_2$ (54 mg, 0.24 mmol) and P(*o*-tolyl)$_3$ (147 mg, 0.48 mmol) were added a second time and the reaction continued for an additional 12 h. Chromatographic purification of the crude product using 98:2 chloroform/methanol gave 65 mg (12%) of **3b** as a yellow–orange solid; mp 214–217 °C; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6H), 3.04 (t, 2H, $J = 7.0$), 4.10 (s, 3H), 4.17 (s, 3H), 4.69 (t, 2H, $J = 7.0$), 7.77 (s, 1H), 8.00 (s, 1H), 8.37 (dd, 1H, $J = 9.5$, $J = 2.4$), 8.82 (d, 1H, $J = 9.5$), 9.06 (d, 1H, $J = 2.4$), 9.72 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.9, 49.4, 56.5, 56.5, 57.9, 97.2, 102.4, 109.0, 113.8, 119.1, 120.4, 122.6, 126.3, 126.7, 140.6, 147.2, 147.9, 148.0, 151.4, 154.5, 163.5; HRMS calcd for $C_{22}H_{22}O_5N_4H$: 423.1668; found: 423.1684.

4.1.3. 8,9-Dimethoxy-3-nitro-5-[2-(*N*,*N*-dimethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (3c). Prepared from **8c** (660 mg, 1.2 mmol), reaction time 1 h. Chromatographic purification of the crude product using 99:1 chloroform/methanol gave 220 mg (42%) of the cyclized product as a bright yellow solid; mp 232–234 °C; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 6H), 3.19 (t, 2H, $J = 7.2$), 4.07 (s, 3H), 4.14 (s, 3H), 4.65 (t, 2H, $J = 7.2$), 7.71 (s, 1H), 7.92 (s, 1H), 8.28 (d, 1H, $J = 9.2$), 8.48 (dd, 1H, $J = 9.2$, $J = 2.2$), 9.64 (d, 1H, $J = 2.2$), 9.68 (s, 1H);

^{13}C NMR ($CDCl_3$) δ 46.1, 49.8, 56.4, 56.5, 57.4, 102.2, 108.9, 112.9, 118.1, 120.0, 122.2, 122.6, 126.8, 132.1, 142.1, 145.0, 148.9, 150.8, 151.3, 154.6, 163.4; HRMS calcd for $C_{22}H_{22}O_5N_4H$: 423.1668; found: 423.1663.

4.1.4. 8,9-Dimethoxy-4-nitro-5-[2-(*N*,*N*-dimethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-one (3d). Prepared from **8d** (880 mg, 1.6 mmol), reaction time 1 h. Chromatographic purification of the crude product using 98:2 chloroform/methanol gave 325 mg (48%) of the **3d** as an orange solid; mp 194–195 °C; ^{1}H NMR ($CDCl_3$) δ 1.75 (s, 6H), 2.30 (dd, 2H, $J = 6.9$, $J = 5.4$), 3.96 (dt, 1H, $J = 12.9$, $J = 6.9$), 4.10 (s, 3H), 4.16 (s, 3H), 4.74 (dt, 1H, $J = 12.9$, $J = 5.4$), 7.71 (s, 1H), 7.76 (dd, 1H, $J = 8.3$, $J = 7.7$), 7.95 (s, 1H), 8.29 (dd, 1H, $J = 7.7$, $J = 1.4$), 8.34 (dd, 1H, $J = 8.3$, $J = 1.4$), 9.57 (s, 1H);^{13}C NMR ($CDCl_3$) δ 45.0, 47.5, 56.4, 56.5, 57.0, 102.5, 109.3, 110.8, 114.8, 120.7, 124.3, 126.1, 127.1, 135.7, 139.3, 146.8, 147.7, 148.8, 151.2, 154.3, 161.6; HRMS calcd for $C_{22}H_{22}O_5N_4H$: 423.1668; found: 423.1668.

4.1.5. 8,9-Dimethoxy-5-[2-(*N*,*N*-dimethylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-one (5). Prepared from **8e** (606 mg, 1.2 mmol), reaction time 25 min. Chromatographic purification of the crude product using 99:1 chloroform/methanol gave 189 mg (42%) of **5**; mp 202.5–203.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6H), 3.01 (t, 2H, $J = 7.2$), 4.04 (s, 3H), 4.11 (s, 3H), 4.69 (t, 2H, $J = 7.2$), 7.58 (m, 1H), 7.67 (s, 1H), 7.71 (m, 1H), 7.87 (s, 1H), 8.16 (d, 1H, $J = 8.0$), 8.47 (d, 1H, $J = 8.8$), 9.51 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.8, 48.9, 56.3, 56.3, 57.6, 102.1, 108.8, 111.8, 118.9, 119.6, 124.6, 125.9, 127.5, 129.1, 130.5, 140.8, 145.6, 148.7, 150.5, 154.2, 163.7; HRMS calcd for $C_{22}H_{23}N_3O_3H$: 378.1818; found: 378.1820.

4.2. General procedure for the reduction of nitro 5-[2-(*N*,*N*-dimethylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-ones to amino 8,9-dimethoxy-5-[2-(*N*,*N*-dimethylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-ones (4a, 4b, and 4c).

4.2.1. 1-Amino-8,9-dimethoxy-5-[2-(*N*,*N*-dimethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (4a). A mixture of $SnCl_2{\cdot}2\ H_2O$ (150 mg, 0.66 mmol) and granular tin (5 mg, 0.042 mmol) in ethanol (1.0 mL) and concentrated HCl (2.0 mL) was cooled to 0 °C and **3a** (40 mg, 0.09 mmol) was added in small portions with stirring. The starting material completely dissolved, and stirring was continued from 20 min at 0 °C and then for 4 h at room temperature. Water (5 mL) was added and the mixture was neutralized by addition of solid sodium bicarbonate, and then the mixture was extracted with chloroform (6 × 10 mL), washed with water (3 × 10 mL), dried ($MgSO_4$), and evaporated under vacuum to give 30 mg (81%) **4a** as a yellow solid; mp 226–229 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 6H), 3.03 (t, 2H, $J = 7.3$), 4.08 (s, 3H), 4.14 (s, 3H), 4.78 (t, 2H, $J = 7.3$), 6.99 (dd, 1H, $J = 7.8$, $J = 1.2$), 7.40 (dd, 1H, $J = 8.4$, $J = 7.8$), 7.73 (s, 1H), 7.75 (dd, 1H, $J = 8.4$, $J = 1.2$), 7.92 (s, 1H), 9.43 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.7, 46.7, 56.3, 56.3, 57.5, 102.1, 108.8, 110.5, 112.4, 113.0, 119.4, 119.7, 126.8, 127.7, 138.4, 141.1, 142.5, 144.8, 150.5, 154.2, 164.0; HRMS calcd for $C_{22}H_{24}O_3N_4H$: 393.1927; found: 393.1923.

4.2.2. 2-Amino-8,9-dimethoxy-5-[2-(*N*,*N*-dimethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (4b). Prepared from **3b** (17 mg, 0.04 mmol), reaction time 10 h at room temperature. During the course of the reaction the product precipitated from solution as a yellow solid. Extraction of neutralized reaction mixture as detailed above provided 10 mg (63%) of **4b** as a yellow solid; mp 208–210 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H), 3.04 (t, 2H, $J = 7.4$), 4.06 (s, 3H), 4.12 (s, 3H), 4.69 (t, 2H, $J = 7.4$), 7.04 (dd, 1H, $J = 9.1$, $J = 2.5$), 7.32 (d, 1H, $J = 2.5$), 7.67 (s, 1H), 7.89 (s, 1H), 8.35 (d, 1H, $J = 9.1$), 9.42 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.9, 48.8, 56.3, 56.3, 57.6, 101.7, 108.8, 109.8, 110.9, 111.9, 117.2, 118.7, 126.1, 128.2, 141.4, 146.1, 147.3, 150.0, 150.9, 154.3, 163.9; HRMS calcd for $C_{22}H_{24}O_3N_4H$: 393.1927; found: 393.1916.

4.2.3. 3-Amino-8,9-dimethoxy-5-[2-(*N*,*N*-dimethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (4c). Prepared from **3c** (26 mg, 0.06 mmol) reaction time was 2 h at room temperature. During the course of the reaction the product precipitated from the solution as a bright yellow solid material. Extraction of neutralized reaction mixture as detailed above provided 17 mg (71%) of **4b** as a yellow solid; mp 213–215 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H), 3.06 (t, 2H, $J = 7.0$), 4.07 (s, 3H), 4.13 (s, 3H), 4.68 (t, 2H, $J = 7.0$), 7.18 (dd, 1H, $J = 8.8$, $J = 2.4$), 7.72 (s, 1H), 7.76 (d, 1H, $J = 2.4$), 7.91 (s, 1H), 7.99 (d, 1H, $J = 8.8$), 9.34 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.9, 48.5, 56.3, 56.4, 58.1, 102.2, 106.2, 108.7, 112.2, 119.6, 120.3, 120.4, 127.8, 131.7, 139.1, 142.1, 143.6, 144.7, 150.4, 154.1, 163.9; HRMS calcd for $C_{22}H_{24}O_3N_4H$: 393.1927; found: 393.1928.

4.2.4. 4-Amino-8,9-dimethoxy-5-[2-(*N*,*N*-dimethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-one (4d). To a stirred solution of **3d** (37 mg, 0.088 mmol) in ethanol (8 mL) was added 1 pinch of Raney Nickel and five drops of hydrazine hydrate, and the mixture was stirred at ambient temperature for 2 h. The mixture was filtered through Celite and the filtrate was evaporated. The residue was dissolved in chloroform (25 mL) and washed with brine (25 mL), dried ($MgSO_4$), and evaporated, giving 28 mg (81%) as a yellow solid; mp 239–241 °C; ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 6H), 2.10 (m, 2H), 4.05 (s, 3H), 4.13 (s, 3H), 4.48 (m, 3H), 5.07 (m, 1H), 6.79 (dd, 1H, $J = 6.3$, $J = 2.5$), 7.52 (m, 2H), 7.64 (s, 1H), 7.87 (s, 1H), 9.38 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.1, 50.5, 56.3, 56.4, 57.4, 102.0, 109.1, 109.6, 111.3, 112.6, 118.7, 119.5, 127.7, 130.0, 141.0, 142.6, 144.8, 149.8, 150.0, 154.3, 164.5; HRMS calcd for $C_{22}H_{24}O_3N_4H$: 393.1927; found: 393.1925.

4.2.5. 4-Chloro-7-nitroquinoline (6b). 7-Nitro-4-quinolone (3.5 g, 18.4 mmol) was refluxed in $POCl_3$ (15 mL) for 1 h. The reaction mix was cooled and $POCl_3$ was removed in vacuo. Water (50 mL) was added to the crude product, and after any residual $POCl_3$ had been hydrolyzed, the mixture was made basic (pH 9) using NH_4OH and extracted into chloroform (5×100 mL), washed with water (3×100 mL), dried ($MgSO_4$), and the solvent evaporated in vacuo, to give 2.4 g (63%) of **6b** as a yellow solid; mp 166–167 °C (lit.[49] mp 156–160 °C); ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 1H, $J = 4.7$), 8.44 (m, 2H), 8.98 (d, 1H, $J = 4.7$), 9.06 (d, 1H, $J = 1.4$); ^{13}C NMR ($CDCl_3$) δ 121.1, 124.0, 126.0, 126.4, 129.1, 133.7, 143.2, 148.1, 152.2.

4.2.6. 7-Nitro-4-quinolone. 3-Carboxy-7-nitro-4-quinolone was converted to its silver salt prior to decarboxylation. To 3-carboxy-7-nitro-4-quinolone (34 g, 0.145 mol) in boiling water (350 mL) was added NH_4OH (2.46 g, 0.145 mol) to obtain a clear solution. To this solution was added a saturated solution of silver nitrate (25.0 g, 0.145 mmol) in water, and the mixture was refluxed for 30 min, then filtered to provide the silver salt as a grey solid. This material was dried and then heated to reflux with vigorous stirring in phenyl ether (300 mL) for 3 h, and then cooled and filtered. The solid material was heated to reflux in ethanol with stirring and filtered, and the filtrate was evaporated. The residue was washed with ethyl ether to remove traces of diphenyl ether and dried to provide 5 g (18%) of product. The solid material (28 g, 57% recovery) that did not dissolve in ethanol was the silver salt of 3-carboxy-7-nitro-4-quinolone. Correcting for recovered starting material, the yield was 42%; mp 325–329 °C (dec.) (lit.[49] mp 314–317 °C (dec.)); ^{1}H NMR (DMSO-d_6) δ 6.17 (d, 1H, $J = 7.4$), 8.02 (dd, 1H, $J = 8.8$, $J = 2.2$), 8.11 (d, 1H, $J = 7.4$), 8.27 (d, 1H, $J = 8.8$), 8.41 (d, 1H, $J = 2.2$); ^{13}C NMR (DMSO-d_6) δ 110.0, 115.3, 117.4, 128.1, 129.6, 140.4, 142.0, 149.7, 176.7.

4.2.7. 4-Chloro-6-nitroquinoline (6c). 6-Nitro-4-quinolone (2.0 g, 10.5 mmol) was refluxed in $POCl_3$ (5 mL) for 5 h. The reaction mix was cooled and poured onto ice. After complete hydrolysis of phosphoryl chloride, the mixture was neutralized by addition of solid sodium acetate, and then extracted into chloroform (3×125 mL), washed with water (3×100 mL), dried ($MgSO_4$), and the solvent was evaporated in vacuo and the product chromatographed in 9:1 hexanes/ethyl acetate, to give 1.6 g (73%) of the chloroquinoline as a light yellow solid; mp 144–145 °C (lit.[50] mp 144–145 °C); ^{1}H NMR ($CDCl_3$) δ 7.68 (d, 1H, $J = 4.7$), 8.29 (d, 1H, $J = 9.2$), 8.55 (dd, 1H, $J = 9.2$, $J = 2.6$), 8.98 (d, 1H, $J = 4.7$), 9.20 (d, 1H, $J = 2.6$); ^{13}C NMR ($CDCl_3$) δ 121.4, 123.0, 123.9, 126.0, 132.1, 144.6, 146.5, 151.1, 153.3.

4.2.8. 6-Nitro-4-quinolone. A solution of 4-quinolone (2.3 g, 15.9 mmol) in H_2SO_4 (12 mL) was cooled to 0 °C, and a mixture of H_2SO_4 (1.2 mL) and HNO_3 (1.2 mL) was added slowly, maintaining the temperature at 0–5 °C. After the addition was complete, the mixture was allowed to warm to room temperature with stirring for an additional 2 h. The solution was then poured onto ice, and the resulting precipitate was filtered to give 2.15 g (75%) of the nitroquinolone as a bright yellow solid; mp 310–314 °C (lit.[50] mp 315–318 °C); ^{1}H NMR (DMSO-d_6) δ 6.27 (d, 1H, $J = 7.6$), 7.77 (d, 1H, $J = 9.2$), 8.12 (d, 1H, $J = 7.6$), 8.44 (dd, 1H, $J = 9.2$, $J = 2.4$), 8.84 (d, 1H, $J = 2.4$); ^{13}C NMR (DMSO-d_6) δ 110.8, 121.1, 122.3, 125.2, 126.7, 142.0, 143.6, 144.4, 176.9.

4.2.9. 4-Chloro-8-nitroquinoline (6a) and 4-chloro-5-nitroquinoline (6d). 4-Chloroquinoline (10.0 g, 61.3 mmol) was added in small portions to sulfuric acid (45 mL) taking care to maintain the temperature at or below 15 °C. Then the solution was cooled and maintained at −5 °C during the addition of fuming nitric acid (9 mL). The mixture was allowed to warm to room temperature and stirred for an additional 3 h. The reaction mix was poured on ice and basified (pH 9) with NH_4OH. The resulting precipitate was filtered, washed well with water, dried, and recrystallized from methanol to provide 7.5 g (59%) of **6a** as golden-brown needles; mp 128–129 °C (lit.[48] mp 129–130 °C); ^{1}H NMR ($CDCl_3$) δ 7.67 (d, 1H, $J = 4.5$), 7.75 (dd, 1H, $J = 8.6$, $J = 7.6$), 8.10 (dd, 1H, $J = 7.6$, $J = 1.3$), 8.48 (dd, 1H, $J = 8.6$, $J = 1.3$), 8.94 (d, 1H, $J = 4.5$); ^{13}C NMR ($CDCl_3$) δ 123.0, 124.4, 126.5, 127.5, 128.3, 140.6, 143.2, 148.7, 152.1. The mother liquor was evaporated and chromatographed in 19:1 hexanes–ethyl acetate, to provide 2.05 g (16%) of the 5-nitro isomer **6d** as a very light-yellow solid; mp 144–146 °C (lit.[47] mp 150 °C); ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 1H, $J = 4.7$), 7.82 (m, 2H), 8.35 (dd, 1H, $J = 2.5$, $J = 7.3$), 8.90 (d, 1H, $J = 4.7$); ^{13}C NMR ($CDCl_3$) δ 118.2, 123.4, 125.1, 128.8, 134.2, 135.6, 139.1, 149.7, 151.2.

4.2.10. 4-Chloroquinoline (6e). 4-Quinolone (10.0 g, 69.0 mmol) was added to phosphorus oxychloride (82.3 g, 0.537 mol), and the stirred mixture was heated to reflux, and maintained at this temperature for 20 min. The reaction mixture was then cooled, and ice was slowly added to the crude residue until the evolution of HCl gas was no longer observed. The mixture was then neutralized by addition of 10% NaOH (pH 7.0) and then extracted into $CHCl_3$ (3×100 mL), washed with water (3×100 mL), dried ($MgSO_4$), and evaporated to give 10.3 g (92%) of the chloroquinoline as a white crystalline solid; mp 28–29 °C (lit.[64] mp 31–32 °C); ^{1}H NMR ($CDCl_3$) δ 7.50 (d, 1H, $J = 4.6$), 7.65 (ddd, 1H, $J = 8.4$, $J = 7.0$, $J = 1.2$), 7.78 (ddd, 1H, $J = 8.4$, $J = 7.0$, $J = 1.4$), 8.14 (dd, 1H, $J = 8.4$, $J = 1.4$), 8.24 (dd, 1H, $J = 8.4$, $J = 1.2$), 8.79 (d, 1H, $J = 4.6$); ^{13}C NMR ($CDCl_3$) δ 121.4, 124.3, 126.7, 127.7, 130.0, 130.5, 142.7, 129.3, 149.9.

4.3. General procedure for the preparation of 4-[[2-(dimethylamino)ethyl]amino]nitroquinolines

4.3.1. 4-[[2-(Dimethylamino)ethyl]amino]-8-nitroquinoline (7a). A mixture of **6a** (1.0 g, 4.8 mmol) and *N*,*N*-di-

methylethylenediamine (6.25 g, 70.9 mmol) was heated to reflux with stirring for 2 h, and was then cooled and the solvent was evaporated in vacuo. The crude residue was dissolved in 5% aqueous HCl (150 mL) and washed with chloroform (3×100 mL), and then basified with 30% NaOH, extracted into chloroform (5×100 mL), dried ($MgSO_4$), evaporated, and chromatographed in 98:2 chloroform–methanol, to provide 480 mg (44%) as an orange crystalline solid; mp 78–79 °C; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 6H), 2.71 (t, 2H, $J = 5.9$), 3.31 (m, 2H), 6.17 (br, 1H), 6.45 (d, 1H, $J = 5.3$), 7.42 (dd, 1H, $J = 8.4$, $J = 7.7$), 7.87 (dd, 1H, $J = 7.6$, $J = 1.4$), 8.01 (dd, 1H, $J = 8.4$, $J = 1.4$), 8.61 (d, 1H, $J = 5.3$); ^{13}C NMR ($CDCl_3$) δ 39.9, 45.1, 56.9, 100.2, 120.4, 122.7, 123.0, 124.1, 140.3, 149.0, 149.8, 153.1; HRMS calcd for $C_{13}H_{16}O_2N_4H$: 261.1352; found: 261.1343.

4.3.2. 4-[[2-(Dimethylamino)ethyl]amino]-7-nitroquinoline (7b). Prepared from **6b** (1.9 g, 9.1 mmol) and *N*,*N*-dimethylethylenediamine (12.5 g, 141.5 mmol), reaction time 2 h, providing 1.8 g (76%) of **7b** as a yellow solid; mp 117–119 °C; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 6H), 2.73 (t, 2H, $J = 5.5$), 3.32 (m, 2H), 6.15 (br, 1H), 6.49 (d, 1H, $J = 5.4$), 7.92 (d, 1H, $J = 9.2$), 8.13 (dd, 1H, $J = 9.2$, $J = 2.4$), 8.65 (d, 1H, $J = 5.4$), 8.80 (d, 1H, $J = 2.4$); ^{13}C NMR ($CDCl_3$) δ 40.0, 45.1, 56.9, 101.1, 117.7, 122.0, 122.5, 125.9, 147.9, 148.0, 149.7, 153.3; HRMS calcd for $C_{13}H_{16}O_2N_4H$: 261.1352; found: 261.1340.

4.3.3. 4-[[2-(Dimethylamino)ethyl]amino]-6-nitroquinoline (7c). Prepared from **6c** (750 mg, 3.6 mmol) and *N*,*N*-dimethylethylenediamine (6.25 g, 70.9 mmol), reaction time 90 min; providing 890 mg (95%) of **7c** as a yellow solid; mp 127–129 °C; ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 6H), 2.73 (t, 2H, $J = 5.9$), 3.32 (m, 2H), 6.30 (br, 1H), 6.50 (d, 1H, $J = 5.5$), 8.04 (d, 1H, $J = 9.2$), 8.40 (dd, 1H, $J = 9.2$, $J = 2.3$), 8.66 (d, 1H, $J = 5.5$), 8.89 (d, 1H, $J = 2.3$); ^{13}C NMR ($CDCl_3$) δ 40.1, 45.2, 56.9, 100.3, 117.9, 118.0, 122.7, 131.3, 143.8, 151.4, 151.5, 154.3; HRMS calcd for $C_{13}H_{16}O_2N_4H$: 261.1352; found: 261.1337.

4.3.4. 4-[[2-(Dimethylamino)ethyl]amino]-5-nitroquinoline (7d). Prepared from **6d** (800 mg, 3.84 mmol) and *N*,*N*-dimethylethylenediamine (6.25 g, 70.9 mmol), reaction time 90 min, providing 730 mg (73%) of **7d**, as an oily semi-solid.[65] ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H), 2.64 (t, 2H, $J = 5.9$), 3.24 (m, 2H), 5.93 (br, 1H), 6.59 (d, 1H, 5.3), 7.62 (m, 2H), 8.17 (dd, 1H, $J = 5.0$, $J = 4.8$), 8.62 (d, 1H, $J = 5.3$); ^{13}C NMR ($CDCl_3$) δ 41.1, 45.0, 56.7, 102.6, 110.9, 121.3, 127.1, 134.6, 135.6, 148.7, 149.8, 151.8.

4.3.5. 4-[[2-(Dimethylamino)ethyl]amino]quinoline (7e). Compound **6e** (2.3 g, 14.1 mmol) was stirred in boiling phenol (12.0 g, 128 mmol) for 2.5 h. Then the mixture was cooled to 100 °C and *N*,*N*-dimethylethylenediamine (3.0 g, 30.0 mmol) was added, and the reaction was stirred for an additional 16 h. The reaction mixture was cooled and solvent removed under vacuum. The residue was dissolved in chloroform (150 mL) and washed with 10% NaOH (3×75 mL), dried ($MgSO_4$), and evaporated in vacuo to give 2.88 g (96 %) of **7e**; mp 99.5–100 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 6H), 2.65 (t, 2H, $J = 6.0$), 3.26 (m, 2H), 5.95 (br, 1H), 6.36 (d, 1H, $J = 5.2$), 7.41 (ddd, 1H, $J = 8.3$, $J = 6.9$, $J = 1.0$), 7.60 (ddd, 1H, $J = 8.5$, $J = 6.9$, $J = 1.2$), 7.81 (dd, 1H, $J = 8.3$, $J = 1.2$), 7.97 (dd, 1H, $J = 8.5$, $J = 1.0$), 8.54 (d, 1H, $J = 5.2$); ^{13}C NMR ($CDCl_3$) δ 40.1, 45.1, 57.2, 98.9, 119.1, 119.9, 124.5, 129.0, 129.8, 148.5, 150.0, 151.1; HRMS calcd for $C_{13}H_{17}N_3O_2H$: 215.1422; found 215.1430.

4.4. General procedure for the preparation of *N*-(quinolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)ethyl]-2-iodo-4,5-dimethoxybenzamides

4.4.1. *N*-(8-Nitroquinolin-4-yl)-N-[2-(*N*,*N*-dimethylamino)-ethyl]-2-iodo-4,5-dimethoxybenzamide (8a). Oxalyl chloride (762 mg, 6.0 mmol) was added to a solution of 3,4-dimethoxy-6-iodobenzoic acid (570 mg, 1.85 mmol) in anhydrous methylene chloride (20 mL), and the stirred mixture was refluxed for 3 h. The mixture was then concentrated to dryness under reduced pressure. The acid chloride was redissolved in 20 mL of anhydrous methylene chloride, and this solution was added to a solution of **7a** (400 mg, 1.54 mmol) and triethylamine (1.14 g, 11.3 mmol) in methylene chloride (20 mL), and the resulting mixture was stirred at reflux overnight. The reaction mix was cooled and washed with saturated sodium bicarbonate (3×75 mL) and extracted with 5% aq HCl (4×100 mL). The combined aqueous extracts were basified with 30% NaOH and then extracted with chloroform (3×100 mL). Combined organic extracts were then dried ($MgSO_4$) and evaporated to give 722 mg (85%) of the amide as a gum; ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 6H), 2.69 (m, 2H), 3.32 (s, 3H), 3.74 (s, 3H), 3.95 (m, 1H), 4.57 (m, 1H), 6.41 (s, 1H), 7.02 (s, 1H), 7.75 (m, 2H), 8.05 (d, 1H, $J = 6.6$), 8.44 (d, 1H, $J = 8.4$), 8.95 (d, 1H, $J = 4.4$); ^{13}C NMR ($CDCl_3$) δ 45.4, 47.3, 55.7, 56.1, 56.5, 82.7, 110.7, 121.9, 123.3, 124.0, 126.1, 126.9, 127.2, 133.1, 140.9, 147.4, 148.4, 149.0, 150.1, 153.0, 169.9; HRMS calcd for $C_{22}H_{23}O_5N_4IH$: 551.0792; found: 551.0803.

4.4.2. *N*-(7-Nitroquinolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)-ethyl]-2-iodo-4,5-dimethoxybenzamide (8b). The acid chloride prepared from 3,4-dimethoxy-6-iodobenzoic acid (985 mg, 3.2 mmol) was redissolved in 20 mL of anhydrous methylene chloride, and this solution was added to a solution of **7b** (680 mg, 2.6 mmol) and triethylamine (2.0 g, 20.0 mmol) in methylene chloride (30 mL). The resulting mixture was stirred at reflux for 2 h and provided 760 mg (53%) of the amide as a gum; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 6H), 2.63 (m, 2H), 3.28 (s, 3H), 3.73 (s, 3H), 3.92 (m, 1H), 4.50 (m, 1H), 6.33 (s, 1H), 7.02 (s, 1H), 7.73 (d, 1H, $J = 4.8$), 8.41 (m, 2H), 8.98 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 45.5, 47.5, 55.6, 56.1, 56.8, 82.9, 110.4, 120.6, 121.8, 124.1, 125.4, 126.6, 129.2, 133.3, 147.5, 148.4, 148.8, 149.9, 152.6, 153.1, 169.7;

HRMS calcd for $C_{22}H_{23}O_5N_4IH$: 551.0792; found: 551.0780.

4.4.3. *N*-(6-Nitroquinolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (8c). The acid chloride prepared from 3,4-dimethoxy-6-iodobenzoic acid (985 mg, 3.2 mmol) was redissolved in 20 mL of anhydrous methylene chloride, and this solution was added to a solution of **7c** (700 mg, 2.7 mmol) and triethylamine (2.0 g, 20.0 mmol) in methylene chloride (30 mL). The resulting mixture was stirred at reflux for 2 h and provided 1.15 g (78%) of **8c** as a gum; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 6H), 2.63 (m, 2H), 3.35 (s, 3H), 3.71 (s, 3H), 3.96 (m, 1H), 4.29 (m, 1H), 6.50 (s, 1H), 6.94 (s, 1H), 7.79 (d, 1H, $J = 5.2$), 8.22 (d, 1H, $J = 9.2$), 8.48 (d, 1H, $J = 9.2$), 9.00 (d, 1H, $J = 5.2$), 9.28 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.5, 48.0, 55.6, 56.1, 56.8, 82.3, 110.7, 120.9, 121.5, 122.9, 123.4, 125.5, 132.2, 133.7, 145.9, 148.4, 149.1, 149.8, 151.5, 154.1, 169.7; HRMS calcd for $C_{22}H_{23}O_5N_4IH$: 551.0792; found: 551.0778.

4.4.4. *N*-(5-Nitroquinolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (8d). The acid chloride prepared from 3,4-dimethoxy-6-iodobenzoic acid (985 mg, 3.2 mmol) was redissolved in 30 mL of anhydrous methylene chloride, and this solution was added to a solution of **7d** (500 mg, 1.9 mmol) and triethylamine (2.0 g, 20.0 mmol) in methylene chloride (20 mL), and the resulting mixture was stirred at reflux for 2 h to provide 1.17 g (81%) of **8d** as a gum. The material was obtained as a mixture of atropisomers and was used as such without separation or further purification; HRMS calcd for $C_{22}H_{23}O_5N_4IH$: 551.0792; found: 551.0791.

4.4.5. *N*-(Quinolin-4-yl)-N-(*N*,*N*-dimethylaminoethyl)-2-iodo-5,6-dimethoxybenzamide (8e). The acid chloride prepared from 3,4-dimethoxy-6-iodobenzoic acid (1.5 g, 4.87 mmol) was redissolved in 40 mL of anhydrous methylene chloride, and this solution was added to a solution of **7e** (870 mg, 4.05 mmol) and triethylamine (4.0 g, 40.0 mmol) in methylene chloride (30 mL), and the resulting mixture was stirred at reflux for 2 h to provide 1.45 g (73%) of **8e**; ^{13}C NMR ($CDCl_3$) δ 45.6, 47.0, 55.3, 56.0, 56.7, 82.5, 110.2, 121.6, 122.8, 125.8, 127.6, 130.0, 130.5, 130.6, 133.8, 146.9, 148.1, 149.6, 149.8, 150.8, 169.8; HRMS calcd for $C_{22}H_{24}O_3N_3IH$: 506.0941; found: 506.0943.

4.5. Computational studies

All computations were performed using Spartan '02 (Wavefunction, Inc.) on a Silicon Graphics Octane 2 workstation. The single-point B3LYP/6-31G*[58,59] computations on the various nitro substituted 4-*N*-methylacetamido quinolines were performed starting from AM1[60] geometries. The values of the potentials at C3 are averages of six points obtained directly from the mesh contour map of the electrostatic potential on the electron density in the region of atom C3.

4.6. Topoisomerase-mediated DNA cleavage assays

Human topoisomerase I was expressed in *E. Coli* and isolated as a recombinant fusion protein using a T7 expression system as described previously.[66] Plasmid YepG was purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium bromide isopycnic centrifugation method as described.[67] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[68] The cleavage assays were performed as previously reported.[66,69] The drug and the DNA in presence of topoisomerase I were incubated for 30 min at 37 °C. After development of the gels, typically 24 h exposure was used to obtain autoradiograms outlining the extent of DNA fragmentation. Topoisomerase I-mediated DNA cleavage values are reported as REC, relative effective concentration, that is concentrations relative to topotecan, whose value is arbitrarily assumed as 1.0, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I.

4.7. Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[70–72] The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan).[60] The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO_2 and maintained by regular passage in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). For determination of IC_{50}, cells were exposed continuously for 4 days to varying concentrations of drug, and MTT assays were performed at the end of the fourth day. Each assay was performed with a control that did not contain any drug. All assays were performed at least twice in six replicate wells.

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