Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 3131–3143
Bioorganic & Medicinal Chemistry

6-Substituted 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones: Reversed lactam analogues of ARC-111 with potent topoisomerase I-targeting activity and cytotoxicity

Shejin Zhu,[a] Alexander L. Ruchelman,[a] Nai Zhou,[b] Angela Liu,[b] Leroy F. Liu[b,c] and Edmond J. LaVoie[a,c,*]

[a]*Department of Pharmaceutical Chemistry, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020, USA*
[b]*Department of Pharmacology, The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA*
[c]*The Cancer Institute of New Jersey, New Brunswick, NJ 08901, USA*

Received 29 August 2005; revised 13 December 2005; accepted 16 December 2005
Available online 18 January 2006

Abstract—6-Substituted 8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones were synthesized and evaluated for topoisomerase I-targeting activity and cytotoxicity. Several of these reversed lactam analogues of ARC-111 exhibited exceptional cytotoxicity with IC_{50} values ranging from 0.5 to 3.0 nM. In contrast to topotecan, no resistance was observed with several of these reversed lactam analogues in tumor cell lines that overexpressed the efflux transporters MDR1 or BCRP.

1. Introduction

Topoisomerases are nuclear enzymes that control the topology of DNA and are critical for replication and transcription. Stabilization of the cleaved complex formed between the enzyme and DNA by TOP1-targeting agents such as camptothecin can be lethal to tumor cells.[1–4] Studies on camptothecin and its structurally related analogues have resulted in the development of two clinical anticancer agents, topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®). These clinical agents possess a similar camptothecin ring system, which incorporates a δ-lactone. Hydrolysis of this lactone results in the loss of TOP1-targeting activity. In addition, the resulting hydroxy acid derivative has a high binding affinity for human serum albumin.[5–7] The instability of this lactone together with data that have identified topotecan and irinotecan as substrates for efflux transporters associated with multi-drug resistance[8–11] have prompted further research into the development of novel TOP1-targeting agents.

Benzo[*i*]phenanthridines and dibenzo[*c*,*h*]cinnolines can exert TOP1-targeting activity and cytotoxicity against several human tumor cell lines.[12–16] Specific 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones and 11*H*-isoquino[4,3-*c*]cinnolin-12-ones have also been identified that possess exceptional TOP1-targeting activity and cytotoxicity.[17–25] The dibenzo[*c*,*h*][1,6]naphthyridin-6-one **1** (ARC-111, topovale®) and the isoquino[4,3-*c*]cinnolin-12-one **2** (Fig. 1) were among the more active compounds that were evaluated. These studies were extended to include the synthesis and evaluation of several 2-aminoethyl esters and amides of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine-12-carboxylic acid.[26,27] In the present study, the structure–activity trends associated with the reversed lactam analogues of **1** were investigated. Specifically, we synthesized several 6-substituted 8,9-dimethoxy-2,3-methylenedioxydibenzo[*c*,*h*][2,6]naphthyridin-6-ones. These structurally related analogues of **1** were assayed for TOP1-targeting activity and cytotoxicity in RPMI8402, as well as its camptothecin-resistant variant, CPT-K5.[28] In addition, the relative cytotoxic activity of these compounds as compared to that of the parent cell line KB3-1 was assessed in KBV-1 cells,[29] which overexpress MDR1 and KBH5.0 cells,[19] which overexpress BCRP. Both MDR1 and BCRP are

Keywords: Topoisomerase I; Cytotoxic; Antitumor; Dibenzo[*c*,*h*][2,6]naphthyridin-5-ones.
* Corresponding author. E-mail: elavoie@rci.rutgers.edu

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.12.028

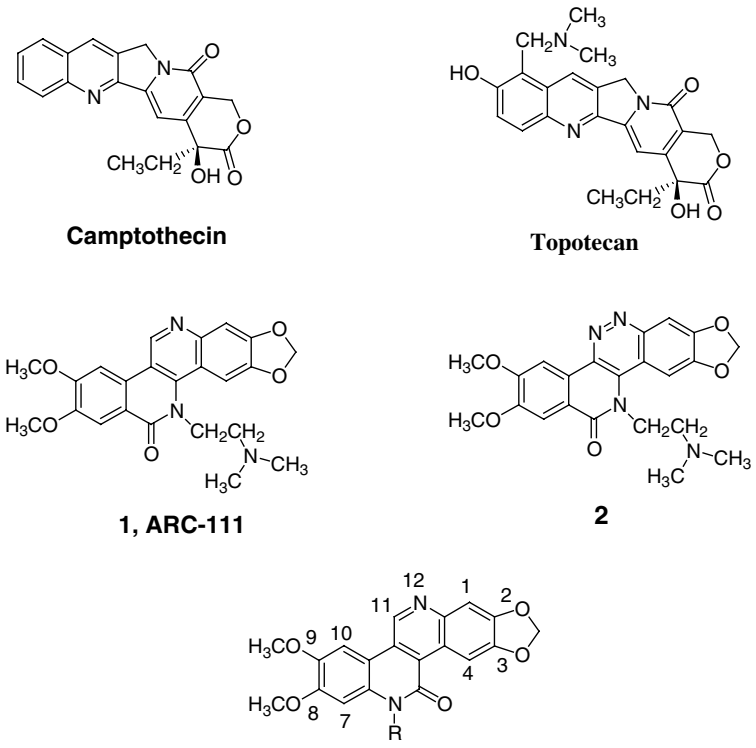


Figure 1.

efflux transporters that have been associated with the multi-drug resistance of tumor cells.[30,31]

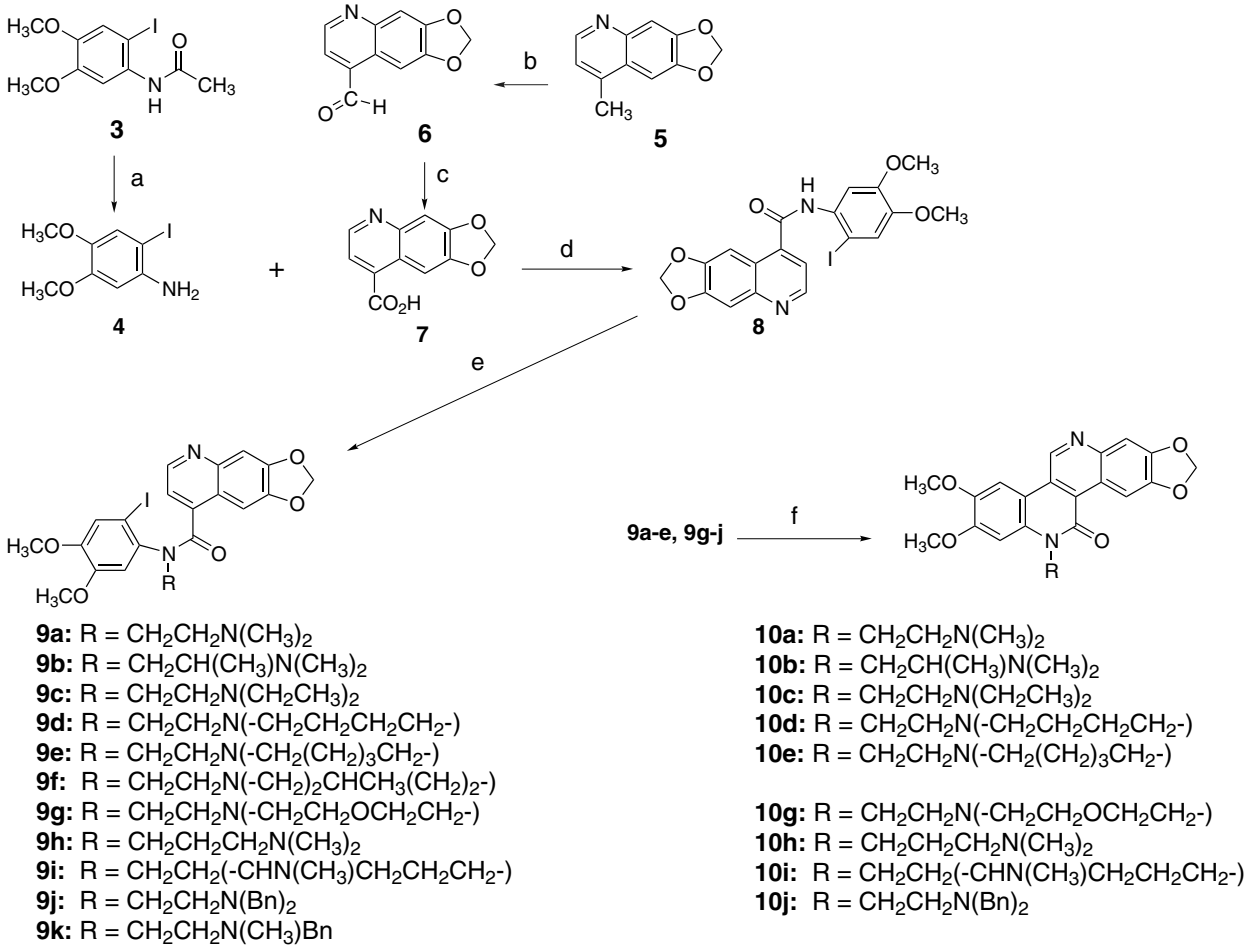
2. Chemistry

Several 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one reversed lactam analogues of ARC-111 were prepared as detailed in Scheme 1. 3,4-Dimethoxyaniline was converted to its acetanilide using acetyl chloride,[32] followed by treatment with iodine monochloride to form the *ortho* iodoacetamide **3**. Compound **4** was prepared by hydrolysis of **3** using aqueous NaOH in ethanol. This compound served as one of the key intermediates used to make the 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones. 4-Formyl-6,7-methylenedioxyquinoline **6** was prepared by oxidation of 4-methyl-6,7-methylenedioxyquinoline[27] with SeO_2 in dioxane-water. 6,7-Methylenedioxy-4-quinolinecarboxylic acid **7** was prepared by treatment of **6** in pyridine with an aqueous solution of $KMnO_4$. Conversion of **7** to the acid chloride in situ was performed using thionyl chloride. Treatment of the acid chloride in CH_2Cl_2 and TEA with freshly prepared **4** and subsequently allowing this mixture to reflux overnight provided the secondary amide **8**. The intermediate **8** was treated with sodium hydride to form the amide anion, which was treated with various substituted alkyl halides to form tertiary amide intermediates **9a**–**k**. 4-Methyl-1-(2-chloroethyl)piperidine and 2-(*N*-benzyl-*N*-methylamino)-1-chloroethane were prepared by treatment of their corresponding alcohols with thionyl chloride using conditions similar to those previously described.[31–33] The other chloroalkanes used to form these tertiary amides were commercially available. Photocyclization of **9a**–**e** and **9g**–**j** using 2% HCl as solvent provided the desired compounds **10a**–**e** and **10g**–**j**.

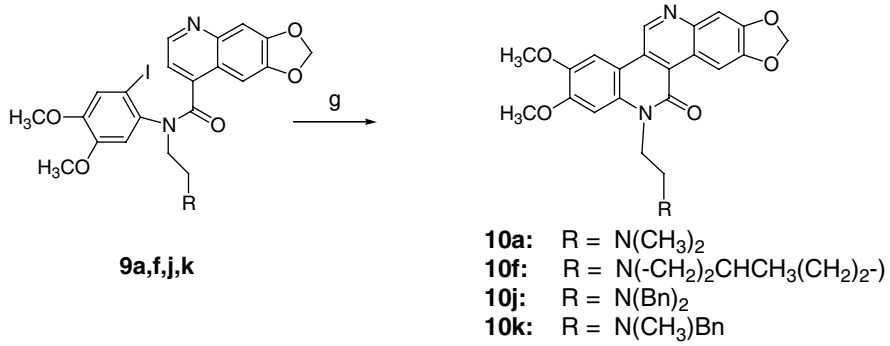
Conversion of **9a** to **10a** employing Heck cyclization conditions did provide the desired product together with undesired side products that were not characterized, Scheme 2. While chromatographic separation of **10a** proved difficult, the desired product could be separated by trituration with small amounts of methylene chloride wherein the by-products had greater solubility. Photocyclization of the *N*-benzyl-substituted analogue **9j** resulted in several unidentified products with only a 5% yield of **10j** and in the case of **9k** little or no detectable amounts of the desired product **10k** were formed. In addition to compound **9a**, Heck reaction conditions were also employed for **9f**, **9j**, and **9k**, which provided acceptable yields of the cyclized products **10f**, **10j**, and **10k**, Scheme 2. No effort was made to identify the side products formed during these Heck cyclization reactions. For both **10j** and **10k**, the benzyl groups were removed in near-quantitative yield using palladium black in acetic acid with formic acid as the hydrogen source, Scheme 3, to provide **10l** and **10m**, respectively.[25]

3. Pharmacology

The relative TOP1-targeting activities of several of these 6-substituted 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones are provided in Table 1. A representative gel of the

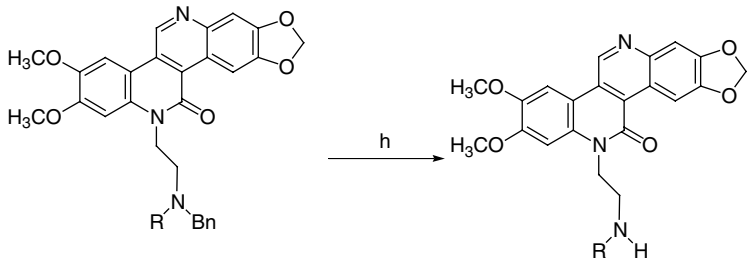


Scheme 1. Reagents and conditions: (a) NaOH, EtOH, H_2O; (b) SeO_2, dioxane, H_2O; (c) $KMnO_4$, pyr, H_2O; (d) first $SOCl_2$, then **4** in CH_2Cl_2, then TEA; (e) NaH, DMF, RCl·HCl for **9a**–**g** and **9i**–**k**; $NaNH_2$, $PhCH_3$, RCl·HCl for **9h**; (f) 2% HCl, hν.



Scheme 2. Reagents: (g) $Pd(OAc)_2$, P(*o*-tol)$_3$, Ag_2CO_3, DMF.

resulting DNA fragmentation that occurs in the presence of a few select compounds and TOP1 is illustrated in Figure 2. We did not observe in our studies on DNA cleavage a noteworthy difference in the pattern of DNA fragments formed as compared to those obtained with camptothecin. In contrast to what had been observed with analogues of ARC-111, elongation of the alkyl chain did not have a dramatic effect on TOP1-targeting activity. Compound **10a**, the reversed lactam of ARC-111, and its propyl homologue **10h** had comparable intrinsic activity to each other, as well as to topotecan, as TOP1-targeting agents. The 6-[2-(*N*-benzylaminoethyl)] analogues **10j** and **10k** exhibited much less activity as TOP1-targeting agents than almost all of the other 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones evaluated in this study. Only the 6-[2-(morphin-4-yl)ethyl] analogue **10g** had weak TOP1-targeting activity that was comparable to that of **10k**. Excluding these *N*-benzyl derivatives, it was observed that the 6-[2-(*N*,*N*-dialkylamino)ethyl] derivatives of 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one, such as **10c**–**e**, that possessed more lipophilic substituents on the 2-aminoethyl group, also had substantially



Scheme 3. Reagents: (h) AcOH, formic acid, Pd black.

Table 1. TOP1-targeting activity and cytotoxicity of 6-substituted 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones

Compound	TOP1-mediated DNA cleavage[a]	Cytotoxicity IC_{50} (μM)				
		RPMI8402	CPT-K5	KB3-1 wt	KBV-1 + MDR1	KBH5.0 + BCRP
1	0.3	0.002	0.90	0.005	0.005	0.006
10a	1.2	0.0007	0.21	0.003	0.004	0.004
10b	1.2	0.003	0.18	0.006	0.015	0.009
10c	0.2	0.003	0.90	0.003	0.012	0.005
10d	0.07	0.003	0.85	0.002	0.004	0.004
10e	0.1	0.003	1.70	0.003	0.005	0.004
10f	3.0	0.004	0.40	0.004	0.023	0.022
10g	9.0	0.035	>5.0	0.045	0.120	0.150
10h	1.4	0.0005	0.20	0.004	0.006	0.005
10i	0.45	0.030	0.30	0.03	0.090	0.050
10j	>100	0.050	2.10	0.04	0.250	0.170
10k	13	0.023	1.90	0.038	0.120	0.070
10l	0.35	0.002	0.30	0.004	0.016	0.020
10m	0.15	0.002	0.40	0.006	0.065	0.085
CPT	0.2	0.006	>10	0.015	0.025	0.026
Topotecan	1.0	0.021	>10	0.04	0.44	0.44

[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 on the plasmid DNA in the presence of human topoisomerase I.

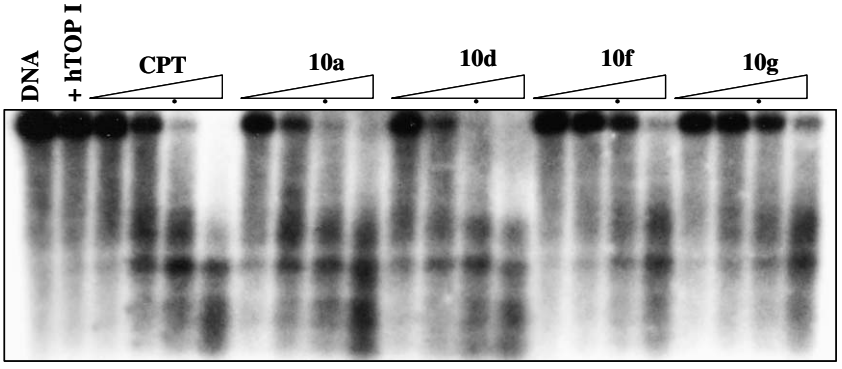


Figure 2. Stimulation of enzyme-mediated DNA cleavage by camptothecin (CPT), **10a**, **10d**, **10f**, and **10g**, using human TOP1. The first lane is the DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each)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

greater potency as TOP1-targeting agents. These reversed lactams were among the more potent TOP1-targeting agents that have been synthesized in our laboratory with potencies exceeding that of camptothecin. These data suggest that the presence of lipophilic substituents, such as the *N*,*N*-diethyl groups or the pentamethylene associated with the piperidine moiety, can significantly enhance TOP1-targeting activity. In contrast to the piperidin-1-yl derivative **10e** appended to the 2-ethyl side chain, it is noteworthy that the 4-meth-

ylpiperidin-1-yl derivative **10f** and in particular the morpholin-4-yl derivative **10g** exhibited significantly reduced intrinsic activity as TOP1-targeting agents. These data suggest that steric or electronic factors associated with substituents attached to the 2-position of the ethyl group can reduce relative TOP1-targeting activity.

The relative cytotoxic activities of several of the 6-substituted 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones in RPMI8402 tumor cells and the camptothecin-resistant variant cell line CPT-K5 are listed in Table 1.

Compounds **10a** and **10h** were among the more cytotoxic analogues that were evaluated. Both of these derivatives exhibited IC_{50} values in RPMI8402 cells that were at or below 0.7 nM. Compounds **10b–f**, **10l**, and **10m** had similar cytotoxic activity with IC_{50} values in RPMI8402 cells ranging from 2 to 4 nM.

A significant loss in cytotoxic activity was observed for **10g** (IC_{50} = 35 nM) wherein a morpholin-4-yl moiety replaced the piperidin-1-yl substituent of **10e** attached to the 2-position of the ethyl side chain. This is consistent with its significantly lower intrinsic TOP1-targeting activity. Lower cytotoxic activities were also observed for the 2-(1-methylpyrrolidin-2-yl)ethyl derivative **10i**, and the 2-(*N*,*N*-dibenzylamino)ethyl analogue **10j**, and the 2-(*N*-benzyl-*N*-methylamino)ethyl analogue **10k**, which had IC_{50} values in RPMI8402 cells of 30, 50, and 23 nM, respectively.

These reversed lactam derivatives were also evaluated in KB3-1 cells, and in KBV-1 and KBH5.0 cells, which overexpress MDR1 and BCRP, respectively (Table 1). No notable differences in cytotoxicity were observed for **10a**, **10d**, **10e**, and **10h** in these three cell lines. Comparison of their relative cytotoxicity in these three cell lines indicates that several reversed lactam analogues, **10b**, **10c**, **10g**, **10i**, **10k**, and **10l** were not substrates for these efflux transporters, with less than a 5-fold difference observed in cytotoxicity in the variant cell lines relative to the parent. Both **10f** and **10j** did appear to be weak substrates for MDR1 with a 5- to 6-fold difference observed in relative IC_{50} values relative to the parent cell line. Compound **10f** was also 5-fold less cytotoxic in KBH5.0 cells relative to KB3-1 cells, indicating that it was also a weak substrate from BCRP. Only **10m** had a difference in cytotoxicity for either KBV-1 cells or KBH5.0 cells relative to their parent KB3-1 cell line that was at least an order of magnitude indicating that this compound was a substrate for both MDR1 and BCRP efflux transport.

The in vitro data on these various 6-substituted 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones indicated that several compounds possess exceptional TOP1-targeting activity and cytotoxicity. In several instances, the potent pharmacological activity observed with certain analogues, such as **10a** and **10d**, exceeds that of ARC-111. ARC-111 has proved to be efficacious after parenteral and oral administration. Studies are in progress to assess the in vivo relative antitumor efficacy of select 6-substituted 6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones by both these routes of administration.

4. Experimental

Melting points were determined with a 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. Infrared spectral data were obtained using a Thermo-Nicolet Avatar 360 Fourier transform spectrometer and are reported in cm^{-1}. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance was 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. All starting materials and reagents were purchased from Aldrich. Solvents were purchased from Fisher Scientific and were of ACS grade or HPLC grade. Methylene chloride was freshly distilled from calcium hydride. All other solvents were used as provided without further purification. 2-(*N*,*N*-Dimethylamino)ethylchloride hydrochloride, 3-(*N*,*N*-dimethylamino)propylchloride hydrochloride, 2-chloro-(*N*,*N*-dimethylpropylamine hydrochloride, 2-(*N*,*N*-diethylamino)ethylchloride hydrochloride, 1-(2-chloroethyl)pyrrolidine hydrochloride, 1-(2-chloroethyl)piperidine hydrochloride, 4-(2-chloroethyl)morpholine hydrochloride, 2-(2-chloroethyl)-1-methylpyrrolidine hydrochloride, and *N*-(2-chloroethyl)dibenzylamine were obtained from Aldrich. 4-(2-Chloroethyl)-1-methylpiperidine was prepared from 4-(2-hydroxyethyl)-1-methylpiperidine[33,34] and 2-(*N*-benzyl-*N*-methylamino)-1-chloroethane hydrochloride was prepared from 2-(*N*-benzyl-*N*-methylamino)ethanol.[35]

4.1. 1-(2-Hydroxyethyl)-4-methylpiperidine

To 4-methylpiperidine (27.8 g, 0.28 mol) was added dropwise, while stirring ethylene chlorohydrin (11.26 g, 0.14 mol). After boiling the reaction mixture for 3 h, it was cooled and stirred several times with a total of 300 mL ether. Precipitated 4-methylpiperidine hydrochloride was filtered off and the filtrate evaporated. The residue obtained was then distilled under vacuum. There was obtained 16.6 g of 1-(2-hydroxyethyl)-4-methylpiperidine[33] in 85% yield as a brown liquid, which was used in subsequent steps without further purification. ^1H NMR ($CDCl_3$) δ 0.94 (d, 3H, J = 6.2), 1.25 (m, 3H), 1.66 (m, 2H), 2.05 (m, 2H), 2.51 (t, 2H, J = 5.4), 2.90 (m, 3H), 3.60 (t, 2H, J = 5.4).

4.2. 1-(2-Chloroethyl)-4-methylpiperidine hydrochloride

4-Methyl-1-(2-hydroxyethyl)piperidine (10 g, 70 mmol) was dissolved in 56 mL anhydrous benzene, and while stirring, so mixed with a solution of 16.8 g thionyl chloride in 12 mL anhydrous benzene such that the temperature of the mixture remained between 25 and 30 °C. After boiling for a further 30 min, the hydrochloride

was filtered and washed with ether to give 13.4 g of 1-(2-chloroethyl)-4-methylpiperidine hydrochloride[34] in 97% yield of a white solid and used in subsequent steps without further purification. ^{1}H NMR ($CDCl_3$) δ 0.99 (d, 3H, J = 6.2), 1.62 (m, 1H), 1.88 (m, 4H), 2.85 (m, 2H), 3.38 (m, 2H), 3.59 (m, 2H), 4.08 (t, 2H, J = 6.6).

4.3. 2-(*N*-Benzyl-*N*-methylamino)-1-chloroethane hydrochloride

To 2-(*N*-benzyl-*N*-methylamino)ethanol (16.5 g, 0.1 mol) was added dropwise HCl (15%) to pH < 2. Benzene (83 mL) was added and the emulsion was refluxed under Dean–Stark conditions to remove water. The benzene solution was evaporated. The oily residue was cooled in an ice bath and thionyl chloride was added dropwise. The reaction mixture was then refluxed for 3 h. Excess thionyl chloride was removed under vacuum. The residue was washed with cold ethanol (45 mL) and dried in a vacuum oven overnight at room temperature to give 20.6 g of 2-(*N*-benzyl-*N*-methylamino)-1-chloroethane hydrochloride[35] in 94% yield as a white solid, which was used in subsequent steps without further purification. ^{1}H NMR ($CDCl_3$) δ 2.82 (d, 3H, J = 4.8), 3.29 (m, 1H), 3.54 (m, 1H), 4.09 (t, 2H, J = 6.6), 4.35 (m, 2H), 7.48 (m, 3H), 7.63 (m, 2H).

4.4. 4,5-Dimethoxy-2-iodoacetanilide (3)

A 1.0 M solution of iodine monochloride in methylene chloride (41.7 mL) was added dropwise to a solution of *N*-(3,4-dimethyoxyphenyl)acetamide[32] (7.4 g, 37.9 mmol) in methylene chloride (45 mL) and acetic acid (7.5 mL). The mixture was stirred under nitrogen overnight and then washed with saturated sodium thiosulfate (2× 150 mL) and brine (150 mL). The methylene chloride solution was dried ($MgSO_4$) and evaporated, and the crude residue was chromatographed using 19:1 chloroform/hexanes, to provide 6.2 g of **1** as a white solid, in 52% yield; mp 140–141.5 °C; IR ($CHCl_3$) 3397, 1687; ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H), 3.86 (s, 3H), 3.90 (s, 3H), 7.17 (s, 1H), 7.26 (br, 1H), 7.86 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.8, 56.1, 56.4, 77.6, 106.4, 120.4, 132.4, 146.6, 149.7, 168.4; HRMS calcd for $C_{10}H_{12}IO_3N$: 320.9862; found: 320.98.61.

4.5. 4,5-Dimethoxy-2-iodoaniline (4)

A mixture of **1** (1.0 g, 3.12 mmol) and NaOH (6.25 g, 156 mmol) in ethanol (125 mL) and water (30 mL) was heated to reflux with stirring for 4 h. The mixture was cooled and the solvent was removed under vacuum. The residue was partitioned between chloroform (100 mL) and water (100 mL). The organic phase was washed with water (2× 100 mL), dried ($MgSO_4$), and evaporated under vacuum to give 810 mg of **2** in 93% yield, as a light pink oil; ^{1}H NMR ($CDCl_3$) δ 3.81 (s, 3H), 3.83 (s, 3H), 6.39 (s, 1H), 7.08 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 55.9, 56.8, 71.2, 99.7, 121.7, 141.3, 142.8, 150.7; HRMS calcd for $C_8H_{10}IO_2N$: 278.9756; found: 278.9763.

4.6. 4-Methyl-6,7-methylenedioxyquinoline (5)

Iron (III) chloride (54.2 g, 0.2 mol) was dissolved in glacial acetic acid (600 mL) with warming to 60 °C. 3,4-Methylenedioxyaniline (27.4 g, 0.2 mol) was added and the mixture was stirred for 5 min. Methyl vinyl ketone (17.4 mL, 0.21 mol) was added dropwise over five min. Following the completion of addition, the mixture was heated to reflux with stirring for 1.5 h. The mixture was cooled and the precipitate was filtered and washed with additional acetic acid. This material was then neutralized by addition to cold 30% NaOH, and the resulting mixture was filtered and air-dried. The crude material was then extracted with chloroform (7× 200 mL). The combined extracts were washed with 10% K_2CO_3 (3× 300 mL), dried ($MgSO_4$), and concentrated under vacuum. The residue was recrystallized from ethyl ether, yielding 16.6 g of **5** as a fluffy light beige solid, in 44% yield; mp 100.5–101.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3H), 6.04 (s, 2H), 7.02 (d, 1H, J = 4.4), 7.13 (s, 1H), 7.32 (s, 1H), 8.52 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 19.1, 99.3, 101.7, 106.3, 120.6, 125.0, 142.9, 146.3, 147.8, 147.9, 150.2; HRMS calcd for $C_{11}H_9O_2N$: 187.0633; found: 187.0627.

4.7. 4-Formyl-6,7-methylenedioxyquinoline (6)

A mixture of **5** (5.01 g, 27.0 mmol) in 30 mL dioxane was heated to 75 °C, and then a solution of SeO_2 in 5:1 dioxane/H_2O (36 mL) was added dropwise. The mixture was heated to reflux with stirring for 4.5 h. The cooled reaction mixture was filtered and the filtrate evaporated. The residue was dissolved in chloroform (50 mL), washed with water (3× 50 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed, eluting with chloroform to provide 3.48 g of **6** in 65% yield; mp 146–147.5 °C; IR ($CHCl_3$) 1702; ^{1}H NMR ($CDCl_3$) δ 6.18 (s, 2H), 7.45 (s, 1H), 7.63 (d, 1H, J = 4.4), 8.41 (s, 1H), 8.96 (d, 1H, J = 4.4), 10.35 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 100.4, 102.3, 106.3, 121.4, 124.7, 135.7, 148.0, 148.3, 150.8, 151.0, 193.4; HRMS calcd for $C_{11}H_7O_3N$: 201.0426; found: 201.0437.

4.8. 6,7-Methylenedioxyquinoline-4-carboxylic acid (7)

A solution of **6** (4.8 g, 23.8 mmol) in pyridine (150 mL) was cooled to −5 °C. The mixture was maintained at this temperature as a solution of potassium permanganate (10.0 g, 63.3 mmol in 150 mL of water) was added dropwise over the course of 1 h. The mixture was stirred at −5 °C for an additional hour and then left to stir overnight. The mixture was filtered and the filtrate was evaporated under vacuum. The solid on the filter was extracted with 100 mL of water with heating to 80 °C, and the aqueous extract was added to the residue resulting from evaporation of the acetone solution. This mixture was acidified to pH 5 using HCl. The precipitated free acid was filtered and washed well with water, ethanol, ethyl acetate, and ethyl ether sequentially, and then dried under vacuum for 2 days to provide 4.6 g of **7** in 90% yield; mp >300 °C; IR (KBr) 3446, 1689; ^{1}H NMR (DMSO-d_6) δ 6.27 (s, 2H), 7.45 (s, 1H), 7.79 (d, 1H, J = 4.8), 8.08 (s, 1H), 8.79 (d, 1H, J = 4.8); ^{13}C

NMR ($CDCl_3$ + 1 drop TFA-*d*) δ 98.1, 102.0, 104.9, 122.2, 128.6, 139.2, 139.7, 140.1, 153.6, 156.5, 166.6; HRMS calcd for $C_{11}H_7O_4N$: 217.0375; found: 217.0371.

4.9. 6,7-Methylenedioxyquinoline-4-carboxylic acid *N*-(2-iodo-4,5-dimethoxyphenyl)amide (8)

A suspension of **7** (500 mg, 2.3 mmol) in thionyl chloride (30 mL) was heated at reflux for 2 h, during which time the starting material completely dissolved. The mixture was cooled and then evaporated to dryness under vacuum. The acid chloride was dissolved in anhydrous methylene chloride (30 mL) and triethylamine (3.0 g, 30 mmol) was added. A solution of **4** (535 mg, 1.9 mmol) in methylene chloride (15 mL) was added, and the resulting mixture was refluxed under nitrogen overnight. The mixture was cooled and additional methylene chloride was added, bringing the total volume up to 100 mL. This solution was washed with saturated sodium bicarbonate (2× 100 mL) and brine (100 mL), dried ($MgSO_4$) and evaporated under vacuum. The crude residue was chromatographed in chloroform to provide 512 mg of **8** as a very pale yellow solid, in 56% yield; mp 210–211 °C; IR ($CHCl_3$) 3375, 1680; ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H), 4.00 (s, 3H), 6.17 (s, 2H), 7.25 (s, 1H), 7.47 (s, 1H), 7.55 (d, 1H, J = 4.4), 7.77 (s, 1H), 7.90 (br, 1H), 8.11 (s, 1H), 8.84 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 56.3, 56.5, 78.3, 100.9, 102.2, 106.4, 111.9, 116.9, 120.5, 121.9, 131.9, 139.9, 147.7, 147.9, 149.3, 149.8, 151.3, 165.6; HRMS calcd for $C_{19}H_{15}IN_2O_5H$: 479.0104; found: 479.0081.

4.10. General method for the preparation of tertiary amide derivatives of 6,7-methylenedioxyquinoline-4-carboxylic acid

4.10.1. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(*N*,*N*-dimethylamino)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9a). A mixture of **8** (350 mg, 0.73 mmol) and 2-(dimethylamino)ethyl chloride HCl (120 mg, 0.83 mmol) in DMF (15 mL) was cooled to 0 °C and sodium hydride (160 mg of a 60% mineral oil suspension, 4.0 mmol) was added in small portions over 5 min. Cooling was removed. The mixture was allowed to warm to room temperature with stirring for 45 min. The reaction flask was then transferred to an oil bath that had been preheated to 65 °C, and the mixture was stirred at this temperature for 3 h. TLC was used to monitor the reaction. The mixture was cooled to room temperature and quenched by addition of a few drops of water. The solvent was removed under vacuum. The crude product was dissolved in 1 N HCl (50 mL). The aqueous solution was washed with chloroform (3× 50 mL), then made basic by the addition of 30% NaOH, and extracted with chloroform (3× 75 mL). The organic layers were combined, dried ($MgSO_4$) and evaporated under vacuum. The residue was chromatographed using 98:2 chloroform/methanol to provide 357 mg of **9a** as a sticky semi-solid glue in 89% yield; IR ($CHCl_3$) 1647 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 6H), 2.51 (m, 2H), 3.19 (m, 1H), 3.33 (s, 3H), 3.73 (s, 3H), 4.92 (m, 1H), 6.08 (s, 2H), 6.76 (s, 1H), 7.04 (s, 1H), 7.22 (d, 1H, J = 4.4), 7.27 (s, 1H), 7.66 (s, 1H), 8.47 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 45.1, 45.6, 55.5, 56.1, 56.3, 88.1, 101.5, 101.9, 106.2, 114.2, 115.3, 120.8, 121.8, 135.7, 142.2, 146.7, 147.4, 148.3, 148.7, 149.2, 150.6, 168.7; HRMS calcd for $C_{23}H_{24}IN_3O_5H$: 550.0839; found: 550.0817.

4.10.2. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(*N*,*N*-dimethylamino)isopropyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9b). Prepared from **8** (800 mg, 1.67 mmol), sodium iodide (375 mg, 2.50 mmol), and 2-(dimethylamino)isopropyl chloride HCl (308 mg, 1.90 mmol) in DMF (36 mL) and sodium hydride (201 mg of a 60% mineral oil suspension, 5.01 mmol) to provide 613 mg of **9b** as a sticky semi-solid glue in 89% yield. IR ($CHCl_3$) 1643 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.97 (d, 3H, J = 6.6), 2.49 (s, 6H), 3.38 (s, 3H), 3.51 (m, 1H), 3.76 (s, 3H), 3.97 (m, 1H), 4.95 (m, 1H), 6.12 (s, 2H), 6.72 (s, 1H), 6.75 (s, 1H), 7.11 (d, 1H, J = 4.4), 7.30 (s, 1H), 7.72 (s, 1H), 8.50 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 16.6, 45.9, 49.8, 55.5, 55.8, 56.0, 92.9, 101.6, 102.6, 106.1, 114.6, 116.3, 120.8, 121.4, 132.4, 142.1, 146.6, 147.6, 147.9, 148.4, 149.0, 150.3, 169.5; HRMS calcd for $C_{24}H_{26}IN_3O_5H$: 564.0996; found: 564.0984.

4.10.3. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(*N*,*N*-diethylamino)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9c). Prepared from **8** (350 mg, 0.73 mmol), sodium iodide (164 mg, 1.10 mmol), 2-(diethylamino)ethyl chloride HCl (145 mg, 0.83 mmol) in DMF (15 mL) and sodium hydride (88 mg of a 60% mineral oil suspension, 2.19 mmol) to give 372 mg of **9c** as a sticky semi-solid glue in 88% yield; IR ($CHCl_3$) 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.11 (t, 6H, J = 7.4), 2.79 (m, 6H), 3.27 (m, 1H), 3.34 (s, 3H), 3.76 (s, 3H), 4.88 (m, 1H), 6.11 (s, 2H), 6.71 (s, 1H), 7.06 (s, 1H), 7.23 (d, 1H, J = 4.4), 7.30 (s, 1H), 7.68 (s, 1H), 8.49 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 11.1, 45.6, 46.2, 50.5, 55.6, 56.2, 88.0, 101.5, 101.9, 106.3, 114.3, 115.5, 120.9, 121.8, 136.2, 142.1, 146.7, 147.5, 148.4, 148.7, 149.1, 150.6, 168.6; HRMS calcd for $C_{25}H_{28}IN_3O_5H$: 578.1153; found: 578.1146.

4.10.4. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(pyrrolidin-1-yl)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9d). A mixture of **8** (500 mg, 1.05 mmol), sodium iodide (236 mg, 1.58 mmol), and 1-(2-chloroethyl)pyrrolidine HCl (210 mg, 1.20 mmol) in DMF (22 mL) was cooled to 0 °C and sodium hydride (126 mg of a 60% mineral oil suspension, 3.15 mmol) was added in small portions over 5 min. The reaction mixture was then allowed to warm to room temperature and to stir for 45 min. The reaction flask was then transferred to an oil bath that had been preheated to 65 °C and stirred at this temperature for 3 h. TLC showed there was still a significant amount of starting material left. To this mixture were added more 1-(2-chloroethyl)pyrrolidine HCl (210 mg, 1.20 mmol), sodium hydride (126 mg of a 60% mineral oil suspension, 3.15 mmol), and sodium iodide (236 mg, 1.58 mmol) at this temperature. The mixture was heated for another 3 h, and cooled to room temperature, and quenched by addition of a few drops of water. The solvent was

removed under vacuum. The crude product was dissolved in 1 N HCl (100 mL). The aqueous solution was washed with chloroform (3× 60 mL), then made basic by the addition of 30% NaOH, and extracted with chloroform (3× 75 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated under vacuum. The residue was chromatographed using 98:2 chloroform/methanol to provide 550 mg of **9d** as a sticky semi-solid glue in 92% yield; IR ($CHCl_3$) 1648 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.94 (m, 4H), 2.57 (m, 3H), 2.89 (m, 3H), 3.22 (m, 1H), 3.32 (s, 3H), 3.75 (s, 3H), 4.98 (m, 1H), 6.10 (s, 2H), 6.80 (s, 1H), 7.05 (s, 1H), 7.25 (d, 1H, J = 4.4), 7.29 (s, 1H), 7.70 (s, 1H), 8.49 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 23.8, 45.6, 52.5, 54.3, 55.5, 56.1, 88.2, 101.5, 101.9, 106.2, 114.2, 115.2, 120.7, 121.8, 135.4, 142.4, 146.7, 147.5, 148.3, 148.6, 149.2, 150.6, 168.7; HRMS calcd for $C_{25}H_{26}IN_3O_5H$: 576.0996; found: 576.1006.

4.10.5. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(piperidin-1-yl)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9e). Prepared from **8** (500 mg, 1.05 mmol), sodium iodide (236 mg, 1.58 mmol), 1-(2-chloroethyl)piperidine HCl (225 mg, 1.20 mmol) in DMF (22 mL), and sodium hydride (126 mg of a 60% mineral oil suspension, 3.15 mmol) The residue was chromatographed using 97.5:2.5 chloroform/methanol to provide 520 mg of **9e** as a sticky semi-solid glue in 84% yield; IR ($CHCl_3$) 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (m, 2H), 1.74 (m, 4H), 2.37 (m, 4H), 2.79 (m, 2H), 3.19 (m, 1H), 3.34 (s, 3H), 3.74 (s, 3H), 5.00 (m, 1H), 6.10 (s, 2H), 6.80 (s, 1H), 7.03 (s, 1H), 7.26 (d, 1H, J = 4.4), 7.27 (s, 1H), 7.81 (s, 1H), 8.49 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 24.6, 26.1, 43.8, 54.9, 55.5, 55.9, 56.1, 88.0, 101.7, 101.9, 106.2, 114.3, 115.3, 120.8, 121.7, 135.4, 142.4, 146.7, 147.5, 148.3, 148.6, 149.2, 150.6, 168.7; HRMS calcd for $C_{26}H_{28}IN_3O_5H$: 590.1153; found: 590.1148.

4.10.6. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(4-methylpiperidin-1-yl)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9f). Prepared from **8** (500 mg, 1.0 mmol), sodium iodide (235 mg, 1.5 mmol), 4-methyl-1-(2-chloroethyl)piperidine hydrochloride (224 mg, 1.14 mmol) in DMF (20 mL), and sodium hydride (125 mg of a 60% suspension, 3.0 mmol). The reaction flask was then transferred to an oil bath that had been preheated to 60 °C, and was stirred at this temperature for 3 h. TLC showed there was still starting material left. More 4-methyl-1-(2-chloroethyl)piperidine hydrochloride (75 mg, 0.38 mmol) and sodium hydride (42 mg of a 60% suspension, 1.05 mmol) were added. The mixture was heated for another 1.5 h, and was cooled to room temperature, and quenched by addition of a few drops of water. The solvent was removed under vacuum and the crude product was dissolved in dilute 1 N HCl (70 mL) and was washed with chloroform (3× 60 mL), and then made basic by the addition of 30% NaOH. The resulting mixture was extracted into chloroform (3× 75 mL), dried ($MgSO_4$), and evaporated under vacuum. The residue was chromatographed using 98:2–96:4 chloroform/methanol to provide 550 mg of **9f** as a sticky semi-solid glue, in 91% yield. IR ($CHCl_3$) 1649; ^1H NMR ($CDCl_3$) δ 1.01 (d, 3H, J = 5.0), 1.47 (m, 3H), 1.78 (m, 2H), 1.95 (t, 1H, J = 11.0), 2.15 (t, 1H, J = 8.8), 2.44 (m, 2H), 2.73 (m, 1H), 3.21 (m, 1H), 3.34 (s, 3H), 3.54 (m, 1H), 3.75 (s, 3H), 5.05 (m, 1H), 6.12 (s, 2H), 6.80 (s, 1H), 7.05 (s, 1H), 7.27 (d, 1H, J = 4.4), 7.29 (s, 1H), 7.80 (s, 1H), 8.49 (s, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 21.9, 31.1, 34.3, 34.7, 43.7, 53.2, 55.5, 56.1, 88.0, 101.7, 101.9, 106.3, 114.2, 115.2, 120.7, 121.7, 135.2, 142.5, 146.6, 147.6, 148.3, 148.6, 149.1, 150.6, 168.8; HRMS $(M+Li)^+$calcd for $C_{27}H_{30}IN_3O_5Li$: 610.1390; found: 610.1371.

4.10.7. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(morpholin-4-yl)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9g). Prepared from **8** (500 mg, 1.05 mmol), sodium iodide (236 mg, 1.58 mmol), 2-(morpholin-4-yl)ethyl chloride HCl (224 mg, 1.20 mmol) in DMF (22 mL), and sodium hydride (126 mg of a 60% mineral oil suspension, 3.15 mmol). The reaction flask was then transferred to an oil bath that had been preheated 60 °C and stirred at this temperature for 3 h. TLC showed there was still a significant amount of starting material left. To this mixture was added more 2-(morpholin-4-yl)ethyl chloride HCl (224 mg, 1.20 mmol), sodium hydride (126 mg of a 60% mineral oil suspension, 3.15 mmol), and sodium iodide (236 mg, 1.58 mmol) at this temperature. The mixture was heated for another 3 h, cooled to room temperature, and quenched by addition of a few drops of water. The solvent was removed under vacuum. The crude product was dissolved in 1 N HCl (100 mL). The aqueous solution was washed with chloroform (3× 60 mL), then made basic by the addition of 30% NaOH, and extracted with chloroform (3× 75 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated under vacuum. The residue was chromatographed using 98.5:1.5 chloroform/methanol to provide 500 mg of **9g** as a sticky semi-solid glue in 81% yield; IR ($CHCl_3$) 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.50 (m, 4H), 2.94 (m, 2H), 3.22 (m, 1H), 3.31 (s, 3H), 3.75 (s, 3H), 3.91 (t, 4H, J = 4.4), 5.05 (m, 1H), 6.12 (s, 2H), 6.73 (s, 1H), 7.07 (s, 1H), 7.26 (d, 1H, J = 4.4), 7.30 (s, 1H), 7.82 (s, 1H), 8.50 (d, 1H, J = 4.4); ^{13}C NMR ($CDCl_3$) δ 43.2, 54.0, 55.6, 55.8, 56.2, 67.0, 88.1, 101.4, 102.0, 106.4, 114.1, 115.2, 121.0, 121.7, 135.2, 142.2, 146.6, 147.6, 148.4, 148.7, 149.3, 150.7, 168.8; HRMS calcd for $C_{25}H_{26}IN_3O_6H$: 592.0945; found: 592.0933.

4.10.8. 6,7-Methylenedioxyquinoline-4-carboxylic acid *N*-[3-(*N*,*N*-dimethylamino)propyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9h). Prepared from **8** (478 mg, 1.0 mmol), 3-(dimethylamino)propyl chloride HCl (190 mg, 1.2 mmol), and sodium amide (156 mg, 4.0 mmol) in toluene (25 mL) heated to reflux with stirring under nitrogen for 4 h. At this time, an equivalent amount of 3-(dimethylamino)propyl chloride HCl and sodium amide was added, and stirring was continued for an additional 3 h. The mixture was cooled to room temperature and quenched by addition of a few drops of water. The solvent was removed under vacuum, and the crude product was dissolved in 1 N HCl (50 mL). This aqueous solution was washed with chloroform (3× 50 mL), then made basic by the addition of 30% NaOH, and

extracted with chloroform (3× 75 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated under vacuum. The residue was chromatographed using 97:3 chloroform/methanol to provide 498 mg of **9h** as a sticky semi-solid glue in 88% yield; IR ($CHCl_3$) 1648 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27 (s, 6H), 2.45 (m, 4H), 3.37 (s, 3H), 3.74 (s, 3H), 3.93 (m, 1H), 4.55 (m, 1H), 6.10 (s, 2H), 6.44 (s, 1H), 7.07 (s, 1H), 7.18 (d, 1H, $J = 4.6$), 7.29 (s, 1H), 7.39 (s, 1H), 8.46 (d, 1H, $J = 4.6$); ^{13}C NMR ($CDCl_3$) δ 26.0, 45.7, 47.6, 55.8, 56.2, 57.4, 87.8, 100.8, 102.0, 106.4, 113.7, 116.03, 121.4, 121.8, 136.5, 141.4, 146.9, 147.3, 148.5, 149.1, 149.2, 150.6, 168.3; HRMS calcd for $C_{24}H_{26}IN_3O_5H$: 564.0995; found: 564.0997.

4.10.9. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(1-methylpyrrolidin-2-yl)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9i). Prepared from **8** (250 mg, 0.52 mmol), sodium iodide (118 mg, 0.79 mmol), 2-(2-chloroethyl)-1-methylpyrrolidine HCl (111 mg, 0.60 mmol) in DMF (11 mL), and sodium hydride (63 mg of a 60% mineral oil suspension, 1.58 mmol). The reaction flask was then transferred to an oil bath that had been preheated to 60 °C and stirred at this temperature for 3 h. TLC showed there was a significant amount of starting material left. To this mixture were then added more 2-(2-chloroethyl)-1-methylpyrrolidine HCl (28 mg, 0.15 mmol), sodium hydride (16 mg of a 60% mineral oil suspension, 0.4 mmol) and sodium iodide (30 mg, 0.2 mmol) at this temperature. The mixture were then heated for another 3 h, cooled to room temperature, and quenched by addition of a few drops of water. The solvent was removed under vacuum. The crude product was dissolved in 1 N HCl (50 mL). The aqueous solution was washed with chloroform (3× 30 mL), then made basic by the addition of 30% NaOH, and extracted with chloroform (3× 40 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated under vacuum. The residue was chromatographed using 95.5:4.5 chloroform/methanol to provide 230 mg of **9i** as a sticky semi-solid glue in 75% yield; IR ($CHCl_3$) 1649 cm^{-1}, ^1H NMR ($CDCl_3$) δ 1.94 (m, 4H), 2.17 (m, 2H), 2.59 (m, 6H), 3.47 (s, 3H), 3.76 (s, 3H), 3.93 (m, 1H), 4.55 (m, 1H), 6.13 (s, 2H), 6.52 (s, 1H), 7.06 (s, 1H), 7.20 (d, 1H, $J = 4.4$), 7.31 (s, 1H), 7.37 (s, 1H), 8.48 (d, 1H, $J = 4.4$); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.2, 29.8, 30.3, 30.5, 40.0, 46.8, 56.1, 56.6, 87.8, 100.7, 102.1, 106.5, 113.7, 116.2, 121.4, 121.8, 136.3, 140.8, 146.9, 147.2, 148.6, 149.3, 149.4, 150.8, 168.5; HRMS calcd for $C_{26}H_{28}IN_3O_5Li$: 596.1234; found: 596.1212.

4.10.10. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(*N*,*N*-dibenzylamino)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9j). Prepared from **8** (461 mg, 0.96 mmol), sodium iodide (217 mg, 1.45 mmol), and *N*-(2-chloroethyl)dibenzylamine HCl (333 mg, 1.10 mmol) in DMF (20 mL) and sodium hydride (116 mg of a 60% mineral oil suspension, 2.89 mmol). The reaction flask was then transferred to an oil bath that had been preheated to 60 °C, and was stirred at this temperature for 3 h. TLC showed there was still a significant amount of starting material left. To this mixture were added more *N*-(2-chloroethyl)dibenzylamine HCl (333 mg, 1.10 mmol), sodium hydride (116 mg of a 60% mineral oil suspension, 2.89 mmol), and sodium iodide (217 mg, 1.45 mmol) at this temperature. The mixture was then heated for another 3 h, cooled to room temperature, and quenched by addition of a few drops of water. The solvent was removed under vacuum. The crude product was dissolved in 1 N HCl (100 mL). The aqueous solution was washed with chloroform (3× 60 mL), then made basic by the addition of 30% NaOH, and extracted with chloroform (3× 75 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated under vacuum. The residue was chromatographed using 99:1 chloroform/methanol to provide 677 mg of **9j** as a sticky semi-solid glue in 85% yield; IR ($CHCl_3$) 1649 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.93 (m, 2H), 3.19 (s, 3H), 3.55 (m, 1H), 3.75 (m, 7H), 4.88 (m, 1H), 6.13 (s, 2H), 6.26 (s, 1H), 7.05 (s, 1H), 7.14 (d, 1H, $J = 4.4$), 7.34 (m, 11H), 7.42 (s, 1H), 8.46 (d, 1H, $J = 4.4$); ^{13}C NMR ($CDCl_3$) δ 46.4, 51.1, 55.9, 56.2, 56.3, 58.2, 87.6, 101.1, 102.0, 106.4, 114.1, 116.1, 121.2, 121.8, 127.2, 128.3, 128.5, 129.3, 136.6, 138.8, 141.3, 146.9, 147.2, 148.5, 148.9, 149.1, 150.7, 168.4; HRMS calcd for $C_{35}H_{32}IN_3O_5Li$: 708.1547; found: 708.1568.

4.10.11. 6,7-Methylenedioxyquinoline-4-carboxylic acid, *N*-[2-(*N*-benzyl-*N*-methylamino)ethyl]-*N*-(2-iodo-4,5-dimethoxyphenyl) amide (9k). Prepared from **8** (500 mg, 1.0 mmol), sodium iodide (235 mg, 1.5 mmol), *N*-benzyl-*N*-methyl-2-chloroethylamine hydrochloride (261 mg, 1.14 mmol) in DMF (20 mL), and sodium hydride (125 mg of a 60% suspension, 3.0 mmol). The residue was chromatographed in 98:2 to 96:4 chloroform/methanol providing 550 mg of **9k** as a sticky semi-solid glue, in 84% yield; IR ($CHCl_3$) 1649; ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H), 2.76 (m, 2H), 3.22 (s, 3H), 3.36 (m, 1H), 3.61 (s, 2H), 3.75 (s, 3H), 4.95 (m, 1H), 6.14 (s, 2H), 6.70 (s, 1H), 7.05 (s, 1H), 7.31 (m, 6H), 7.59 (s, 1H), 7.02 (s, 1H), 8.49 (d, 1H, $J = 4.8$); ^{13}C NMR ($CDCl_3$) δ 41.7, 45.6, 55.2, 55.7, 56.2, 62.9, 87.8, 101.2, 102.0, 106.4, 114.3, 115.8, 120.9, 121.8, 127.2, 128.4, 129.3, 136.1, 138.3, 141.8, 146.8, 147.4, 148.4, 148.8, 149.2, 150.6, 168.6; HRMS $(M+Li)^+$ calcd for $C_{29}H_{28}IN_3O_5Li$: 632.1234; found: 632.1238.

4.11. Methods for the formation of 6-substituted 8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-ones

4.11.1. 6-[2-(*N*,*N*-Dimethylamino)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10a). A solution of **9a** (275 mg, 0.5 mmol) in 950 mL of 2% HCl was transferred to the photoreactor apparatus and degassed by nitrogen purge for 30 min. The solution was irradiated through a Vycor filter for 90 min. The mixture was basified (30% NaOH) and extracted with ethyl acetate (4× 50 mL). The combined organic extracts were evaporated and the residue was chromatographed on silica eluting with 98:2 chloroform/methanol to provide 70 mg of **10a** as a yellow solid in 34% yield; mp 253–255 °C; IR ($CHCl_3$) 1639 cm^{-1}; ^1H NMR ($CDCl_3$ + 1 drop CD_3OD) δ 2.49 (s, 6H), 2.79 (t, 2H, $J = 7.9$), 3.97 (s, 3H), 3.99 (s, 3H), 4.57 (t, 2H, $J = 7.9$), 6.08 (s, 2H), 7.01 (s, 1H), 7.32 (s, 1H),

7.73 (s, 1H), 9.32 (s, 1H), 9.44 (s, 1H); ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD) δ 41.0, 45.0, 55.0, 56.3, 56.4, 98.3, 102.2, 103.5, 104.8, 105.7, 110.3, 121.9, 122.3, 125.9, 132.6, 143.3, 144.4, 145.9, 149.9, 150.1, 152.2, 161.1; HRMS calcd for $C_{23}H_{23}N_3O_5H$: 422.1716; found: 422.1703.

4.11.2. 6-[2-(*N*,*N*-Dimethylamino)isopropyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10b). Prepared from **9b** (200 mg, 0.36 mmol) in 900 mL of 2% HCl using a photoreactor apparatus to provide 51 mg of **10b** as a yellow solid in 29% yield; mp: 262–264 °C; IR (KBr) 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$ + 3 drops CD_3OD) δ 1.07 (d, 3H, J = 6.6), 2.46 (s, 6H), 3.23 (m, 1H), 4.07 (s, 6H), 4.24 (m, 1H), 5.03 (m, 1H), 6.18 (s, 2H), 7.17 (s, 1H), 7.49 (s, 1H), 7.84 (s, 1H), 9.52 (s, 1H), 9.60 (s, 1H); ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD) δ 10.8, 40.7, 45.9, 56.2, 56.4, 57.4, 99.0, 102.2, 103.8, 104.7, 105.7, 110.4, 122.1, 122.5, 125.9, 133.0, 143.3, 144.5, 145.8, 149.9, 150.1, 151.8, 161.5; HRMS calcd for $C_{24}H_{25}N_3O_5H$: 436.1873; found: 436.1852.

4.11.3. 6-[2-(*N*,*N*-Diethylamino)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10c). Prepared from **9c** (200 mg, 0.36 mmol) in 900 mL of 2% HCl using a photoreactor apparatus. The residue was chromatographed on silica eluting with 98.5:1.5 chloroform/methanol to provide 40 mg of **10c** as a yellow solid in 25% yield; mp: 218–220 °C; IR (KBr) 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$ + 3 drops CD_3OD) δ 1.20 (t, 6H, J = 6.6), 2.82 (m, 6H), 4.05 (s, 3H), 4.07 (s, 3H), 4.64 (m, 2H), 6.16 (s, 2H), 7.16 (s, 1H), 7.46 (s, 1H), 7.83 (s, 1H), 9.48 (s, 1H), 9.57 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops CD_3OD) δ 11.3, 41.7, 47.5, 49.2, 56.5, 98.5, 102.2, 103.8, 104.8, 106.1, 110.4, 122.0, 122.3, 125.9, 132.9, 143.4, 144.7, 145.9, 149.8, 150.1, 152.1, 161.2; HRMS calcd for $C_{25}H_{27}N_3O_5H$: 450.2030; found: 450.2009.

4.11.4. 6-[2-(Pyrrolidin-1-yl)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10d). Prepared from **9d** (450 mg, 0.78 mmol) in 900 mL of 2% HCl using a photoreactor apparatus to provide 110 mg of **10d** as a yellow solid in 34% yield; mp: 240–242 °C; IR (KBr) 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$ + 3 drops CD_3OD) δ 1.92 (m, 4H), 2.87 (m, 6H), 4.04 (s, 3H), 4.08 (s, 3H), 4.67 (m, 2H), 6.15 (s, 2H), 7.16 (s, 1H), 7.44 (s, 1H), 7.82 (s, 1H), 9.45 (s, 1H), 9.55 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops CD_3OD) 23.5, 42.1, 52.2, 54.3, 56.5, 98.5, 102.2, 103.7, 104.8, 105.9, 110.3, 122.0, 122.3, 125.9, 132.8, 143.4, 144.5, 145.9, 149.8, 150.1, 152.2, 161.1; HRMS calcd for $C_{25}H_{25}N_3O_5H$: 448.1873; found: 448.1872.

4.11.5. 6-[2-(Piperidin-1-yl)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10e). Prepared from **9e** (480 mg, 0.82 mmol) in 900 mL of 2% HCl using a photoreactor apparatus to provide 110 mg of **10e** as a yellow solid in 30% yield; mp: 247.5–249.5 °C; IR (KBr) 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$ + 1 drop CD_3OD) δ 1.51 (m, 2H), 1.69 (m, 4H), 2.65 (m, 6H), 4.03 (s, 3H), 4.06 (s, 3H), 4.62 (m, 2H), 6.14 (s, 2H), 7.14 (s, 1H), 7.42 (s, 1H), 7.79 (s, 1H), 9.44 (s, 1H), 9.52 (s, 1H); ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD) δ 23.9, 25.6, 41.1, 54.8, 56.5, 98.6, 102.2, 103.7, 104.8, 106.0, 110.4, 122.4, 125.9, 132.9, 143.4, 144.6, 145.9, 149.9, 150.1, 152.2, 161.2; HRMS calcd for $C_{26}H_{27}N_3O_5H$: 462.2030; found: 462.2026.

4.11.6. 6-[2-(4-Methylpiperidin-1-yl)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10f). A mixture of **9f** (500 mg, 0.83 mmol), $Pd(PPh_3)_4$ (195 mg, 0.166 mmol), and Ag_2CO_3 (457 mg, 1.66 mmol) in DMF (36 mL) was heated to reflux for 45 min. The mixture was cooled, diluted with $CHCl_3$, and filtered through Celite. The filtrate was evaporated, triturated with 15 mL of 50% CH_2Cl_2 in ether, and filtered to give 161 mg of **10f** in 41% yield as a yellow solid. mp (dec.): 245.5–246.5 °C; IR (KBr) 1637; ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 3H, J = 5.8), 1.32 (m, 3H), 1.70 (m, 2H), 2.53 (m, 2H), 2.72 (m, 2H), 3.07 (m, 2H), 4.04 (s, 6H), 4.58 (m, 2H), 6.14 (s, 2H), 7.10 (s, 1H), 7.41 (s, 1H), 7.77 (s, 1H), 9.44 (s, 1H), 9.50 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.8, 30.5, 34.2, 41.4, 54.4, 54.9, 56.4, 98.5, 102.2, 103.8, 104.6, 106.0, 110.3, 121.9, 122.3, 125.8, 132.9, 143.4, 144.6, 145.8, 149.8, 150.1, 152.0, 161.1; HRMS calcd HRMS $(M+Li)^+$ calcd for $C_{27}H_{29}N_3O_5Li$: 482.2267; found: 482.2265.

4.11.7. 6-[2-(Morpholin-4-yl)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10g). Prepared from **9g** (450 mg, 0.76 mmol) in 900 mL of 2% HCl using a photoreactor to provide 100 mg of **10g** as a yellow solid in 31% yield; mp 273–275 °C; IR (KBr) 1636 cm^{-1}; ^{1}H NMR ($CDCl_3$ + 3 drops CD_3OD) δ 2.66 (m, 4H), 3.73 (m, 4H), 4.00 (m, 8H), 4.55 (m, 2H), 6.08 (s, 2H), 7.03 (s, 1H), 7.32 (s, 1H), 7.74 (s, 1H), 9.31 (s, 1H), 9.44 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops CD_3OD) δ 41.1, 53.8, 55.1, 56.4, 66.8, 98.4, 102.2, 103.6, 104.8, 105.8, 110.4, 122.3, 125.9, 132.7, 143.3, 144.5, 145.9, 149.9, 150.1, 152.0, 161.2; HRMS calcd for $C_{25}H_{25}N_3O_6H$: 464.1823; found: 464.1821.

4.11.8. 6-[3-(*N*,*N*-Dimethylamino)propyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10h). Prepared from **9h** (200 mg, 0.42 mmol) in 950 mL of 2% HCl using a photoreactor to provide 51 mg of **10h** as a yellow solid in 29% yield; mp 238–240 °C; IR ($CHCl_3$) 1638 cm^{-1}; ^{1}H NMR ($CDCl_3$ + 1 drop CD_3OD) δ 2.07 (m, 2H), 2.38 (s, 6H), 2.65 (t, 2H, J = 7.3), 4.04 (s, 6H), 4.48 (t, 2H, J = 7.5), 6.16 (s, 2H), 7.02 (s, 1H), 7.44 (s, 1H), 7.81 (s, 1H), 9.46 (s, 1H), 9.54 (s, 1H); ^{13}C NMR ($CDCl_3$ + 1 drop CD_3OD) δ 25.1, 41.9, 45.1, 56.3, 56.5, 56.8, 98.4, 102.2, 103.8, 104.8, 106.1, 110.5, 122.2, 122.4, 125.9, 132.8, 143.4, 144.7 145.9, 149.8, 150.1, 152.0, 161.3; HRMS calcd for $C_{24}H_{25}N_3O_5H$: 436.1872; found: 436.1883.

4.11.9. 6-[2-(1-Methylpyrrolidine-2-yl)ethyl]-8,9-dimethoxy-2,3- methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10i). Prepared from **9i** (200 mg, 0.34 mmol) in 900 mL of 2% HCl using a photoreactor apparatus to provide 38 mg of **10i** as a beige solid in 24% yield; mp: 272–274 °C; IR (KBr) 1637 cm^{-1}; ^{1}H

NMR ($CDCl_3$ + 3 drops CD_3OD) δ 2.07 (m, 4H), 2.52 (m, 3H), 2.84 (m, 2H), 3.42 (m, 1H), 3.82 (m, 2H), 4.03 (s, 3H), 4.10 (s, 3H), 4.59 (m, 2H), 6.15 (s, 2H), 6.95 (s, 1H), 7.42 (s, 1H), 7.77 (s, 1H), 9.34 (s, 1H), 9.51 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops CD_3OD) δ 21.7, 29.2, 30.2, 39.6, 40.6, 56.2, 56.5, 56.8, 68.0, 98.3, 102.3, 103.5, 104.9, 106.2, 110.3, 122.2, 126.0, 132.3, 143.4, 144.6, 146.2, 149.8, 150.1, 152.4, 161.7; HRMS calcd for $C_{26}H_{27}N_3O_5H$: 462.2029; found: 462.2017.

4.11.10. 6-[(2-*N*,*N*-Dibenzylamino)propyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10j). Prepared from **9j** (200 mg, 0.28 mmol) in 900 mL of 2% HCl using a photoreactor to provide 8 mg of **10j** as a yellow solid in 5% yield.

4.11.11. 6-[(2-*N*,*N*-Dibenzylamino)propyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10j). A mixture of **9j** (560 mg, 0.8 mmol), $Pd(PPh_3)_4$ (188 mg, 0.16 mmol), and Ag_2CO_3 (442 mg, 1.6 mmol) in DMF (35 mL) was heated to reflux for 1 h. The mixture was cooled, diluted with $CHCl_3$, and filtered through Celite. The filtrate was evaporated, triturated with 15 mL of 30% CH_2Cl_2 in ether, and filtered to give 65 mg of **10j** as a yellow solid. The filtrate was evaporated. To the residue added $Pd(PPh_3)_4$ (188 mg, 0.16 mmol), Ag_2CO_3 (442 mg, 1.6 mmol), and DMF (35 mL). The reaction mixture was heated to reflux for 1 h, cooled, diluted with chloroform, and filtered through Celite. The filtrate was evaporated, triturated with 15 mL of 30% methylene chloride in ether, and filtered to give another 40 mg of **10j**, total 105 mg of **10j** in 23% yield as a yellow solid; mp: 215–217 °C; IR (KBr) 1640 cm^{-1}; ^1H NMR ($CDCl_3$ + 3 drops CD_3OD) δ 2.90 (m, 2H), 3.54 (s, 4H), 3.79 (s, 3H), 4.01 (s, 3H), 4.57 (m, 2H), 6.15 (s, 2H), 6.50 (s, 1H), 7.24 (m, 11H), 7.68 (s, 1H), 9.43 (s, 1H), 9.46 (s, 1H); ^{13}C NMR ($CDCl_3$ + 3 drops CD_3OD) δ 41.9, 50.4, 55.8, 56.5, 59.0, 98.1, 102.1, 104.0, 104.7, 106.6, 110.2, 122.3, 127.1, 128.4, 128.8, 129.2, 139.6, 143.5, 145.1, 145.6, 149.7, 150.0, 161.2; HRMS calcd for $C_{35}H_{31}N_3O_5Li$: 580.2424; found: 580.2409.

4.11.12. 6-[2-(*N*-Benzyl-*N*-methylamino)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10k). A mixture of **9k** (500 mg, 0.8 mmol), $Pd(PPh_3)_4$ (188 mg, 0.16 mmol), and Ag_2CO_3 (442 mg, 1.6 mmol) in DMF (35 mL) was heated to reflux for 1 h. The mixture was cooled, diluted with chloroform, and filtered through Celite. The filtrate was evaporated, triturated with 15 mL of 30% methylene chloride in ether, and filtered to give 50 mg of **10k** as a yellow solid. The filtrate was evaporated. To the residue were added $Pd(PPh_3)_4$ (188 mg, 0.16 mmol), Ag_2CO_3 (442 mg, 1.6 mmol), and DMF (35 mL). The reaction mixture was heated to reflux for 1 h, cooled, diluted with chloroform, and filtered through Celite. The filtrate was evaporated, triturated with 15 mL of 30% methylene chloride in ether, and filtered to give another 35 mg of **10k**, total 85 mg of **10k** in 21% yield as a yellow solid; mp (dec.): 222.5–223.5 °C; IR ($CHCl_3$) 1638; ^1H NMR ($CDCl_3$) δ 2.42 (s, 3H), 2.73 (m, 2H), 3.57 (s, 2H), 3.74 (s, 3H), 3.93 (s, 3H), 4.51 (m, 2H), 6.06 (s, 2H), 6.80 (s, 1H), 7.11 (m, 5H), 7.32 (s, 1H), 7.72 (s, 1H), 9.35 (s, 1H), 9.44 (s, 1H); HRMS $(M+Li)^+$ calcd for $C_{29}H_{27}IN_3O_5Li$: 504.2111; found: 504.2108.

4.11.13. 6-[2-(Amino)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10l). Compound **10j** (50 mg, 0.09 mmol) was dissolved in acetic acid (46 mL). Formic acid (2.6 mL) and palladium black (93 mg) were then added while stirring. After 30 min, more palladium black (70 mg) was added. The whole mixture was stirred for another 30 min, at which time additional palladium black (50 mg) was then added and the reaction mixture was allowed to stir for another 30 min. The reaction mixture was diluted with chloroform (30 mL), filtered through Celite, and evaporated. The residue was partitioned between chloroform (50 mL) and 10% NaOH (20 mL). The aqueous phase was washed by chloroform three times. Combined organic phases were washed with water (2 × 20 mL), and brine (20 mL), evaporated, and chromatographed with chloroform/methanol/triethylamine (96:4:0.2) providing 28 mg of **10l** in 82 % yield as a light orange solid; mp (dec.): 218.5–219.5 °C; IR (KBr) 1637; ^1H NMR ($CDCl_3$) δ 3.11 (m, 2H), 4.01 (s, 6H), 4.50 (m, 2H), 6.12 (s, 2H), 6.95 (s, 1H), 7.37 (s, 1H), 7.76 (s, 1H), 9.37 (s, 1H), 9.48 (s, 1H); HRMS $(M+Li)^+$ calcd for $C_{21}H_{19}N_3O_5Li$: 400.1485; found: 400.1480.

4.11.14. 6-[2-(*N*-Methylamino)ethyl]-8,9-dimethoxy-2,3-methylenedioxy-6*H*-dibenzo[*c*,*h*][2,6]naphthyridin-5-one (10m). Formic acid (1.06 mL) and palladium black (24 mg) were added with stirring to a solution of **10k** (40 mg, 0.08 mmol) in acetic acid (27 mL). After 30 min, 20 mg of palladium black (26 mg) was added. After an additional 30 min, 20 mg of palladium black was added and the reaction mixture was then allowed to stir for another 30 min. The reaction mixture was diluted with chloroform (30 mL), filtered through Celite, and evaporated. The residue was partitioned between chloroform (50 mL) and 10% NaOH (20 mL). The aqueous phase was extracted with chloroform (3× 50 mL). The combined organic phases were washed with water (2× 20 mL), and brine (20 mL), evaporated, and the residue was triturated with ethyl ether to give 28 mg of **10m** in 88% yield as a yellow solid; mp (dec.): 221–222.5 °C; IR (KBr) 1635; ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H), 2.99 (t, 2H, J = 6.8), 4.02 (s, 6H), 4.53 (t, 2H, J = 6.8), 6.12 (s, 2H), 6.98 (s, 1H), 7.37 (s, 1H), 7.74 (s, 1H), 9.37 (s, 1H), 9.46 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 29.7, 36.1, 42.9, 56.3, 56.4, 98.2, 102.3, 103.6, 104.6, 105.9, 110.3, 121.9, 122.3, 125.8, 132.7, 143.3, 144.5, 145.8, 149.8, 150.1, 151.9, 161.4; HRMS $(M+Li)^+$calcd for $C_{22}H_{21}N_3O_5Li$: 414.1641; found: 414.1649.

4.12. 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.[36] Plasmid YepG was purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium bromide isopycnic centrifugation method as described.[37] The end-labeling of the plasmid was accom-

plished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[38] The cleavage assays were performed as previously reported.[36,39] The drug and the DNA in the 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.

4.13. Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[40–42] 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).[28] The KB3-1 cell line and its multidrug-resistant variant KBV-1 were obtained from K.V. Chin (The Cancer Institute of New Jersey, NJ).[29] The KBH5.0 cell line was derived from KB3-1 by stepwise selection against Hoechst 33342.[19] 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 four 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.

Acknowledgments

This study was supported by Grant CA098127 (E.J.L.), Grant CA39662 (L.F.L.), and Grant CA077433 (L.F.L.) from the National Cancer Institute.

References and notes

1. Wang, J. C. *Annu. Rev. Biochem.* **1985**, *54*, 665.
2. Liu, L. *Annu. Rev. Biochem.* **1989**, *58*, 351.
3. Chen, A. Y.; Liu, L. F. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191.
4. Haiang, Y.; Lihou, M.; Liu, L. *Cancer Res.* **1989**, *49*, 5077.
5. Wall, J. G.; Burris, H. A.; Von-Hoff, D. D.; Rodriquez, G.; Kneuper-Hall, R.; Shaffer, D.; O'Rourke, T.; Brown, T.; Weiss, G.; Clark, G., et al. *Anti-cancer Drugs* **1992**, *3*, 337.
6. Burke, T. G.; Mi, Z. *J. Med. Chem.* **1993**, *36*, 2580.
7. Burke, T. G.; Mi, Z. *Anal. Biochem.* **1993**, *212*, 285.
8. Chen, A. Y.; Yu, C.; Potmesil, M.; Wall, M. E.; Wani, M. C.; Liu, L. F. *Cancer Res.* **1991**, *51*, 6039.
9. Chu, X.; Kato, Y.; Sugiyama, Y. *Cancer Res.* **1997**, *57*, 1934.
10. Kawabata, S.; Oka, M.; Shiozawa, K.; Tsukamoto, K.; Nakatomi, K.; Soda, H.; Fududa, M.; Ikegami, Y.; Sugahara, K.; Yamada, Y.; Kamihira, S.; Doyle, L. A.; Ross, D. D.; Kohno, S. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 1216.
11. Yang, C. H.; Schneider, E.; Kuo, M. L.; Volk, E. L.; Roccchi, E.; Chen, Y. C. *Biochem. Pharmacol.* **2000**, *60*, 831.
12. Makhey, D.; Li, D.; Zhao, B.; Sim, S.-P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 1809.
13. Li, D.; Zhao, B.; Sim, S.-P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 521.
14. Li, D.; Zhao, B.; Sim, S.-P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 3795.
15. Yu, Y.; Singh, S. K.; Liu, A.; Li, T.-K.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 1474.
16. Singh, S. K.; Ruchelman, A. L.; Zhou, N.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Med. Chem. Res.* **2003**, *12*, 1.
17. Ruchelman, A. L.; Singh, S. K.; Wu, X.; Ray, A.; Yang, J.-M.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3333.
18. Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X.; Yang, J.-M.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2061.
19. Li, T.-K.; Houghton, P. J.; Desai, S. D.; Daroui, P.; Liu, A. A.; Hars, E. S.; Ruchelman, A. L.; LaVoie, E. J.; Liu, L. F. *Cancer Res.* **2003**, *63*, 8400.
20. Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X.; Yang, J.-M.; Zhou, N.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2004**, *12*, 795.
21. Singh, S. K.; Ruchelman, A. L.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *J. Med. Chem.* **2003**, *46*, 2254.
22. Kerrigan, J. E.; Pilch, D. S.; Ruchelman, A. L.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3395.
23. Ruchelman, A. L.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2004**, *12*, 3731.
24. Ruchelman, A. L.; Liu, A.; Zhou, N.; Liu, L. F.; LaVoie, E. J. *Lett. Drug Des. Discovery* **2004**, *1*, 198.
25. Ruchelman, A. L.; Houghton, P. J.; Liu, A.; Zhou, N.; Liu, L. F.; LaVoie, E. J. *J. Med. Chem.* **2005**, *48*, 792.
26. Ruchelman, A. L.; Zhu, S.; Zhou, N.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5585.
27. Zhu, S.; Ruchelman, A. L.; Zhou, N.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2005**, *13*, 6782.
28. Tamura, H.; Kohchi, C.; Yamada, R.; Ikeda, T.; Koiwai, O.; Patterson, E.; Keene, J. D.; Okada, K.; Kjeldsen, E.; Nishikawa, K.; Andoh, T. *Nucleic Acids Res.* **1990**, *19*, 69.
29. Shen, D. W.; Fojo, A.; Chin, J. E.; Roninson, I. B.; Richert, N.; Pastan, I.; Gottesman, M. M. *Science* **1986**, *232*, 643.
30. Hoffmann, U.; Kroemer, H. K. *Drug Metab. Rev.* **2004**, *36*, 669.
31. Rasheed, Z. A.; Rubin, E. H. *Oncogene* **2003**, 7296.
32. Whiteley, C. G. *Bioorg. Med. Chem.* **2002**, *10*, 1221.
33. Daoud, K. M.; Ibrahim, M. N.; Sarrd, M. S. *J. Indian Chem. Soc.* **1989**, *66*, 316.
34. Tron-Loisel, H.; Brossier, P.; Compagnon, O.; Grosjean, B.; Compagnon, P. L.; Branceni, D. *Eur. J. Med. Chem.* **1977**, *12*, 379; Elz, S.; Keller, A. *Arch. Pharm.* **1995**, *328*, 585.
35. Boehme, T. M.; Keim, C.; Kreutzmann, K.; Linder, M.; Dingermann, T.; Dannhardt, G.; Mutschler, E.; Lambtrcht, G. *J. Med. Chem.* **2003**, *46*, 856.
36. Gatto, B.; Sanders, M. M.; Yu, C.; Wu, H.-Y.; Makhey, D.; LaVoie, E. J.; Liu, L. F. *Cancer Res.* **1996**, *56*, 2795.

37. Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning, a Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor NY, 1982, pp 149–185.
38. Tewey, K. M.; Rowe, T. C.; Yang, L.; Hallogan, B. C.; Liu, L. F. *Science* **1984**, *226*, 466.
39. Wang, H.; Mao, Y.; Chen, A.; Zhou, N.; LaVoie, E. J.; Liu, L. F. *Biochemistry* **2001**, *40*, 3316.
40. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
41. Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
42. Denizot, F.; Lang, R. J. *J. Immunol. Methods* **1986**, *89*, 271.