5-(2-Aminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones: Variation of *N*-Alkyl Substituents Modulates Sensitivity to Efflux Transporters Associated with Multidrug Resistance

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5*H*-8,9-Dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c*,*h*]-[1,6]naphthyridin-6-one (ARC-111) has potent TOP1-targeting activity and pronounced antitumor activity. Several analogues of ARC-111 were synthesized with NH_2, *N*-alkyl, *N*,*N*-dialkyl, pyrrolidinyl, piperidinyl, and piperazinyl substituents at the 2-position of the 5-ethyl group. The relative TOP1-targeting activity and cytotoxicity of these structural analogues were assessed in RPMI8402 and P388 tumor cells and their camptothecin-resistant variants CPT-K5 and P388/CPT45, respectively. Potent TOP1-targeting activity was retained within a series of mono *N*-alkyl analogues that included $NHCH_2CH_3$, $NHCH(CH_3)_2$, and $NHC(CH_3)_3$. TOP1-targeting activity was diminished by the presence of a *N*-benzyl moiety. In a comparison of a series of *N*-alkyl-*N*-isopropyl analogues, activity decreased in the order $CH_3 > CH_2CH_3 > CH(CH_3)_2$. Cytotoxicity in RPMI8402 and P388 did correlate with TOP1-targeting activity. Cytotoxic activity was also determined in KB3-1 cells and its variants KB/V-1 and KBH5.0. As KB/V-1 cells overexpress MDR1 and KBH5.0 cells overexpress BCRP, decreased cytotoxicity in these cell lines relative to the parent cell line is indicative of compounds that are substrates for these efflux transporters. In view of their diminished cytotoxicity in KB/V-1 cells, it appears that the likely demethylated metabolites of ARC-111, i.e., where NH_2 or $NHCH_3$ replaces the $N(CH_3)_2$ at the 2-position of the 5-ethyl substituent, are substrates for MDR1. In contrast, no significant difference in cytotoxicity among these three cell lines was observed with other *N*-alkyl analogues, including NHC_2H_5, $NHCH(CH_3)_2$, $NHC(CH_3)_3$, $N(CH_3)_2$, $N(CH_2CH_3)_2$, $NCH_3(CH(CH_3)_2)$, and either the pyrrolidinyl or the piperidinyl analogues. The 2-(piperazinyl) analogues were associated with diminished cytotoxicity in KB/V-1 cells, suggesting that the second basic amino substituent is associated with their recognition as substrates by MDR1. Comparative studies on the antitumor activity of ARC-111 and its N-demethylated derivatives (the $NHCH_3$ and NH_2 analogues) against SJ-BT45 medulloblastoma xenografts in scid mice revealed that the secondary amine metabolite is at least as active as ARC-111 in vivo, although the primary amine derivative was significantly less potent.

Introduction

Topoisomerases are enzymes that regulate the topology of DNA and are critical to replication and transcription. Two major subtypes, topoisomerase I (TOP1) and topoisomerase II (TOP2), are distinguished based upon differences in their primary sequence and initial mechanisms, wherein either a single- or double-stranded DNA break is implicated.[1−4] Topoisomerase-targeting agents that stabilize the cleavable complex formed between the enzyme and DNA have proven to be effective in the treatment of cancer. Molecules capable of stabilizing the topoisomerase−DNA cleavable complex in effect convert these enzymes into cellular poisons. Camptothecin (CPT) was the first molecule identified as a TOP1-targeting agent.[5] It has recently been shown that formation of the TOP1−DNA cleavable complex in the presence of camptothecin (CPT) induces downregulation of TOP1 via an ubiquitin/26S proteosomal pathway.[6,7] However, this CPT-induced downregulation of TOP1 is impaired to a variable degree in many tumor cell lines. Therefore, it is believed that detoxification by the ubiquitin/26S proteosomal pathway might be responsible for the tumor selectivity as well as resistance observed with CPT in some tumor cells.

Topotecan (Hycamtin) and irinotecan (CPT-11/Camptosar) are two clinical anticancer agents developed from the research performed with camptothecin and its structurally related analogues. These camptothecin-based drugs have incorporated within their structures a δ-lactone, which is susceptible to hydrolysis. Hydrolysis of this lactone results in the formation of an inactive derivative that possesses high affinity for human serum

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albumin.[8−10] Susceptibility to transporter-mediated cellular efflux limits intracellular accumulation of several camptothecin analogues. Overexpression of MDR1 (P-glycoprotein, P-gp) confers resistance to camptothecins, although to a lesser degree than that observed for TOP2-targeting drugs such as doxorubicin or etoposide.[11,12] The camptothecins that are in clinical use are good substrates for the efflux transporter, breast cancer resistance protein (BCRP),[13,14] which may represent a more clinically relevant source of resistance.[15] Although topotecan and irinotecan have good utility as a result of their extraordinary potency, their metabolic instability coupled with their susceptibility to efflux transport have prompted further studies on the development of novel TOP1-targeting agents. A chemotherapeutic agent structurally unrelated to CPT could in theory avoid the pharmacological drawbacks outlined above and might also realize a different pattern of biodistribution. Such a drug could be clinically useful in the treatment of tumor types not affected by camptothecins or could be used in combination with a CPT derivative.

Several novel non-camptothecin TOP1-targeting agents have been identified. These include derivatives of bi- and terbenzimidazoles,[16−18] benz[*a*]anthracenes,[19] benzo[*c*]-phenanthridine and protoberberine alkaloids,[20,21] indolocarbazoles,[22] the fungal metabolites bulgarein[23] and saintopin,[24] and several indenoisoquinolines[25−27] and benzophenazines.[28] Previous studies in our laboratory identified 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]-phenanthridine, **1**,[29] and 2,3-dimethoxy-8,9-methylenedioxydibenzo[*c,h*]cinnoline, **2**,[30] as lead compounds with good TOP1-targeting activities (Figure 1). Further work led to the development of solubilized derivatives such as 8,9-dimethoxy-2,3-methylenedioxy-5-[2-(*N*,*N*-dimethylamino)ethyl]-5*H*-dibenzo[*c,h*][1,6]naphthyridin-6-one, **3**, and 2,3-dimethoxy-8,9-methylenedioxy-11-[2-(*N*,*N*-dimethylamino)ethyl]-11*H*-isoquino[4,3-*c*]-cinnolin-12-one, **4**.[31] Studies of structure−activity relationships (SAR) among these families of compounds elucidated those structural features that were associated with potent TOP1-targeting activity. Enhanced TOP1-targeting activity and cytotoxicity are associated with (1) the presence of methoxy substituents at both the 2- and 3-position of the A-ring (benzo[*i*]phenanthridine numbering), (2) a 8,9-methylenedioxy moiety within the D-ring, and (3) heteroatom substitution adjacent to the benzo ring that incorporates the methylenedioxy substituent. For families of compounds represented by **3** and **4**, the alkyl linker between the dialkylamino moiety and the fused-ring polycyclic nucleus should be two carbons in length for optimal activity. The dialkylamino group can be a cyclic amine such as pyrrolidine, but increasing the size beyond piperidine results in analogues with decreased activity.

Compound **3** is not a substrate for either MDR1 or BCRP (see below for further discussion). When assayed in NCR-nude-nude mice against the breast tumor xenograft MDA-MB-435, **3** was found to have in vivo efficacy comparable to that of CPT-11.[32] Results from an unpublished study revealed the secondary amine (M1 metabolite) resulting from N-dealkylation is a major metabolite in Sprague−Dawley rats. In total, 46.5% of **3** is converted to the M1 metabolite, which reaches a maximum plasma concentration (8.54 ng/mL) at 240 min postdosing in vivo. The M1 metabolite resulting from N-dealklyation retains very potent activity in cell-based assays. However, this minor structural modification renders the M1 metabolite sensitive to BCRP-mediated cellular clearance. Although unlikely to trigger clinical resistance to **3**, the ability of the M1 metabolite to be a substrate for BCRP-mediated transport could potentially have a detrimental impact on its activity. The focus of the present study is to develop analogues of **3** in which the dialkylamino moiety has been modified to reduce the rate of N-dealkylation or to provide N-dealkylation metabolites that are not substrates for efflux transporters.

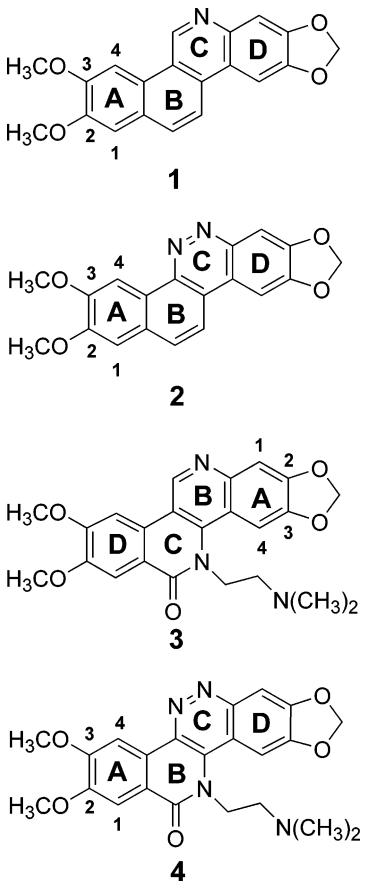
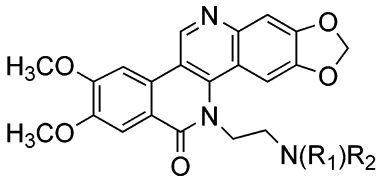


Figure 1. Structure and numbering of 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine, **1**, 2,3-dimethoxy-2,3-methylenedioxydibenzo[*c,h*]cinnoline, **2**, 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c,h*][1,6]naphthyridin-6-one, **3**, and 11*H*-2,3-dimethoxy-11-(2-*N*,*N*-dimethylaminoethyl)-8,9-methylenedioxy-5,6,1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

Compounds synthesized to investigate the "structure−sensitivity relationships" relevant to the BCRP transporter are listed in Figure 2. The variable within this series is the identity of the *N*-alkyl, *N*,*N*-dialkylamino, or cyclic amino group. Compounds **5e**−**g** contain identical *N*,*N*-dialkyl substituents (benzyl, ethyl, and isopropyl groups). Several unsymmetrical dialkyl substitutients were also evaluated including **5a**−**d** and **7a**−**b**. Benzylamines **5a**−**e** and **6e** provide a means for assessing the effect of benzyl substitution on cytotoxicity, TOP1-targeting activity, and sensitivity to MDR1 and BCRP. These benzylamines also served as useful precursors to the respective secondary amines **6a**−**e** and the primary amine **6f.** The secondary amines **6a**−**e** as



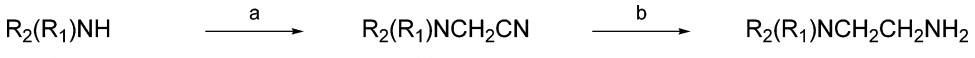
3 R_1=R_2=Me
5a R_1=Bn; R_2=Me
5b R_1=Bn; R_2=Et
5c R_1=Bn; R_2=*i*-Pr
5d R_1=Bn; R_2=*t*-Bu
5e R_1=R_2=Bn
5f R_1=R_2=Et
5g R_1=R_2=*i*-Pr

6a R_1=Me; R_2=H
6b R_1=Et; R_2=H
6c R_1=*i*-Pr; R_2=H
6d R_1=*t*-Bu; R_2=H
6e R_1=Bn; R_2=H
6f R_1=R_2=H
7a R_1=*i*-Pr; R_2=Me
7b R_1=*i*-Pr; R_2=Et

8a R_1,R_2 = -$CH_2CH_2CH_2CH_2$-
8b R_1,R_2 = -$CH_2CH_2CH_2CH_2CH_2$-
8c R_1,R_2 = -$CH_2CH_2CH(CH_3)CH_2CH_2$-
8d R_1,R_2 = -$CH_2CH_2N(Bn)CH_2CH_2$-
8e R_1,R_2 = -$CH_2CH_2NHCH_2CH_2$-
8f R_1,R_2 = -$CH_2CH_2N(CH_3)CH_2CH_2$-

Figure 2. Structures of 5-(2-aminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-one and variously *N*-alkyl substituted analogues synthesized and evaluated for TOP1-targeting activity and cytotoxicity.

Scheme 1[a]



9 **10** **11**

a R_1=Bn; R_2=Me
b R_1=Bn; R_2=Et
c R_1=Bn; R_2=*i*-Pr
d R_1=Bn; R_2=*t*-Bu
e R_1=R_2=Bn

j R_1,R_2 = -$CH_2CH_2CH(CH_3)CH_2CH_2$-
k R_1,R_2 = -$CH_2CH_2N(Bn)CH_2CH_2$-

[a] Conditions: (a) **9a**−**b** $ClCH_2CN$, TEA, $PhCH_3$; **9c**−**e**, **9j**, **9k** $ClCH_2CN$ or $BrCH_2CN$, NaI, K_2CO_3, CH_3CN; (b) $LiAlH_4$, THF.

well as the primary amine **6f** were synthesized to explore the sensitivity of steric bulk on efflux transport. In addition, the effect of replacement of the 2-*N*,*N*-dialkylamino substituent with pyrrolidine as well as piperidine and piperazine derivatives (**8a**−**f**) was also evaluated.

Chemistry

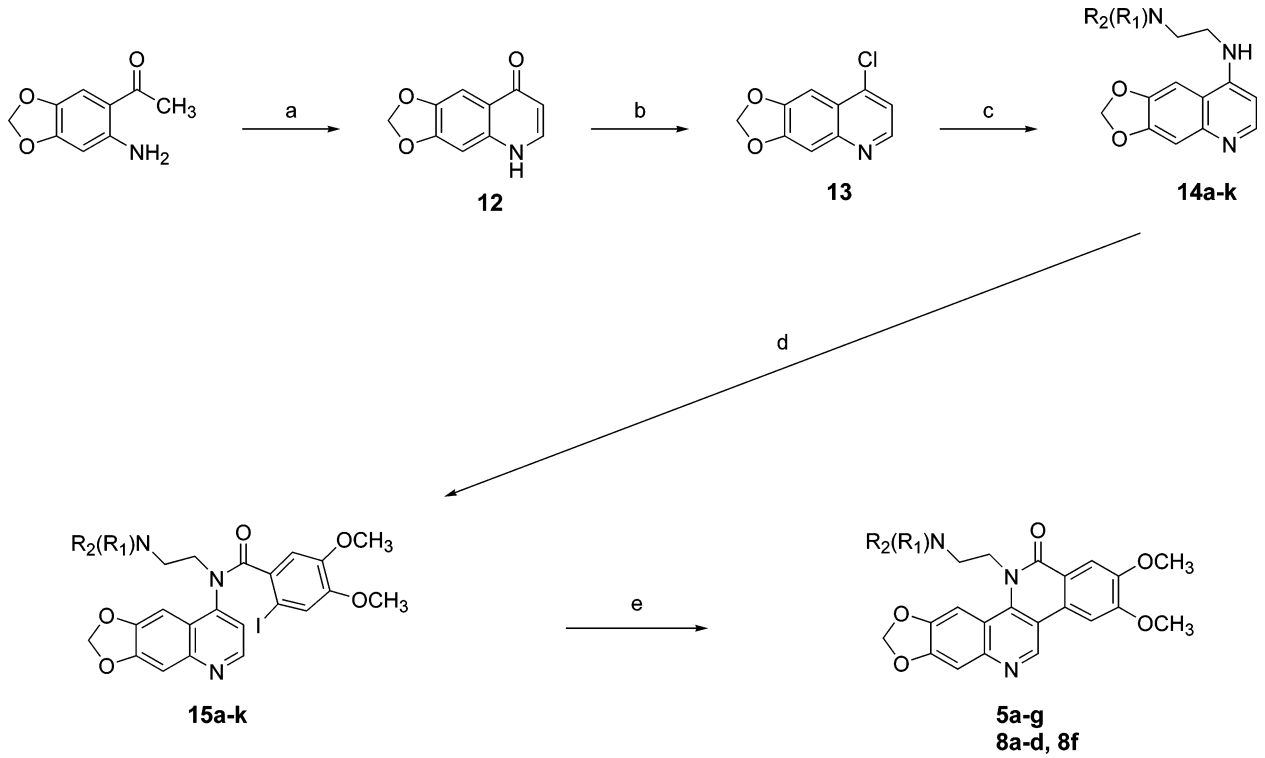
The synthetic methodology reported previously for the synthesis of **3** was applicable to the new compounds described herein. Dialkylaminoethylamines **11a**−**e**, 1-(2-aminoethyl)-4-methylipiperidine, **11j**, and the 1-(2-aminoethyl)-4-benzylpiperazine, **11k**, were not commercially available and were synthesized as described previously,[33−38] (Scheme 1). Alkylation of dialkylamines or a nitrogen heterocycle **9a**−**e**, **9j,** and **9k** with chloroacetonitrile or bromoacetonitrile provided intermediates **10a**−**e**, **10j**, and **10k**, which were reduced to the corresponding dialkylaminoethylamines. *N*,*N*-Diethylethylenediamine, **11f**, *N*,*N*-diisopropylethylenediamine, **11g**, 1-(2-aminoethyl)pyrrolidine, **11h**, and 1-(2-aminoethyl)piperidine, **11i** were available commercially.

The targeted 5-[2-(*N*,*N*-dialkylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones were synthesized as shown in Scheme 2. An improved synthesis of 4-hydroxy-6,7-methylenedioxyquinoline, **12**, recently developed in our laboratory, involves treating the *o*-aminoacetophenone with sodium hydride in ethyl formate. This process is considerably simpler to execute than the previously reported literature procedure[39] and the yield is improved (89% versus 41% over four steps starting from 3,4-methylenedioxyaniline). Chlorination with phosphoryl chloride provided 4-chloroquinoline, **13**, which when heated with ethylenediamines **11a**−**k** in the presence of phenol[40] gave the 4-aminoquinolines **14a**−**14k**. These intermediates, upon reaction with 2-iodo-4,5-dimethoxybenzoyl chloride, yielded *o*-iodobenzamides **15a**−**k**. Palladium-assisted biaryl coupling[41−43] then provided the dibenzo[*c*,*h*][1,6]naphthyridin-6-ones (**5a**−**g**, **8a**−**d**, and **8f**). Secondary amines **6a**−**6e** and **8e** were obtained by debenzylation of the corresponding tertiary benzylamines (Scheme 3). Deprotection proved refractory to most reaction conditions tried, including catalytic hydrogenolysis over 10% Pd−C or Raney nickel using 70 psi of H_2 gas at ambient temperature or with heating as well as transfer hydrogenolysis using cyclohexadiene/10% Pd−C, cyclohexene/20% Pd(OH) (Pearlman's catalyst), and ammonium formate/10% Pd−C. Benzyl groups were removed in near-quantitative yield using palladium black in acetic acid with formic acid as the hydrogen source.[44] In the hydrogenation of the dibenzylamino compound, allowing the reaction to proceed for 90 min gave the primary amine **6f** (85%). Alternatively, quenching the reaction after 15 min gave predominantly the monobenzyl analogue **6e** (54%). *N*-Methyl- and *N*-ethyl-*N*-isopropyl-5-(2-aminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones, **7a** and **7b**, were obtained by reductive alkylation of the secondary isopropylamine **6c** with paraformaldehyde and acetaldehyde, respectively (Scheme 4).

Results and Discussion

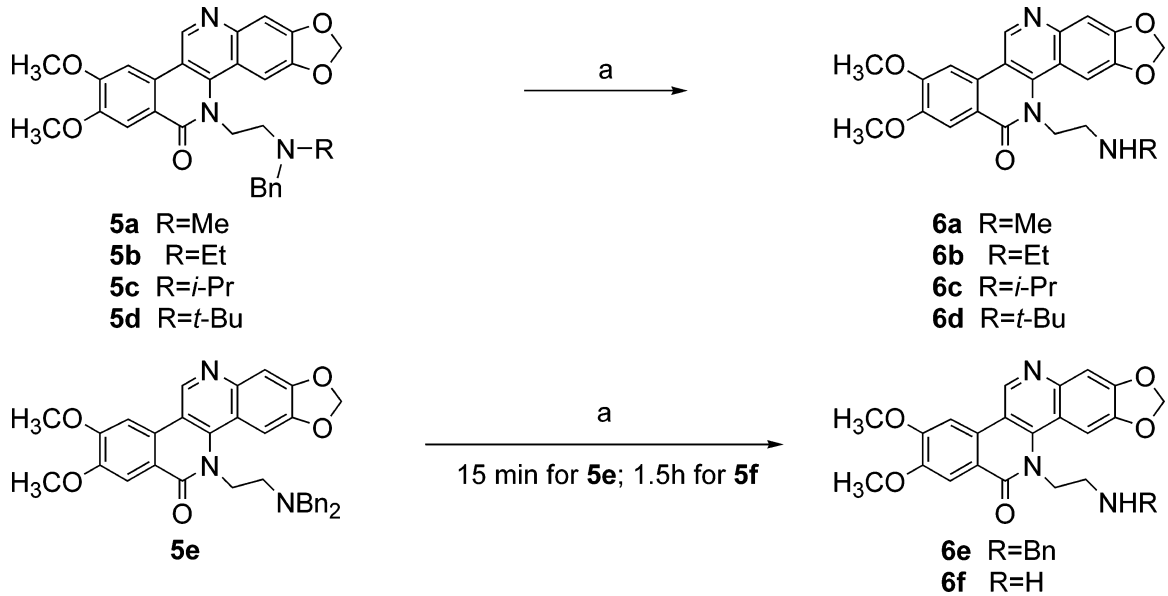
The relative TOP1-targeting activities of several 5-[(2-monoalkylamino)ethyl]-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones **6a**−**e** and 5-(2-aminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-one, **6f**, are listed in Table 1. The pharmacological activities of ARC-111 **3** and its *N*,*N*-diethyl analogue **5f** are also provided in Table 1. The DNA

Scheme 2[a]



[a] Conditions: (a) NaH, ethyl formate; (b) $POCl_3$, reflux; (c) refluxing phenol, then cool to 135 °C and add **11a**−**l**; (d) 4,5-dimethoxy-2-iodobenzoyl chloride, TEA, CH_2Cl_2; (e) $Pd(OAc)_2$, $P(o\text{-tol})_3$, Ag_2CO_3, DMF.

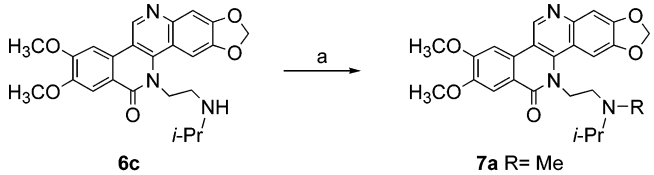
Scheme 3[a]



[a] Conditions: (a) AcOH, formic acid, Pd black.

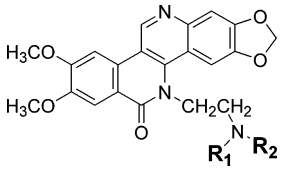
fragmentation pattern observed for those analogues that possessed significant TOP1-targeting activity was similar to that observed with topotecan.[31,32] The unsubstituted 2-aminoethyl analogue **6f** exhibited the most potent TOP1-targeting activity. With the exception of the 2-(*N*-benzylamino)ethyl analogue **6e**, the other 2-(monoalkylamino)ethyl analogues, **6a**−**d**, had comparable TOP1-targeting activity to **3** and **5f**. The relative TOP1-targeting activity of a series of *N*-isopropyl-*N*-alkyl derivatives, **7a**, **7b**, and **5g** in Table 2, revealed that activity decreases with the increase in size of the second alkyl group, i.e., CH_3 > CH_2CH_3 > $CH(CH_3)_2$. In a series of *N*-benzyl-*N*-alkyl derivatives, **5a**−**e**, the

Scheme 4[a]



[a] Conditions: (a) $NaCNBH_3$, EtOH, with $(CH_2O)_n$ for **7a** or CH_3CHO for **7b**.

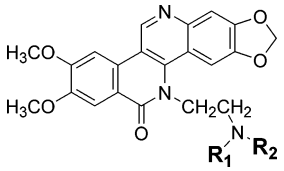
Table 1. Topoisomerase I-Targeting Activity and Cytotoxicity of 5-[2-(Amino)ethyl)]-, 5-[2-(*N*-Alkylamino)ethyl)]-, 5-[2-(*N*,*N*-Dimethylamino)ethyl)]-, and 5-[2-(*N*,*N*-Diethylamino)ethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-ones



				cytotoxicity IC_{50} (μM)[b]						
compd	R1	R2	TOP1-mediated cleavage[a]	RPMI8402	CPT-K5	P388	P388/CPT45	KB3-1 parent	KBV-1 mdr-1	KBH5.0 bcrp
6f	H	H	0.02	0.004	0.25	0.003	0.027	0.005	0.015	0.012
6a	CH_3	H	0.1	0.0003	0.25	0.0003	0.07	0.0004	0.022	0.0005
6b	CH_2CH_3	H	0.1	0.002	0.17	0.0007	0.03	0.001	0.016	0.005
6c	$CH(CH_3)_2$	H	0.3	0.003	0.18	0.001	0.03	0.005	0.005	0.006
6d	$C(CH_3)_3$	H	0.1	0.002	0.22	0.002	0.037	0.003	0.016	0.006
6e	$CH_2C_6H_5$	H	0.5	0.016	0.28	0.016	0.18	0.006	0.035	0.010
3	CH_3	CH_3	0.3	0.002	0.900	0.001	0.23	0.005	0.005	0.006
5f	CH_2CH_3	CH_2CH_3	1.4	0.006	0.85	0.004	0.15	0.004	0.015	0.005
topotecan			1.0	0.021	> 10	0.045	> 10	0.040	0.440	0.440

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentrations, i.e., 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. [b] See Experimental Section for details.

Table 2. Topoisomerase I-Targeting Activity and Cytotoxicity of 5-[2-(*N*-Alkyl-*N*-benzylamino)ethyl)]dibenzo[*c*,*h*][1,6]naphthyridin-6-ones



				cytotoxicity IC_{50} (μM)[b]						
compd	R1	R2	TOP1-mediated cleavage[a]	RPMI 8402	CPT-K5	P388	P388/CPT45	KB3-1 parent	KBV-1 mdr-1	KBH5.0 bcrp
7a	$CH(CH_3)_2$	CH_3	0.03	0.008	0.63	0.006	0.071	0.003	0.005	0.004
7b	$CH(CH_3)_2$	CH_2CH_3	0.17	0.005	0.31	0.004	0.12	0.003	0.021	0.008
5g	$CH(CH_3)_2$	$CH(CH_3)_2$	4.7	0.008	0.60	0.006	0.20	0.005	0.04	0.04
5a	$CH_2C_6H_5$	CH_3	15.8	0.053	2.0	0.038	0.45	0.05	0.30	0.15
5b	$CH_2C_6H_5$	CH_2CH_3	12.3	0.077	7.5	0.05	0.36	0.18	0.40	0.33
5c	$CH_2C_6H_5$	$CH(CH_3)_2$	7.4	0.30	5.9	0.23	0.65	0.80	4.0	0.5
5d	$CH_2C_6H_5$	$C(CH_3)_3$	>1000	0.85	5.75	0.20	1.85	0.19	2.5	0.5
5e	$CH_2C_6H_5$	$CH_2C_6H_5$	15	0.38	1.95	0.25	0.50	0.30	0.36	0.30

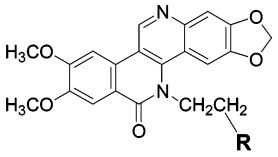
[a] Topoisomerase I cleavage values are reported as REC, relative effective concentrations, i.e., concentrations relative to topotecan, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I. [b] See Experimental Section for details.

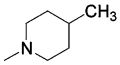
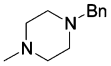
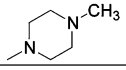
presence of a second alkyl group significantly decreased activity relative to **6e**, with the *N*-benzyl-*N*-*tert*-butyl analogue, **5d**, not exhibiting any significant TOP1-targeting activity. Table 3 summarizes the results observed for several cyclic amines at the 2-position of the 5-ethyl substituent within a series of dibenzo[*c*,*h*]-[1,6]naphthyridin-6-ones. In the case of either a piperidine or a piperazine moiety at this position, the presence of a 4-methyl group on these heterocycles resulted in decreased cytotoxic activity, i.e., **8c** and **8e** relative to **8b** and **8f**, respectively. In the case of **8e** and **8f**, there was also a precipitous drop in TOP1-targeting activity. This observation is consistent with the difference in activity observed between **8d** and **8e** and suggests that an unfavorable steric interaction may, in general, be associated with substitution at the 4-position of the 2-piperazine moiety.

Tables 1−3 also list the relative cytotoxicity in RPMI8402 and P388 cell lines as well as their camptothecin-resistant variants CPT-K5 and P388/CPT45, respectively. In the case of CPT-K5, resistance to camptothecin is associated with a mutant form of topoisomerase I.[45] The camptothecin resistance of P388/CPT45 is associated with the decreased expression of TOP1 in this variant cell line.[46] The cytotoxicity data summarized in Table 1 reflects a good correlation between relative TOP1-targeting activity and cytotoxicity. The most cytotoxic analogue in this table is **6a** and the least is **6e**. As would be anticipated for compounds that primarily exert their cytotoxicity by stabilizing TOP1-DNA cleavable complexes, all of these analogues were less active in the camptothecin-resistant variants CPT-K5 or P388/CPT45 as compared to their parent cell lines.

The cytotoxicity data for KB3-1 cells and for the variants KB/V-1 and KBH5.0 are also listed in Tables 1−3. KB/V-1 cells overexpress the efflux transporter MDR1, [47] and KBH5.0 cells overexpress the efflux transporter BCRP.[48] The decreased cytotoxicity against KB/V-1 and KBH5.0 cells observed with CPT-11 and

Table 3. Topoisomerase I-Targeting Activity and Cytotoxicity of 5-[2-Pyrrolidin-1-yl]ethyl]-, 5-[2-Piperidin-1-yl]-, and 5-[2-Piperazin-1-yl]ethyl]dibenzo[*c*,*h*][1,6]naphthyridin-6-ones



compd	R	TOP1-mediated cleavage[a]	cytotoxicity IC_{50} (μM)[b]						
			RPMI 8402	CPT-K5	P388	P388/CPT45	KB3-1 parent	KBV-1 mdr-1	KBH5.0 bcrp
8a		0.3	0.003	0.39	0.006	0.06	0.003	0.008	0.004
8b		0.6	0.007	0.40	0.006	0.05	0.004	0.013	0.009
8c		>100	0.03	0.45	0.014	0.19	0.03	0.05	0.04
8d		1000	0.40	0.70	0.25	0.20	0.50	0.70	0.75
8e		4.3	0.09	2.7	0.06	0.30	0.10	1.9	0.53
8f		1000	0.30	5.9	0.23	0.65	0.50	2.5	0.40

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentrations, i.e., 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. [b] See Experimental Section for details.

topotecan relative to KB3-1 cells is indicative of drugs that are substrates for both MDR1 and BCRP efflux transporters. Among the dibenzo[*c*,*h*][1,6]naphthyridin-6-ones in Table 1, the absence of any significant difference in the cytotoxicity observed in KB3-1, KBV-1, and KBH5.0 cells for **3**, **5f**, and **6c**−**f** indicates that these analogues are not substrates for either MDR1 or BCRP efflux transporters. In the case of **6a** and **6b**, there were significant differences (>10-fold) in their relative cytotoxicity between KB3-1 and KBV-1 cell lines, indicating that these specific analogues are substrates for the efflux transporter, MDR1. The absence of a significant difference in the cytotoxicity of **6a** and **6b** in KBV-1 and KBH5.0 cells, however, indicates that these analogues are not substrates for BCRP.

The only compounds in Table 2 that were cytotoxic below 10 nM in RPMI8402 and P388 cells were **7a**, **7b**, and **5g**. This is consistent with their greater TOP1-targeting activity relative to **5a**−**e**. As indicated by the data in Table 2, as relative TOP1-targeting activity decreases, the differences in cytotoxicity in camptothecin-sensitive and camptothecin-resistant cell lines are also reduced. For **5d** and **5e**, it is likely that mechanisms other than targeting TOP1 contribute to their cytotoxicity. In the case of **5g**, the data do suggest that as steric bulk or lipophilicity are increased these compounds may tend toward becoming substrates for MDR1 and BCRP efflux transporters. Among the cyclic amines listed in Table 3, it is again evident that cytotoxicity of individual analogues correlates with its relative potency as a TOP1-targeting drug. Although both **8e** and **8f** are not potent TOP1-targeting agents, it is evident that the presence of a second basic nitrogen atom within these heterocycles is associated with their recognition as substrates by the MDR1 efflux transporter based upon their decreased cytotoxicity in KBV-1 relative to KB3-1 cells.

It is anticipated that both **6a**, a known major metabolite of ARC-111, and **6f** contribute to the antitumor activity observed with ARC-111. Although **6a** is a substrate for MDR1, **6f** is not. To assess the relative potency of these N-demthylated analogues of ARC-111, the antitumor activity of these compounds was evaluated against SJ-BT45 medulloblastoma xenografts in scid mice. The results of this study are illustrated in Figure 3.

Both **3** and **6a** had significant antitumor activity when administered at a dose of 9 mg per week. It is evident that the primary amine **6f** was significantly less potent in vivo than either ARC-111 or its monodemethylated metabolite, **6a**, at this dose. This is likely associated with an increased metabolic instability associated with **6f**. These data suggest that **6a** could contribute to a greater extent to the overall antitumor activity observed with ARC-111, especially in tumors that do not overexpress MDR1.

In light of the data summarized in Tables 1 and 2, analogues such as **5f**, **7a**, and **7b** may not readily undergo N-dealkylation to form active metabolites that would be substrates for MDR1. To the degree that N-dealkylation may occur in the case of **7a** and **7b**, one would most likely observe the *N*-isopropyl analogue **6c**, which is not a substrate for MDR1, as a primary metabolite. Such analogues may represent a potential therapeutic advantage relative to ARC-111 in those instances where the ability of one of its major metabolites to serve as a substrate for the MDR1 efflux transporter has an adverse impact on overall antitumor activity.

Experimental Section

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, Germany) using the solvent systems

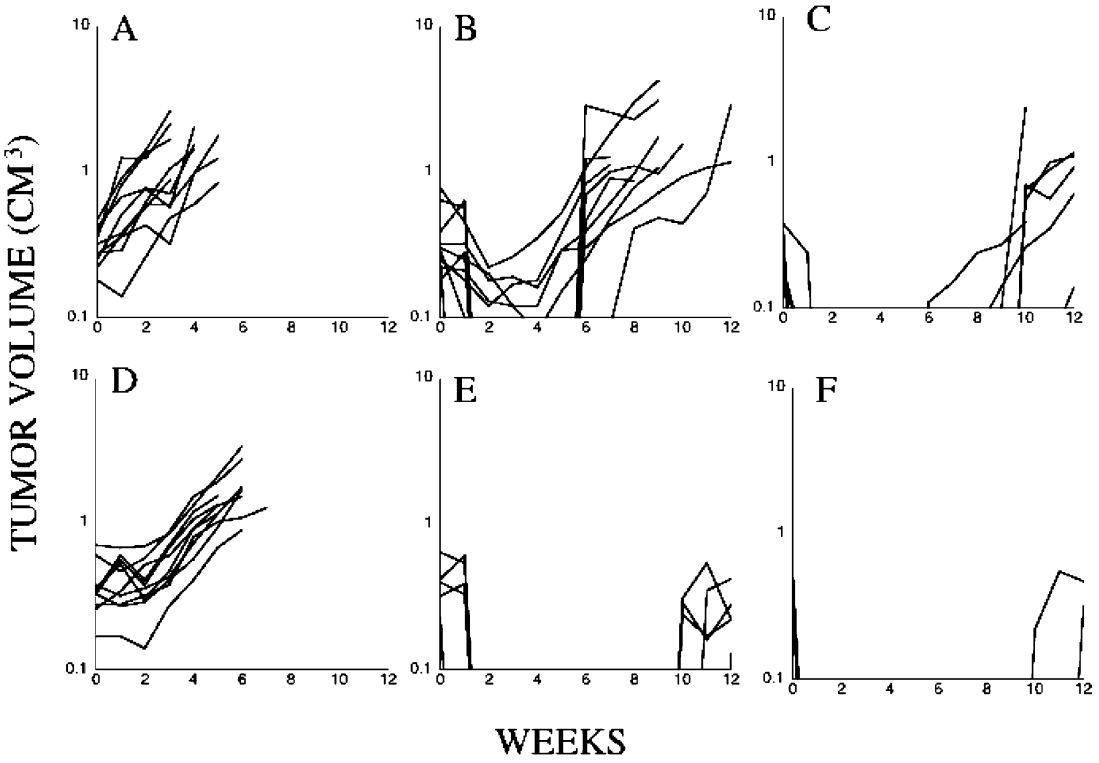


Figure 3. Comparison of the antitumor activity against SJ-BT45 medulloblastoma xenografts in scid mice. (A) Vehicle-treated control (10 mice). (B) Compound **3**, 3 mg/(kg/dose) (10 mice). (C) Compound **3,** 2 mg/(kg/dose) (5 mice). (D) Compound **6f,** 3 mg/(kg/dose) (10 mice). (E) Compound **6a,** 3 mg/(kg/dose) (5 mice). (F) Compound **6a,** 2 mg/(kg/dose) (5 mice). Each compound was administered by intravenous injection 3 times per week (Monday, Wednesday, Friday) for 2 consecutive weeks. The cycle of therapy was repeated at 21 days for a total of two cycles. Each curve shows the growth of an individual tumor in a female scid mouse.

indicated. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier transform spectrometer. NMR spectra (200 MHz ^{1}H and 50 MHz ^{13}C) were recorded in the deuterated solvent indicated with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz (Hz). Mass spectra were obtained from Washington University Resource for Biomedical and Bioorganic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO. Elemental analyses were obtained from Atlantic Microlabs, Norcross, GA, and were within 0.4% of theory. All starting materials and reagents were purchased from Aldrich. Solvents were purchased from Fisher Scientific and were ACS or HPLC grade. Methylene chloride was freshly distilled from calcium hydride. All other solvents were used as provided without further purification. The preparation of compounds **3**, **8a**, and **8f** have been reported.[31,32] Compounds **11a**,[33] **11b**,[34] **11c**,[35] **11d**,[36] **11e**,[34] **11j**,[37] and **11k**[38] were synthesized as previously described. *N*,*N*-Diethylethylenediamine, **11f**, *N*,*N*-diisopropylethylenediamine, **11g**, 1-(2-aminoethyl)pyrrolidine, **11h**, and 1-(2-aminoethyl)piperidine, **11i,** were commercially available from Aldrich.

General Procedure for the Preparation of 5-[2-(*N*,*N*-Dialkylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones 5a−5g and the Piperaidinyl and Piperazinyl Derivatives 8b−e. 8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-benzyl-*N*-methylamino)ethyl]-5*H*-dibenzo[*c*,*h*]-[1,6]naphthyridin-6-one (5a). A mixture of **15a** (720 mg, 1.2 mmol), $Pd(OAc)_2$ (54 mg, 0.24 mmol), P(*o*-tolyl)$_3$ (147 mg, 0.48 mmol), and Ag_2CO_3 (660 mg, 2.4 mmol) in dimethylformamide (DMF) (36 mL) was heated to reflux with stirring for 30 min. The reaction mixture was cooled, diluted with chloroform, and filtered through Celite, and the solvent was removed under vacuum. The crude residue was chromatographed in 99:1 chloroform/methanol to provide 210 mg (35%) of the cyclized product as a white solid: mp 224−226 °C. IR ($CHCl_3$): 1648. ^{1}H NMR ($CDCl_3$) δ: 2.19 (s, 3H), 3.06 (t, 2H, J = 6.4), 3.51 (s, 2H), 4.06 (s, 3H), 4.14 (s, 3H), 4.67 (t, 2H, J = 6.9), 6.21 (s, 2H), 7.20 (m, 5H), 7.45 (s, 1H), 7.67 (s, 1H), 7.86 (s, 1H), 7.89 (s, 1H), 9.36 (s, 1H).^{13}C NMR ($CDCl_3$) δ: 42.4, 49.2, 56.0, 56.3, 56.4, 63.1, 101.4, 101.9, 102.2, 107.1, 108.8, 111.7, 114.9, 119.3, 127.1, 127.7, 128.2, 129.0, 138.8, 141.0, 143.5, 147.3, 147.7, 149.9, 150.2, 154.1, 164.1. HRMS Calcd for $C_{29}H_{27}O_5N_3H$: 498.2029. Found: 498.2005. Anal. ($C_{29}H_{27}O_5N_3$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(N-benzyl-*N*-ethylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (5b) was prepared from **15b** (639 mg, 1.0 mmol), reaction time 30 min. Chromatographic purification using chloroform gave 204 mg (40%) as a white solid: mp 237.5−238.5 °C. IR ($CHCl_3$): 1648. ^{1}H NMR ($CDCl_3$) δ: 0.92 (t, 3H, J = 7.1), 2.46 (q, 2H, J = 7.1), 3.09 (t, 2H, J = 6.4), 3.57 (s, 2H), 4.07 (s, 3H), 4.15 (s, 3H), 4.67 (t, 2H, J = 6.4), 6.21 (s, 2H), 7.17 (m, 5H), 7.47 (s, 1H), 7.69 (s, 1H), 7.88 (s, 1H), 7.90 (s, 1H), 9.38 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 11.6, 47.9, 49.4, 52.3, 56.3, 56.4, 58.9, 101.4, 101.9, 102.2, 107.0, 108.9, 111.8, 115.0, 119.4, 126.9, 127.7, 128.1, 128.8, 139.3, 141.1, 143.5, 147.2, 147.7, 149.9, 150.2, 154.1, 164.1. HRMS Calcd for $C_{30}H_{29}N_3O_5H$: 512.2185. Found: 512.2195. Anal. ($C_{30}H_{29}N_3O_5\cdot0.5H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-benzyl-*N*-isopropylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (5c) was prepared from **15c** (653 mg, 1.0 mmol), reaction time 30 min. Chromatographic purification eluting with chloroform gave 225 mg (43%) as a white solid: mp 218.5−220.0 °C. IR ($CHCl_3$): 1647. ^{1}H NMR ($CDCl_3$) δ: 0.91 (d, 6H, J = 6.7), 2.80 (m, 1H), 3.08 (t, 2H, J = 6.5), 3.58 (s, 2H), 4.06 (s, 3H), 4.14 (s, 3H), 4.56 (t, 2H, J = 6.5), 6.19 (s, 2H), 7.12 (m, 5H), 7.45 (s, 1H), 7.67 (s, 1H), 7.81 (s, 1H), 7.86 (s, 1H), 9.36 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 17.7, 48.5, 50.1, 50.6, 55.0, 56.3, 56.4, 101.4, 102.0, 102.1, 107.0, 108.9, 111.8, 114.9, 119.5, 126.6, 127.7, 128.1, 128.4, 140.4, 141.1, 143.4, 147.3, 147.5, 149.8, 150.2, 154.1, 164.0. HRMS Calcd for $C_{31}H_{31}N_3O_5Li$: 532.2424. Found: 532.2402. Anal. ($C_{31}H_{31}N_3O_5\cdot0.5H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-benzyl-*N*-*tert*-butylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-

6-one (5d) was prepared from **15d** (500 mg, 0.75 mmol), reaction time 30 min. Chromatographic purification eluting with chloroform gave 223 mg (55%) as a white solid: mp 212−213 °C. IR ($CHCl_3$): 1648. ^1H NMR ($CDCl_3$) δ: 1.21 (s, 9H), 3.20 (t, 2H, J = 7.2), 3.78 (s, 2H), 4.06 (s, 3H), 4.12 (s, 3H), 4.25 (t, 2H, J = 6.5), 6.22 (s, 2H), 7.13 (m, 5H), 7.44 (s, 1H), 7.65 (s, 1H), 7.81 (s, 1H), 7.86 (s, 1H), 9.32 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 27.2, 50.0, 51.9, 55.1, 55.7, 56.3, 56.4, 101.6, 102.0, 102.1, 106.9, 108.9, 111.6, 114.8, 119.5, 126.2, 127.6, 127.8, 127.9, 140.7, 142.5, 143.2, 147.2, 147.4, 149.9, 150.2, 154.1, 163.9. HRMS Calcd for $C_{32}H_{33}N_3O_5H$: 540.2498. Found: 540.2492. Anal. ($C_{32}H_{33}N_3O_5$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*,*N*-dibenzylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (5e) was prepared from **15e** (701 mg, 1.0 mmol), reaction time 30 min. Chromatographic purification eluting with chloroform gave 361 mg (63%) as a white solid: mp 213-215 °C. IR ($CHCl_3$): 1647. ^1H NMR ($CDCl_3$) δ: 3.06 (t, 2H, J = 5.9), 3.49 (s, 4H), 4.05 (s, 3H), 4.14 (s, 3H), 4.68 (t, 2H, J = 5.9), 6.23 (s, 2H), 7.15 (m, 10H), 7.46 (s, 1H), 7.66 (s, 1H), 7.72 (s, 1H), 7.79 (s, 1H), 9.35 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 49.0, 53.1, 56.3, 56.4, 59.2, 101.3, 102.0, 102.3, 106.6, 109.0, 111.8, 114.8, 119.4, 127.0, 127.5, 128.2, 128.9, 138.7, 141.2, 142.9, 146.6, 147.7, 150.0, 150.4, 154.2, 163.8. HRMS Calcd for $C_{35}H_{31}N_3O_5H$: 574.2342. Found: 574.2314. Anal. ($C_{35}H_{31}N_3O_5 \cdot 0.5H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*,*N*-diethylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (5f) was prepared from **15f** (577 mg, 1.0 mmol), reaction time 30 min. Chromatographic purification eluting with 99:1 chloroform/methanol gave 250 mg (56%) as a white solid: mp 221−223 °C (dec). IR ($CHCl_3$): 1648. ^1H NMR ($CDCl_3$) δ: 0.95 (t, 6H, J = 7.0), 2.80 (q, 4H, J = 7.0), 3.04 (t, 2H, J = 6.7), 4.06 (s, 3H), 4.13 (s, 3H), 4.63 (t, 2H, J = 6.7), 6.17 (s, 2H), 7.46 (s, 1H), 7.68 (s, 1H), 7.90 (s, 1H), 7.96 (s, 1H), 9.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 12.0, 47.6, 49.6, 51.7, 56.3, 101.4, 102.0, 102.2, 107.0, 108.9, 111.8, 115.0, 119.5, 127.7, 141.1, 143.5, 147.3, 147.7, 149.9, 150.3, 154.2, 164.2. HRMS Calcd for $C_{25}H_{27}O_5N_3H$: 450.2029. Found: 450.2032. Anal. ($C_{25}H_{27}O_5N_3$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*,*N*-diisopropylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (5g) was prepared from **15g** (605 mg, 1.0 mmol), reaction time 35 min. Chromatographic purification eluting with 98:2 chloroform/methanol gave 240 mg (50%) as a light beige solid: mp 257−260 °C (dec). IR ($CHCl_3$): 1650. ^1H NMR ($CDCl_3$) δ: 0.88 (d, 12H, J = 6.2), 2.94 (m, 4H), 4.07 (s, 3H), 4.14 (s, 3H), 4.61 (t, 2H, J = 6.7), 6.18 (s, 2H), 7.46 (s, 1H), 7.68 (s, 1H), 7.91 (s, 1H), 7.96 (s, 1H), 9.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 20.7, 43.8, 48.4, 51.0, 56.3, 56.4, 101.6, 101.9, 102.2, 107.0, 108.9, 111.9, 114.9, 119.6, 127.7, 141.2, 143.4, 147.2, 147.5, 149.9, 150.2, 154.1, 164.2. HRMS Calcd for $C_{27}H_{31}O_5N_3$-Li: 484.2424. Found: 484.2438. Anal. ($C_{27}H_{31}O_5N_3$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(piperidinyl)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (8b) was prepared from **15i** (294.5 mg, 0.5 mmol), reaction time 25 min. Chromatograpic purification with 99:1 chloroform/methanol gave 82 mg (36%) of a light beige solid: mp 269−270 °C. ^1H NMR ($CDCl_3$) δ: 1.41 (t, 6H, J = 7.4), 2.38 (t, 4H, J = 5.4), 2.92 (t, 2H, J = 6.6), 4.06 (s, 3H), 4.13 (s, 3H), 4.65 (t, 2H, J = 6.6), 6.18 (s,2H), 7.46 (s,1H), 7.68 (s,1H), 7.89 (s,1H), 8.04 (s,1H), 9.36 (s,1H). ^{13}C NMR ($CDCl_3$) δ: 24.3, 26.0, 49.0, 54.8, 56.3, 57.4, 101.5, 101.9, 102.1, 106.9, 108.9, 111.8, 115.0, 119.5, 127.6, 141.2, 143.4, 147.2, 147.6, 149.8, 150.2, 154.1, 164.3. HRMS Calcd for $C_{26}H_{28}N_3O_5H$: 462.2029. Found: 462.2029. Anal. ($C_{26}H_{27}O_5N_3 \cdot 0.5H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(4-methylpiperidin-1-yl)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (8c) was prepared from **15j** (603 mg, 1.0 mmol), reaction time 45 min. Chromatograpic purification with 99:1 chloroform/methanol gave 166 mg (34%) of a colorless crystalline solid: mp 247.0−248.5 °C. ^1H NMR ($CDCl_3$) δ: 0.89 (d, 3H, J = 5.8), 1.12 (m, 3H), 1.55 (d, 2H, J = 11.0), 2.08 (m, 2H), 2.82 (d, 2H, J = 11.0), 2.96 (t, 2H, J = 6.6), 4.06 (s, 3H), 4.13 (s, 3H), 4.66 (t, 2H, J = 6.6), 6.19 (s, 2H), 7.45 (s, 1H), 7.67 (s, 1H), 7.89 (s, 1H), 8.00 (s, 1H), 9.36 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 22.0, 30.6, 34.2, 49.0, 54.3, 56.3, 56.4, 57.0, 101.5, 101.9, 102.2, 107.0, 108.8, 111.8, 114.9, 119.4, 127.7, 141.1, 146.4, 147.2, 147.7, 149.9, 150.2, 154.1, 164.3. IR ($CHCl_3$): 1648. HRMS Calcd for $C_{27}H_{29}N_3O_5H$: 476.2185. Found: 476.217. Anal. ($C_{27}H_{32}O_5N_3 \cdot 0.5H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(4-benzylpiperazinyl)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (8d) was prepared from **15k** (1.02 g, 1.5 mmol), reaction time 30 min. Chromatographic purification eluting with 99:1 chloroform/methanol gave 365 mg (44%) of a colorless crystalline solid: mp 230.5−231 °C. ^1H NMR ($CDCl_3$) δ: 2.34 (t, 4H, J = 4.4), 2.49 (t, 4H, J = 4.4,), 2.96 (t, 2H, J = 6.6), 3.46 (s, 2H), 4.07 (s, 3H), 4.14 (s, 3H), 4.65 (t, 2H, J = 6.6), 6.17 (s, 2H), 7.30 (m, 5H), 7.45 (s, 1H), 7.67 (s, 1H), 7.88 (s, 1H), 7.93 (s, 1H), 9.35 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 48.8, 53.1, 53.4, 56.3, 56.4, 56.6, 63.1, 101.3, 101.9, 102.2, 107.2, 108.9, 111.9, 115.0, 119.5, 127.1, 127.6, 128.3, 129.2, 138.2, 141.1, 143.4, 147.2, 147.7, 149.9, 150.3, 154.1, 164.3. IR ($CHCl_3$): 1649. HRMS Calcd for $C_{32}H_{32}N_4O_5H$: 553.2451. Found: 553.2443. Anal. ($C_{26}H_{32}O_5N_4$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(piperazinyl)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (8e). Formic acid (1.2 mL) and then palladium black (25 mg) were added to a solution of **8d** (50 mg, 0.074 mmol) in acetic acid (30 mL). The mixture was stirred at room temperature for 10 h, then filtered, and evaporated under vacuum. The residue was partitioned between $CHCl_3$ (50 mL) and 10% NaOH (50 mL), and the aqueous phase was extracted with additional $CHCl_3$ (2 × 25 mL). The combined organic phases were washed with water (2 × 75 mL) and brine (75 mL), dried ($MgSO_4$), and evaporated under vacuum, yielding 27 mg (79%) of a white solid: mp 229−230 °C. ^1H NMR ($CDCl_3$) δ: 1.75 (1H, br), 2.40 (t, J = 4.6, 4H), 2.73 (t, J = 4.6, 4H), 2.93 (t, J = 6.4, 2H), 4.07 (s, 3H), 4.14 (s, 3H), 4.67 (t, J = 6.4, 2H), 6.19 (s, 2H), 7.46 (s, 1H), 7.68 (s, 1H), 7.89 (s, 1H), 7.99 (s, 1H), 9.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 46.1, 48.7, 54.7, 56.3, 56.4, 57.2, 101.3, 101.9, 102.2, 107.0, 108.8, 111.9, 115.0, 119.4, 127.6, 141.2, 143.4, 147.2, 147.7, 149.9, 150.2, 154.1, 164.4. IR ($CHCl_3$): 1648. HRMS Calcd for $C_{25}H_{25}N_4O_5H$: 463.1981. Found: 463.1962. Anal. ($C_{25}H_{26}O_5N_4 \cdot 0.5CHCl_3$) C, H, N.

General Procedure for the Preparation of 5-[2-(*N*-Alkylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones 6a−f. 8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-methylamino)ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (6a). Compound **5a** (100 mg, 0.02 mmol) was dissolved in acetic acid (60 mL). Formic acid (2.4 mL) was added, and then palladium black (50 mg) was added. The mixture was stirred at room temperature for 90 min, and was then filtered through Celite, and the filtrate was evaporated under vacuum. The residue was partitioned between $CHCl_3$ (75 mL) and 10% NaOH (75 mL), and the aqueous phase was extracted with additional $CHCl_3$ (2 × 30 mL). The combined organic phases were evaporated under vacuum, and the residue was chromatographed through a short column of silica eluting with 97:3 chloroform/methanol, yielding 78 mg of a light yellow solid, in 95% yield: mp 254−257 °C (dec). IR ($CHCl_3$): 3330, 1648. ^1H NMR ($CDCl_3$) δ: 2.50 (s, 3H), 3.30 (t, 2H, J = 6.6), 4.07 (s, 3H), 4.14 (s, 3H), 4.61 (t, 2H, J = 6.6), 6.18 (s, 2H), 7.47 (s, 1H), 7.70 (s, 2H), 7.89 (s, 1H), 9.38 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 36.5, 50.6, 51.2, 56.3, 56.4, 100.8, 102.0, 102.2, 107.2, 108.7, 111.7, 114.8, 119.3, 127.7, 140.9, 143.5, 147.3, 147.6, 150.0, 150.3, 154.2, 164.3. HRMS Calcd for $C_{22}H_{21}N_3O_5H$: 408.1559. Found: 408.1550. Anal. ($C_{22}H_{21}O_5N_3 \cdot 0.5H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-ethylamino)-ethyl]-5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-one (6b) was prepared from **5b** (20 mg, 0.04 mmol), reaction time 90 min. Short column chromatographic purification of the crude product using 97:3 chloroform/methanol gave 14 mg (85%) as a white solid: mp 245−247 °C (dec). IR ($CHCl_3$): 3308, 1647. ^1H NMR ($CDCl_3$) δ: 1.14 (t, 3H, J = 7.0), 2.06 (br, 1H), 2.75 (q, 2H, J = 7.1), 3.35 (t, 2H, J = 6.4), 4.07 (s, 3H), 4.14 (s, 3H), 4.59 (t, 2H, J = 6.4), 6.18 (s, 2H), 7.46 (s, 1H), 7.68 (s,

1H), 7.70 (s, 1H), 7.89 (s, 1H), 9.37 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 15.3, 44.0, 48.8, 50.8, 56.3, 56.4, 100.9, 102.0, 102.2, 107.2, 108.8, 111.7, 114.8, 119.3, 127.7, 140.9, 143.5, 147.4, 147.7, 149.9, 150.4, 154.3, 164.3. HRMS Calcd for $C_{23}H_{23}N_3O_5H$: 422.1716. Found: 422.1705. Anal. ($C_{23}H_{23}N_3O_5$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-isopropylamino)ethyl]-5*H*-dibenzo[*c,h*][1,6]naphthyridin-6-one (6c) was prepared from **5c** (30 mg, 0.06 mmol), reaction time 90 min. Short column chromatographic purification of the crude product using 97:3 chloroform/methanol gave 23 mg (94%) as a white solid: mp 275−278 °C (dec). IR ($CHCl_3$): 3306, 1647. ^{1}H NMR ($CDCl_3$) δ: 1.16 (d, 6H, J = 6.2), 2.96 (m, 1H), 3.40 (t, 2H, J = 6.4), 4.07 (s, 3H), 4.14 (s, 3H), 4.58 (t, 2H, J = 6.4), 4.79 (br, 1H), 6.18 (s, 2H), 7.47 (s, 1H), 7.66 (s, 1H), 7.69 (s, 1H), 7.89 (s, 1H), 9.39 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 22.6, 46.3, 48.8, 50.9, 56.3, 56.4, 100.9, 102.1, 102.2, 107.1, 108.9, 111.7, 114.8, 119.2, 127.7, 141.0, 143.4, 147.3, 147.8, 150.0, 150.4, 154.4, 164.4. HRMS Calcd for $C_{24}H_{25}N_3O_5H$: 436.1872. Found: 436.1855. Anal. ($C_{24}H_{25}N_3O_5$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-*tert*-butylamino)ethyl]-5*H*-dibenzo[*c,h*][1,6]naphthyridin-6-one (6d) was prepared from **5d** (30 mg, 0.06 mmol), reaction time 90 min. Short column chromatographic purification of the crude product using 97:3 chloroform/methanol gave 22 mg (88%) as a white solid: mp 258−260 °C. IR ($CHCl_3$): 3298, 1650. ^{1}H NMR ($CDCl_3$) δ: 1.19 (s, 9H), 3.36 (t, 2H, J = 6.7), 4.07 (s, 3H), 4.14 (s, 3H), 4.54 (t, 2H, J = 6.7), 6.18 (s, 2H), 7.47 (s, 1H), 7.67 (s, 1H), 7.77 (s, 1H), 7.90 (s, 1H), 9.40 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 28.9, 42.0, 51.0, 51.5, 56.3, 56.4, 101.2, 102.0, 102.2, 107.1, 108.9, 111.6, 114.8, 119.2, 127.8, 141.0, 143.5, 147.4, 147.8, 149.9, 150.4, 154.3, 164.3. HRMS Calcd for $C_{25}H_{27}N_3O_5H$: 450.2029. Found: 450.2027. Anal. ($C_{25}H_{27}N_3O_5 \cdot 0.5H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-benzylamino)ethyl]-5*H*-dibenzo[*c,h*][1,6]naphthyridin-6-one (6e) was prepared from **5e** (100 mg, 0.17 mmol), reaction time 15 min. Chromatographic purification of the crude product eluting with 97:3 chloroform/methanol gave 46 mg (54%) as a pale yellow solid: mp 244−247 °C. IR ($CHCl_3$): 3307, 1648. ^{1}H NMR ($CDCl_3$) δ: 3.33 (t, 2H, J = 6.5), 3.84 (s, 2H), 4.07 (s, 3H), 4.14 (s, 3H), 4.64 (t, 2H, J = 6.5), 6.20 (s, 2H), 7.28 (s, 5H), 7.47 (s, 1H), 7.69 (s, 1H), 7.76 (s, 1H), 7.88 (s, 1H), 9.38 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 48.4, 50.7, 53.9, 56.3, 56.4, 101.1, 102.0, 102.2, 107.2, 108.9, 111.8, 114.9, 119.3, 127.1, 127.7, 128.2, 128.4, 140.0, 141.0, 143.5, 147.3, 147.7, 149.9, 150.3, 154.3, 164.3. HRMS Calcd for $C_{28}H_{25}N_3O_5H$: 484.1872. Found: 484.1854. Anal. ($C_{28}H_{25}N_3O_5$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-(2-aminoethyl)-5*H*-dibenzo[*c,h*][1,6]naphthyridin-6-one (6f) was prepared from **5e** (200 mg, 0.35 mmol), reaction time 90 min. Short column chromatographic purification of the crude product using 96:4 chloroform/methanol gave 110 mg (80%) as a white solid: mp 298−299 °C (dec). IR ($CHCl_3$): 3391, 1649. ^{1}H NMR ($CDCl_3$) δ: 1.48 (br, 2H), 3.38 (t, 2H, J = 6.6), 4.07 (s, 3H), 4.14 (s, 3H), 4.58 (t, 2H, J = 6.6), 6.18 (s, 2H), 7.47 (s, 1H), 7.65 (s, 1H), 7.69 (s, 1H), 7.90 (s, 1H), 9.38 (s, 1H). ^{13}C NMR (DMSO-d_6) δ: 40.7, 52.4, 56.4, 57.1, 101.7, 103.1, 104.2, 107.0, 109.0, 112.1, 114.9, 119.2, 128.0, 140.9, 145.0, 147.5, 147.8, 150.3, 150.6, 154.8, 163.8. HRMS Calcd for $C_{21}H_{19}N_3O_5H$: 394.1403. Found: 394.1402; Anal. ($C_{21}H_{19}N_3O_5 \cdot H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-isopropyl-*N*-methylamino)ethyl]-5*H*-dibenzo[*c,h*][1,6]naphthyridin-6-one (7a). A mixture of **6c** (44 mg, 0.1 mmol), paraformaldehyde (29 mg), and sodium cyanoborohydride (21 mg, 0.3 mmol) in ethanol (35 mL) was heated to reflux under nitrogen for 20 h. The mixture was filtered, and the filtrate was evaporated. The residue was partitioned between chloroform (30 mL) and 10% NaOH (30 mL), and the aqueous phase was extracted with chloroform (2 × 30 mL). The combined organic phases were evaporated and chromatographed through a short column of silica eluting with in 97:3 chloroform/methanol to provide 41 mg (91%) as a cream colored solid: mp 238−240 °C. IR ($CHCl_3$): 1648. ^{1}H NMR ($CDCl_3$) δ: 0.98 (d, 6H, J = 6.6), 2.27 (s, 3H), 2.80 (m, 1H), 3.06 (t, 2H, J = 6.9), 4.06 (s, 3H), 4.13 (s, 3H), 4.60 (t, 2H, J = 6.9), 6.18 (s, 2H), 7.47 (s, 1H), 7.69 (s, 1H), 7.91 (s, 1H), 7.99 (s, 1H), 9.38 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 18.0, 37.5, 49.9, 51.7, 54.6, 56.3, 56.4, 101.5, 102.0, 102.2, 107.0, 108.9, 111.7, 115.0, 119.5, 127.7, 141.2, 143.5, 147.3, 147.7, 149.9, 150.3, 154.2, 164.2. HRMS Calcd for $C_{25}H_{27}O_5N_3Li$: 456.2111. Found: 456.2106. Anal. ($C_{25}H_{27}O_5N_3 \cdot 0.5H_2O$) C, H, N.

8,9-Dimethoxy-2,3-methylenedioxy-5-[2-(*N*-ethyl-*N*-isopropylamino)ethyl]-5*H*-dibenzo[*c,h*][1,6]naphthyridin-6-one (7b). A mixture of **6c** (44 mg, 0.1 mmol), acetaldehyde (44 mg, 1.0 mmol), and sodium cyanoborohydride (21 mg, 0.3 mmol) in ethanol (15 mL) was heated to 75 °C in a sealed tube for 6 h. The mixture was filtered, and the filtrate was evaporated. The residue was partitioned between chloroform (30 mL) and 10% NaOH (30 mL), and the aqueous phase was extracted with chloroform (2 × 30 mL). The combined organic phases were evaporated and chromatographed through a short column of silica eluting with in 97:3 chloroform/methanol to provide 33 mg (76%) as a white solid: mp 223−225 °C. IR ($CHCl_3$): 1647. ^{1}H NMR ($CDCl_3$) δ: 0.91 (d, 6H, J = 6.2), 1.20 (m, 3H), 2.46 (m, 2H), 2.84 (m, 1H), 3.01 (t, 2H, J = 6.6), 4.07 (s, 3H), 4.14 (s, 3H), 4.63 (t, 2H, J = 6.6), 6.18 (s, 2H), 7.47 (s, 1H), 7.69 (s, 1H), 7.91 (s, 1H), 7.99 (s, 1H), 9.38 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 13.9, 18.1, 44.2, 48.0, 48.2, 50.4, 56.3, 56.4, 101.5, 102.0, 102.2, 107.0, 109.0, 111.9, 115.0, 119.6, 127.7, 141.2, 143.5, 147.3, 147.7, 149.9, 150.3, 154.2, 164.3. HRMS Calcd for $C_{26}H_{29}O_5N_3Li$: 470.2267. Found: 470.2277. Anal. ($C_{26}H_{29}O_5N_3 \cdot 0.5H_2O$) C, H, N.

6,7-Methylenedioxy-4-quinolone (12). To a mixture of 6′amino-3′4′-methylenedioxyacetophenone (16.0 g, 89.4 mmol) in ethyl formate (300 mL) in a 1 L flask equipped with a condenser was added sodium hydride (14.3 g, 0.358 mol). After a short induction period, the mixture began to react vigorously and soon began refluxing. The mixture was stirred for an additional 30 min and was then cooled to 0 °C. The reaction was quenched by the addition of water (3 mL) and then acetic acid (5 mL). The solid material that had precipitated during the reaction was filtered and washed with water, ethanol, ethyl acetate, and ethyl ether, consecutively. Drying under high vacuum provided 15.0 g of an off-white solid in 89% yield: mp 285−289 °C (lit.[36] mp 276 °C). ^{1}H NMR (DMSO-d_6) δ: 5.95 (d, 1H, J = 7.3), 6.13 (s, 2H), 6.97 (s, 1H), 7.38 (s, 1H), 7.77 (d, 1H, J = 7.3). ^{13}C NMR (DMSO-d_6) δ: 97.5, 102.1, 102.6, 108.7, 119.4, 122.0, 130.8, 138.7, 145.8, 151.7.

4-Chloro-6,7-methylenedioxyquinoline (13). Compound **12** (5.0 g, 26.5 mmol) was boiled in $POCl_3$ (75 mL) for 2 h and then cooled. Excess phospohoryl chloride was removed under reduced pressure, and ice−water (100 mL) was added to hydrolyze any residual phosphorus oxychloride. The mixture was basified (pH 9) with ammonium hydroxide, and the solid precipitate was filtered. This material was dissolved in chloroform (250 mL), washed with water (3 × 250 mL), dried ($MgSO_4$), and evaporated to provide 4.98 g of a white solid in 91% yield: mp 127.5−128 °C (lit.[36] mp 129 °C). ^{1}H NMR ($CDCl_3$) δ: 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 ($CDCl_3$) δ: 99.8, 102.2, 106.1, 119.9, 123.7, 129.8, 141.2, 147.7, 149.1, 151.4.

General Procedure for the Preparation of 4-[[2-(Dialkylamino)ethyl]amino]-6,7-methylenedioxyquinolines 14a−k. 4-[[2-(*N*-Benzyl-*N*-methylamino)ethyl]amino]-6,7-methylenedioxyquinoline (14a). Compound **13** (1.0 g, 4.83 mmol) was stirred in refluxing phenol for 2.5 h. The bath temperature was lowered to 128° C, and **11a** (1.7 g, 9.66 mmol) was added. The mixture was stirred at this temperature for 20h, then phenol was removed by Kugelrohr distillation, and the resulting crude residue was partitioned between chloroform (150 mL) and 10% NaOH (100 mL). The organic phase was washed with water (3 × 100 mL) and was then extracted with dilute aqueous HCl (3 × 100 mL). The combined aqueous layers were washed with chloroform (2 × 100 mL), basified (30% NaOH), and back-extracted into chloroform (3 × 100 mL). The combined organic layers were dried ($MgSO_4$) and evaporated, and the residue was triturated with ethyl ether and

filtered to provide 1.05 g as a white solid in 65% yield: mp 162−165° C. ^{1}H NMR ($CDCl_3$) δ: 2.24 (s, 3H), 2.59 (t, 2H, J = 7.2), 3.16 (m, 2H), 3.55 (s, 2H), 5.75 (d, 1H, J = 5.6), 5.98 (s, 2H), 6.80 (s, 1H), 7.29 (m, 5H), 7.58 (s, 1H), 7.72 (d, 1H, J = 5.6). ^{13}C NMR ($CDCl_3$) δ: 40.0, 42.1, 54.6, 62.4, 96.2, 98.8, 101.72, 106.4, 114.43, 127.55, 128.6, 129.1, 138.83, 146.2, 146.8, 148.8, 149.6, 150.1. HRMS Calcd for $C_{20}H_{21}O_2N_3$: 335.1634. Found: 335.1633.

4-[[2-(*N*-Benzyl-*N*-ethylamino)ethyl]amino]-6,7-methylenedioxyquinoline (14b) was prepared from **13** (1.0 g, 4.83 mmol) and **11b** (1.78 g, 10.0 mmol), reaction time 20 h, to provide 1.0 g of a light beige solid in 60% yield: mp 143−144 °C. ^{1}H NMR ($CDCl_3$) δ: 1.16 (t, 3H, J = 7.1), 2.66 (q, 2H, J = 7.1), 2.85 (t, 2H, J = 5.7), 3.22 (m, 2H), 3.65 (s, 2H), 5.42 (br, 1H), 6.12 (s, 2H), 6.28 (d, 1H, J = 5.1), 6.96 (s, 1H), 7.34 (m, 6H), 8.37 (d, 1H, J = 5.1). ^{13}C NMR ($CDCl_3$) δ: 12.1, 40.0, 47.4, 51.0, 58.0, 96.1, 98.9, 101.6, 106.7, 114.4, 127.4, 128.7, 129.1, 139.5, 146.5, 146.7, 149.2, 149.5, 149.9. HRMS Calcd for $C_{21}H_{23}N_3O_2$: 349.1790. Found: 349.1779.

4-[[2-(*N*-Benzyl-*N*-isopropylamino)ethyl]amino]-6,7-methylenedioxyquinoline (14c) was prepared from **13** (1.0 g, 4.83 mmol) and **11c** (1.90 g, 10.0 mmol), reaction time 20 h, to provide 1.19 g of a light beige solid in 68% yield: mp 155−156 °C. ^{1}H NMR ($CDCl_3$) δ: 1.14 (d, 6H, J = 6.6), 2.86 (t, 2H, J = 5.7), 3.11 (m, 3H), 3.60 (s, 2H), 5.36 (br, 1H), 6.10 (s, 2H), 6.23 (d, 1H, J = 5.2), 6.90 (s, 1H), 7.32 (m, 6H), 8.35 (d, 1H, J = 5.2). ^{13}C NMR ($CDCl_3$) δ: 18.1, 40.2, 47.4, 49.4, 53.6, 96.2, 98.9, 101.6, 106.7, 114.5, 127.3, 128.7, 128.8, 140.5, 146.5, 146.7, 149.1, 149.5, 149.9. HRMS Calcd for $C_{22}H_{25}N_3O_2$: 363.1947. Found: 363.1934.

4-[[2-(*N*-Benzyl-*N*-*tert*-butylamino)ethyl]amino]-6,7-methylenedioxyquinoline (14d) was prepared from **13** (1.0 g, 4.83 mmol) and **11d** (2.06 g, 10.0 mmol), reaction time 20 h, to provide 1.24 g of a light beige solid in 69% yield: mp 112−115 °C. ^{1}H NMR ($CDCl_3$) δ: 1.24 (s, 9H), 2.97 (m, 4H), 3.75 (s, 2H), 5.08 (br, 1H), 6.01 (d, 1H, J = 5.4), 6.10 (s, 2H), 6.82 (s, 1H), 7.24 (m, 6H), 8.26 (d, J = 5.4, 1H). ^{13}C NMR ($CDCl_3$) δ: 27.6, 42.8, 49.5, 55.5, 55.7, 96.1, 98.9, 101.5, 106.7, 114.5, 126.9, 128.0, 128.5, 142.3, 146.5, 146.6, 149.0, 149.4, 149.8. HRMS Calcd for $C_{23}H_{27}N_3O_2$: 377.2103. Found: 377.2090.

4-[[2-(*N*,*N*-Dibenzylamino)ethyl]amino]-6,7-methylenedioxyquinoline (14e) was prepared from **13** (1.0 g, 4.83 mmol) and **11e** (2.40 g, 10.0 mmol), reaction time 20 h, to provide 1.48 g of a light beige solid in 75% yield: mp 132−133 °C. ^{1}H NMR ($CDCl_3$) δ: 2.87 (t, 2H, J = 5.5), 3.23 (m, 2H), 5.19 (br, 1H), 6.14 (s, 2H), 6.23 (d, 1H, J = 5.4), 6.92 (s, 1H), 7.33 (m, 11H), 8.34 (d, 1H, J = 5.4). ^{13}C NMR ($CDCl_3$) δ: 40.2, 51.4, 58.7, 96.2, 98.8, 101.6, 106.6, 114.3, 127.6, 128.7, 129.2, 139.1, 146.4, 146.8, 149.0, 149.4, 150.0. HRMS Calcd for $C_{26}H_{25}N_3O_2$: 411.1947. Found: 411.1953.

4-[[2-(*N*,*N*-Diethylamino)ethyl]amino]-6,7-methylenedioxyquinoline (14f) was prepared from **13** (1.0 g, 4.83 mmol) and *N*,*N*-diethylethylenediamine (1.16 g, 10.0 mmol), reaction time 20 h, to provide 793 mg of an off-white solid in 8% yield: mp 201−202 °C. ^{1}H NMR ($CDCl_3$) δ: 1.09 (t, 6H, J = 7.2), 2.61 (q, 4H, J = 7.2), 2.82 (t, 2H, J = 5.8), 3.26 (m, 2H), 5.71 (br, 1H), 6.08 (s, 2H), 6.35 (d, 1H, J = 5.2), 7.03 (s, 1H), 7.31 (s, 1H), 8.40 (d, 1H, J = 5.2). ^{13}C NMR ($CDCl_3$) δ: 12.2, 40.1, 46.7, 51.0, 96.1, 99.0, 101.5, 106.7, 114.5, 146.5, 146.7, 149.1, 149.6, 149.9. HRMS Calcd for $C_{16}H_{21}N_3O_2$: 287.1634. Found: 287.1631.

4-[[2-(*N*,*N*-Diisopropylamino)ethyl]amino]-6,7-methylenedioxyquinoline (14 g) was prepared from **13** (1.0 g, 4.83 mmol) and *N*,*N*-diisopropylethylenediamine (1.44 g, 10.0 mmol), reaction time 20 h, to provide 943 mg of an off-white solid in 62% yield: mp 195−196 °C. ^{1}H NMR ($CDCl_3$) δ: 1.11 (d, 6H, J = 6.6), 2.90 (t, 2H, J = 5.8), 3.13 (m, 4H), 5.77 (br, 1H), 6.08 (s, 2H), 6.35 (d, 1H, J = 5.2), 7.00 (s, 1H), 7.31 (s, 1H), 8.40 (d, J = 5.2, 1H). ^{13}C NMR ($CDCl_3$) δ: 21.0, 40.4, 42.3, 47.4, 95.9, 99.1, 101.5, 106.8, 114.6, 146.6, 146.7, 149.2, 149.6, 149.9. HRMS Calcd for $C_{18}H_{25}N_3O_2$: 315.1947. Found: 315.1944.

4-[[2-(Piperidin-1-yl)ethyl]amino]-6,7-methylenedioxyquinoline (14i) was prepared from **13** (1.0 gm, 5.0 mmol) and 2-aminoethylpiperidine, **11i** (0.9 gm, 7.0 mmol), reaction time 12 h, to provide 600 mg of a light brown solid in 40% yield: mp 203−204 °C. ^{1}H NMR ($CDCl_3$) δ: 1.25−1.50 (m, 2H), 1.59−1.64 (m, 4H), 2.42 (t, 4H, J = 5.2), 2.67 (t, 2H, J = 5.4), 3.23 (m, 2H), 5.7 (br, 1H), 6.0 (s, 2H), 6.32 (d, 1H, J = 5.2), 7.06 (s,1H), 7.29 (s,1H), 8.37 (d, 1H, J = 5.6). ^{13}C NMR ($CDCl_3$) δ: 24.4, 26.2, 30.9, 39.3, 54.1, 56.4, 96.1, 98.9, 101.5, 106.6, 114.4, 146.5, 146.7, 149.1, 149.5, 149.9. HRMS Calcd for$C_{17}H_{21}N_3O_2$: 299.1634. Found: 299.1629.

4-[[2-(4-Methylpiperidin-1-yl)ethyl]amino]-6,7-methylenedioxyquinoline (14j) was prepared from **13** (1.0 g, 4.8 mmol) and **11j** (1.56 g, 10.0 mmol), reaction time 20 h, to provide 1.29 g of an off-white solid in 85% yield: mp 193.5−194 °C. ^{1}H NMR ($CDCl_3$) δ: 0.97 (d, J = 6.2, 3H), 1.29 (m, 3H), 1.67 (d, J = 11.4, 2H), 2.05 (m, 2H), 2.73 (t, J = 5.9, 2H), 2.90 (d, J = 11.4, 2H), 3.28 (t, J = 5.9, 2H), 6.09 (s, 2H), 6.34 (d, J = 5.2, 1H), 7.05 (s, 1H), 7.30 (s, 1H), 8.40 (d, J = 5.2, 1H). HRMS Calcd for $C_{18}H_{23}N_3O_2$: 313.1790. Found: 313.1780.

4-[[2-(4-Benzylpiperazin-1-yl)ethyl]amino]-6,7-methylenedioxyquinoline (14k) was prepared from **13** (1.2 g, 5.8 mmol) and **11k** (10 g, 106 mmol), reaction time 2.5 h, to provide 1.65 g of a light brown solid in 73% yield: mp 244−247 °C. ^{1}H NMR ($CDCl_3$) δ: 2.56 (s, 8H), 2.77 (t, J = 5.9, 2H), 3.30 (m, 2H), 3.56 (s, 2H), 5.61 (br, 1H), 6.10 (s, 2H), 6.35 (d, J = 5.2, 1H), 7.06 (s, 1H), 7.32 (m, 6H), 8.40 (m, J = 5.2, 1H). ^{13}C NMR ($CDCl_3$) δ: 39.2, 52.8, 53.4, 55.8, 63.1, 96.1, 99.0, 101.6, 106.7, 114.4, 127.2, 128.3, 129.2, 138.3, 146.5, 146.8, 149.1, 149.5, 150.0. HRMS Calcd for $C_{23}H_{26}N_4O_2H$: 391.2134. Found: 391.2135.

General Procedure for the Preparation of *N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(*N*,*N*-dialkylamino)-ethyl]-2-iodo-4,5-dimethoxybenzamides 15a−l. *N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(N-benzyl-*N*-methylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (15a). Oxalyl chloride (1.37 g, 10.8 mmol) was added to a mixture of 2-iodo-4,5-dimethoxybenzoic acid (1.5 g, 3.25 mmol) in methylene chloride (40 mL), and the mixture was heated to reflux under nitrogen with stirring for 4 h. The mixture was concentrated to dryness under vacuum. The acid chloride was redissolved in 40 mL of methylene chloride, and a solution of **14a** (906 mg, 2.7 mmol) added, then triethylamine (2.0 mL, 27 mmol) was added. The mixture was heated to reflux with stirring for 16 h and was then cooled to room temperature. The mixture was washed with saturated $NaHCO_3$ (3 × 100 mL) and extracted into dilute aqueous HCl (3 × 100 mL). The combined aqueous phases were washed with chloroform (2 × 100 mL), basified (30% NaOH), and extracted with chloroform (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and evaporated, yielding 1.44 g in 85% yield as a light brown sticky glue. IR ($CHCl_3$): 1652. ^{1}H NMR ($CDCl_3$) δ: 2.20 (s, 3H), 2.77 (m, 2H), 3.31 (s, 3H), 3.46 (m, 1H), 3.61 (s, 2H), 4.60 (m, 1H), 6.13 (s, 2H), 6.39 (s, 1H), 7.02 (s, 1H), 7.32 (m, 8H), 8.50 (d, 1H, J = 4.8). ^{13}C NMR ($CDCl_3$) δ: 42.3, 44.3, 46.8, 55.5, 56.0, 62.5, 82.8, 98.4, 102.2, 106.7, 110.4, 120.3, 121.6, 122.8, 127.1, 128.2, 129.1, 133.7, 138.7, 145.9, 148.0, 148.3, 148.4, 149.0, 149.6, 151.0, 169.9. HRMS Calcd for $C_{29}H_{28}IN_3O_5H$: 626.1152. Found: 626.1117.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(*N*-benzyl-*N*-ethylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (15b)** was prepared from 2-iodo-4,5-dimethoxybenzoic acid (0.9 g, 2.60 mmol) and **14b** (0.75 g, 2.14 mmol), reaction time 16 h, to provide 916 mg of a light brown sticky glue in 89% yield. IR ($CHCl_3$): 1649. ^{1}H NMR ($CDCl_3$) δ: 1.04 (t, 3H, J = 7.0), 2.57 (q, 2H, J = 7.0), 2.87 (m, 2H), 3.30 (s, 3H), 3.47 (m, 1H), 3.59 (s, 2H), 3.73 (s, 3H), 4.55 (m, 1H), 6.14 (s, 2H), 6.36 (s, 1H), 7.02 (s, 1H), 7.14 (d, 1H, J = 4.8), 7.17 (m, 5H), 7.32 (s, 1H), 7.36 (s, 1H), 8.47 (d, 1H, J = 4.8). ^{13}C NMR ($CDCl_3$) δ: 11.9, 47.2, 47.9, 51.2, 55.5, 56.1, 58.3, 82.8, 98.3, 102.2, 106.8, 110.4, 120.2, 121.6, 122.6, 126.9, 128.1, 129.0, 133.6, 139.2, 146.1, 148.0, 148.3, 148.4, 149.0, 149.6, 151.0, 169.9. HRMS Calcd for $C_{30}H_{30}IN_3O_5H$: 640.1308. Found: 640.1248.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(*N*-benzyl-*N*-isopropylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (15c)** was prepared from 2-iodo-4,5-dimethoxybenzoic acid (1.1 g, 3.30 mmol) and **14c** (1.0 g, 2.75 mmol), reaction time

16 h, to provide 1.34 g of a light brown sticky glue in 75% yield. IR ($CHCl_3$): 1650. ^1H NMR ($CDCl_3$) δ: 1.05 (m, 6H), 2.76 (m, 1H), 3.00 (m, 2H), 3.27 (s, 3H), 3.55 (s, 2H), 3.71 (s, 3H), 3.89 (m, 1H), 4.40 (m, 1H), 6.13 (s, 2H), 6.31 (s, 1H), 7.07 (s, 1H), 7.05 (d, 1H, $J = 4.8$), 7.09 (m, 5H), 7.20 (s, 1H), 7.35 (s, 1H), 8.45, (d, 1H, $J = 4.8$). ^{13}C NMR ($CDCl_3$) δ: 18.4, 48.0, 48.9, 51.5, 55.1, 55.5, 56.0, 82.8, 98.3, 102.2, 106.7, 110.6, 120.0, 121.8, 122.4, 126.6, 128.0, 128.6, 133.7, 140.6, 146.2, 148.1, 148.2, 148.3, 148.9, 149.7, 150.9, 169.8. HRMS Calcd for $C_{31}H_{32}IN_3O_5Li$: 660.1547. Found: 660.1531.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(*N*-benzyl-*N*-*tert*-butylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (15d)** was prepared from 2-iodo-4,5-dimethoxybenzoic acid (1.5 g, 3.25 mmol) and **14d** (1.02 g, 2.7 mmol), reaction time 16 h, to provide 1.48 g of a white solid in 82% yield. IR ($CHCl_3$): 1649. ^1H NMR ($CDCl_3$) δ: 1.19 (s, 9H), 2.58 (m, 1H), 2.83 (m, 1H), 3.23 (s, 3H), 3.41 (m, 2H), 3.69 (s, 3H), 3.90 (m, 2H), 4.13 (m, 1H), 6.12 (s, 2H), 6.24 (s, 1H), 6.84 (m, 5H), 7.00 (m, 3H), 7.33 (s, 1H), 8.42, (d, 1H, $J = 4.6$). ^{13}C NMR ($CDCl_3$) δ: 27.5, 47.1, 50.5, 54.8, 55.1, 55.5, 56.0, 82.8, 98.1, 102.1, 106.6, 110.4, 119.6, 121.7, 122.3, 126.2, 127.6, 127.7, 128.5, 133.7, 141.5, 145.9, 148.1, 148.2, 148.8, 149.7, 150.9, 169.8. HRMS Calcd for $C_{32}H_{34}IN_3O_5Li$: 674.1703. Found: 674.1706.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(*N*,*N*-dibenzylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (15e).** This reaction was considerably more sluggish than the other acylations described in this section, and the procedure was modified accordingly. The acid chloride prepared from 2-iodo-4,5-dimethoxybenzoic acid (1.5 g, 3.25 mmol) was added to **14e** (1.11 g, 2.7 mmol). After 16 h at reflux, an equivalent amount of acid chloride was added, and after an additional 16 h refluxing another equivalent of acid chloride was added. The workup was performed as described above, except that it was followed by purification by column chromatography eluting with chloroform, to provide 1.70 g of a fluffy white solid in 90% yield. IR ($CHCl_3$): 1650. ^1H NMR ($CDCl_3$) δ: 2.79 (m, 1H), 2.94 (m, 1H), 3.30 (s, 3H), 3.64 (s, 4H), 3.72 (s, 3H), 3.96 (m, 1H), 4.67 (m, 1H), 6.15 (s, 2H), 6.34 (s, 1H), 6.90 (d, 1H, $J =$ 4.8), 7.18 (m, 13H), 8.39, (d, 1H, $J = 4.8$). ^{13}C NMR ($CDCl_3$) δ: 46.8, 51.6, 55.6, 56.1, 58.7, 82.8, 98.2, 102.2, 106.8, 110.6, 120.1, 121.7, 122.5, 127.2, 128.3, 128.5, 129.2, 133.6, 138.8, 145.8, 148.1, 148.3, 149.0, 149.8, 151.0, 169.9. HRMS Calcd for $C_{35}H_{32}I\ N_3O_5H$: 702.1465. Found: 702.1465.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(*N*,*N*-diethylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (15f)** was prepared from 2-iodo-4,5-dimethoxybenzoic acid (820 mg, 2.6 mmol) and **14f** (640 mg, 2.2 mmol), reaction time 16 h, to provide 1.10 g of a light brown sticky glue in 86% yield. IR ($CHCl_3$): 1651. ^1H NMR ($CDCl_3$) δ: 0.96 (t, 6H, $J = 7.2$), 2.54 (q, 4H, $J = 7.2$), 2.82 (m, 2H), 3.29 (s, 3H), 3.71 (s, 3H), 3.92 (m, 1H), 4.46 (m, 1H), 6.12 (s, 2H), 6.37 (s, 1H), 7.00 (s, 1H), 7.27 (d, 1H, $J = 4.8$), 7.33 (s, 1H), 7.39 (s, 1H), 8.52 (d, 1H, J = 4.8). ^{13}C NMR ($CDCl_3$) δ: 11.8, 47.1, 47.5, 50.7, 55.5, 56.1, 82.7, 98.5, 102.2, 106.7, 110.6, 120.1, 121.8, 122.7, 133.7, 146.3, 148.1, 148.3, 148.5, 149.0, 149.7, 151.0, 170.0. HRMS Calcd for $C_{25}H_{28}O_5N_3IH$: 578.1152. Found: 578.1153.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-N-[2-(*N*,*N*-diisopropylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (15g)** was prepared from 2-iodo-4,5-dimethoxybenzoic acid (820 mg, 2.6 mmol) and **14g** (700 mg, 2.2 mmol), reaction time 16 h, to provide 1.15 g of a light brown sticky glue in 86% yield. ^1H NMR ($CDCl_3$) δ: 0.95 (m, 12H), 2.70 (m, 1H), 2.97 (m, 2H), 3.27 (s, 3H), 3.71 (s, 3H), 3.90 (m, 2H), 4.37 (m, 1H), 6.12 (s, 2H), 6.35 (s, 1H), 7.00 (s, 1H), 7.23 (d, 1H, $J = 4.8$), 7.33 (s, 2H), 8.52 (d, 1H, $J = 4.8$). ^{13}C NMR ($CDCl_3$) δ: 20.8, 42.9, 49.4, 51.3, 55.5, 56.1, 82.8, 98.3, 102.3, 106.7, 110.4, 119.7, 121.7, 122.5, 133.7, 146.5, 148.1, 148.3, 148.5, 149.0, 149.7, 151.0, 169.9. HRMS Calcd for $C_{27}H_{32}O_5N_3ILi$: 612.1547. Found: 612.1573.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(piperidin-1-yl)ethyl]-2-iodo-4,5-dimethoxybenzamide (15i)** was prepared from 2-iodo-4,5-dimethoxybenzoic acid and **14i** (450 mg, 1.5 mmol), reaction time 16 h, to provide 350 mg of a light brown solid in 40% yield: mp 91−92 °C. ^1H NMR ($CDCl_3$) δ: 1.42−1.52 (m, 6H), 2.42 (m, 4H), 2.65 (t, 2H, $J = 6.8$), 3.33 (s, 3H), 3.73 (s, 3H), 3.94 (m, 1H), 4.42−4.52 (m, 1H), 6.15 (s, 2H), 6.41 (s, 1H), 7.02 (s, 1H), 7.35 (s, 1H), 7.38 (s, 1H), 7.45 (s, 1H), 8.54 (d, 1H, $J = 4.8$). ^{13}C NMR ($CDCl_3$) δ: 24.3, 25.9, 46.2, 54.6, 55.5, 56.0, 56.1, 56.4, 98.6, 102.2, 106.6, 110.4, 120.2, 121.5, 122.9, 133.7, 146.1, 148.0, 148.2, 148.3, 148.9, 149.5, 150.9, 169.9. HRMS Calcd for $C_{26}H_{29}IN_3O_5H$: 590.1152. Found: 590.1157.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(4-methylpiperidin-1-yl)ethyl]-2-iodo-4,5-dimethoxybenzamide (15j)** was prepared from 2-iodo-4,5-dimethoxybenzoic acid (1.4 g, 4.54 mmol) and **14j** (1.0 g, 3.19 mmol) reaction time 16 h, to provide 1.40 g of a white solid in 73% yield as a sticky glue. IR ($CHCl_3$): 1648. ^1H NMR ($CDCl_3$) δ: 0.86 (d, $J = 6.2$, 3H), 1.17 (m, 3H), 1.54 (d, $J = 12.8$, 2H), 1.96 (m, 2H), 2.61 (m, 2H), 2.81 (d, $J = 12.8$, 2H), 3.30 (s, 3H), 3.69 (s, 3H), 3.92 (m, 1H), 4.42 (m, 1H), 6.10 (s, 1H), 6.37 (s, 1H), 6.98 (s, 1H), 7.27 (m, 2H), 7.43 (s, 1H), 8.52 (d, $J = 4.8$, 1H). ^{13}C NMR ($CDCl_3$) δ: 22.0, 30.8, 34.5, 46.5, 53.7, 54.5, 55.5, 56.0, 82.7, 98.7, 102.2, 106.6, 110.4, 120.2, 121.5, 122.9, 133.8, 146.1, 148.0, 148.2, 148.3, 148.9, 149.5, 150.9, 169.9. HRMS Calcd for $C_{27}H_{30}IN_3O_5H$: 604.1308. Found: 604.1288.

***N*-(6,7-Methylenedioxyquinolin-4-yl)-*N*-[2-(4-benzylpiperazinyl)ethyl]-2-iodo-4,5-dimethoxybenzamide (15k)** was prepared from 2-iodo-4,5-dimethoxybenzoic acid (1.4 g, 4.61 mmol) and **14k** (1.5 g, 3.84 mmol), reaction time 16h, to provide 1.32 g in 51% yield as a light brown sticky glue. IR ($CHCl_3$): 1650. ^1H NMR ($CDCl_3$) δ: 2.46 (s, 8H), 2.62 (m, 2H), 3.31 (s, 3H), 3.50 (s, 2H), 3.70 (s, 3H) 3.87 (m, 1H), 4.43 (m, 1H), 6.11 (s, 1H), 6.12 (s, 1H), 6.39 (s, 1H), 7.00 (s, 1H), 7.25 (m, 5H), 7.33 (d, $J = 4.7$, 1H), 7.46 (s, 1H), 8.53 (d, $J = 4.7$, 1H). ^{13}C NMR ($CDCl_3$) δ: 45.9, 53.0, 53.2, 55.5, 56.0, 56.1, 63.1, 82.8, 98.7, 102.2, 106.6, 110.6, 120.3, 121.7, 123.0, 127.1, 128.3, 129.3, 133.8, 138.0, 146.0, 148.0, 148.2, 148.3, 148.9, 149.6, 151.0, 169.9. HRMS Calcd for $C_{32}H_{33}IN_4O_5H$: 681.1574. Found: 681.1590.

Topoisomerase-Mediated DNA Cleavage Assays. Human topoisomerase I was expressed in *E. coli* and isolated as a recombinant fusion protein using a T7 expression system as described previously.[49] Plasmid YepG was purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium bromide isopycnic centrifugation method as described.[50] The end labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end filling with Klenow polymerase as previously described.[51] The cleavage assays were performed as previously reported.[49,52] 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, i.e., 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.

Cytotoxicity Assays. The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[53−55] 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).[56] The P388 mouse leukemia cell line and its CPT-resistant TOP1-deficient variant P388/CPT45 were obtained from Michael R. Mattern and Randal K. Johnson (GlaxoSmithKline, PA).[57] The U937 cell line and its CPT-resistant variant U937/CR were obtained from Dr. Eric H. Rubin (The Cancer Institute of New Jersey, NJ).[58] 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).[59] The KBH5.0 cell line was derived from KB3-1 by stepwise selection against Hoechst 33342.[48] The CEM cell line and its VP-16-resistant variants CEM/V-1 and CEM/V-5 were obtained from William Beck (University of Illinois, Chicago).[60] The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO_2

and maintained by regular passage in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). For determination of IC_{50} values, cells were exposed continuously for 4 days to varying concentrations of drug, and MTT assays were performed at the end of the fourth day. Each assay was performed with a control that did not contain any drug. All assays were performed at least twice in six replicate wells.

Antitumor Activity Against Scid Mice Carrying Human Tumor Xenografts. CB17/Icr female scid mice were subcutaneously implanted with a single tumor fragment. Tumor-bearing mice were randomized into groups of 5 or 10 animals prior to therapy. All mice were maintained under barrier conditions. Mice bearing subcutaneous xenografts were treated with D5W when tumors were approximately 0.2−1 cm in diameter. The procedures have been reported previously.[61] Briefly, two perpendicular diameters were determined at 7-day intervals using digital Vernier calipers interfaced with a Macintosh computer. Tumor volumes were calculated assuming tumors to be spherical using the formula $[(\pi/6)d^3]$, where d is the mean diameter, and mice were followed for up to 12 weeks after starting treatment. Compounds **3**, **6a**, and **6f** were administered 3 times per week for 2 consecutive weeks as their monocitrate salts dissolved in 5% dextrose solution U. S. P. Cycles of treatment were repeated every 21 days for a total of two cycles of treatment.

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Supporting Information Available: Elemental analysis data on new compounds evaluated for biological activity. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Li, T.-K.; Liu, L. F. Tumor cell death induced by topoisomerase-targeting drugs. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *41*, 53−77.
(2) Wang, J. C. DNA topoisomerases. *Annu. Rev. Biochem.* **1985**, *54*, 665−697.
(3) Liu, L. F. DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.* **1989**, *58*, 351−375.
(4) Chen, A. Y.; Liu, L. F. DNA topoisomerases: essential enzymes and lethal targets. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191−218.
(5) Hsiang, Y. H.; Lihou, M.; Liu, L. Arrest or replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.* **1989**, *49*, 5077−5082.
(6) Desai, S. D.; Li, T.-K.; Rodriguez-Bauman, A., Rubin, E. H., Liu, L. F. Ubiquitin/26S proteosome-mediated degradation of topoisomerase I as a resistance mechanism to camptothecin in tumor cells. *Cancer Res*. **2001**, *61*, 5926−5932.
(7) Liu, L. F., Desai, S. D., Li, T.-K., Mao, Y., Sun, M., Sim, S.-P. Mechanism of Action of Camptothecins. *Ann. N. Y. Acad. Sci.* **2000**, *922*, 1−8.
(8) 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. A phase I clinical and pharmacokinetic study of the topoisomerase I inhibitor topotecan (SK&F 104864). *Anticancer Drugs* **1992**, *3*, 337−345.
(9) Burke, T. G.; Mi, Z. Ethyl substitution at the 7 position extends the half-life of 10-hydroxycamptothecin in the presence of human serum albumin. *J. Med. Chem.* **1993**, *36*, 2580−2582.
(10) Burke, T. G.; Mi, Z. Preferential binding of the carboxylate form of camptothecin by human serum albumin. *Anal. Biochem.* **1993**, *212*, 285−287.
(11) Chen, A. Y.; Yu, C., Potmesil, M.; Wall, M. E.; Wani, M. C.; Liu, L. F. Camptothecin overcomes MDR1-mediated resistance in human KB carcinoma cells. *Cancer Res.* **1991**, *51*, 6039−6044.
(12) Chu, X., Kato, Y., Sugiyama, Y. Multiplicity of biliary excretion mechanisms for irinotecan, CPT-11, and its metabolites in rats. *Cancer Res.* **1997** *57*, 1934−1938.
(13) 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. Breast cancer resistance protein directly confers SN-38 resistance of lung cancer cells. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 1216−1223.
(14) Yang, C. H.; Schneider, E.; Kuo, M. L.; Volk, E. L.; Roccchi; E.; Chen, Y. C.; BCRP/MXR/ABCP expression in topotecan-resistant human breast cancer cells, *Biochem. Pharmacol.* **2000**, *60*, 831−837.
(15) Scehllens, J. H. M., Melirpaard, M., Scheper, R. J., Scheffer, G. L., Jonker, J. W., Smit, J. W., Beijnen, J. H., and Schinkel, A. H. Transport of topoisomerase I inhibitors by the breast cancer resistance protein. *Ann. N. Y. Acad. Sci.* **2000**, *922*, 188−194.
(16) Kim, J. S.; Gatto B.; Yu, C.; Liu, A.; Liu, L. F.; LaVoie, E. J. Terbenzimidazoles: Influence of 2″-, 4-, and 5-substituents on cytotoxicity and relative potency as topoisomerase I poisons. *J. Med. Chem.* **1997**, *40*, 2818−2824.
(17) Ranagarajan, M.; Kim, J. S.; Sim, S.-P.; Liu, A.; Liu, L. F.; LaVoie, E. J. Topoisomerase I inhibition and cytotoxicity of 5-bromo and 5-phenylterbenzimidazoles. *Bioorg. Med. Chem.* **2000**, *11*, 2591−2600.
(18) Jin, S.; Kim, J. S.; Sim, J. S.; Sim, S.-P.; Liu, A.; Pilch, D. S.; Liu, L. F.; LaVoie, E. J. Heterocyclic bibenzimidazole derivatives as topoisomerase I inhibitors. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 719−723.
(19) Makhey, D.; Yu, C.; Liu, A.; Liu, L. F.; LaVoie, E. J. Substituted benz[*a*]acridines and benz[*c*]acridines as mammalian topoisomerase poisons. *Bioorg. Med. Chem.* **2000**, *8*, 1171−1182.
(20) Janin, Y. L.; Croisy, A.; Rious, J.-L.; Bisagni, E. Synthesis and evaluation of new 6-amino-substituted benzo[*c*]phenanthridine derivatives. *J. Med. Chem.* **1993**, *36*, 3686−3692.
(21) Makhey, D.; Gatto, B.; Yu, C.; Liu, A.; Liu, L. F.; LaVoie, E. J. Protoberberine alkaloids and related compounds as dual inhibitors of mammalian topoisomerase I and II. *Med. Chem. Res.* **1995**, *5*, 1−12.
(22) Yamashita, Y.; Fujii, N.; Murakaya C.; Ashizawa, T.; Okabe, M.; Nakano, H. Induction of mammalian DNA topoisomerase I mediated DNA cleavage by antitumor indolocarbazole derivatives. *Biochemistry* **1992**, *31*, 12069−12075.
(23) Fujii, N.; Yamashita, Y.; Saitoh; Y.; Nakano H. Induction of mammalian DNA topoisomerase I-mediated DNA cleavage and DNA winding by bulgarein. *J. Biol. Chem.* **1993**, *268*, 13160−13165.
(24) Yamashita, Y.; Kawada, S.; Fujii, N.; Nakano, H. Induction of mammalian DNA topoisomerase I and II mediated DNA cleavage by saintopin, a new antitumor agent from fungus. *Biochemistry* **1991**, *30*, 5838−5845.
(25) Jayaraman, M.; Fox, B. M.; Hollingshead, M.; Kohlhagen, G.; Pommier, Y.; Cushman, M. Synthesis of new dihydroindeno[1,2-*c*]indeno[1, 2-*c*]isoquinoline and indenoisoquinolinium chloride topoisomerase I inhibitors having high in vivo anticancer activity in the hollow fiber animal model. *J. Med. Chem.* **2002**, *45*, 242−249.
(26) Cushman, M.; Jayaraman, M.; Vroman, J. A.; Fukunaga, A. K.; Foc, B. M.; Kohlhagen, G.; Strumberg, D.; Pommier, Y. Synthesis of new indeno[1,2-*c*]isoquinolines; cytotoxic noncamptothecin topoisomerase I inhibitors. *J. Med. Chem.* **2000**, *43*, 3688−3698.
(27) Ruchelman, A. L.; Singh, S. K.; Liu, A.; Zhou, N.; Liu, L. F.; LaVoie E. J. Cytotoxicity of 5*H*-Dibenzo[*c,h*][1,6]naphthyridin-6-ones and 6*H*-indeno[1,2-*c*]isoquinolin-5, 11-diones in tumor cells sensitive and resistant to camptothecin analogues. *Lett. Drug Des. Disc.* **2004**, *1*, 198−202.
(28) Vicker, N.; Burgess, L.; Chuckowree, I. S.; Dodd, R.; Folkes, A. J.; Hardick, D.; Hancox, T. C.; Miller, W.; Milton, J.; Sohal, S.; Wang, S.; Wren, S. P.; Charlton, P. A.; Dangerfield, W.; Liddle, C.; Mistry, P.; Stewart, A. J.; Denny, W. A. Novel angular benzophenazines: dual topoisomerase I and topoisomerase II inhibitors as potential anticancer agents. *J. Med. Chem.* **2002**, *45*, 721−739.
(29) Li, D.; Zhao, B.; Sim, S.-P.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. 8,9-Methylenedioxybenzo[*i*]phenanthridines: Topoisomerase I-targeting activity and cytotoxicity. *Bioorg. Med. Chem.* **2003**, *11*, 3795−3805.
(30) Yu, Y.; Singh, S. K.; Liu, A.; Li, T.-K.; Liu, L. F.; LaVoie, E. J. Substituted Dibenzo[*c,h*]cinnolines: Topoisomerase I-targeting anticancer agents. *Bioorg. Med. Chem.* **2003**, *11*, 1475−1491.
(31) Ruchelman, A. L.; Singh, S. K.; Wu, X.; Ray, A.; Yang, J.-M.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. Diaza- and triazachrysenes: Potent topoisomerase-targeting agents with exceptional antitumor activity against the human tumor xenograft, MDA-MB-435. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3333−3336.
(32) Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X.; Yang, J.-M.; Li, T.-K.; Liu, A.; Liu L. F.; LaVoie, E. J. 5*H*-Dibenzo[*c,h*]1,6-naphthyridin-6-ones: Novel topoisomerase I-targeting anticancer agents with potent cytotoxic activity. *Bioorg. Med. Chem.* **2003**, *11*, 2061−2073.
(33) Contreras, J.-M.; Rival, Y. M.; Chayer, S.; Bourguignon, J.-J.; Wermuth. C. G.; Aminopyridazines as acetylcholinesterase inhibitors. *J. Med. Chem.* **1999**, *42*, 730−741.

(34) Iwanami, S.; Takashima, M.; Hirata, Y.; Hasegawa, O.; Usuda, S. Synthesis and neuroleptic activity of benzamides. cis-N−(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-(methylamino)benzamide and related compounds. *J. Med. Chem.* **1981**, *24*, 1224−1230.
(35) Meyer, R. F.; Stratton, C. D.; Hastings, S. G.; Corey, R. M. β-Adrenergic blocking agents. Nitrogen heteroaryl-substituted 2-propanolamines and ethanolamines. *J. Med. Chem.* **1973**, *16*, 1113−16.
(36) Leclerc, E.; Vrancken, E.; Mangeney, P. Diastereoselective aldolization of α-aminonitriles. diastereoselective synthesis of β-amino alcohols and β,γ-diamino alcohols. *J. Org. Chem.* **2002**, *67*, 8928−8937.
(37) Pasini, C., Coda, S.; Colo, V.; Ferrini, R.; Glaesser, A. H. Pharmacological effects of some guanidine and guanethidine derivatives. *Farmaco Ed. Sci.* **1965**, *20*, 673−685.
(38) Saxena, M.; Agarwal, A.; Shiv, K.; Patnaik, G. K.; Saxena, A. K. Synthesis, biological evaluation, and quantitative structure−activity relationship analysis of [β-(aroylamino)ethyl]piperazines and -piperidines and [2-[(aryamino)carbonyl]ethyl]piperazines, -piperidines, -pyrazinopyridoindoles, and -pyrazinoisoquinolines. A new class of potent H1 antagonists. *J. Med. Chem.* **1990**, *33*, 2970−2976.
(39) Gall-Istok, K.; Sterk, L.; Deak, G. Notes on the Synthesis of 4-Amino-6,7-di-*sec*-butoxyquinoline, −6,7-Methylenedioxyquinoline and its N-alkylaminoacetyl derivatives. *Acta Chim. Hung.* **1983**, *112*, 241−247.
(40) Wright, G. C.; Watson, E. J.; Ebetino, F. F.; Lougheed, G.; Stevenson, B. F.; Winterstein, A. Synthesis and hypotensive properties of new 4-aminoquinolines. *J. Med. Chem.* **1971**, *14*, 1060−1066.
(41) Grigg, R.; Sridharan, V.; Stevenson, P.; Sukirthalingham, S.; Worakun, T. The synthesis of fused ring nitrogen heterocycles via regiospecific intramolecular Heck reactions. *Tetrahedron* **1990**, *46*, 4003−4018.
(42) Harayama, T.; Akiyama, H.; Kawano, K.; Abe, H.; Takeuchi, Y. Total synthesis of benzo[*c*]phenanthridine alkaloids, chelerythrine and 12-methoxydihydrochelerythrine, by a palladium-assisted internal biaryl coupling reaction. *Synthesis* **2001**, 444−450.
(43) Harayama, T.; Sato, T.; Nakano, Y.; Abe, H.; Takeuchi, Y. Concise synthesis of fagaridine and decarine, phenolic benzo[*c*]-phenanthridine alkaloids, using the palladium-assisted biaryl coupling reaction. *Heterocycles* **2003**, *59*, 293−301.
(44) Jacobi, P. A.; Martinelli, M. J.; Polanc, S. Total synthesis of (±)-saxitoxin. *J. Am. Chem. Soc.* **1984**, *106*, 5594−5598.
(45) Tamura, H.; Kohchi, C.; Yamada, R., Ikeda, T.; Koiwai, O.; Patterson, E.; Keene, J. D.; Okada, K.; Kjeldsen, E.; Nishikawa, K.; Andoh, T. Molecular cloning of a cDNA of a camptothecin-resistant human DNA topoisomerase I and identification of mutation sites, *Nucleic Acids Res.* **1990**, *19*, 69−75.
(46) Woessner, R. D.; Eng, W. K.; Hofmann, G. A.; Rieman, D. J.; McCabe, F. L.; Hertzberg, R. P.; Mattern, M. R.; Tan, K. B., Johnson, R. K. Camptothecin hyper-resistant P388 cells: drug-dependent reduction in topoisomerase I content. *Oncol. Res.* **1992**, *4*, 481−488.
(47) Shen, D. W.; Fojo, A.; Chin, J. E.; Roninson, I. B.; Richert, N.; Pastan, I.; Gottesman, M. M. Human multidrug-resistant cell lines: increased mdr1 expression can precede gene amplification, *Science* **1986**, *232*, 643−645.
(48) 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. Characterization of ARC-111 as a novel topoisomerase I-targeting anticancer agent, *Cancer Res.* **2003**, *63*, 8400−8407.
(49) Gatto, B.; Sanders, M. M.; Yu C.; Wu, H.-Y.; Makhey, D.; LaVoie, E. J.; Liu, L. F. Identification of topoisomerase I as the cytotoxic target for the protoberberine alkaloid coralyne. *Cancer Res.* **1996**, *56*, 2795−2800.
(50) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning, a Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY 1982; pp 149−185.
(51) Tewey, K. M.; Rowe, T. C.; Yang, L.; Hallogan, B. C.; Liu, L. F. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* **1984**, *226*, 466−468.
(52) Wang, H.; Mao, Y.; Chen, A.; Zhou, N.; LaVoie, E. J.; Liu, L. F. Stimulation of topoisomerase II-mediated DNA damage via a mechanism involving protein thiolation. *Biochemistry* **2001**, *40*, 3316−3323.
(53) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(54) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936−942.
(55) Denizot, F.; Lang, R. J. Rapid colorimetric assay for cellular growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J. Immunol. Methods* **1986**, *89*, 271−277.
(56) Andoh, T.; Ishii, K.; Suzuki, Y.; Ikegami, Y.; Kusunoki, Y.; Takemoto, Y.; Okada, K. Characterization of a mammalian mutant with a camptothecin-resistant DNA topoisomerase I. *Proc. Natl. Acad. Sci., U.S.A.* **1987**, *84*, 5565−5569.
(57) Woessner, R. D.; Eng, W. K.; Hofmann, G. A.; Rieman, D. J.; McCabe, F. L.; Hertzberg, R. P.; Mattern, M. R.; Tan, K. B.; Johnson, R. K. Camptothecin hyper-resistant P388 cells: drug-dependent reduction in topoisomerase I content. *Oncol. Res.* **1992**, *4*, 481−488.
(58) Rubin, E.; Pantazis, P.; Bharti, A.; Toppmeyer, D.; Giovanella, B.; Kufe, D. Identification of a mutant human topoisomerase I with intact catalytic activity and resistance to 9-nitro-camptothecin. *J. Biol. Chem.* **1994**, *269*, 2433−2439.
(59) Gervasoni, J. E., Jr.; Fields, S. Z.; Krishna, S.; Baker, M. A.; Rosado, M.; Thuraisamy, K.; Hindenburg, A. A., and Taub, R. N. Subcellular distribution of daunorubicin in P-glycoprotein-positive and -negative drug-resistant cell lines using laser-assisted confocal microscopy. *Cancer Res.* **1991**, *51*, 4955−4963.
(60) Fernandes, D. J.; Danks, M. K.; Beck, W. T. Decreased nuclear matrix DNA topoisomerase II in human leukemia cells resistant to VM-26 and m-AMSA. *Biochemistry* **1990**, *29*, 4235−4241.
(61) Houghton, P. J.; Cheshire, P. J.; Hallman, J. D.; Lutz, L.; Friedman, H. S.; Danks, M. K.; Houghton, J. A. Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. *Cancer Chem.Pharm.* **1995**, *36*, 393−403.

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