Synthesis and Cytotoxic Activity of Carboxamide Derivatives of Benzo[*b*][1,6]naphthyridines

Leslie W. Deady,*,† Thomas Rodemann,† Li Zhuang,‡ Bruce C. Baguley,‡ and William A. Denny*,‡

Chemistry Department, La Trobe University, Victoria 3086, Australia, and Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand

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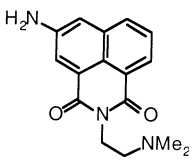
The reaction of 4-dimethylaminomethylene-6-methyl-4*H*-pyrano[4,3-*b*]quinoline-1,3-dione with a range of primary amines gave rise to a series of 2-substituted 6-methyl-1-oxo-1,2-dihydrobenzo-[*b*][1,6]naphthyridine-4-carboxylic acids. The derived 4-*N*-[2-(dimethylamino)ethyl]carboxamides were tested for growth inhibitory properties against murine P388 leukemia, Lewis lung carcinoma (LLTC), and human Jurkat leukemia cell lines. Most compounds were potent cytotoxins, with some having IC_{50} values less than 10 nM. Five were tested in vivo against subcutaneous colon 38 tumors in mice, and a single dose (3.9 mg/kg) proved to be curative for the 2-methyl and 2-(3,4-dimethoxyphenyl) derivatives in this refractory model.

Introduction

A number of compounds comprising tricyclic chromophores bearing a flexible cationic side chain are known to show cytotoxic effects. These include benziso-quinolinediones (e.g., amonafide, **1**[1,2]), anthraquinones (e.g., mitoxantrone, **2**[3]), and acridine-4-carboxamides (e.g., DACA, **3**[4,5]). Their utility as anticancer drugs is considered to be primarily through their ability to inhibit topoisomerase enzymes. With respect to DACA, it has been found that the peri arrangement between the amide function and the central ring nitrogen is essential for anticancer activity.[4]



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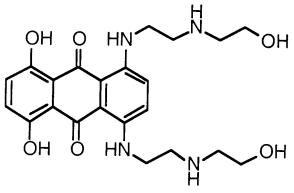

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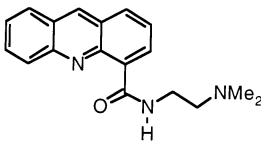

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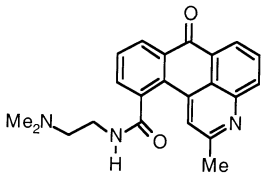

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We recently reinvestigated the reaction of homophthalic acid derivatives with Vilsmeier reagent and extended this to formation of quinoline analogues **5**.[6] We have now found that **5** reacts readily with a range of primary amines to form 2-substituted 1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxylic acids **6** (Scheme 1). Subsequently, new carboxamides **7** derived from these were obtained, where the peri arrangement of amide and central ring nitrogen seen in DACA is duplicated. Their synthesis, growth inhibitory properties in a panel of tumor cell lines, and discovery of remarkably potent in vivo activity are discussed.

Chemistry

N2- and carbon-substituted derivatives of the benzo-[*b*][1,6]naphthyridin-2(1*H*)-one system are known.[7−10] Dibenzo[*b*,*h*][1,6]naphthyridin-6(5*H*)-ones have also been reported,[7,11] but the synthetic route in the present work differs from these previous examples.

Compound **6d** was formed previously by refluxing **5b** with phosphoryl chloride for 48 h.[6] However, reaction with methylamine in tetrahydrofuran/dimethylformamide at room temperature brought about the same conversion in an experimentally preferable procedure and opened up the possibility of the analogous reaction with other amines. The related reaction of isocoumarins with ammonia to give isoquinolones (the Gabriel reaction[12]) is long known and has been extended in related systems to reactions with alkylamines[13] and arylamines.[14] Compounds **5** reacted with a selection of alkylamines under very mild conditions to give **6a**−**h** (Scheme 1) in generally good yields, while reaction with *N*,*N*′-bis(2-aminoethyl)-*N*,*N*′-dimethylpropane-1,3-diamine gave an example of a bis compound **6i**.

A slight anomaly occurred when ammonia was used instead of an alkylamine, since the product that separated from the reaction mixture in this case alone contained some ammonium species; a triplet at 7.1 ppm (broad or sharp with $J = 51$ Hz, depending on solvent and conditions) was evident in the ^{1}H NMR spectrum, though the NH (δ 12.2) and CO_2H (δ 16.0) proton singlets could also be seen. Liberation of **6c** required treatment of this material with acid (this turned out to be important to avoid complications in further reac-

* To whom correspondence should be addressed. For L.W.D.: phone, 61 3 9479 2561; fax, 61 3 9479 1399; e-mail, L.Deady@latrobe.edu.au. For W.A.D.: phone, 64 9 3737 599, extension 6144; fax, 64 9 3737 502; e-mail, b.denny@auckland.ac.nz.
† La Trobe University.
‡ The University of Auckland.

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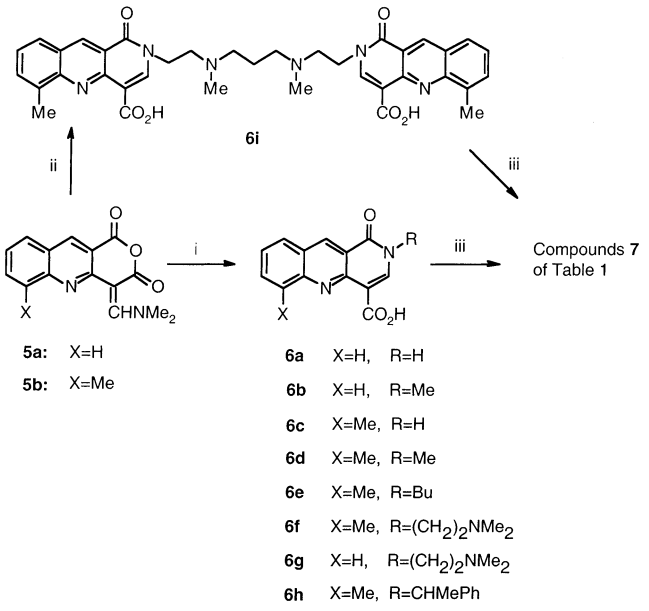
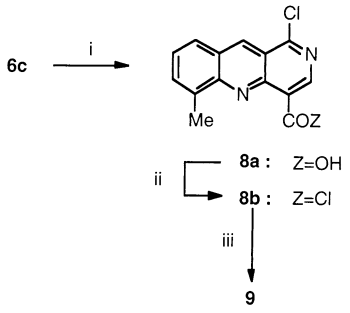
Scheme 1[a]



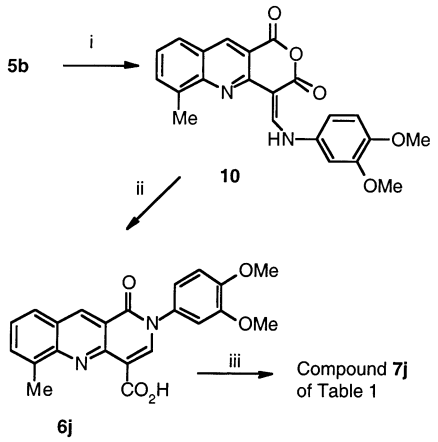
[a] (i) RNH_2, DMF, 20 °C; (ii) $CH_2[CH_2NMe(CH_2)_2NH_2]_2$ (0.5 mol equiv), DMF, 20 °C; (iii) $SOCl_2$, reflux, then $Me_2N(CH_2)_2NH_2$, CH_2Cl_2.

Scheme 2[a]



[a] (i) $POCl_3$, reflux, aqueous workup; (ii) $SOCl_2$, reflux; (iii) excess $Me_2N(CH_2)_2NH_2$, CH_2Cl_2, reflux.

Scheme 3[a]



[a] (i) 4-aminoveratrole, NEt_3, DMF, 20 °C; (ii) 4-aminoveratrole, NEt_3, pyridine, reflux 8 h; (iii) $SOCl_2$, reflux, then $Me_2N(CH_2)_2NH_2$, CH_2Cl_2.

tions). Further chemistry on **6c** was possible (Scheme 2). Reaction with phosphoryl chloride replaced the oxo function, though complete conversion was not obtained and prolonged reflux gave complex mixtures. An aqueous workup afforded **8a**. This was converted to the acid chloride **8b**, and then reaction with an excess of *N*,*N*-dimethylethylenediamine displaced both chlorines to give compound **9** of Table 1.

Reaction of **5b** with the arylamine 3,4-dimethoxyaniline failed in the absence of triethylamine, and it seems that moderate base catalysis of this reaction is required. Alkylamines behave as both nucleophile and base, but the arylamine required the presence of the more basic, nonnucleophilic triethylamine. Even then, reaction did not go to completion probably because of the insolubility of the initial product **10** (Scheme 3). Hot pyridine was found to be a suitable solvent, and reflux of a mixture of **10**, more 3,4-dimethoxyaniline, and triethylamine in this solvent for 8 h gave **6j** in 78% yield.

The carboxamides of Table 1 were prepared by reaction of the intermediate acid chlorides (from thionyl chloride reaction with the appropriate **6** and not isolated) with *N*,*N*-dimethylethylenediamine.

Results and Discussion

The compounds were evaluated for growth inhibitory properties, measured as IC_{50} values against murine P388 leukemia cells, Lewis lung carcinoma cells (LLTC), and human Jurkat leukemia cells (JL_C), together with their amsacrine- and doxorubicin-resistant derivatives (JL_A and JL_D, respectively), which were obtained and cultured as previously described.[15,16] The results are summarized in Table 1, along with comparable data for some reference compounds.

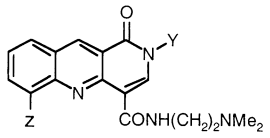
The JL_A line is resistant to the DNA intercalator amsacrine and similar agents because of a reduced level of topo II enzyme. The JL_D line is resistant to doxorubicin, primarily by virtue of altered levels of topo II but probably also by additional mechanisms. The ratios of the IC_{50} values of a drug in the parent line to one of the sublines ($IC_{50}[JL_A]/IC_{50}[JL_C]$ and ($IC_{50}[JL_D]/IC_{50}[JL_C]$) therefore provide some indication of the mechanism of cytotoxicity. Classical topo II inhibitors such as amsacrine, doxorubicin, and etoposide have large ratios (10- to 90-fold), whereas topo I inhibitors such as camptothecin and mixed topo I/II inhibitors such as DACA **3** have ratios of only about 2-fold. Values of these ratios of less than about 1.5−2 therefore suggest cytotoxicity by a non-topo-II-mediated mechanism.

Since the chemistry allowed the introduction of a range of N2 substituents in the 1-oxo series (Table 1, structure **A**), the main interest was in this aspect of structure−activity relationships and all compounds were prepared with the same carboxamide function as DACA. The noteworthy feature is the generally high level of cytotoxicity compared with that of DACA and **4** (a recently discovered tetracyclic carboxamide with remarkable in vivo activity in early results;[17] see below). Thus, for a diverse array of substituents from Y = H (**7c**) to Y = dimethoxyphenyl (**7j**), potency is high. The exception is **7h**, where branching at the α-carbon is evidently not tolerated.

Two pairs (**7b**/**7d** and **7g**/**7f**) allowed the effect of a 6-methyl substituent to be assessed. As expected from results with DACA analogues where small 5-substituents gave greater cytotoxicity,[18] the methyl group here too provided enhanced activity.

The bis example **6i** was included because there is much current interest in the anticancer properties of

Table 1. Growth Inhibitory Activity of 1-Oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxamides and Reference Compounds



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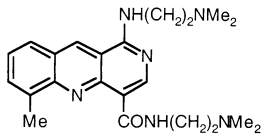

B

compd	form	Y	Z	IC_{50} [a] (nM) P388[c]	LL[d]	JL_C[e]	IC_{50} ratio[b] JL_A/JL_C	JL_D/JL_C
7b	A	Me	H	14				
7c	A	H	6-Me	11	10	26	1.9	2.6
7d	A	Me	6-Me	2.1	1.7	6.7	5.6	7.9
7e	A	Bu	6-Me	14	15	51	2.3	3.0
7f	A	$Me_2N(CH_2)_2$	6-Me	6.8	3.7	8.5	0.7	0.9
7g	A	$Me_2N(CH_2)_2$	H	54	50	123	0.8	1.1
7h	A	(*S*)-PhCH(Me)	6-Me	590	161	1070	0.8	1.1
7j	A	3,4-$(MeO)_2C_6H_3$	6-Me	12	4.4	9.4	1.3	1.5
7i	A[f]	$(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2$	6-Me	22	4.7	1.0	0.3	0.4
9	B			2.4				
doxorubicin				15	22	9.6	4.4	13
etoposide				150	180	160	13	90
camptothecin				13	33	5.6	2.0	1.4
3 (DACA)[g]				71	190	580	1.9	2.3
4[h]				100	101	424	2.5	2.5

[a] IC_{50}: concentration of drug to reduce cell number to 50% of control cultures. [b] IC_{50} values for the Jurkat lines JL_A and JL_D, respectively, relative to JL_C. See text. [c] Murine P388 leukemia. [d] Murine Lewis lung carcinoma. [e] Human Jurkat leukemia. [f] A bis compound. [g] Data from ref 18. [h] Data from ref 17.

Table 2. In Vivo Activity of 1-Oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxamides and Reference Compounds against Subcutaneous Colon 38 Tumors in Mice

drug	dose, mg kg^{-1} day^{-1}	schedule[a]	growth delay days	cures[b]
7c	30[c]	sd	14	0/5
7d	8.9[c]	sd	>20	4/4
7d	5.9	sd	>20	10/10
7d	3.9	sd	>20	4/4
7d	2.6	sd	10	0/5
7e	13.3[c]	sd	>20	5/5
7e	5.9	sd	18	0/5
7f	5.9	sd	14	0/5
7j	3.9[c]	sd	>20	4/5
7j	2.6	sd	16	0/5
9	8.9[c]	sd	6	0/5
doxorubicin	2.6	q4d × 3	8	0/5
daunorubicin	3.9	q4d × 3	0	0/5
amsacrine	13.3	q4d × 3	2	0/5
mitoxantrone	3.9	q4d × 3	2	0/5
DACA	200	q7d × 2	13	0/5
etoposide	45	q4d × 3	1.5	0/5
irinotecan	65	q4d × 3	7	0/5
4[d]	65	q2d × 2	>20	5/5

[a] q2d × 2 = every 2 days × 2; q4d × 3 = every 4 days × 3; q7d × 2 = every 7 days × 2; sd = single dose. [b] Number of mice with no measurable tumor 20 days after commencement of treatment divided by total number of mice. [c] Highest dose that did not produce evidence of toxicity. [d] Data from ref 17.

compounds comprising two DNA-intercalating chromophores joined by a flexible cationic or dicationic linker chain.[19]

Compound **9**, the lone example of structure **B**, is a direct 2-aza analogue of an acridine derivative reported recently to have high in vitro cytotoxicity against colon adenocarcinoma and ovarian carcinoma cell lines.[20] The P388 result indicates that **9** too is a potent cytotoxic agent.

Five examples were then evaluated in vivo against subcutaneously implanted colon 38 tumors in mice with the results summarized in Table 2. Noteworthy is the finding of some curative effects at low, single-dose schedules. Colon 38 is relatively refractory to antimetabolites, alkylating agents, and other topoisomerase-directed agents; of the established compounds listed in Table 2, growth delays vary, but none produced cures. We recently reported that **4** at 65 mg/kg in a single repeat-dose schedule was therefore strikingly effective in this regard,[17] but compounds in the present set are even more potent. Thus, the 2-methyl compound **7d** produced 100% cures at a single dose of 3.9 mg/kg. The 2-butyl analogue **7e** also produced cures but was slightly less potent, while the 2-H compound **7c** was much less active. Compound **7f**, bearing the extra cationic side chain, was also less effective. The wide tolerance of the nature of the 2-substituent noted in the in vitro testing was retained in the colon 38 results; the aryl-substituted **7j** also produced cures at a low dosage, though the indication is that this compound is more toxic than is **7d**. Compound **9**, with the cationic side chain in place of the 1-oxo group of compounds **7**, produced only a small growth delay at the highest tolerated dose.

Conclusions

The 4-(2-(dimethylamino)ethylcarboxamide derivatives of 1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridines are an interesting new class of antitumor agents, with some showing curative in vivo activity in the sc 38 model in a single dosing protocol. The range of 2-substituents that produce significant activity is apparently wide. Further investigation of the scope of this variable and the mechanism of action of these compounds is in progress.

Experimental Section

Melting points are uncorrected. NMR spectra were recorded on a Bruker AM-300 spectrometer operating at 300.13 MHz (^1H) and 75.47 MHz (^{13}C) and on a Bruker DRX-400 spectrom-

eter operating at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C). Chemical shifts are reported as δ values (ppm) relative to Me_4-Si. Various standard techniques were used to identify proton-bound carbons in ^{13}C NMR spectra. Full assignments of the spectra for **6e** were obtained from further COSY, HMQC, and HMBC experiments using appropriate pulse programs from the Bruker library. Electrospray mass spectra (ESMS) were recorded on a VG Bio-Q triple quadrupole mass spectrometer using methanol or acetonitrile with formic acid (1%) as mobile phase. EI and LSI (3-nitrobenzyl alcohol as liquid matrix) mode high-resolution mass spectra were obtained by Dr. N. Davies, University of Tasmania, Australia. Microanalyses, indicated by symbols of the elements, were confined to the final products, compounds of Table 1, along with one example of a precursor acid (**6d**) and were within ±0.4% of the theoretical values. They were carried out at the Campbell Microanalytical Laboratory, University of Otago, New Zealand.

N,*N*′-Bis(2-aminoethyl)-*N*,*N*′-dimethylpropane-1,3-diamine was available,[19] and the other amines were commercial samples.

4-Dimethylaminomethylene-6-methyl-4*H*-pyrano[4,3-*b*]quinoline-1,3-dione (5b). Phosphoryl chloride (13 mL) was added, with stirring at 0 °C, to dimethylformamide (40 mL). After a further 20 min, a solution of ethyl (3-carboxy-8-methylquinolin-2-yl)acetate[6] (10.0 g, 0.037 mol) in dimethylformamide (10 mL) was added in a single portion and the mixture was stirred at room temperature for a further 1.5 h. The precipitate was collected by filtration and washed with cold acetone to give the product as an orange solid (10.0 g, 97%), mp >295 °C (decomposed, formed needles above 280 °C). ^{1}H NMR (DMSO-d_6): δ 2.69 (s, 3 H, CH_3), 3.30 (s, 3 H, NCH_3), 3.59 (s, 3 H, NCH_3), 7.36 (t, J = 7.6 Hz, 1 H, H-3), 7.68 (d, J = 7.0 Hz, 1 H), 7.89 (d, J = 8.1 Hz, 1 H), 8.81 (s, 1 H), 8.93 (s, 1 H).

4-Dimethylaminomethylene-4*H*-pyrano[4,3-*b*]quinoline-1,3-dione (5a). 5a was similarly prepared from acetanilide, as an orange solid (87%), mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 3.33 (s, 3 H, NCH_3), 3.64 (s, 3 H, NCH_3), 7.55 (t, J = 7.4 Hz, 1 H), 7.91 (t, J = 7.3 Hz, 1 H), 8.08 (d, J = 7.9 Hz, 1 H), 8.15 (d, J = 7.8 Hz, 1 H), 8.94 (s, 1 H), 9.13 (s, 1 H).

2,6-Dimethyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]-naphthyridine-4-carboxylic Acid (6d). A solution of 2 M methylamine in tetrahydrofuran (7.0 mL, 14 mmol) was added to a suspension of **5b** (0.8 g, 2.8 mmol) in dimethylformamide (20 mL), and the whole was stirred for 16 h at room temperature. The reactant dissolved and was soon replaced by a new solid. This was collected by filtration and washed with a little cold acetone to give the product as a bright-yellow solid (0.60 g, 79%), mp >300 °C (formed cubic crystals above 290 °C). ^{1}H NMR (DMSO-d_6): δ 2.75 (s, 3 H, CH_3), 3.67 (s, 3 H, NCH_3), 7.67 (t, J = 7.7 Hz, 1 H, H-8), 7.95 (d, J = 6.6 Hz, 1 H), 8.25 (d, J = 8.1 Hz, 1 H), 8.83 (s, 1 H), 9.52 (s, 1 H), 16.03 (s, 1 H, COOH). Anal. ($C_{15}H_{12}N_2O_3$·0.2H_2O) C, H, N.

2-Methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxylic Acid (6b). This was prepared from **5a** and methylamine, as for **6d**, and obtained as a yellow solid (69%), mp >305 °C (formed needles above 230 °C). ^{1}H NMR (DMSO-d_6): δ 3.61 (s, 3 H, N−CH_3), 7.71 (t, J = 7.7 Hz, 1 H), 8.00 (t, J = 8.2 Hz, 1 H), 8.16 (d, J = 8.6 Hz, 1 H), 8.33 (d, J = 8.1 Hz, 1 H), 8.66 (s, 1 H), 9.46 (s, 1 H), 16.00 (s, 1 H, COOH).

2-Butyl-6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]-naphthyridine-4-carboxylic Acid (6e). This was prepared from **5b** and butylamine, as for **6d**, and obtained as a bright-yellow solid (82%), mp 259 °C. ^{1}H NMR ($CDCl_3$): δ 0.98 (t, J = 7.2 Hz, 3 H, δ-CH_3), 1.42 (sextet, J = 7.6 Hz, 2 H, γ-CH_2), 1.81 (quintet, J = 7.7 Hz, 2 H, β-CH_2), 2.83 (s, 3 H, C6−CH_3), 4.10 (t, J = 7.4 Hz, 2 H, N−CH_2), 7.57 (t, J = 7.7 Hz, 1 H, H-8), 7.81 (d, J = 7.0 Hz, 1 H, H-7), 7.93 (d, J = 8.3 Hz, 1 H, H-9), 8.59 (s, 1 H, H-3), 9.35 (s, 1 H, H-10), 16.09 (br s, 1 H, COOH). ^{13}C NMR ($CDCl_3$): δ 13.6 (δ-CH_3), 18.2 (C6−CH_3), 19.9 (γ-CH_2), 31.3 (β-CH_2), 49.8 (N−CH_2), 105.2 (C-4), 119.1 (C-10a), 126.4 (C-9a), 127.4 (C-8), 127.6 (C-9), 134.2 (C-7), 134.9 (C-6), 141.3 (C-10), 145.2 (C-3), 146.7 (C-5a), 148.7 (C-4a), 161.8 (C-1), 166.3 (COOH).

2-[(2-Dimethylamino)ethyl]-6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxylic Acid (6f). This was prepared from **5b** and *N*,*N*-dimethylethylenediamine, as for **6d**, and obtained as a yellow solid (78%), mp 254−255 °C. ^{1}H NMR (DMSO-d_6): δ 2.21 (s, 6 H, $N(CH_3)_2$), 2.60 (t, J = 6.0 Hz, 2 H), 2.73 (s, 3 H, CH_3), 4.23 (t, J = 6.0 Hz, 2 H), 7.65 (t, J = 7.6 Hz, 1 H, H-8), 7.93 (d, J = 6.8 Hz, 1 H), 8.21 (d, J = 8.3 Hz, 1 H), 8.74 (s, 1 H, H-3), 9.48 (s, 1 H, H-10).

2-[(2-Dimethylamino)ethyl]-1-oxo-1,2-dihydrobenzo[*b*]-[1,6]naphthyridine-4-carboxylic Acid (6g). This was prepared from reaction of *N*,*N*-dimethylethylenediamine with **5a**, as for **6d**, and obtained as a yellow solid (52%), mp 232−233 °C. ^{1}H NMR (DMSO-d_6): δ 2.20 (s, 6 H, $N(CH_3)_2$), 2.58 (t, J = 5.9 Hz, 2 H), 4.22 (t, J = 6.0 Hz, 2 H), 7.77 (t, J = 7.4 Hz, 1 H), 8.06 (t, J = 7.6 Hz, 1 H), 8.23 (d, J = 8.6 Hz, 1 H), 8.39 (d, J = 8.3 Hz, 1 H), 8.74 (s, 1 H, H-3), 9.54 (s, 1 H, H-10).

(*S*)-6-Methyl-1-oxo-2-(1-phenylethyl)-1,2-dihydrobenzo-[*b*][1,6]naphthyridine-4-carboxylic Acid (6h). This was prepared from **5b** and (*S*)-(−)-α-methylbenzylamine, as for **6d**, except that the product remained in solution. After 16 h at 20 °C, water was added and the resulting precipitate was collected by filtration and washed with water to give the product as a yellow solid (79%), mp 234−235 °C. ^{1}H NMR (DMSO-d_6): δ 1.84 (d, J = 7.1 Hz, 3 H, CH−CH_3), 2.73 (s, 3 H, C6−CH_3), 6.29 (q, J = 7.1 Hz, 1 H, N−CH), 7.3−7.5 (m, 5 H), 7.66 (t, J = 7.7 Hz, 1 H, H-8), 7.94 (d, J = 6.8 Hz, 1 H), 8.23 (d, J = 8.2 Hz, 1 H), 8.43 (s, 1 H), 9.53 (s, 1 H, H-10), 16.00 (br s, 1 H, COOH).

2-(3,4-Dimethoxyphenyl)-6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxylic Acid (6j). A mixture of 4-aminoveratrole (0.12 g, 0.78 mmol), **5b** (0.20 g, 0.7 mmol), dimethylformamide (5 mL), and triethylamine (1 mL) was stirred at room temperature for 16 h. Solid was present at all times, and it was collected by filtration and washed with a little cold dichloromethane to give the intermediate 4-[(3,4-dimethoxyphenyl)aminomethylene]-6-methyl-4*H*-pyrano[4,3-*b*]quinoline-1,3-dione (**10**) as a yellow solid (0.25 g, 90%), mp 280−281 °C. ^{1}H NMR (DMSO-d_6): δ 2.81 (s, 3 H, CH_3), 3.79 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 7.06 (s, 2 H), 7.21 (s, 1 H), 7.52 (t, J = 7.5 Hz, 1 H, H-8), 7.83 (d, J = 7.0 Hz, 1 H), 8.02 (d, J = 7.9 Hz, 1 H), 8.81 (s, 1 H, J = 13.2 Hz, 1 H, CHN), 9.09 (s, 1 H, H-10), 13.83 (d, J = 13.2 Hz, 1 H, NH).

A mixture of **10** (0.23 g), 4-aminoveratrole (0.46 g), pyridine (15 mL), and triethylamine (1 mL) was heated under reflux for 8 h (dissolution occurred during the heating). The reaction mixture was allowed to stand at −10 °C overnight, and the resulting precipitate was collected by filtration and washed with a little cold acetone to give the product as a yellow solid (0.18 g, 78%), mp 297−298 °C (after forming needles above 285 °C). ^{1}H NMR (DMSO-d_6): δ 2.73 (s, 3 H, CH_3), 3.78 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 7.12 (s, 2 H), 7.23 (s, 1 H), 7.67 (t, J = 7.4 Hz, 1 H, H-8), 7.95 (d, J = 6.7 Hz, 1 H), 8.23 (d, J = 8.2 Hz, 1 H), 8.45 (s, 1 H), 9.50 (s, 1 H, H-10), 15.98 (br s, 1 H, COOH).

2,2′-[1,3-Propanediylbis[(methylimino)-2,1-ethanediyl]]-bis[6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxylic Acid] (6i). *N*,*N*′-Bis(2-aminoethyl)-*N*,*N*′-dimethylpropane-1,3-diamine (0.10 g, 0.54 mmol) was added to a suspension of **5b** (0.30 g, 1.06 mmol) in dimethylformamide (7.5 mL) and triethylamine (0.4 mL), and the resulting solution was stirred for 16 h at room temperature. The precipitate, which formed during the reaction, was collected by filtration and was stirred in ethyl acetate (10 mL) for 5 min. The solid was collected by filtration to give the product as a yellow solid (0.15 g, 43%), mp 224−228 °C. ^{1}H NMR (DMSO-d_6): δ 1.45−1.49 (m, 2 H), 2.13 (s, 6 H, 2 × NCH_3, 2.28−2.34 (m, 4 H), 2.48−2.55 (m, 10 H), 4.01−4.05 (m, 4 H), 7.36 (t, J = 7.4 Hz, 2 H), 7.62 (d, J = 6.5 Hz, 2 H), 7.93 (d, J = 7.7 Hz, 2 H), 8.49 (s, 2 H), 9.16 (s, 2 H).

6-Methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxylic Acid (6c). A mixture of **5b** (1.0 g) in dimethylformamide (25 mL) was stirred under a stream of ammonia for 4 h at room temperature. The precipitate, which formed

after dissolution of the starting material, was collected by filtration and washed thoroughly with acetone to give a yellow solid (0.70 g).

This ammonium-containing species (see text) (0.15 g) in 5% hydrochloric acid (20 mL) was heated under reflux until a clear solution was obtained (10 min). After being cooled, the solution was adjusted to pH 4 with 10% sodium hydroxide. The precipitate was collected by filtration to give the acid as a yellow solid (0.13 g), mp >300 °C (after forming needles above 255 °C). ^{1}H NMR (DMSO-d_6): δ 2.67 (s, 3 H, CH_3), 7.58 (br s, 1 H, H-8), 7.89 (d, J = 4.9 Hz, 1 H), 8.14 (d, J = 7.0 Hz, 1 H), 8.31 (s, 1 H, H-3), 9.35 (s, 1 H, H-10), 12.19 (br s, 1 H, NH), 15.90 (br s, 1 H, COOH).

***N*-[(2-Dimethylamino)ethyl]-1-[2-((dimethylamino)ethyl)amino]-6-methylbenzo[*b*][1,6]naphthyridine-4-carboxamide (9).** Acid **6c** (0.5 g) was heated under reflux in phosphoryl chloride (50 mL) for 8 h. The excess of phosphoryl chloride was removed at reduced pressure and ice/water (30 mL) was carefully added to the residue. The precipitate was collected by filtration, washed with water, and dried. The solid was heated under reflux in thionyl chloride (20 mL) for 1 h. The thionyl chloride was removed at reduced pressure, and a solution of *N*,*N*-dimethylethylenediamine (1 mL) in dichloromethane (20 mL) was added to the residue. The resulting solution was heated under reflux for 16 h and then cooled and washed with 10% sodium carbonate and twice with water. The solvent was removed, and preparative TLC of the residue (alumina; chloroform/methanol, 20:1) gave the crude product, R_f = 0.6. This was recrystallized from toluene to give the product as a yellow solid (0.27 g, 35%), mp 219−220 °C. ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 6 H, $N(CH_3)_2$), 2.36 (s, 6 H, $N(CH_3)_2$), 2.64 (t, J = 4.8 Hz, 2 H), 2.70 (t, J = 5.9 Hz, 2 H), 2.86 (s, 3 H, CH_3), 3.71−3.78 (m, 4 H), 6.89 (br s, 1 H, NH), 7.45 (dd, J = 7.9, 6.9 Hz, 1 H, H-8), 7.70 (d, J = 7.0 Hz, 1 H), 7.86 (d, J = 8.3 Hz, 1 H, H-9), 8.75 (s, 1 H), 9.26 (s, 1 H, H-10), 11.02 (br s, 1 H, CONH). ESMS *m*/*z*: 198 [(M + 2)/2, 100%], 395 (M + 1, 25%). Anal. ($C_{22}H_{30}N_6O$) C, H, N.

Preparation of *N*-[2-(Dimethylamino)ethyl]-2,6-dimethyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]-naphthyridine-4-carboxamide (7d). An Example of the General Method for Amide Formation. Acid **6d** (0.40 g) was heated under reflux in thionyl chloride (20 mL) for 30 min. The excess of thionyl chloride was removed at reduced pressure, and a solution of *N*,*N*-dimethylethylenediamine (0.5 mL) in dichloromethane (20 mL) was added to the residue. The resulting solution was stirred at room temperature for 16 h, and the solution was washed with 10% sodium carbonate and water (× 2). The solvent was removed at reduced pressure to give the product as a yellow solid (0.48 g, 95%), mp 167−170 °C (acetonitrile). ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 6 H, $N(CH_3)_2$), 2.61 (t, J = 6.3 Hz, 2 H), 2.82 (s, 3 H, CH_3), 3.67−3.73 (m, 5 H), 7.46 (t, J = 7.6 Hz, 1 H, H-8), 7.70 (d, J = 6.9 Hz, 1 H), 7.82 (d, J = 8.2 Hz, 1 H), 8.56 (s, 1 H, H-3), 9.21 (s, 1 H, H-10), 10.91 (br s, 1 H, CONH). ^{13}C NMR ($CDCl_3$): δ 18.5 (C6-**C**H_3), 37.2 (N−CH_3), 37.6 (CH_2), 45.4 ($N(CH_3)_2$), 58.8 (CH_2), 109.5 (C), 119.3 (C), 125.9 (C), 126.6 (CH), 127.3 (CH), 132.8 (CH), 135.9 (C), 139.8 (CH), 143.6 (CH), 148.2 (C), 148.8 (C), 162.8 (C), 164.5 (C). ESMS *m*/*z*: 339 (M + 1). Anal. ($C_{19}H_{22}N_4O_2$) C, H, N.

The following amides were made in a similar manner.

***N*-[2-(Dimethylamino)ethyl]-2-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxamide (7b). 7b** was prepared from acid **6b** and was obtained as yellow plates (67%), mp 192−194 °C (acetonitrile). ^{1}H NMR ($CDCl_3$): δ 2.40 (s, 6 H, $N(CH_3)_2$), 2.63 (t, J = 6.1 Hz, 2 H), 3.6−3.7 (m, 5 H), 7.60 (t, J = 7.1 Hz, 1 H), 7.87 (t, J = 7.5 Hz, 1 H), 8.01 (d, J = 8.2 Hz, 1 H), 8.10 (d, J = 8.5 Hz, 1 H), 8.58 (s, 1 H), 9.31 (s, 1 H, H-10), 11.30 (br s, 1 H, CONH). ^{13}C NMR ($CDCl_3$): δ 36.8 (N−CH_3), 37.0 (CH_2), 44.9 ($N(CH_3)_2$), 57.7 (CH_2), 109.0 (C), 119.1 (C), 125.5 (C), 126.4 (CH), 128.0 (CH), 128.8 (CH), 132.5 (CH), 139.1 (CH), 143.2 (CH), 148.5 (C), 149.3 (C), 162.4 (C), 164.0 (C). Anal. ($C_{18}H_{20}N_4O_2$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-butyl-6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxamide (7e). 7e** was prepared from acid **6e** and was obtained as a yellow solid (89%), mp 127−128 °C [light petroleum (bp 90−120 °C)]. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.3 Hz, 3 H, CH_2−C**H**$_3$), 1.41 (sextet, J = 7.3 Hz, 2 H, C**H**$_2$−CH_3), 1.80 (quintet, J = 7.4 Hz, 2 H, N2−CH_2C**H**$_2$), 2.29 (s, 6 H, $N(CH_3)_2$), 2.62 (t, J = 6.5 Hz, 2 H, C**H**$_2$−$N(CH_3)_2$), 2.85 (s, 3 H, C6−CH_3), 3.72 (q, J = 6.3 Hz, 2 H, CONH−C**H**$_2$), 4.08 (t, J = 7.4 Hz, 2 H, N2−CH_2), 7.47 (t, J = 7.9 Hz, 1 H, H-8), 7.71 (d, J = 6.8 Hz, 1 H), 7.85 (d, J = 8.2 Hz, 1 H), 8.59 (s, 1 H, H-3), 9.27 (s, 1 H, H-10), 10.97 (br s, 1 H, CONH). ^{13}C NMR ($CDCl_3$): δ 13.6 (CH_2−**C**H_3), 18.5 (C6−**C**H_3), 19.8 (CH_2), 31.3 (CH_2), 37.6 (CH_2), 45.4 ($N(CH_3)_2$), 49.4 (CH_2), 58.8 (CH_2), 109.5 (C), 119.5 (C), 125.9 (C), 126.5 (CH), 127.3 (CH), 132.8 (CH), 135.9 (C), 140.0 (CH), 143.0 (CH), 148.2 (C), 148.8 (C), 162.4 (C), 164.6 (C). ESMS *m*/*z*: 381 (M + 1, 100%), 191 [(M + 2)/2, 20%]. Anal. ($C_{22}H_{28}N_4O_2$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-[2-(dimethylamino)ethyl]-6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]-naphthyridine-4-carboxamide (7f). 7f** was prepared from acid **6f** and was obtained as a yellow solid (84%), mp 141−143 °C [light petroleum (bp 90−120 °C)]. ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 6 H, $N(CH_3)_2$), 2.38 (s, 6 H, $N(CH_3)_2$), 2.67−2.76 (m, 4 H), 2.86 (s, 3 H, C6−CH_3), 3.78 (q, J = 6.3 Hz, 2 H, CH_2), 4.18 (t, J = 6.6 Hz, 2 H, CH_2), 7.49 (t, J = 7.4 Hz, 1 H, H-8), 7.73 (d, J = 6.8 Hz, 1 H), 7.87 (d, J = 8.2 Hz, 1 H), 8.61 (s, 1 H, H-3), 9.29 (s, 1 H, H-10), 11.07 (br s, 1 H, CONH). ^{13}C NMR ($CDCl_3$): δ 18.6 (C6−**C**H_3), 37.1 (CH_2), 45.0 ($N(CH_3)_2$), 45.6 ($N(CH_3)_2$), 47.2 (CH_2), 57.7 (CH_2), 58.5 (CH_2), 109.3 (C), 119.5 (C), 126.0 (C), 126.6 (CH), 127.4 (CH), 133.0 (CH), 135.9 (C), 140.1 (CH), 143.4 (CH), 148.3 (C), 148.8 (C), 162.5 (C), 165.0 (C). ESMS *m*/*z*: 198.5 [(M + 2)/2, 100%], 396 (M + 1, 60%). Anal. ($C_{22}H_{29}N_5O_2$·0.25H_2O) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-[2-(dimethylamino)ethyl]-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxamide (7g). 7g** was prepared from acid **6g** and was obtained as a yellow solid (80%), mp 143−144 °C (toluene). ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 6 H, $N(CH_3)_2$), 2.42 (s, 6 H, $N(CH_3)_2$), 2.65−2.71 (m, 4 H), 3.70 (q, J = 6.0 Hz, 2 H, CH_2), 4.17 (t, J = 6.5 Hz, 2 H, CH_2), 7.60 (dd, J = 8.4 Hz, 1 H, 7.4 Hz, 1 H), 7.88 (t, J = 7.3 Hz, 1 H), 8.02 (d, J = 8.2 Hz, 1 H), 8.12 (d, J = 8.6), 8.60 (s, 1 H, H-3), 9.32 (s, 1 H, H-10), 11.32 (br s, 1 H, CONH). ESMS *m*/*z*: 382 (M + 1, 100%), 191.5 [(M + 2)/2, 90%]. Anal. ($C_{21}H_{27}N_5O_2$) C, H, N.

(*S*)-*N*-[2-(Dimethylamino)ethyl]-6-methyl-1-oxo-2-(1-phenylethyl)-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxamide (7h). 7h was prepared from acid **6h** and was obtained as a yellow semisolid (86%). ^{1}H NMR ($CDCl_3$): δ 1.05 (d, J = 7.2 Hz, 3 H, CH−C**H**$_3$), 2.27 (s, 6 H, $N(CH_3)_2$), 2.59 (t, J = 6.5 Hz, 2 H, C**H**$_2$−$N(CH_3)_2$), 2.85 (s, 3 H, C6−CH_3), 3.67 (q, J = 6.7 Hz, 2 H, CONH−C**H**$_2$), 6.45 (q, J = 7.1 Hz, 1 H, N2−CH), 7.27−7.41 (m, 5 H), 7.45 (dd, J = 8.1, 7.3 Hz, 1 H, H-8), 7.69 (d, J = 7.0 Hz, 1 H), 7.84 (d, J = 8.2 Hz, 1 H), 8.62 (s, 1 H, H-3), 9.30 (s, 1 H, H-10), 10.96 (br s, 1 H, CONH). ^{13}C NMR ($CDCl_3$): δ 18.5 (CH_3), 19.1 (CH_3), 37.4 (CH_2), 45.4 ($N(CH_3)_2$), 53.4 (CH), 58.7 (CH_2), 110.0 (C), 119.4 (C), 126.0 (C), 126.5 (CH), 127.2 (CH), 127.3 (CH), 128.2 (CH), 128.9 (CH), 132.8 (CH), 135.9 (C), 139.56 (CH), 139.61 (CH), 140.3 (CH), 148.2 (C), 148.4 (C), 162.4 (C), 164.6 (C). HRMS (LSI) calcd for $C_{26}H_{29}N_4O_2$: ((M + H)$^+$) 429.2292. Found: 429.2298.

***N*-[2-(Dimethylamino)ethyl]-2-(3,4-dimethoxyphenyl)-6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxamide (7j). 7j** was prepared from acid **6j** and was obtained as a yellow solid (92%), mp 199−201 °C (toluene). ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 6 H, $N(CH_3)_2$), 2.70 (t, J = 6.3 Hz, 2 H, C**H**$_2$−$N(CH_3)_2$), 2.89 (s, 3 H, C6−CH_3), 3.77 (q, J = 6.4 Hz, 2 H, CONH−C**H**$_2$), 3.90 (s, 3 H, OCH_3), 3.94 (s, 3 H, OCH_3), 6.96−6.99 (m, 3 H), 7.52 (t, J = 7.5 Hz, 1 H, H-8), 7.76 (d, J = 7.0 Hz, 1 H), 7.89 (d, J = 8.1 Hz, 1 H), 8.71 (s, 1 H, H-3), 9.34 (s, 1 H, H-10), 11.07 (br s, 1 H, CONH). ^{13}C NMR ($CDCl_3$): δ 18.6 (C6−**C**H_3), 37.3 (CH_2), 45.2 ($N(CH_3)_2$), 56.1 (2 × OCH_3), 58.5 (CH_2), 109.7 (C), 110.6 (CH), 111.2 (CH), 118.9 (CH), 119.8 (C), 126.1 (C), 126.9 (CH), 127.4 (CH), 133.0 (C), 133.2 (CH), 136.0 (C), 140.5 (CH), 143.8 (CH), 148.4 (C), 148.6 (C), 149.3 (C), 149.4 (C), 162.5 (C), 164.7 (C). ESMS *m*/*z*: 461 (M + 1,

100%), 231 [(M + 2)/2, 25%). Anal. ($C_{26}H_{28}N_4O_4 \cdot 0.5H_2O$) C, H. N requires 11.9; found 11.3.

2,2′-[1,3-Propanediylbis[(methylimino)-2,1-ethanediyl]]-bis[*N*-(2-(dimethylamino)ethyl)-6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]naphthyridine-4-carboxamide] (7i). 7i was prepared from acid **6i** and was obtained as a yellow solid (75%), mp 97−102 °C [light petroleum (bp 90−120 °C)]. ^{1}H NMR ($CDCl_3$): δ 1.55−1.63 (m, 2 H), 2.23 (s, 6 H), 2.42−2.84 [m, 30 H, including 2.50 (s, 12 H), 2.72 (s, 6 H)], 3.78 (q, J = 6.2 Hz, 4 H, CH_2), 4.08 (t, J = 5.9 Hz, 4 H, CH_2), 7.31 (t, J = 7.8 Hz, 2 H), 7.53 (d, J = 6.8 Hz, 2 H), 7.71 (d, J = 8.2 Hz, 2 H), 8.51 (s, 2 H), 9.11 (s, 2 H), 10.96 (br s, 2 H, 2 × CONH). ^{13}C NMR ($CDCl_3$): δ 18.5 (CH_3), 24.8 (CH_2), 36.9 (CH_2), 42.1 (NCH_3), 44.9 ($N(CH_3)_2$), 46.8 (CH_2), 54.9 (CH_2), 56.5 (CH_2), 58.2 (CH_2), 108.6 (C), 119.2 (C), 125.6 (C), 126.4 (CH), 127.1 (CH), 132.6 (CH), 135.6 (C), 139.7 (CH), 143.8 (CH), 147.8 (C), 148.4 (C), 162.1 (C), 164.8 (C). ESMS *m/z*: 402.3 [(M + 2)/2, 100%], 803.5 (M + 1, 50%). Anal. ($C_{45}H_{58}N_{10}O_4 \cdot 1.5H_2O$) C, H. N requires 16.9; found 16.3.

***N*-[(2-Dimethylamino)ethyl]-6-methyl-1-oxo-1,2-dihydrobenzo[*b*][1,6]-naphthyridine-4-carboxamide Perchlorate Salt (7c). 7c** was prepared from the ammonium salt of **6c** and was obtained in crude form as a yellow solid (69%). The perchlorate salt was prepared in ethanol, recrystallized from "moist" methanol, and obtained as a brown solid, mp 218−219 °C (explosive decomposition above 250 °C). ^{1}H NMR (MeOD-d_4): δ 2.91 (s, 9 H, C6−CH_3 + $N(CH_3)_2$), 3.19 (t, J = 6.2 Hz, 2 H, CH_2), 3.45 (t, J = 6.2 Hz, 2 H), 6.96−7.00 (m, 2 H), 7.21 (br s, 1 H), 7.37 (br s, 1 H), 7.67 (br s, 1 H). ESMS *m/z*: 325 (M + 1, 100%), 163 [(M + 2)/2, 12%]. Anal. ($C_{18}H_{20}N_4O_2 \cdot HClO_4 \cdot 2H_2O$) C, H, N.

In Vitro Cytotoxicity Assays. Murine P388 leukemia cells, Lewis lung carcinoma cells (LL), and human Jurkat leukemia cells (JL_C), together with their amsacrine and doxorubicin-resistant derivatives (JL_A and JL_D, respectively), were obtained and cultured as described.[21] Growth inhibition assays were performed by culturing cells at 4.5×10^3 (P388), 10^3 (LL), and 3.75×10^3 (Jurkat lines) per well in microculture plates (150 mL per well) for 3 (P388) or 4 days in the presence of drug. Cell growth was determined by [3H]TdR uptake (P388)[22] or the sulforhodamine assay.[23] Independent assays were performed in duplicate.

In Vivo Tumor Assays. Colon 38 tumors were grown subcutaneously from 1 mm^3 fragments implanted in one flank of BDF1 mice (anesthetized with pentobarbitone, 90 mg/kg). When tumors reached a diameter of approximately 4−6 mm (7−8 days), mice were divided into control and drug treatment groups (five mice per group) with similar average tumor volumes in each group. Drugs were administered as solutions of the hydrochloride salts in distilled water (perchlorate salt for **7c**) and were injected intraperitoneally in a volume of 0.01 mL/g of body weight, using either single-dose or intermittent (q4d × 3 or qd2 × 2) schedules. The mice were monitored closely, and tumor diameters were measured with calipers three times a week. Tumor volumes were calculated as $0.52a^2b$, where a and b are the minor and major tumor axes and data are plotted on a semilogarithmic graph as mean tumor volumes (±SEM) versus time after treatment. The growth delay was calculated as the time taken for tumors to reach a mean volume 4-fold higher than their pretreatment volume.

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