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5*H*-Dibenzo[*c*,*h*]1,6-naphthyridin-6-ones: Novel Topoisomerase I-Targeting Anticancer Agents with Potent Cytotoxic Activity

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Abstract—5*H*-Dibenzo[*c*,*h*]1,6-naphthyridine-6-ones can exhibit potent antitumor activity. The effect of varied substituents at the 5-position of 5*H*-8,9-dimethoxy-2,3-methylenedioxydibenzo[*c*,*h*]1,6-naphthyridine on relative cytotoxicity and topoisomerase I-targeting activity was evaluated. Potent TOP-1-targeting activity is observed when the 5-position is substituted with either a 2-(*N*,*N*-dimethylamino)ethyl group, as in **3a,** or a 2-(pyrrolidin-1-yl)ethyl substituent, **3c**. In contrast, the addition of a β-methyl group or a β-hydroxymethyl group to compound **3a**, as in **3b** and **3j**, results in a loss of significant TOP1-targeting activity. While the presence of a 3-(*N*,*N*-dimethylamino)propyl substituent at the 5-position or a methyl(2-tetrahydrofuranyl) group allows for retention of TOP1-targeting activity, the 2-(4-methyl-1-piperazinyl)ethyl analogue, **3d**, did not exhibit significant activity. Replacement of the *N*,*N*-dimethylamino group of **3a** with either C_2H_5 or OH, as in **3f** and **3h**, respectively, also had a negative impact on both cytotoxicity and TOP1-targeting activity. Treatment of **3a** with LAH gave the 5,6-dihydrodibenzo[*c*,*h*]naphthyridine, **4a**. This dihydro derivative has approximately 2/3 the potency of **3a** as a TOP1-targeting agent. Compounds **3a**, **3b**, **3h**, **3i**, and **4a** were evaluated for antitumor activity in the human tumor xenograft model using athymic nude mice. The non-estrogen responsive breast tumor cell line, MDA-MB-435, was used in these assays. Compound **3a** proved to be effective in regressing tumor growth in vivo when administered either by ip injection or orally 3× week at a dose of 2.0 mg/kg. Compound **4a** when administered orally 5× weekly at a dose of 40 mg/kg also suppressed tumor growth.

Introduction

Topoisomerases are nuclear enzymes that 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 a single or double-stranded DNA break is implicated.[1–3] Topoisomerase-targeting agents that stabilize the cleaveable complex formed between the enzyme and DNA have proved to be effective in the treatment of cancer. This drug-induced stabilization of the enzyme–DNA cleavable complex effectively converts these nuclear enzymes into cellular poisons.

Camptothecin was the first agent identified as a TOP1-targeting agent.[4] There are two clinical agents, topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®), that have been developed from the extensive studies on camptothecin and its structurally related analogues. These camptothecin-based drugs have incorporated within their structures a γ-lactone, which is prone to hydrolysis. Ring-opening of the lactone moiety of camptothecin results in the formation of an inactive derivative that possesses high affinity for human serum albumin.[5–7] 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.[8–11]

There have been several reports on novel non-camptothecin TOP1-targeting agents. These include derivatives of bi- and terbenzimidazoles,[12,13] benz[*a*]anthracenes,[14]

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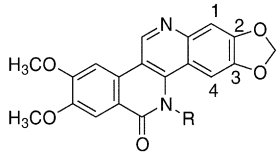
benzo[*c*]phenanthridine and protoberberine alkaloids,[15,16] indolocarbazoles,[17] the fungal metabolites bulgarein[18] and saintopin,[19] and several indenoisoquinolines[20] and benzophenazines.[21] These studies were performed in an effort to develop TOP1-targeting agents with the potential for improved efficacy (Fig. 1).



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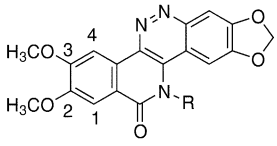

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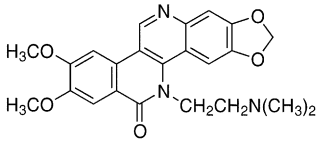


3a

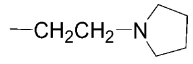
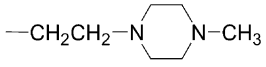
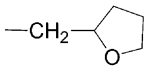
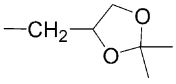
Figure 1. Structure and numbering of 5*H*-dibenzo[*c,h*]1,6-naphthyridin-6-ones, **1**, 11*H*-5,6,11-triazachrysen-12-ones, **2** and 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c,h*]1,6-naphthyridin-6-one, **3a**.

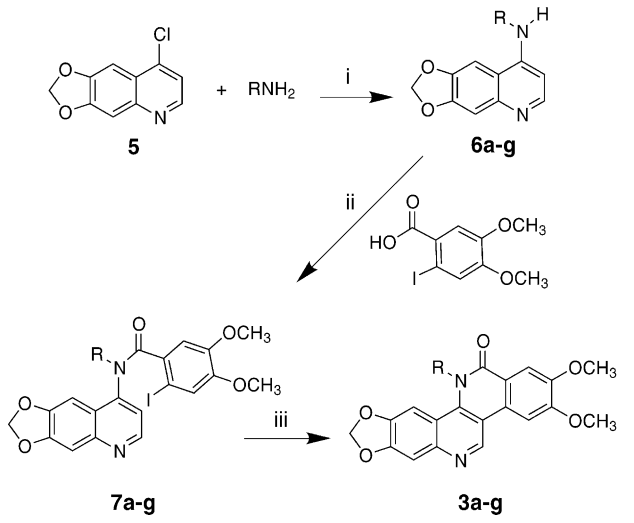
Studies in our laboratory have demonstrated that substituted 5*H*-dibenzo[*c,h*]1,6-naphthyridin-6-ones, **1**, and 11-*H*-5,6,11-triazachrysen-12-ones, **2**, can exhibit potent TOP1-targeting activity and pronounced cytotoxicity.[22] In the case of 5*H*-8,9-dimethoxy-5-(2-N,N-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c,h*]1,6-naphthyridin-6-one, **3a**, potent antitumor activity was observed in vivo in athymic nude mice with human tumor xenografts by both oral and parenteral administration. These data have prompted further studies on the structure–activity relationships associated with various 5*H*-dibenzo[*c,h*]1,6-naphthyridines. In the present study, the synthesis and pharmacological evaluation of these agents are discussed.

Chemistry

The 5-substituted 8,9-dimethoxy-2,3-methylenedioxydibenzo[*c,h*]1,6-naphthyridines that were synthesized are summarized in Table 1. The common intermediate for preparation of compounds **3a–k** was 4-chloro-6,7-methylenedioxyquinoline,[23] which was prepared as previously reported. Compounds **3a–g** were synthesized by conversion of **5** to the appropriately substituted 4-aminoquinolines, **6a–g**, using phenol in the presence of the requisite primary amine as outlined in Scheme 1.[24,25] Treatment of 4,5-dimethoxy-2-iodobenzoic acid with oxalyl chloride provided the acid chloride, which was treated with **6a–g** to yield the benzamide derivatives, **7a–g**. These *o*-iodobenzamides were cyclized to **3a–g**

Table 1. Relative TOP1-targeting activity and cytotoxicity of 5-substituted dibenzo[*c,h*]1,6-naphthyridines

Compd	Alkyl group at N-5	TOP1-mediated DNA cleavage	Cytotoxicity IC_{50} (μM)	
			RPMI8402	CPT-K5
3a	$CH_2CH_2N(CH_3)_2$	0.5	0.003	0.8
3b	$CHCH_3CH_2N(CH_3)_2$	>1000	0.57	3.4
3c		0.3	0.003	0.39
3d		1000	0.34	10
3e	$CH_2CH_2CH_2N(CH_3)_2$	1.0	0.03	0.9
3f	$CH_2CH_2CH_2CH_3$	1000	0.08	>10
3g		10	0.15	>10
3h	CH_2CH_2OH	50	0.02	>10
3i	$CH_2CH_2OCH_2CH_2OH$	1.0	0.026	>10
3j	$CH(CH_2OH)CH_2N(CH_3)_2$	>1000	1.6	12.5
3k	$CH_2CHOHCH_2OH$	0.8–1.0	0.044	>10
4a	$CH_2CH_2N(CH_3)_2$	0.8	0.017	4.0
4b	$CHCH_3CH_2N(CH_3)_2$	100	0.16	0.4
9k		10	0.039	>10
CPT		0.5–0.8	0.005	>10
CPT-11		25	0.57	>10
Topotecan		1.0	0.012	>10
VM-26		>1000	0.22	0.28



Scheme 1. (i) C_6H_5OH, reflux initially, then 100 °C; (ii) $COCl_2$, CH_2Cl_2, then TEA, CH_2Cl_2; (iii) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Ag_2CO_3, DMF.

using conditions similar to those reported by Harayama et al. for the preparation of benzo[*c*]phenanthridine alkaloids.[26]

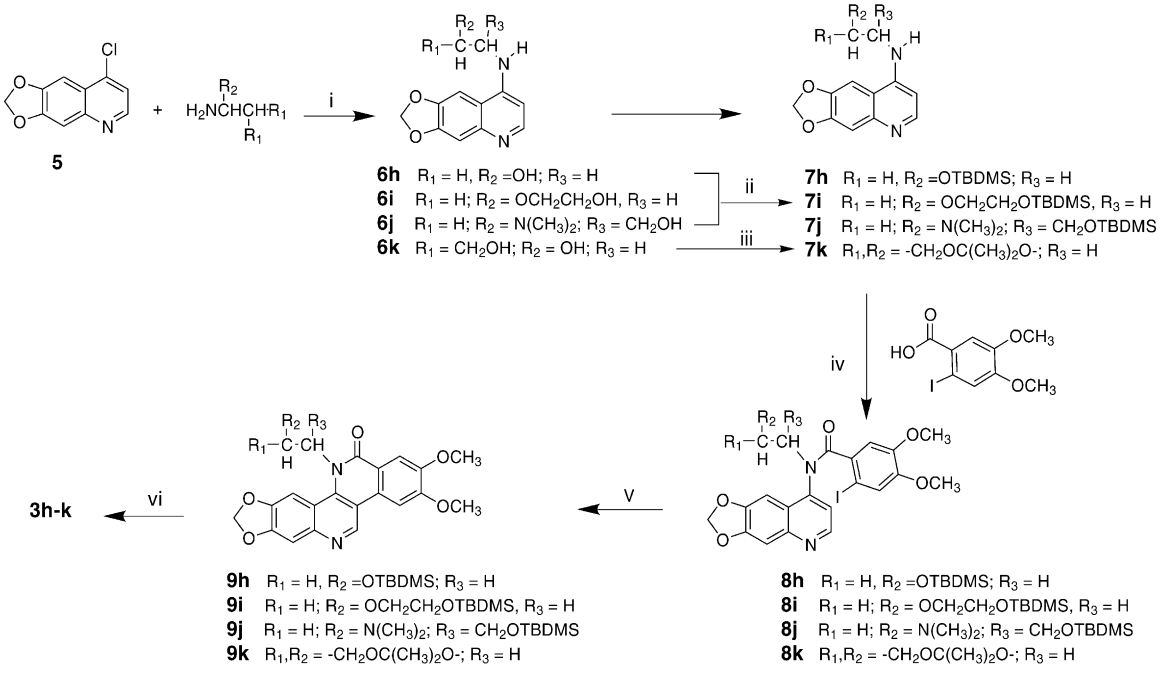
The 5-(hydroxyalkyl) substituted analogues, **3h–k**, were similarly prepared by treating ethanolamine, aminoethylethoxyethanol, 2,3-dihydroxypropylamine, 2-(4-methyl-1-piperazinyl)ethylamine[27] and *N*,*N*-dimethyl-2-(hydroxymethyl)ethylenediamine[21] with 4-chloro-6,7-methylenedioxyquinoline[23] to form the 4-substituted aminoquinoline derivatives, **6h–k** (Scheme 2). Prior to treating these intermediates with 4,5-dimethoxy-2-iodobenzoyl chloride,[28] the hydroxyl groups in the case of **6h–j** were protected by forming either the TBDMS ethers **7h–j** or, in the case of the diol **6k**, the acetonide, **7k**. The intermediates **7h–k** were then converted to their 2-iodo-4,5-dimethoxybenzamides, **8h–k** and were subjected to Heck cyclization conditions to provide **9h–k**. The silyl ethers **9h–j** were cleaved at room temperature under acidic conditions to provide **3h–j**. The acetonide **9k** was converted to the diol **3k**, using 80% AcOH.

Treatment of **3a** and **3b** with LAH in THF, as illustrated in Scheme 3, provided the 5,6-dihydro-8,9-dimethoxy-2,3-methylenedioxydibenzo[*c*,*h*]1,6-naphthyridines, **4a** and **4b**.

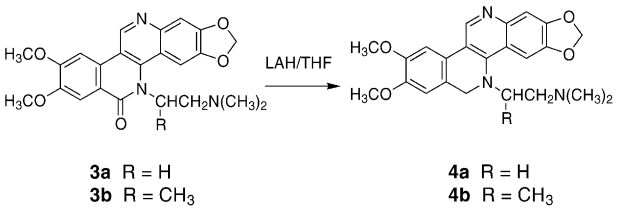
Pharmacology

The relative TOP1-targeting activity and cytotoxicity of the 5-substituted 5*H*-2,3-methylenedioxy-8,9-dimethoxydibenzo[*c*,*h*]1,6-naphthyridines synthesized are listed in Table 1. Several compounds had TOP1-targeting activity equivalent to or greater than CPT. The data indicate that **3a** and **3c** are among the more potent TOP1-targeting compounds. A representative gel of the TOP1-mediated cleavage observed with compounds **3a**, **3e**, **4a**, and **4b** is illustrated in Figure 2. The exceptional potency of **3a** and **3c** as TOP1-targeting agents also correlates with their greater cytotoxic activity. CPT-K5, which is camptothecin resistant, is a variant of RPMI8402 and expresses mutant TOP1. Significant cross-resistance to CPT-K5 (>10-fold difference in IC_{50} values) is indicative of TOP1-targeting activity being associated with cytotoxicity.

In the case of **3a** and **3c**, both compounds were cross-resistant to CPT-K5 cells. Compound **3b**, wherein a β-methyl substituent has been added to **3a**, has no significant TOP1-targeting activity and is more than two orders of magnitude less cytotoxic than **3a**. This intolerance of additional substituents on the carbon



Scheme 2. (i) C_6H_5OH, reflux initially, then 100 °C (ii) TBDMS chloride, imidazole, DMF; (iii) pTSA, $CH_3C(OCH_3)_2CH_3$, DMF, 80 °C; (iv) $COCl_2$, CH_2Cl_2, then TEA, CH_2Cl_2; (v) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Ag_2CO_3, DMF; (vi) for **8h–k** AcOH/THF/H_2O (3:1:1) or for **8k** 80% AcOH, reflux.



Scheme 3.

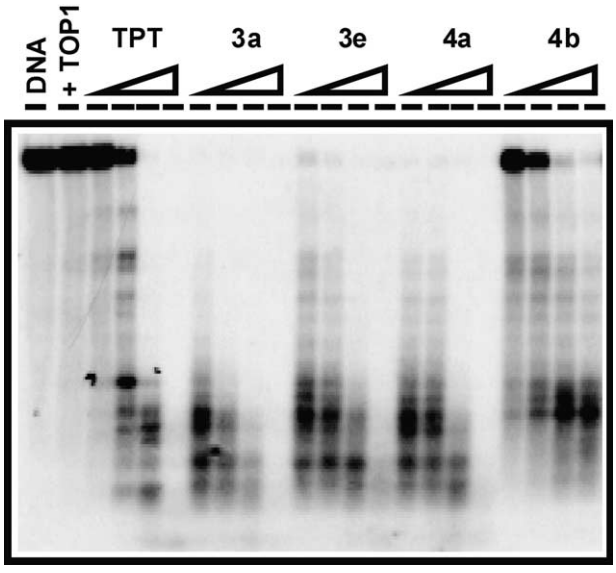


Figure 2. Stimulation of enzyme-mediated DNA cleavage by topotecan (TPT), **3a**, **3e**, **4a** and **4b** using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.001 to 1.0 μM.

attached to the N-5 position is also observed in the case of **3j**, which is the β-hydroxymethyl derivative of **3a**. Compound **3j** also did not exhibit any significant TOP1-targeting activity. There was also a dramatic effect on relative cytotoxic activity. While **3a** had an IC_{50} of 3 nM towards RPMI8402, the IC_{50} value observed with **3j** was 1.6 μM.

The length of the alkyl chain attached to N-5 also influenced relative TOP1-targeting activity and cytotoxicity. Insertion of a methylene group into the side chain of **3a**, as is the case for **3e**, did result in a decrease in intrinsic TOP1-targeting activity (≈1/2 that of **3a**) and cytotoxicity. Compound **3d**, which has a 2-(*N*-methylpiperidinyl) moiety attached to the N-5 ethyl side chain, did not possess significant TOP1-targeting activity and was two orders of magnitude less cytotoxic than **3a**. These data suggest that either the presence of a two tertiary amines attached to the N-5 side chain or the increased steric bulk associated with this *N*-methylpiperazinyl moiety adversely affect activity. Further studies are needed to determine the steric constraints associated with retention of TOP1-targeting activity. It is of interest to note that **3i**, which extends six atoms in length from the N-5 position and has substantial conformational flexibility, does retain good TOP1-targeting activity and cytotoxic activity.

The presence of an alkylamino or cycloalkylamino group on the side chain attached to the N-5 position had a pronounced effect on water solubility and activity. The *n*-butyl derivative, **3f**, did not exhibit significant TOP1-targeting activity. Despite this result, it did exhibit significant cytotoxicity and was cross-resistant to CPT-K5 cells. While water solubility could influence the relative activity of the compounds evaluated in these in vitro assays, it is difficult to reconcile these data in the case of **3f**. The results observed for compounds **3i** and **3k** do suggest that such alkylamino groups on the side chain are not a requirement for maintaining TOP1-targeting activity or potent cytotoxicity. While not as potent **3a** or **3c,** the presence of hydroxyl groups on the side chain, as in the case of **3i** and **3k**, was associated with significant topoisomerase-targeting activity and cytotoxicity.

Comparison of relative biological activities of **3a** with it dihydro derivative, **4a**, reveals that **3a** is more active as a TOP1-targeting agent and has greater cytotoxicity. This may be related to their differences in their molecular geometry. In the case of **3a**, the interaction between the β-CH_2 and the hydrogen atom at the 4-position cause a molecular distortion that results in a torsion angle of 17.4° between atoms C4–C4a–C4b–N5 (dihedral angle I) and an angle of 7.5° between atoms C10–C10a–C10b–C11 (dihedral angle II).[29] As shown in Figure 3, in the case of **4a**, these torsion angles are 5.8 and 19.2° for dihedral angles I and II, respectively. In the case of **3b** with **4b** greater TOP1-targeting activity was observed with the dihydro derivative, **4b**, suggesting that the factors that contribute to the decreased activity of β-methyl substituted analogue of **3a** may be somewhat reduced in the case of **4b**. The adverse effect of steric bulk in close proximity to the N-5 heteroatom on TOP1-targeting activity and cytotoxicity is also evident when comparing the biological activities of **3i** with its acetonide precursor, **3k**. In contrast to either the six atom linear alkyl side of **3i** or a 2,3-dihydroxypropyl group at N-5 as in **3k**, the presence of an isopropylidene at the 2,3-positions of a propyl moiety at N-5, as is the case with **9k**, results in a dramatic loss of TOP1-targeting activity (Fig. 4).

Compounds **3a**, **3b**, **3h**, **3i**, and **4a** were evaluated for antitumor activity using the human tumor xenograft, MDA-MB-435 in athymic nude mice. Table 2 summarizes the results of these assays. Two assays were performed using female NCR/NU NU mice. A third study was initiated using male NCR/NU NU as outlined in Table 2. In each bioassay, CPT-11 was

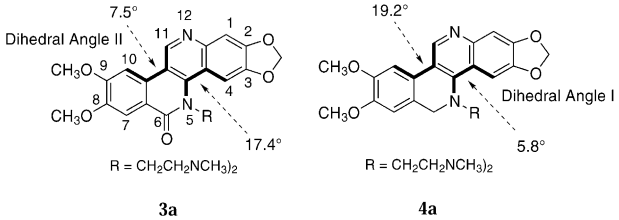


Figure 3. Dihedral angles formed between atoms C4–C4a–C4b–N5 (dihedral angle I) and C10–C10a–C10b–C11 (dihedral angle II).

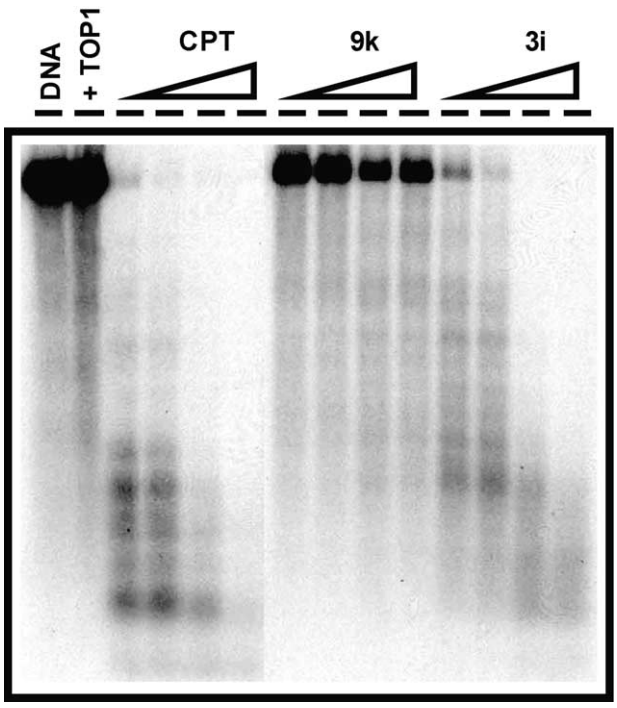


Figure 4. Stimulation of enzyme-mediated DNA cleavage by camptothecan (CPT), **9k** and **3i** using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.001 to 1.0 μM.

employed as the positive control. The vehicle controls in these studies consisted of 0.1% citric acid in water. The data in Table 2 and illustrated in Figure 5 indicate that **3a** administered ip or po exhibits antitumor activity in this human tumor xenograft model at approximately 1/20th the total dose of CPT-11 used in these assays. While CPT-11 is not effective orally, **3a** exhibits similar antitumor activity whether administered ip or po. The dihydro derivative, **4a**, was better tolerated when administered either ip or po than **3a**. The relative antitumor activity of **4a** in this model, however, was less than **3a**. Compound **4a** was less effective as an antitumor agent at doses 15–30× higher than the doses used to administer **3a** by the ip and po routes, respectively. As illustrated in Figure 6, only at the higher dose that was administered po did **4a** have significant antitumor activity. Compounds **3h** and **3i** were administered ip. Because of their poor solubility, the dose levels of **3h** and **3i** that could be administered were limited. These compounds were administered as a suspension at a dose of 10 mg/kg 5 days per week. Neither **3h** nor **3i** exhibited significant antitumor activity using this schedule and dose. In the one bioassay performed using male mice, both CPT-11 and **3a** appeared to be less responsive. Compound **3b**, which is devoid of TOP1-targeting activity, was initially administered at a dose of 1.0 mg/kg and the dose increased to 10 mg/kg. Under these assay conditions, **3b** did not exhibit significant antitumor activity in this animal model.

Table 2. Antitumor activity observed in athymic nude mice with the human tumor xenograft MDA-MB-435

Compd	Assay[a]	No. of Mice and Sex	Route	Average tumor volume (mm^3)				Total dose (mg/kg)/mouse
				Day 7	Day 13	Day 21	Day 31	
3a	A	7♀[b]	po	159	148	99	62	0.78
3a	A	7♀[b]	ip	123	84	65	40	0.72
3h	B	7♀[c]	ip	132	176	249	351	4.89
3i	B	6♀[c]	ip	132	143	211	245	5.05
4a	B	7♀[d]	po	117	96	108	142	23.90
4a	B	7♀[e]	ip	95	122	194	247	11.07
CPT-11	A	6♀[e]	ip	118	116	36	11	13.89
	B	7♀[e]	ip	99	98	40	14	17.38
Vehicle	A	6♀[f]	ip	199	234	356	472	
	B	7♀[f]	ip	133	152	188	298	
3a	C	7♂[b]	ip	105	100	104	113	1.00
3b	C	7♂[g]	ip	114	136	174	222	3.51
CPT-11	C	7♂[e]	ip	111	102	69	19	17.29
Vehicle	C	7♂[f]	ip	124	156	224	235	

[a]Three separate assays were performed. Assay A and B used female NCR/NU NU mice. Assay C used male NCR/NU NU mice.
[b]Initial dose was 2.0 mg/kg qd × 4/week. Administration was adjusted to approximately 3 × week in view of weight loss. Dosing was withheld from some mice with notable weight loss.
[c]Administration at 10 mg/kg qd × 5/week. Dose administered was limited by the solubility of compound.
[d]Administered at 40 mg/kg qd × 5/week.
[e]Administered at a dose of 20 mg/kg × 5/week. Toxicity was observed at a dose of 40 mg/kg × 5/week.
[f]Vehicle consisted of 0.1% citrate in H_2O.
[g]Initial dose was 1.0 mg/kg qd × 5/week and was increased to 10 mg/kg × 5/week.

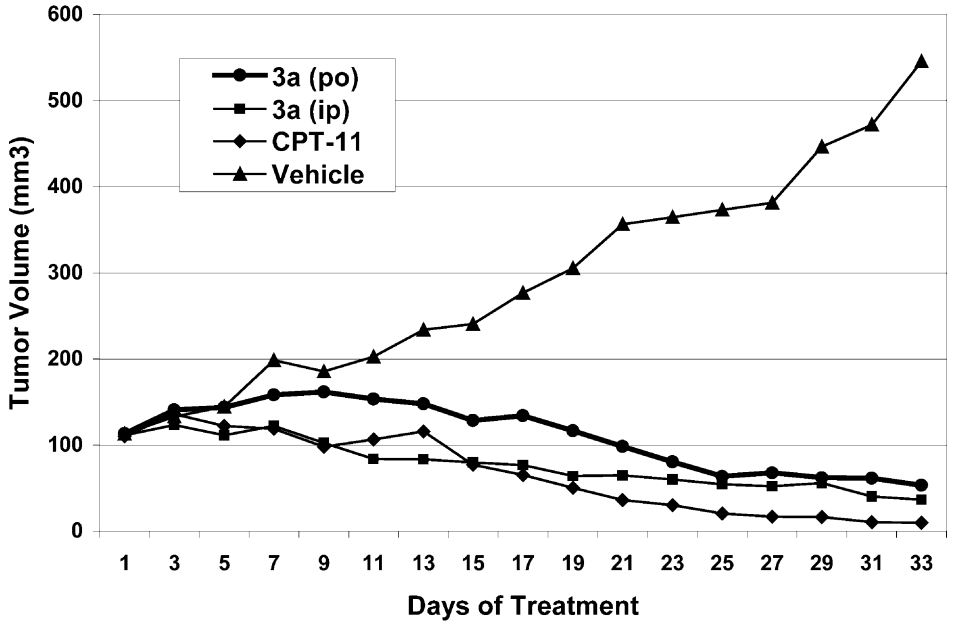


Figure 5. Antitumor activity of **3a** and CPT-11.

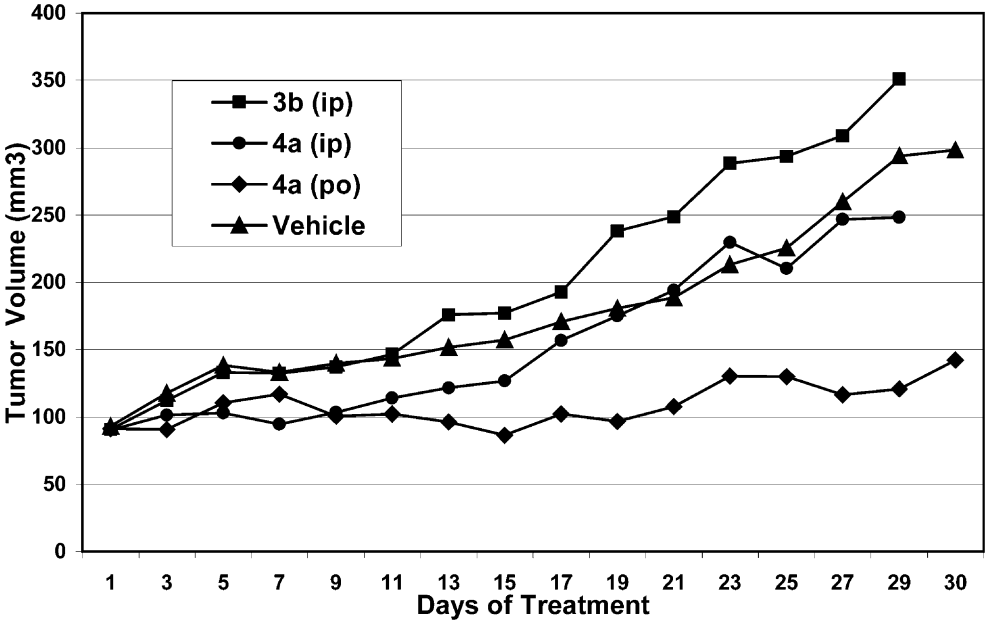


Figure 6. Evaluation of the antitumor activity of **3b** and **4a**.

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, Germany) using the solvent systems indicated. Infrared spectral data (IR) were obtained on a Thermo Nicolet Avatar 380 Fourier transform spectrophotometer and are reported in cm^{-1}. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in the deuterated solvent indicated with chemical shifts reported in δ units downfield from tetramethylsilane (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, USA.

General procedure for the cyclization of *o*-iodobenzamides

A mixture of the 4-amino-6,7-methylenedioxyquinoline *o*-iodobenzamide derivative (1.0 mmol equiv), $Pd(OAc)_2$ (0.2 mmol equiv), P(*o*-tolyl)$_3$ (0.4 mmol equiv), and Ag_2CO_3 (2.0 mmol equiv) was heated to reflux in DMF

(30 mL per mmol equiv) with stirring. The reaction mixture was allowed to cool to room temperature, diluted with $CHCl_3$, and filtered through Celite. The sicciate was extensively washed with 10% CH_3OH in $CHCl_3$. The filtrate was concentrated in vacuo and the residue chromatographed on silica gel using chloroform/methanol.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*,*N*-dimethylamino)ethyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3a). Prepared from **7a**; (41% yield); reaction time 25 min; mp 283–285 °C (dec.); IR ($CHCl_3$) 1653; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H), 3.04 (t, 2H, J = 7.2), 4.07 (s, 3H), 4.14 (s, 3H), 4.64 (t, 2H, J = 7.2), 6.18 (s, 2H), 7.47 (s, 1H), 7.68 (s, 1H), 7.89 (s, 2H), 9.37 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.9, 49.2, 56.3, 56.3, 57.9, 101.2, 102.0, 102.3, 107.1, 108.8, 111.7, 114.8, 119.3, 127.6, 140.9, 143.5, 147.3, 147.7, 149.9, 150.3, 154.2, 164.1; HRMS calcd for $C_{23}H_{23}N_3O_5H$: 422.1716; found 422.1710.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*,*N*-dimethylamino)-1-methylethyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3b). Prepared from **7b**; (30% yield); reaction time 30 min; mp 186–187 °C; IR (KBr) 1649; ^{1}H NMR ($CDCl_3$); δ 1.95–1.98 (m, 9H), 2.77 (dd, 1H, J = 12.0, 8.0), 3.21 (dd, 1H, J = 12.0, 8.0), 4.06 (s, 3H), 4.13 (s, 3H), 4.84–4.92 (m, 1H), 6.17 (s, 2H), 7.46 (s, 1H), 7.66 (s, 1H), 7.77 (s, 1H), 7.87 (s, 1H), 9.35 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.7, 45.5, 56.2, 56.3, 59.5, 63.1, 100.9, 101.9, 102.1, 107.0, 108.7, 112.4, 115.2, 120.5, 127.3, 142.6, 143.3, 147.0, 147.3, 149.9, 150.1, 154.0, 164.9; HRMS calcd for $C_{24}H_{25}N_3O_5H$: 436.1794; found 436.1863.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(pyrrolidin-1-yl)-ethyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3c). Prepared from **7c**; (36% yield); reaction time 30 min; mp 255–257 °C (dec.); IR ($CHCl_3$) 1653; ^{1}H NMR ($CDCl_3$) δ 1.79 (m, 4H), 2.64 (m, 4H), 3.20 (t, 2H, J = 7.1), 4.07 (s, 3H), 4.14 (s, 3H), 4.69 (t, 2H, J = 7.1), 6.18 (s, 2H), 7.46 (s, 1H), 7.68 (s, 1H), 7.89 (s, 1H), 7.95 (s, 1H), 9.37 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.7, 49.6, 54.3, 56.3, 56.4, 56.4, 101.3, 102.0, 102.3, 107.0, 108.7, 111.7, 114.8, 119.3, 127.7, 140.9, 143.4, 147.3, 147.8, 150.0, 150.3, 154.2, 164.2; HRMS calcd for $C_{25}H_{25}N_3O_5H$: 448.1872; found 448.1872.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(4-methylpiperazin-1-yl)ethyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3d). Prepared from **7d**; (18% yield); reaction time 25 min; mp 244–246 °C; IR ($CHCl_3$) 1651; ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H), 2.51 (m, 8H), 2.95 (t, 2H, J = 6.2), 4.07 (s, 3H), 4.15 (s, 3H), 4.69 (t, 2H, J = 6.2), 6.19 (s, 2H), 7.48 (s, 1H), 7.70 (s, 1H), 7.91 (s, 2H), 7.92 (s, 1H), 9.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.8, 45.9, 48.6, 53.0, 55.0, 56.4, 56.4, 101.2, 102.0, 102.2, 107.1, 108.9, 112.0, 115.0, 119.5, 127.6, 141.2, 143.4, 147.2, 147.4, 150.0, 150.3, 154.1, 164.4; HRMS calcd for $C_{26}H_{28}N_4O_5H$: 477.2138; found 477.2139.

8,9-Dimethoxy-2,3-methylenedioxy-5-[3-(*N*,*N*-dimethylamino)propyl] - 5*H* - dibenzo[*c*,*h*]1,6 - naphthyridin - 6 - one (3e). Prepared from **7e**; (45% yield); reaction time 30 min; mp 262–264 °C (dec.); IR ($CHCl_3$) 1648; ^{1}H NMR ($CDCl_3$) δ 2.29 (m, 8H), 2.45 (m, 2H), 4.07 (s, 3H), 4.14 (s, 3H), 4.53 (t, 2H, J = 7.4), 6.19 (s, 2H), 7.48 (s, 1H), 7.65 (s, 1H), 7.69 (s, 1H), 7.90 (s, 1H), 9.40 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.9, 45.3, 49.2, 56.3, 56.4, 56.9, 100.8, 101.9, 102.3, 107.1, 108.7, 111.6, 114.9, 119.4, 127.5, 141.0, 143.6, 147.2, 147.7, 149.9, 150.3, 154.1, 164.1; HRMS calcd for $C_{24}H_{25}N_3O_5H$: 436.1872; found 436.1878.

8,9-Dimethoxy-2,3-methylenedioxy-5-[butyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3f). Prepared from **7f**; (24% yield); reaction time 45 min; mp 224 °C (dec.); IR (KBr) 1654; ^{1}H NMR ($CDCl_3$); δ 0.99 (t, 3H, J = 7.4), 1.62 (m, 2H), 2.09 (m, 2H), 4.07 (s, 3H), 4.14 (s, 3H), 4.49 (m, 2H), 6.19 (s, 2H), 7.50 (s, 1H), 7.61 (s, 1H), 7.70 (s, 1H), 7.92 (s, 1H), 9.40 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 20.2, 31.2, 50.6, 56.3, 56.4, 100.7, 102.0, 102.2, 107.4, 108.8, 111.7, 114.9, 119.5, 127.4, 141.1, 143.7, 147.1, 147.5, 149.8, 150.3, 154.1, 164.0; HRMS calcd for $C_{23}H_{22}N_2O_5H$: 406.1529; found 406.1534.

8,9-Dimethoxy-2,3-methylenedioxy-5-(2-tetrahydofuranyl)methyl-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3g). Prepared from **7g**; (22% yield); reaction time 30 min; mp 270–273 °C; IR ($CHCl_3$) 1648; ^{1}H NMR ($CDCl_3$) δ 1.87 (m, 4H), 3.72 (m, 2H), 4.07 (s, 3H), 4.14 (s, 3H), 4.68 (m, 3H), 6.18 (s, 2H), 7.48 (s, 1H), 7.69 (s, 1H), 7.90 (s, 1H), 8.04 (s, 1H), 9.39 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.6, 30.3, 54.7, 56.3, 56.4, 68.1, 77.3, 101.7, 102.2, 102.3, 107.0, 109.0, 112.1, 115.2, 119.5, 127.7, 141.2, 143.5, 147.2, 147.4, 149.9, 150.3, 154.2, 164.6; HRMS calcd for $C_{24}H_{22}N_2O_6H$ 435.1556; found 435.1566.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(hydroxy)ethyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3h). Prepared from **9h** by treatment with AcOH, THF, H_2O (3:1:1) at room temperature; (84% yield); reaction time 48 h; mp 285–286 °C; IR (KBr); 1653, 3448; ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 3H), 4.04 (s, 3H), 4.54 (t, 2H, J = 4.4), 4.96 (t, 2H, J = 4.0), 6.26 (s, 2H), 7.44 (s, 1H), 7.71 (s, 1H), 7.98 (s, 1H), 8.03 (s, 1H), 9.64 (s, 1H); ^{13}C NMR (DMSO-d_6); δ 52.6, 56.4, 57.0, 59.5, 101.9, 103.0, 104.0, 106.8, 108.8, 111.9, 114.8, 119.1, 128.0, 141.2, 144.9, 147.4, 147.7, 150.2, 150.5, 154.6, 163.7; HRMS calcd (M^+-OH)for $C_{21}H_{17}O_5N_2$ 377.1137; found 377.1121.

Compound **9h** was prepared from **8h** (36% yield); reaction time 30 min; mp 271–273 °C; IR (KBr) 1658; ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 6H), 0.68 (s, 9H), 4.04 (s, 3H), 4.12 (s, 3H), 4.24 (t, 2H, J = 8.0), 4.65 (t, 2H, J = 8.0), 6.18 (s, 2H), 7.44 (s, 1H), 7.64 (s, 1H), 7.85 (s, 1H), 8.01 (s, 1H), 9.29 (s, 1H); HRMS calcd for $C_{27}H_{33}ISiN_2O_6H$: 637.1153; found 637.1212.

8,9 - Dimethoxy - 2,3 - methylenedioxy - 5 - [2 - (2 - hydroxyethoxy)ethyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3i). Prepared from **9i** by treatment with AcOH, THF, H_2O (3:1:1) at room temperature; (76% yield); reaction time 18 h; mp 235 °C; IR (KBr) 1654; ^{1}H NMR ($CDCl_3$) δ 3.61 (t, 2H, J = 5.2), 3.73 (t, 2H, J = 5.2), 4.07 (s, 3H), 4.14 (s, 3H), 4.22 (t, 2H, J = 5.6), 4.71 (t, 2H, J = 5.6), 6.2 (s, 2H), 7.53 (s, 1H), 7.69 (s, 1H), 7.88 (s, 1H), 8.05 (s, 1H), 9.39 (s, 1H). HRMS calcd for $C_{23}H_{22}N_2O_7H$: 439.1506; found 439.1499.

Compound **9i** was prepared from **8i**; (75% yield); reaction time 18 h; mp 238 °C (dec.); IR (KBr): 1639; ^{1}H NMR ($CDCl_3$); δ 0.00 (s, 6H), 0.85 (s, 9H), 3.54 (t, 2H, J = 5.2), 3.70 (t, 2H, J = 5.2), 4.07 (s, 3H), 4.14 (s, 3H), 4.16 (t, 2H, J = 6.0), 4.71 (t, 2H, J = 6.0), 6.17 (s, 2H), 7.48 (s, 1H) 7.70 (s, 1H), 7.94 (s, 1H), 9.39 (s, 1H); HRMS calcd for $C_{23}H_{23}N_2O_7H$: 439.1505; found 439.1506.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-*N*,*N*-dimethylamino-1-(hydroxymethyl)ethyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3j). Prepared from **9j** by treatment with 5N HCl in isopropanol at room temperature for 30 min; (57% yield); reaction time 30 min; mp 132 °C; IR (KBr) 1647; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.72–2.81 (m, 1H), 3.16–3.26 (m, 1H), 4.05 (s, 3H), 4.12 (s, 3H), 4.20–4.28 (m, 1H), 4.65–4.73 (m, 1H), 4.98 (m, 1H), 6.17 (q, 2H, J = 1.2), 7.44 (s, 1H), 7.51 (s, 1H), 7.64 (s, 1H), 7.82 (s, 1H), 7.82 (s, 1H); 9.33 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.6, 56.2, 56.3, 60.0, 64.1, 65.2, 100.9, 101.8, 102.3, 106.6, 108.5, 112.5, 115.0, 119.6, 127.5, 141.1, 143.0, 147.1, 147.5, 149.9, 150.0, 154.1, 165.0; HRMS calcd for $C_{24}H_{25}N_3O_6Li$: 458.1903; found 458.1905.

Compound **9j** was prepared from **8j** (95% yield); reaction time 45 min; ^{1}H NMR ($CDCl_3$); δ −0.13 (s, 6H), 0.69 (s, 9H), 1.97 (s, 6H), 1.92 (s, 6H), 2.52 (m, 1H), 2.80 (m, 1H) 3.20 (m, 1H), 4.01 (s, 3H), 4.09 (s, 3H), 4.50 (m, 1H), 4.90 (m, 1H), 6.11 (m, 2H), 7.30 (s, 1H), 7.61 (s, 1H), 7.79 (s, 1H), 8.19 (s, 1H), 9.32 (s, 1H); HRMS calcd for $C_{30}H_{39}N_3O_6SiH$; 566.2686; found 566.2692.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2,3-dihydroxypropyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (3k). Prepared from **9k** by treatment 80% AcOH at reflux for 2 h. The reaction mixture was allowed to cool, and then concentrated in vacuo. The crude residue was triturated with chloroform (1.5 mL), filtered, and washed with additional chloroform (10 mL), to provide 16.5 mg of pure material, in 60% yield; mp 272–274 °C (dec.); IR (KBr) 1631, 3407; ^{1}H NMR (DMSO-d_6) δ 3.31 (d, 2H, J = 8.0), 3.95 (s, 3H), 4.07 (s, 3H), 4.63 (m, 3H), 6.33 (s, 2H), 7.55 (s, 1H), 7.72 (s, 1H), 8.06 (s, 2H), 8.21 (s, 1H), 9.79 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 54.4, 56.5, 57.3, 64.9, 68.8, 103.2, 103.8, 104.6, 108.9, 109.0, 112.6, 115.5, 119.3, 127.3, 138.5, 140.6, 148.2, 151.0, 151.3, 151.8, 154.8, 163.9; HRMS calcd for $C_{22}H_{20}N_2O_7H$: 425.1350; found 425.1359.

8,9-Dimethoxy-2,3-methylenedioxy-5-[[2,2-dimethyl[1,3]-dioxolan-4-yl]methyl]-5*H*-dibenzo[*c*,*h*]1,6-naphthyridin-6-one (9k). Prepared from **8k** (22% yield); reaction time 45 min; mp 241–244 °C (dec.); IR ($CHCl_3$) 1652; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3H), 1.36 (s, 3H), 3.95 (m, 2H), 4.08 (s, 3H), 4.14 (s, 3H), 4.35 (m, 1H), 4.55 (m, 1H), 4.77 (m, 1H), 6.19 (s, 2H), 7.48 (s, 1H), 7.70 (s, 1H), 7.87 (s, 2H), 8.05 (s, 1H), 9.40 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.5, 26.5, 54.0, 56.3, 56.4, 69.4, 75.5, 101.6, 102.1, 102.3, 107.0, 108.7, 109.7, 111.8, 114.9, 119.1, 127.8, 141.1, 143.5, 147.4, 147.7, 150.1, 150.4, 154.4, 164.6; HRMS calcd for $C_{25}H_{24}N_2O_7H$: 465.1662; found 435.1677.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*,*N*-dimethylamino)ethyl]-5,6-dihydro-dibenzo[*c*,*h*]1,6-naphthyridine (4a). To a solution of **3a** (160 mg, 0.38 mmol) in THF (650 mL) was added $LiAlH_4$ (75 mg, 2.0 mmol), and the mixture was stirred under nitrogen at reflux. After 2 h, an additional 2.0 mmol of $LiAlH_4$ was added. The reaction was refluxed for an additional 3 h, then allowed to cool to room temperature. The reaction was quenched by the sequential addition of water (five drops), 10% NaOH (five drops), and water (five drops). The mixture was filtered through Celite and evaporated, and the crude mixture was chromatographed on silica in 98:2 chloroform–methanol, to give 132 mg of the reduced product, in 85% yield; mp 271–273 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 6H), 2.58 (t, 2H, J = 6.8), 3.12 (t, 2H, J = 6.8), 3.97 (s, 3H), 4.02 (s, 3H), 4.27 (s, 2H), 6.13 (s, 2H), 6.79 (s, 1H), 7.38 (s, 2H), 7.61 (s, 1H), 9.05 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 46.0, 50.6, 51.2, 56.2, 56.3, 58.4, 99.6, 101.7, 105.7, 106.6, 110.0, 120.7, 123.1, 124.8, 131.1, 144.1, 146.9, 148.0, 149.0, 149.4, 149.8, 150.2; HRMS calcd for $C_{23}H_{25}N_3O_4$: 407.1845; found 407.1848.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*,*N*-dimethylamino)-1-methylethyl]-5,6-dihydro-dibenzo[*c*,*h*]1,6-naphthyridine (4b). Prepared by treating a solution of **3b** (80 mg, 0.18 mmol) in THF (150 mL) with $LiAlH_4$ (50 mg, 1.3 mmol). The mixture was refluxed with stirring under nitrogen for 4 h. The reaction was quenched by the sequential addition of water (five drops), 10% NaOH (five drops), and water (five drops). The mixture was filtered through Celite and evaporated, and the crude mixture was chromatographed on silica in 1.0% methanol in chloroform to give 35 mg of the reduced product, in 45% yield; mp 153–154 °C; ^{1}H NMR ($CDCl_3$) δ 1.16 (d, 3H, J = 8.0), 2.38 (dd, 2H, J = 12.2, 8.0), 3.68–3.80 (m, 1H), 3.88 (s, 3H), 4.24 (s, 2H), 6.16 (s, 2H), 6.64 (s, 1H), 7.24 (s, 1H), 7.40 (s, 2H), 7.62 (s, 1H), 8.88 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 17.7, 45.6, 46.0, 56.2, 56.4, 57.8, 64.2, 100.1, 101.7, 105.8, 106.4, 108.5, 120.5, 120.6, 123.6, 126.9, 143.4, 146.6, 147.7, 148.9, 149.5, 149.6, 150.0; HRMS calcd for $C_{24}H_{27}N_3O_4H$: 422.2002; found 422.2081.

4-Chloro-6,7-methylenedioxyquinoline (5). Prepared from 4-hydroxy-6,7-methylenedioxyquinoline using methods as previous described in the literature for the conversion of 4-hydroxyquinoline to 4-chloroquinoline. Compound **5** had: mp 127.5–128 °C (lit.[28] mp 129 °C); ^{1}H NMR ($CDCl_3$) 6.15 (s, 2H), 7.35 (d, 1H, J = 4.7), 7.39 (s, 1H), 7.49 (s, 1H), 8.56 (d, 1H, J = 4.7); ^{13}C NMR ($CDCl_3$) 99.8, 102.2, 106.1, 119.9, 123.7, 129.8, 141.2, 147.7, 149.1, 151.4.

General procedure for the formation of 4-amino-6,7-methylenedioxyquinolines

Intermediate **5** was stirred in refluxing phenol (5.5 mol equiv) for 2.5 h. The temperature was lowered to 100 °C and the primary amine (2.0 mol equiv) was added with stirring. The reaction was stirred at 100 °C for several hours, and the phenol removed by Kugelrohr distillation under reduced pressure. In the case of those derivatives that have an alkylamine incorporated in their structure, the residue was partitioned between $CHCl_3$ and 10% NaOH. The aqueous layer was repeatedly

extracted with $CHCl_3$. All of the $CHCl_3$ solutions (initial partition and extracts) were combined and dried ($MgSO_4$). All other 4-amino-6,7-methylenedioxyquinoline derivatives, with the exception of **6i** and **6j**, were purified by column chromatography.

***N'*-(6,7-Methylenedioxyquinolin-4-yl)-*N*,*N*-dimethylethane-1,2-diamine (6a).** Prepared from *N*,*N*-dimethylethylenediamine (2.55 g, 29 mmol) in 54% yield with a reaction time of 24 h. Compound **6a** had: mp 193–194 °C; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6H), 2.70 (t, 2H, J = 6.6), 3.29 (m, 2H), 5.62 (br, 1H), 6.10 (s, 2H), 6.36 (d, 1H, J = 5.3), 7.10 (s, 1H), 7.34 (s, 1H), 8.40 (d, 1H, J = 5.3); ^{13}C NMR ($CDCl_3$) δ 40.1, 45.2, 57.2, 96.3, 98.9, 101.6, 106.5, 114.4, 145.2, 146.8, 148.9, 149.7, 150.1; HRMS calcd for $C_{14}H_{17}N_3O_2$: 260.1399; found 260.1377.

***N'*-(6,7-Methylenedioxyquinolin-4-yl)-*N*,*N*-dimethylpropane-1,2-diamine (6b).** Prepared from 2-methyl-2-(*N*,*N*-dimethylamino)ethylamine (2.55 g, 29 mmol) in 31% yield with a reaction time of 24 h. Compound **6b** had: mp 71–72 °C; ^{1}H NMR (CD_3OD) δ 1.26 (d, 3H, J = 5.6), 3.22 (s, 6H), 2.41 (dd, 1H, J = 6.2, 12), 2.65 (dd, 1H, J = 5.8, 12.2), 3.82–3.86 (m, 1H), 6.16 (s, 2H), 6.46 (d, 1H, J = 5.8), 7.16 (s, 1H), 7.45 (s, 1H), 8.20 (d, 1H, J = 6.0); ^{13}C NMR δ 17.1, 44.0, 45.4, 63.6, 96.6, 97.3, 101.3, 101.8, 113.9, 144.8, 146.3, 146.8, 149.7, 150.0; HRMS calcd for $C_{15}H_{19}N_3O_2H$: 273.1484; found 273.1477.

1-[2-[*N*-(6,7-Methylenedioxyquinolin-4-yl)]amino]ethylpyrrolidine (6c). Prepared from 1-(2-aminoethyl)pyrrolidine (1.14 g, 10.0 mmol) in 31% yield with a reaction time of 20 h. Compound **6c** had: mp 179–182 °C; ^{1}H NMR ($CDCl_3$) δ 1.83 (m, 4H), 2.60 (m, 4H), 2.87 (t, 2H, J = 5.9), 3.33 (m, 2H), 5.58 (br, 1H), 6.08 (s, 2H), 6.34 (d, 1H, J = 5.1), 7.08 (s, 1H), 7.31 (s, 1H), 8.40 (d, 1H, J = 5.1); ^{13}C NMR ($CDCl_3$) δ 23.7, 41.4, 53.9, 54.0, 96.3, 98.9, 101.6, 106.6, 114.4, 146.4, 146.7, 149.1, 149.6, 150.0; HRMS calcd for $C_{16}H_{19}N_3O_2$: 285.1477; found 285.1468.

1-[2-[*N*-(6,7-Methylenedioxyquinolin-4-yl)]amino]ethyl-4-methylpiperazine (6d). Prepared from 2-(4-methylpiperidin-1-yl)ethylamine[27] (1.43 g, 10.0 mmol) in 20% yield with a reaction time of 24 h. Compound **6d** had: mp 159–161 °C; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3H), 2.54 (m, 10H), 2.80 (t, 2H, J = 5.9), 5.62 (br, 1H), 6.11 (s, 2H), 6.38 (d, 1H, J = 5.2), 7.05 (s, 1H), 7.33 (s, 1H), 8.41 (d, 1H, J = 5.2); ^{13}C NMR ($CDCl_3$) δ 39.1, 46.2, 52.7, 55.4, 55.7, 96.0, 99.0, 101.6, 106.6, 114.3, 146.8, 146.8, 149.0, 149.5, 150.0; HRMS calcd for $C_{17}H_{22}N_4O_2$: 314.1743; found 314.1738.

***N'*-(6,7-Methylenedioxyquinolin-4-yl)-*N*,*N*-dimethylpropane-1,3-diamine (6e).** Prepared from *N*,*N*-dimethyl-1,3-diaminopropane (1.0 g, 10.0 mmol) in 25% yield with a reaction time of 20 h. Compound **6e** had: mp 178–181 °C; ^{1}H NMR ($CDCl_3$) δ 1.92 (m, 2H), 2.39 (s, 6H), 2.58 (t, 2H, J = 5.5), 3.39 (m, 2H), 6.08 (s, 2H), 6.29 (d, 1H, J = 5.6), 6.95 (s, 1H), 7.31 (s, 1H), 7.52 (br s, 1H), 8.37 (d, 1H, J = 5.6); ^{13}C NMR ($CDCl_3$) δ 24.6, 44.4, 45.7, 59.7, 96.6, 98.0, 101.5, 106.4, 114.5, 146.2, 146.6, 148.9, 149.9, 150.5; HRMS calcd for $C_{15}H_{19}N_3O_2$: 273.1477; found 273.1473.

***N*-(6,7-Methylenedioxyquinolin-4-yl)butylamine (6f).** Prepared from *n*-butylamine (0.53 g, 7.2 mmol) in 34% yield with a reaction time of 24 h. Compound **6f** had: mp 186–187 °C; ^{1}H NMR (CD_3OD) δ 1.02 (t, 3H, J = 7.2), 1.52 (q, 2H, J = 7.2), 1.75 (q, 2H J = 7.2), 3.33 (q, 2H, J = 7.2), 4.88 (br, 1H), 6.08 (s, 2H), 6.40 (d, 1H, J = 5.6), 7.07 (s, 1H), 7.35 (s, 1H), 8.37 (d, 1H, J = 6.0); ^{13}C NMR (CD_3OD) δ 12.2, 19.3, 29.6, 42.3, 96.9, 97.3, 98.8, 102.3, 112.5, 138.7, 141.5, 147.3, 151.6, 152.8; HRMS calcd for $C_{14}H_{16}N_2O_2$: 244.1212; found 244.1222.

2-[[*N*-(6,7-Methylenedioxyquinolin-4-yl)amino]methyl]tetrahydrofuran (6g). Prepared from tetrahydrofurfurylamine (1.01 g, 10.0 mmol) in 84% yield with a reaction time of 20 h. Compound **6g** had: mp 276–278 °C; ^{1}H NMR (CD_3OD) δ 1.77 (m, 1H), 2.07 (m, 3H), 3.61 (m, 2H), 3.86 (m, 2H), 4.26 (m, 1H), 6.28 (s, 2H), 6.90 (d, 1H, J = 7.1), 7.19 (s, 1H), 7.74 (s, 1H), 8.21 (d, 1H, J = 7.1); ^{13}C NMR ($CDCl_3$) δ 24.7, 28.1, 46.6, 67.3, 76.7, 96.5, 97.6, 97.8, 103.1, 112.2, 135.8, 138.6, 148.3, 153.2, 155.1; HRMS calcd for $C_{15}H_{16}N_2O_3$: 272.1161; found 272.1172.

***N*-(6,7-Methylenedioxyquinolin-4-yl)ethanolamine (6h).** Prepared from ethanolamine (0.6 g, 10 mmol) in 54% yield with a reaction time of 24 h. Compound **6h** had: mp 233–234 °C; ^{1}H NMR (DMSO-d_6) δ 3.51 (dd, 2H, J = 10.4, 6.0), 3.69 (t, 2H, J = 6.0), 6.27 (s, 2H), 6.72 (d, 1H, J = 7.0), 7.37 (s, 1H), 8.12 (s, 1H), 8.29 (d, 1H, J = 7.0); ^{13}C NMR (DMSO-d_6); 46.5, 59.5, 98.6, 98.8, 100.3, 103.8, 113.2, 137.6, 141.0, 148.2, 152.8, 155.0 ; HRMS calcd for $C_{12}H_{12}N_2O_3H$: 232.0848; found 232.0881.

2-[2-[*N*-(6,7-Methylenedioxyquinolin-4-yl)]amino]ethoxyethanol (6i). Prepared from 2-[2-(hydroxyethyl)-ethoxy]ethylamine (0.76 g, 7.2 mmol) with a reaction time of 18 h. Compound **6i** was not isolated, but was converted to its *t*-butyldimethylsilanyloxy derivative, **7i**.

2-[*N*-(6,7-Methylenedioxyquinolin-4-yl)amino]-3-(*N*,*N*-dimethylamino)propanol (6j). Prepared from 1-(hydroxymethyl)-2-(*N*,*N*-dimethylethylenediamine (1.13 g, 9.6 mmol) with a reaction time of 48 h. This polar product was not isolated, but was converted to its *t*-butyldimethylsilanyloxy derivative, **7j**.

3-[*N*-(6,7-Methylenedioxyquinolin-4-yl)amino]-1,2-propandiol (6k). Prepared from 3-amino-1,2-propanediol (1.32 g, 14.5 mmol) in 34% yield with a reaction time of 24 h. Compound **6k** had: mp 213–217 °C (dec.); ^{1}H NMR (CD_3OD) δ 3.67 (m, 5H), 6.26 (s, 2H), 6.87 (d, 1H, J = 7.2), 7.19 (s, 1H), 7.71 (s, 1H), 8.21 (d, 1H, J = 7.2); ^{13}C NMR (CD_3OD) δ 45.7, 63.1, 69.4, 96.8, 97.4, 97.8, 103.0, 112.3, 136.1, 138.9, 148.2, 153.0, 155.0; HRMS calcd for $C_9H_7N_3O_2$: 262.0954; found 262.0954.

General procedure for the formation of 2-iodo-4,5-dimethoxybenzamides (7a–g; 8h–k). A 2.0 M solution of oxalyl chloride in CH_2Cl_2 (1.3 equiv) was added to a solution of 2-iodo-5,6-dimethoxybenzoic acid

(1.0 equiv) in anhydrous CH_2Cl_2 (≈60 mL per 10 mmol benzoic acid) and the solution stirred at reflux for 3 h. The mixture was allowed to cool and was then concentrated to dryness in vacuo. To the residue was added a solution of appropriate 4-amino-6,7-dimethoxyquinoline (1.0 equiv), triethylamine (2 equiv) in CH_2Cl_2 (≈60 mL per 4 mmol aminoquinoline). The reaction mixture was then stirred at reflux under N_2 In the case of those derivatives that have an alkylamine incorporated in their structure, the CH_2Cl_2 solution was washed twice with satd aqueous $NaHCO_3$ and then extracted into 10% HCl (3×), which was washed $CHCl_3$ prior to neutraliation with 30% NaOH. The aqueous layer was then repeatedly extracted with $CHCl_3$, dried ($MgSO_4$), and evaporated.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-(*N*,*N*-dimethylaminoethyl)-2-iodo-4,5-dimethoxybenzamide (7a).** Prepared from **6a** (1.0 g, 3.84 mmol) in 71% yield with a reaction time of 3 h, from the acid chloride prepared using 10 mmol of oxalyl chloride and 4.8 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **7a** had: IR ($CHCl_3$) 1652; ^{1}H NMR ($CDCl_3$) δ 2.74 (s, 6H), 2.66 (t, 2H, J = 7.0), 3.33 (s, 3H), 3.74 (s, 3H), 3.96 (m, 1H), 4.49, (m, 1H), 6.15 (s, 2H), 6.41 (s, 1H), 7.03 (s, 1H), 7.34 (d, 1H, J = 4.8), 7.37 (s, 1H), 7.44 (s, 1H), 8.56 (d, 1H, J = 4.8); ^{13}C NMR ($CDCl_3$) δ 45.7, 46.9, 55.5, 56.1, 56.6, 82.7, 98.5, 102.2, 106.7, 110.2, 120.2, 121.5, 122.9, 121.5, 122.9, 133.8, 145.9, 148.0, 148.3, 148.5, 149.0, 149.6, 151.0, 170.0; HRMS calcd for $C_{23}H_{24}IN_3O_5H$: 550.0839; found 550.0823.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)-1-methylethyl)]-2-iodo-4,5-dimethoxybenzamide (7b).** Prepared from **6b** (273 mg, 1.0 mol) in 60% yield with a reaction time of 12 h, from the acid chloride prepared using 4.8 mmol of oxalyl chloride and 1.2 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **7b** had: mp 82–84 °C; IR (KBr) 1648, 3415; HRMS calcd for $C_{24}H_{26}IN_3O_5H$ 564.0917; found 564.0997.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[(2-pyrrolidin-1-yl)ethyl]-2-iodo-4,5-dimethoxybenzamide (7c).** Prepared from **6c** (285 mg, 1.0 mmol), in 87% yield with a reaction time of 12 h, from the acid chloride prepared using 4 mmol of oxalyl chloride and 1.36 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **7c** had: IR ($CHCl_3$) 1650; ^{1}H NMR ($CDCl_3$) δ 1.78 (m, 4H), 2.22 (m, 1H), 2.59 (m, 3H), 2.83 (t, 2H, J = 6.6), 3.33 (s, 3H), 3.74 (s, 3H), 3.96 (d, 1H, J = 4), 4.54 (m, 1H), 6.15 (s, 1H), 6.42 (s, 1H), 7.03 (s, 1H), 7.34 (d, 1H, J = 4.8), 7.36 (s, 1H), 7.44 (s, 1H), 8.55 (d, 1H, J = 4.8); ^{13}C NMR ($CDCl_3$) δ 23.7, 47.7, 52.9, 54.1, 55.5, 56.1, 82.7, 98.4, 102.2, 106.7, 106.7, 120.1, 121.5, 122.9, 133.7, 145.9, 148.0, 148.3, 148.4, 149.0, 149.6, 151.0, 170.0; HRMS calcd for $C_{25}H_{26}IN_3O_5H$: 576.0995; found 576.1003.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(4-methyl-1-piperazinyl)ethyl]-2-iodo-4,5-dimethoxybenzamide (7d).** Prepared from **6d** (290 mg, 0.9 mmol) in 50% yield with a reaction time of 12 h, from the acid chloride prepared using 4.0 mmol of oxalyl chloride and 1.8 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **7d** had: IR ($CHCl_3$) 1649; ^{1}H NMR ($CDCl_3$) δ 2.29 (s, 3H), 2.51 (m, 10H), 3.35 (s, 3H), 3.75 (s, 3H), 3.95 (m, 1H), 4.46 (m, 1H), 6.15 (s, 1H), 6.42 (s, 1H), 7.03 (s, 1H), 7.35 (d, 1H, J = 4.6), 7.36 (s, 1H), 7.48 (s, 1H), 8.57 (d, 1H, J = 4.6); ^{13}C NMR ($CDCl_3$) δ 46.0, 46.2, 53.1, 55.2, 55.5, 55.5, 56.0, 82.7, 98.7, 102.2, 106.7, 110.4, 120.3, 121.6, 123.0, 133.7, 146.0, 148.0, 148.4, 148.4, 148.9, 149.6, 151.0, 170.0; HRMS calcd for $C_{26}H_{29}IN_4O_5H$: 605.1261; found 605.1261.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-iodo-4,5-dimethoxybenzamide (7e).** Prepared from **6e** (273 mg, 1.0 mmol), in 79% yield with a reaction time of 12 h, from the acid chloride prepared using 4.0 mmol of oxalyl chloride and 1.36 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **7e** had: IR ($CHCl_3$) 1650; ^{1}H NMR ($CDCl_3$) δ 1.93 (m, 1H), 2.16 (m, 1H), 2.34 (s, 6H), 2.58 (m, 1H), 3.31 (s, 3H), 3.47 (m, 1H), 3.75 (s, 3H), 3.95 (m, 1H,), 4.55, (m, 1H), 6.16 (s, 1H), 6.39 (s, 1H), 7.04 (s, 1H), 7.28 (d, 1H, J = 5.0), 7.31 (s, 1H), 7.38 (s, 1H), 8.56 (d, 1 h, J = 5.0); ^{13}C NMR ($CDCl_3$) δ 25.8, 45.1, 47.2, 55.5, 56.1, 56.9, 82.7, 98.1, 102.3, 107.0, 110.1, 120.1, 121.5, 122.5, 133.5, 145.5, 148.1, 148.4, 148.6, 149.2, 149.7, 151.1, 170.1; HRMS calcd for $C_{24}H_{26}IN_3O_5H$: 564.0995; found 564.0990.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-(butyl)-2-iodo-4,5-dimethoxybenzamide (7f).** Prepared from **6f** (400 mg, 1.6 mmol) in 46% yield with a reaction time of 72 h, from the acid chloride prepared using 8.2 mmol of oxalyl chloride and 1.9 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **7f** had: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J = 7.4), 1.20–1.91 (m, 4H), 3.22 (s, 3H), 3.65 (s, 3H), 4.45 (m, 2H), 6.08 (d, 2H, J = 1.8), 6.31 (s, 1H), 6.97 (s, 1H), 7.17 (d, 1H, J = 4.8), 7.29 (s, 1H), 7.30 (s, 1H), 8.49 (d, 1H, J = 4.8); HRMS calcd for $C_{23}H_{23}IN_2O_5H$: 535.0684; found 535.0685.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(tetrahydrofuran-2-yl)methyl]-2-iodo-4,5-dimethoxybenzamide (7g).** Prepared from **6g** (272 mg, 1.0 mol) in 36% yield with a reaction time of 16 h, from the acid chloride prepared using 4.0 mmol of oxalyl chloride and 1.36 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **7g** had: IR ($CHCl_3$) 1652; HRMS calcd for $C_{24}H_{23}N_2O_6IH$: 563.0679; found 563.0703.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[(2-(*t*-butyldimethylsilanyloxy)ethyl]-2-iodo-4,5-dimethoxybenzamide (8h).** Prepared from **7h** (400 mg, 1.15 mmol) in 52% yield with a reaction time of 12 h, from the acid chloride prepared using 5.0 mmol of oxalyl chloride and 1.38 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **8h** had: mp 79–80 °C; IR (KBr); 1653 ^{1}H NMR ($CDCl_3$); δ 0.00 (d, 3H, J = 4.2), 0.82 (s, 9H), 3.26 (s, 3H), 3.67 (s, 3H), 3.84–4.02 (m, 4H), 6.13 (d, 2H, J = 4.0), 6.40 (s, 1H), 7.02 (s, 1H), 7.33 (d, 1H, J = 4.2), 7.36 (s, 1H), 7.42 (s, 1H), 8.52 (d, 1H, J = 4.0); HRMS calcd for $C_{27}H_{33}ISiN_2O_6H$: 637.1232; observed 637.1212.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(2-(*t*-butyldimethylsilanyloxy)ethoxy)ethyl]-2-iodo-4,5-dimethoxybenzamide (8i).** Prepared from **7i** (354 mg, 9.0 mmol) in

60% yield with a reaction time of 24 h, from the acid chloride prepared using 4.5 mmol of oxalyl chloride and 1.8 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **8i** had: ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 6H), 0.83 (s, 9H), 3.27 (s, 3H), 3.48 (t, 2H, J = 4.6), 3.67 (t, 2H, J = 5.6), 3.69 (s, 3H), 3.76–4.55 (m, 4H), 6.10 (s, 2H), 6.36 (s, 1H), 6.99 (s, 1H), 7.30–7.32 (three singlets, 3H), 8.52 (d, 1H, J = 4.8).

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[1-[(*t*-butyldimethylsilanyloxy)methyl]-*N*-2-dimethylaminoethyl]-2-iodo-4,5-dimethoxybenzamide (8j).** Prepared from **7j** (0.48 mg, 1.2 mmol) in 55% yield with a reaction time of 18 h, from the acid chloride prepared using 5.9 mmol of oxalyl chloride and 2.4 mmol of 2-iodo-5,6-dimethoxybenzoic acid. Compound **8j** had: IR ($CHCl_3$) 1656; ^{1}H NMR ($CDCl_3$) (unresolved atropisomers in an apparent 57:43 ratio at rt) major atropisomer δ 0.01 (s, 6H), 0.84 (s, 9H), 2.34 (s, 6H), 2.55 (m, 1H), 2.85 (m, 1H); 3.43 (s, 3H), 3.71(s, 3H) 3.86–4.04 (m, 3H), 6.12 (s, 2H), 6.56 (s, 1H), 7.29–7.31 (s, 1H), 7.67 (d, 1H, J = 5.0), 8.00 (s, 1H), 8.59 (d, 1H, J = 4.4); minor atropisomer δ 0.17 (s, 6H), 0.96 (s, 9H), 2.15 (s, 6H), 2.55 (m, 1H), 2.85 (m, 1H), 3.36 (s, 3H), 3.72 (s, 3H) 3.86–4.04 (m, 3H), 6.13 (s, 2H), 6.53 (s, 1H), 7.00 (s, 1H), 7.31 (s, 1H), 7.51 (d, 1H, J = 4.8), 8.25 (s, 1H), 8.55 (d, 1H, J = 5.2); HRMS calcd for $C_{30}H_{40}IN_3O_6SiH$: 694.1809; found 694.1821.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[(2,3-dihydroxy)-propyl]-2-iodo-5,6-dimethoxybenzamide (8k).** Prepared from **7k** (290 mg, 0.9 mmol) in 47% yield with a reaction time of 12 h, from the acid chloride prepared using 30 mmol of oxalyl chloride and 13 mmol of 2-iodo-5,6-dimethoxybenzoic acid. The acid chloride was added as a methylene chloride solution to a solution of **7k** in 125 mL of DME containing triethylamine (3.04 g 30.1 mmol). Compound **8k** had: IR ($CHCl_3$) 1653; ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 3H), 1.33 (s, 3H), 3.33 (s, 3H), 3.76 (s, 3H), 3.94 (m, 3H), 4.61 (m, 2H), 6.18 (s, 1H), 6.39 (s, 1H), 7.05 (s, 1H), 7.31 (d, 1H, J = 4.8), 7.46 (s, 1H), 7.49 (s, 1H), 8.61 (d, 1H, J = 4.8); ^{13}C NMR ($CDCl_3$) δ 25.6, 26.9, 55.6, 56.1, 56.4, 68.2, 73.2, 82.8, 98.2, 98.7, 102.4, 106.1, 110.3, 120.7, 121.7, 124.1, 133.3, 147.5, 148.0, 148.8, 149.5, 150.0, 151.5, 152.3, 167.8; HRMS calcd for $C_{25}H_{25}N_2O_7IH$: 593.0785; found 593.0802.

General procedure for formation of the TBDMS derivatives (7h–j)

A mixture of the 4-amino-6,7-methylenedioxyquinoline derivative (1.0 mmol equiv), imidazole (1.1 mmol equiv) and *t*-butyldimethylsilyl chloride (1.2 mmol equiv) in DMF (15 mL per mmol equiv) was stirred at room temperature for 6 h. DMF was removed in vacuo, water was added to the residue, and the solid was filtered and dried.

4-[*N*-[2-(*t*-Butyldimethylsilanyloxy)]ethyl]amino-6,7-methylenedioxyquinoline (7h). Prepared from **6h** in 48.7% yield; mp 215–216 °C; ^{1}H NMR (DMSO-d_6) δ 0.01 (s, 6H), 0.85 (s, 9H), 3.39 (dd, 2H, J = 6.0, 12.0), 3.80 (t, 2H, J = 6.2), 6.14 (s, 2H), 6.42 (d, 1H, J = 5.4), 7.12 (s, 1H), 7.60 (s, 1H), 8.18 (d, 1H, J = 4.8); HRMS calcd for $C_{18}H_{26}SiO_3$: 3246.1713; found 346.1724.

4-[*N*-[2-[2-(*t*-Butyldimethylsilanyloxy)ethoxy]ethyl]]amino-6,7-methylenedioxyquinoline (7i). Prepared from **6i** in 39% yield (overall yield from **5**); ^{1}H NMR ($CDCl_3$) δ 0.10 (s, 6H), 0.92 (s, 9H), 3.64–3.69 (m, 4H), 3.84 (d, 2H, J = 5.2), 3.93 (d, 2H, J = 5.2), 6.15 (s, 2H), 6.56 (d, 1H, J = 6.4), 7.42 (s, 1H), 7.82 (s, 1H), 8.18 (d, 1H, J = 6.4); HRMS calcd for $C_{20}H_{30}N_2O_4Si$: 390.1975; found 390.1976.

4-[*N*-[2-(*N*,*N*-Dimethylamino)-1-[(*t*-butyldimethylsilanyloxy)methyl]]ethyl]amino - 6,7 - methylenedioxyquinoline (7j). Prepared from **6j** in 25% yield (overall yield from **5**); ^{1}H NMR ($CDCl_3$) (unresolved atropisomers in an apparent 57:43 ratio at rt) major atropisomer δ 0.07 (s, 6H), 0.92–0.94 (s, 9H), 2.24 (s, 6H), 2.45–2.55 (m, 2H), 3.60–4.05 (m, 3H), 5.40 (d, 1H), 6.09 (s, 2H), 6.45 (d, 1H, J = 6.4), 7.02 (s, 1H), 7.30 (s, 1H), 8.18 (d, 1H, J = 6.4); minor atropisomer δ 0.09 (s, 6H), 0.94 (s, 9H), 2.30 (s, 6H), 2.45–2.55 (m, 2H), 3.60–4.05 (m, 3H), 5.40 (d, 1H), 6.0 (s, 2H), 6.45 (d, 1H, J = 6.4), 7.02 (s, 1H), 7.30 (s, 1H), 8.18 (d, 1H, J = 6.4); HRMS calcd for $C_{21}H_{33}N_3O_3Si$: 403.2291; found 403.2298.

4-[*N*-(2,2-Dimethyl-[1,3]dioxolan-4-yl)methyl]amino-6,7-methylenedioxyquinoline (7k). A mixture of **6k** (500 mg, 1.9 mmol), *p*-toluenesulfonic acid (5 mg, 0.02 mg) in DMF (20 mL) and 2,2-dimethoxypropane (5 mL), was heated to 80 °C and stirred at this temperature for 18 h. To the cooled solution was added 1 mL of pyridine and the solvent was evaporated in vacuo. The crude material was chromatographed in 96:4 chloroform–methanol to give 466 mg of the acetonide, in 81% yield; mp 219–221 °C; ^{1}H NMR (CD_3OD) δ 1.35 (s, 3H), 1.38 (s, 3H), 3.74 (m, 3H), 4.19 (m, 1H), 4.49 (m, 1H), 6.28 (s, 2H), 6.94 (d, 1H, J = 7.2), 7.20 (s, 1H), 7.74 (s, 1H), 8.24 (d, 1H, J = 7.2); ^{13}C NMR (CD_3OD) δ 23.5, 25.1, 45.0, 66.0, 73.6, 96.5, 97.7, 97.8, 103.1, 109.1, 112.2, 135.8, 138.6, 148.4, 153.3, 155.3; HRMS calcd for $C_{16}H_{18}N_2O_4$: 302.1267; found 302.1267.

Topoisomerase-mediated DNA cleavage assays

Human topoisomerase I was expressed in *Escherichia coli* and isolated as a recombinant fusion protein using a T7 expression system as described previously.[14] Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described.[30] The 3′ end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[31] The cleavage assays were performed as previously reported.[32,33] The drug and the DNA in presence of topoisomerase I were incubated for 30 min at 37 °C. The reactions were terminated by the addition of 5 μL of 5% SDS and 1 mg/mL protein kinase K with an additional 1 h of incubation at 37 °C. Samples were then alkali denatured by the addition of NaOH, EDTA, sucrose, and bromophenol blue to final concentrations of 75 mM, 2.5%, and 0.05 mg/mL, respectively, prior to loading onto a

neutral agarose gel. 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 camptothecin, 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.

Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[34–36] 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).[37] 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). Each well was plated with either 2000 RPMI8402 cells or 4000 CPT-K5 cells. For determination of IC_{50}, cells were exposed continuously for 4 days to varying concentrations of the 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.

Human tumor xenograft

Two bioassays were performed using female NCR/NU NU mice of approximately 9 weeks of age as obtained from Taconic Farms, Inc. (Germantown, NY, USA). Mice were housed four per cage in laminar flow HEPA filtered microisolator caging (Allentown Cage and Equipment, Allentown, NJ, USA). Mice were fed Purina autoclavable breeder chow #5021 and given drinking water, purified by reverse-osmosis, ad libitum. Five days after delivery, the mice were inoculated on the right flank with 1.5×10^6 MDA-MB-435 tumor cells in 0.1 mL of RPMI 1640 Media by sc injection (25 gauge needle × 5/8 inch). The MDA-MB-435 cells were grown in 75 cm^2 flasks using RPMI 1640 Media and 10% fetal bovine serum. Tumors were of sufficient size at 19–20 days after inoculation. Tumor-bearing mice were matched in each experimental group based on tumor volume, that is, the mice with the larger tumor volumes were placed within each experimental group. Tumor volume was calculated by measuring the tumor with a microcaliper. The length (l) is the maximum two-dimensional distance of the tumor and the width (w) is the maximum distance perpendicular to this length, measured in mm. Tumor volume was calculated using the formula $(l \times w^2)/2$. Every mouse in this study was weighed individually on a daily basis. Tumor volume was determined for each individual mouse every other day. A third in vivo study was performed using male NCR/NU NU mice as obtained from Taconic Farms. Treatment of these mice was similar to that performed with the female mice with the exception that the numbers of mice per cage had to be reduced to 1–2 to prevent excessive fighting. The experimental groups, route of administration and approximate dosing schedule for these studies are outlined in Table 2.

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