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Synthesis of Substituted Indeno[1,2-*b*]quinoline-6-carboxamides, [1]Benzothieno[3,2-*b*]quinoline-4-carboxamides and 10*H*-Quindoline-4-carboxamides: Evaluation of Structure–Activity Relationships for Cytotoxicity

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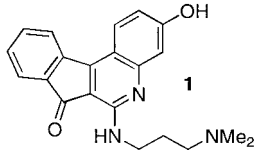
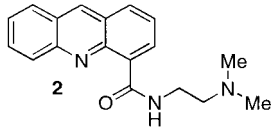
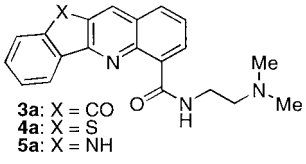
Abstract—New substituted indeno[1,2-*b*]quinoline-6-carboxamides, [1]benzothieno[3,2-*b*]quinoline-4-carboxamides and 10*H*-quindoline-4-carboxamides were prepared from methyl 2-amino-3-formylbenzoate by a new Friedlander synthesis. Evaluation of these carboxamides for cytotoxicity in a panel of cell lines showed that small lipophilic substituents in the non-carboxamide ring, in a pseudo-*peri* position to the side chain, significantly increased cytotoxic potency while retaining a pattern of cytotoxicity consistent with a non-topo II mode of action. The methyl-substituted indeno[1,2-*b*]quinoline-6-carboxamide demonstrated substantial effectiveness (20-day growth delays) in a sub-cutaneous colon 38 in vivo tumor model. This is comparable to that reported for the dual topo I/II inhibitor DACA that is in clinical trial.

Introduction

The neutral-chromophore DNA intercalators TAS 103 (**1**)[1,2] and DACA (**2**),[3,4] currently both in clinical trial as anticancer drugs,[5–7] are dual topoisomerase I/II (topo I/II) inhibitors, although the cytotoxicity of TAS 103 has recently been attributed entirely to its topo II effects.[8] We have previously reported[9–11] on the 11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamides (e.g., **3a**). These tetracyclic quinoline derivatives showed similar patterns of activity to DACA in a panel of topo II-deficient human leukemia lines.[9] They also had similar structure–activity relationships to DACA for ring substitution, with small lipophilic substituents (Me, Cl) on the carbon *peri* to the chromophore nitrogen being the most effective.[10] Substitution with small lipophilic groups at this position is known[3] to give analogues with tighter DNA binding; recent crystallographic studies[12,13] of drug/oligonucleotide complexes suggest this is probably through van der Waals interaction of the substituent with the cytosine forming the intercalation site.







Prompted by the good activity of **3a** and the above understanding of the substituent structure–activity relationships in this and related series, we have now investigated further 4-methyl and 4-methoxy analogues (**3b**–**3g**), exploring the effects of additional ring A substitution, in order to identify a candidate for possible further development. The beneficial effect of a 4-methyl substituent

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has also been evaluated in two closely-related chromophores; benzothieno[3,2-*b*]quinolines (**4a, 4b**) and quindolines (**5a, 5b**) with S and NR respectively in place of the carbonyl group in the B ring of **3a**.

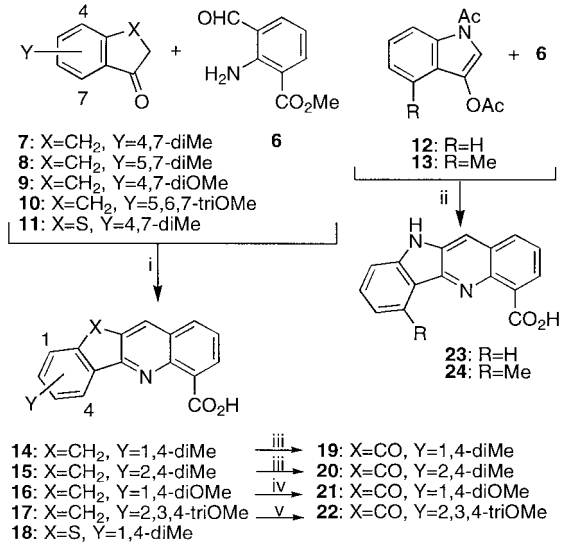
Results and Discussion

Chemistry

In previous studies,[9,10] the tetracyclic quinoline derivatives were constructed by Pfitzinger synthesis between isatin-7-carboxylic acid and the appropriate bicyclic ketone. Some shortcomings of this approach became evident: decarboxylation of the intermediate 10-carboxylic acids were successful only on a small scale, certain methoxy substituted compounds tended to demethylate during the decarboxylation process and formation of [1]benzothieno[3,2-*b*]quinoline analogues were low yielding under the strongly basic Pfitzinger conditions. In a significant improvement, our recent synthesis of methyl 2-amino-3-formylbenzoate[14] **6** allowed the tetracycles reported here to be prepared more successfully, by Friedlander condensation of **6** with indanone analogues under mild, base catalyzed conditions (Scheme 1). 1-Indanones **7**–**10**, a sulfur analogue **11** and precursors **12** and **13** of indole carbonyl compounds were all employed. In the preparation of the carbonyl containing compounds **19**–**22** from the methylene bridged precursors **14**–**17**, no single oxidant sufficed for all, and suitable agents were found by trial and error (see Experimental). The new amides of Table 1 were prepared by generating an intermediate imidazolide in situ from the precursor acids **18**–**24** and 1,1′-carbonyldiimidazole, and reacting this with *N,N*-dimethylethylenediamine (Scheme 2).

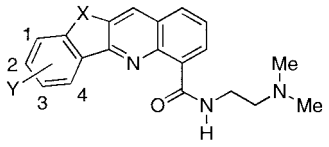
Structure–activity relationships for growth inhibition

The compounds were evaluated in culture using a panel of cell lines; murine P388 leukemia, murine Lewis lung carcinoma,[15] and three human leukemia (Jurkat) lines that have been described in detail previously.[16,17] JL_C is the wild-type (sensitive) line, JL_A is resistant to the topo II agents (85-fold resistant to amsacrine) because of a reduced level of topo II, and JL_D is similarly resistant (13-fold) to doxorubicin. IC_{50} values for the compounds in the P388, LLTC and JL_A lines are given in Table 1, together with ratios of IC_{50} values against JL_C and the other two Jurkat lines (ratios JL_A/JL_C and JL_D/JL_C). Values of these ratios of less than about 2-fold suggest a non-topo II mediated mechanism of action. Thus the dual topo I/II inhibitor DACA (**2**) has ratios of 1.9 and



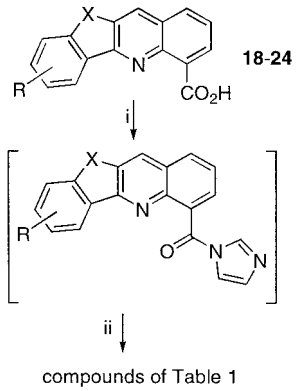
Scheme 1. (i) *t*-BuOK/*t*-BuOH (**14**–**16**), piperidine/EtOH (**18**); (ii) NaOH/aq. EtOH; (iii) $Na_2Cr_2O_7$/3 M H_2SO_4; (iv) SeO_2/dioxan; (v) $KMnO_4$/aq. Na_2CO_3.

Table 1. Cell growth inhibition data for indeno[1,2-*b*]quinoline-6-carboxamides



Compound	X	Y	IC_{50} (nM)[a] P388[b]	LL[c]	JL_C[d]	IC_{50} ratios JL_A/JL_C	JL_D/JL_C
3a[e]	CO	H	110	91	180	1.2	0.9
3b[f]	CO	4-Me	14	15	35	2.1	0.9
3c	CO	1,4-diMe	240	55	122	0.7	0.9
3d	CO	2,4-diMe	700	12	30	0.9	0.8
3e[f]	CO	4-OMe	23	23	71	2.2	0.8
3f	CO	1,4-diOMe	53	27	68	2.4	1.0
3g	CO	2,3,4-triOMe	250	64	118	1.0	0.9
4a[e]	S	H	46	66	170	1.5	1.6
4b	S	1,4-diMe	230	104	171	1.3	1.2
5a[e]	NH	H	370	290	450	1.0	1.1
5b	NH	4-Me	59	33	77	1.1	1.5
2[g]	DACA		98	189	580	1.9	2.3
3	TAS 103 (topo II)		2.5	1.5	5.4	56	75
Amsacrine (topo II)[h]			20	12	37	85	74
Doxorubicin (topo II)[h]			15	22	9.6	4.5	13
Camptothecin (topo I)			13	33	5.6	2.0	1.4

[a]IC_{50}; concentration of drug to reduce cell number to 50% of control cultures (see text).
[b]Murine P388 leukemia.
[c]Murine Lewis lung carcinoma.
[d]Human Jurkat leukemia.
[e]Data from ref 9.
[f]Data from ref 10.
[g]Data from ref 4.
[h]Data from ref 30.



Scheme 2. (i) CDI/dioxan; (ii) $H_2N(CH_2)_3NMe_2$.

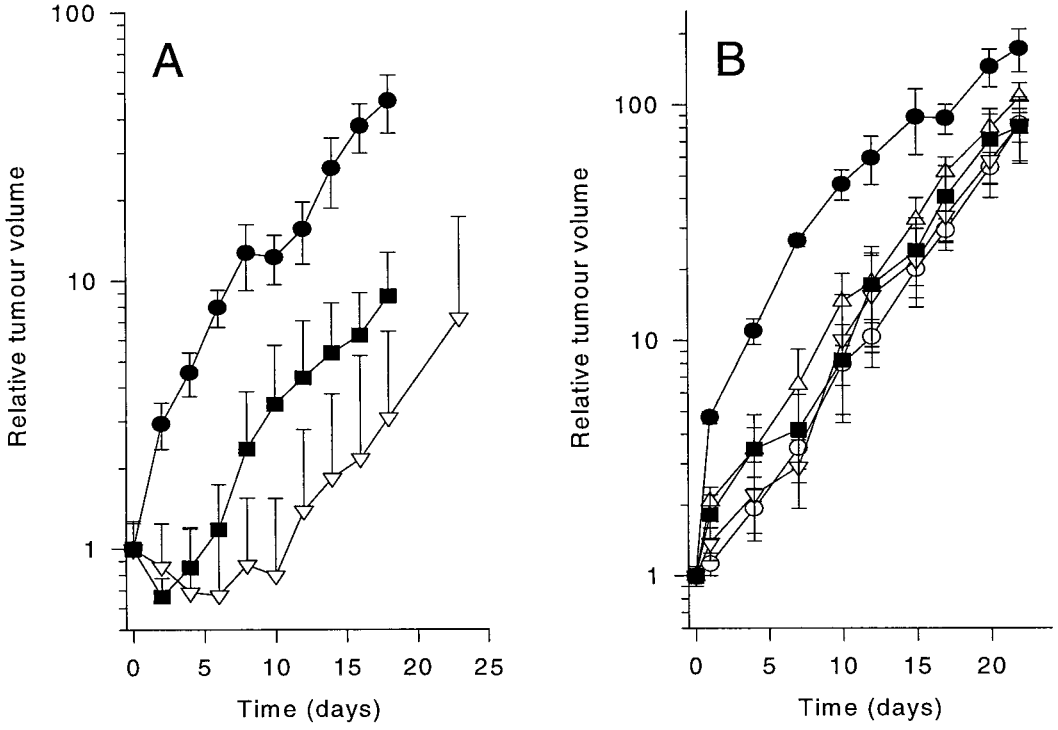


Figure 1. Effect of **3b** (A) and irinotecan (B) on the growth of colon 38 tumors. (A) Mice (with subcutaneously implanted tumors of approximately 4 mm diameter) were treated with either solvent as control (●), or with **3b** at doses of 30 mg/kg (■) or 45 mg/kg (▽) on an intermittent schedule (every 4 days×3). (B) Mice were treated as above with either solvent as control (●), or with irinotecan at doses of 20 (■), 30 (▽), 45 (△) or 65 (○) mg/kg.

2.3 respectively, while the "classical" topo I inhibitor camptothecin shows ratios of 2.0 and 1.4, and TAS 103 (recently[8] shown to functionally be a topo II inhibitor) has much larger ratios (56 and 75) (Table 1).

All of the compounds in Table 1 had similar potencies in both the wild-type and resistant Jurkat lines, suggesting a mode of action not primarily dependent on topo II. As reported previously,[10] substitution of the parent compound **3a** with a 4-methyl group (to give **3b**) resulted in substantially increased potency (5–8-fold) in all the cell lines. This parallels data reported for DACA analogues, where the (structurally equivalent) 5-substituted derivatives were more cytotoxic than DACA itself.[4] Compounds **3c** and **3d** explore the effects of substituents in addition to the 4-Me, and show that the 2,4-diMe (but not the 1,4-diMe) is broadly comparable. Similarly,[10] the 4-OMe (**3e**) was substantially more effective than **3a**, as was the 1,4-diOMe (**3f**) but not the 2,3,4-triOMe (**3g**) derivatives.

Compounds **4b** and **5b** were prepared to evaluate the consistency of the effect of a 4-methyl group in the related [1]benzothieno[3,2-*b*]quinoline-4-carboxamide and 10*H*-quindoline-4-carboxamide subclasses of heterocycles. The parent [1]benzothieno[3,2-*b*]quinoline (**4a**) is more potent than the indenoquinoline,[9] but the 1,4-diMe derivative **4b** was less effective (the 4-Me analogue could not be prepared). In the 10*H*-quindoline subseries, the 4-Me analogue **5b** was substantially more effective than the parent **5a**, but still not as effective as the 4-Me indeno[1,2-*b*]quinoline analogue **3b**.

In vivo studies

Because of the superior growth inhibitory properties of **3b**, this compound was selected for in vivo studies, to further gauge the value of the general class of indeno[1,2-*b*]quinoline-6-carboxamide dual topo I/II inhibitors. Figure 1(A) shows it has substantial effectiveness against subcutaneously implanted colon 38 tumors, with a 20-day growth delay when given at 45 mg/kg on an intermittent dose schedule (3 doses, 4 days apart). This is comparable to that reported for DACA (5.5 days at 65 mg/kg) using the same schedule.[18] The indenoquinoline **3a** is also active in this model, with a growth delay of 7 days at 90 mg/kg, but on a different, single-dose schedule.[9] The pure topo I inhibitor irinotecan gave a growth delay of ca 5 days using the 4 days×3 schedule, with little evidence of a dose-response over the dose range 65–20 mg/kg (Fig. 1(B)). This may be because irinotecan is metabolized in vivo to the active form SN-38.[19]

Conclusions

Substitution of indeno[1,2-*b*]quinoline-6-carboxamide (**3a**) and the related 11*H*-benzothieno[3,2-*b*]quinoline-6-carboxamide (**4a**) and 10*H*-quindoline-4-carboxamide (**5a**) at the pseudo-*peri* position to the carboxamide side chain provided analogues of significantly increased cytotoxic potency. The indeno[1,2-*b*]quinoline-6-carboxamide analogue (**3b**) also had substantial in vivo activity in the refractory colon 38 model. This substitu-

tion pattern seems generally beneficial in the general class of tetracyclic carboxamides.

Experimental

^{1}H NMR spectra were obtained at 300 MHz, in DMSO-d_6 unless stated otherwise, and are referenced to Me_4Si. In the listings, proton counts for aromatic protons (which have not been assigned) are given only for unresolved multiplets; the other aromatic signals are single proton doublets and triplets with $J = 6$–8 Hz, except the pyrido ring proton, a singlet. Microanalyses were performed at the Campbell Microanalytical Laboratory, University of Otago, New Zealand.

2,3,4-Trimethoxy-11*H*-indeno[1,2-*b*]quinoline-6-carboxylic acid[14] **17** and 4,7-dimethoxy-1-indanone[20] **9** were prepared as reported. 4,7-Dimethylbenzothiophen-3(*2H*)-one **11** was prepared by a literature procedure[21] (for benzothiophen-3(*2H*)-one) from 2,5-dimethylthiophenol in 59% overall yield, mp 81–86 °C (from light petroleum (bp 60–90 °C)). 3-Acetoxy-1-acetylindole **12** and 3-acetoxy-1-acetyl-4-methylindole **13**, mp 89–91 °C, were prepared by a general method[22] from the appropriate precursor *N*-(carboxymethyl)anthranilic acids, also available by a general procedure.[23]

5,7-Dimethyl-1-indanone (8). A mixture of dry dimethylamine hydrochloride (7.2 g), paraformaldehyde (5 g), 2,4-dimethylacetophenone (10 g) and concentrated HCl (2 mL) in ethanol (30 mL) was heated under reflux with vigorous stirring for 16 h, and the solvent was then removed under reduced pressure. The residue was treated with 10% aqueous Na_2CO_3 (100 mL) and extracted with ether (3×100 mL). The combined extracts were dried ($MgSO_4$) and the ether was removed under reduced pressure to give 3-(dimethylamino)-1-(2,4-dimethylphenyl)propan-1-one as a golden oil (12.75 g, 92%). This was then reacted as reported[24] for 7-methyl-1-indanone to give **8**, mp 77–78 °C (lit.[25] mp 76–77 °C), in 54% overall yield. ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H, CH_3), 2.54–2.65 (m, 5H), 3.10 (t, 2H, $J = 6$ Hz, CH_2), 6.89 (s), 7.05 (s).

4,7-Dimethyl-1-indanone (7). A solution of *p*-xylene (15 g) and propionyl chloride (10.4 g) was added over 10 min to a stirred and cooled (4 °C) mixture of $AlCl_3$ (10 g) in CS_2 (30 mL). A further portion of $AlCl_3$ (5 g) was then added over 5 min and the whole was immediately poured onto ice/water (100 mL), stirred for 1 h, then extracted with $CHCl_3$. The extract was washed with water, dried ($MgSO_4$), the solvent was removed, and the residue was distilled to give 2′,5′-dimethylpropiophenone (71%), bp 132 °C/20 mm Hg. A Willgerodt reaction (as for propiophenone[26] but with 16 h reflux) gave a mixture of 3-(2,5-dimethylphenyl)propanoic acid and 2,5-dimethylbenzoic acid. Recrystallization from light petroleum (bp 40–70 °C) removed the less soluble benzoic acid, and evaporation of the filtrate gave the propionic acid (36%), mp 45–46 °C (lit.[27] 45–46 °C). This was then converted to the acid chloride and cyclized with $AlCl_3$ to give **7** (92%), mp 77–79 °C (lit.[27] 78–79 °C).

6,9-Dimethyl[1]benzothieno[3,2-*b*]quinoline-4-carboxylic acid (18). To a solution of **6** (0.21 g) and **11** (0.18 g) in EtOH (10 mL) under a nitrogen atmosphere was added piperidine (0.08 g), and this solution was heated under reflux for a further 18 h. The solvent was removed under reduced pressure, and the residue was triturated with light petroleum (bp 40–70 °C). The solid which formed was filtered off (0.32 g), mixed with 10% aqueous NaOH (20 mL), heated under reflux for 5 h, then cooled and the solution was washed with $CHCl_3$ (20 mL). The aqueous layer was acidified with concentrated HCl to give **18** as a purple solid (0.24 g, 77%), mp > 295 °C (forms needles > 240 °C). ^{1}H NMR (DMSO-d_6/1 drop CF_3CO_2H) δ 2.53 (s, 3H, CH_3), 3.02 (s, 3H, CH_3), 7.39 (d), 7.51 (d), 7.86 (t), 8.42 (d), 8.61 (d), 9.36 (s).

2,4-Dimethyl-11*H*-indeno[1,2-*b*]quinoline-6-carboxylic acid (15). To a solution of **6** (0.5 g) and **8** (0.55 g) in *t*-BuOH (not dried) (5 mL) was added 0.35 M *t*-BuOK solution (20 mL). This was refluxed for 30 min, cooled and poured onto light petroleum (bp 40–70 °C). The solid which separated was filtered off, dissolved in water (20 mL) and acidified with concentrated HCl to give **15** as a pale orange solid (0.65 g, 81%), mp 199–201 °C. ^{1}H NMR δ 2.42 (s, 3H, CH_3), 2.81 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 7.19 (s), 7.41 (s), 7.78 (t), 8.35 (d), 8.55 (d), 8.71 (s), 16.9 (br s, 1 H, CO_2H).

1,4-Dimethyl-11*H*-indeno[1,2-*b*]quinoline-6-carboxylic acid (14). This was prepared (from **6** and **7**) as for **15**, as a pale orange solid (84%), mp 277–279 °C. ^{1}H NMR δ 2.32 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 3.87 (s, 2H, CH_2), 7.17 (d), 7.24 (d), 7.70 (t), 8.25 (d), 8.46 (d), 8.61 (s).

1,4-Dimethoxy-11*H*-indeno[1,2-*b*]quinoline-6-carboxylic acid (16). This was prepared (from **6** and **9**) as for **15**, as a pale orange solid (56%), mp 263–265 °C (dec.). ^{1}H NMR δ 3.87 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.01 (s, 2H, CH_2), 7.09 (d), 7.18 (d), 7.77 (t), 8.33 (d), 8.53 (d), 8.72 (s).

10*H*-Quindoline-4-carboxylic acid (23). To a solution of **6** (0.18 g, 1.0 mmol) and 3-acetoxy-1-acetylindole **12** (0.22 g, 1.0 mmol) in ethanol (10 mL) was added 10% sodium hydroxide solution (2 mL) and the mixture was refluxed, under nitrogen, for 1 h. After being cooled, the mixture was taken to pH 2 with concentrated HCl to give **23** (0.23 g, 88%), mp > 300 °C. ^{1}H NMR δ 7.37 (t), 7.66–7.79 (m, 3H), 8.37 (d), 8.50 (d), 8.53 (d), 8.70 (s), 12.0 (s, CO_2H).

6-Methyl-10*H*-quindoline-4-carboxylic acid (24). This was obtained in 80% yield (from **6** and 3-acetoxy-1-acetyl-4-methylindole **13**) as for **23** (reflux 30 min), mp > 300 °C. ^{1}H NMR δ 2.94 (s, 3H, CH_3), 7.09 (d), 7.45 (d), 7.57 (t), 7.69 (t), 8.43 (d), 8.49 (d), 8.59 (s), 11.95 (s, NH), 17.24 (br s, CO_2H).

2,4-Dimethyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxylic acid (20). To a boiling mixture of **15** (0.28 g) in 3 M H_2SO_4 (10 mL) was added, in small portions, a solution of $Na_2Cr_2O_7$ (0.70 g) in 3 M H_2SO_4 (13 mL). The whole was then heated and stirred for a further 3 h,

cooled, water (75 mL) was added and the solid which formed was filtered off to give **20** as a pale yellow solid (0.16 g, 54%), mp >295 °C. ^{1}H NMR δ 2.41 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 7.46 (s), 7.50 (s), 7.79 (t), 8.39 (d), 8.50 (d), 8.79 (s).

1,4-Dimethyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxylic acid (19). This was prepared (from **14**) as for **20**, as a pale yellow solid (64%) (with ca. 5% of **14**), mp >295 °C (with darkening >260 °C). ^{1}H NMR δ 2.52 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 7.28 (d), 7.41 (d), 7.74 (t), 8.34 (d), 8.46 (d), 8.69 (s).

1,4-Dimethoxy-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxylic acid (21). A mixture of **16** (0.2 g) and selenium (IV) oxide (0.2 g) in dioxan (10 mL) was heated under reflux, with vigorous stirring, for 1.5 h and then filtered while hot. The solvent was removed from the filtrate under reduced pressure. The residue was dissolved in $CHCl_3$, filtered, and the filtrate was washed with water, dried and evaporated to dryness. This left **21** as a yellow solid (0.12 g, 57%), mp 198–200 °C. ^{1}H NMR δ 3.91 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 7.30 (d), 7.48 (d), 7.80 (t), 8.38 (d), 8.56 (d), 8.75 (s).

2,3,4-Trimethoxy-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxylic acid (22). Indene **17** (0.2 g) was added to a solution of Na_2CO_3 (0.2 g) in water (20 mL) and stirred at 55 °C until dissolution was complete. Potassium permanganate (0.16 g) was added and the mixture was heated and stirred until a drop on filter paper showed no permanganate color (ca. 2 min). The mixture was filtered through Celite, which was washed with 10% Na_2CO_3, then water and the filtrate was acidified to pH 2 with concentrated HCl. The solid which formed was filtered off to give **22** as a yellow solid (0.08 g, 38%), mp 275–278 °C (formed needles at ca. 240 °C). ^{1}H NMR δ 3.93 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.03 (s, 3H, OCH_3), 7.33 (s), 7.79 (t), 8.38 (d), 8.57 (d), 8.77 (s), 16.37 (br s, 1 H, CO_2H).

Preparation of *N*-[2-(dimethylamino)ethyl]-2,3,4-trimethoxy-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide (3g): example of the amidation reaction. Oxo acid **22** (0.40 g, 1.09 mmol) and 1,1′-carbonyldiimidazole (0.5 g) in dry dioxan (20 mL) were heated under reflux until dissolution was complete. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (30 mL). The organic layer was washed twice with warm water (20 mL), and dried over $MgSO_4$. A solution of *N,N*-dimethylethylenediamine (0.12 g, 1.36 mmol) in CH_2Cl_2 (3 mL) was added and the whole was stirred at room temperature for 16 h then washed with 10% Na_2CO_3 solution (2×20 mL), warm water (2×20 mL), dried ($MgSO_4$) and the solvent was removed. Column chromatography of the residue (alumina; $CHCl_3$) gave **3g** (0.11 g, 24%), R_f = 0.31, mp 189–191 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 2.61 (s, 6H, $N(CH_3)_2$), 2.99 (m, 2H, CH_2N), 3.97–4.04 (m, 8H, OCH_3, CH_2NH), 4.19 (s, 3H, OCH_3), 7.19 (s), 7.60 (t), 7.93 (d), 8.29 (s), 8.80 (d), 11.6 (br s, 1H, NH). Anal. calcd for $C_{24}H_{25}N_3O_5 \cdot 0.25H_2O$: C, 65.5; H, 5.9; N, 9.6. Found: C, 65.4; H, 6.1; N, 9.5.

The following amides were made by this method:

***N*-[2-(Dimethylamino)ethyl]-1,4-dimethyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide (3c).** In 27% yield after chromatography (alumina; $CHCl_3$), R_f = 0.24, mp 196–198 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6H, $N(CH_3)_2$), 2.61–2.68 (m, 5H,CH_3, CH_2N), 2.87 (s, 3H, CH_3), 3.71 (q, 2H, J = 7 Hz, CH_2NH), 7.18 (d), 7.33 (d), 7.62 (t), 7.97 (d), 8.39 (s), 8.85 (d), 10.8 (br s, 1H, NH). Anal. calcd for $C_{23}H_{23}N_3O_2 \cdot 0.75H_2O$: C, 71.4; H, 6.3; N, 10.9. Found: C, 71.1; H, 5.8; N, 10.7.

***N*-[2-(Dimethylamino)ethyl]-2,4-dimethyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide (3d).** In 60% yield, mp 215–217 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 6H, $N(CH_3)$), 2.41 (s, 3H, CH_3), 2.61 (t, 2H, J = 7 Hz, CH_2N), 2.84 (s, 3H, CH_3), 3.71 (q, 2H, J = 7 Hz, CH_2NH), 7.26 (s), 7.48 (s), 7.62 (t), 7.92 (d), 8.35 (s), 8.82 (d), 10.8 (br s, 1H, NH). Anal. calcd for $C_{23}H_{23}N_3O_2$: C, 74.0; H, 6.2; N, 11.3. Found: C, 73.7; H, 6.3; N, 11.3.

***N*-[2-(Dimethylamino)ethyl]-1,4-dimethoxy-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide (3f).** In 21% yield after chromatography (alumina; $CHCl_3$:MeOH, 98:2), R_f = 0.66, mp 185–187 °C (softens 170 °C) (from $CHCl_3$/hexane). ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 6H, $N(CH_3)_2$), 2.98 (m, 2H, CH_2N), 3.9–4.0 (m, 5H, OCH_3, CH_2NH), 4.16 (s, 3H, OCH_3), 7.05 (d), 7.24 (d), 7.62 (t), 7.97 (d), 8.40 (s), 8.83 (d), 11.8 (br s, 1H, NH). Anal. calcd for $C_{23}H_{23}N_3O_4 \cdot H_2O$: C, 65.2; H, 6.0; N, 9.9. Found: C, 65.1; H, 5.8; N, 9.7.

***N*-[2-(Dimethylamino)ethyl]-6,9-dimethyl[1]benzothieno[3,2-*b*]quinoline-4-carboxamide (4b).** In 27% yield after chromatography (alumina; $CHCl_3$), R_f = 0.34, mp 192–194 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 6H, $N(CH_3)_2$), 2.57 (s, 3H, CH_3), 2.65 (t, 2H, J = 7 Hz, CH_2N), 3.16 (s, 3H, CH_3), 3.78 (q, 2H, J = 7 Hz, CH_2NH), 7.28 (d), 7.35 (d), 7.69 (t), 8.03 (d), 8.71 (s), 8.90 (d), 11.2 (br s, 1H, NH). Anal. calcd for $C_{22}H_{23}N_3OS \cdot 0.25H_2O$: C, 69.2; H, 6.2; N, 11.0. Found: C, 69.2; H, 6.0; N, 11.0.

***N*-[2-(Dimethylamino)ethyl]-6-methyl-10*H*-quindoline-4-carboxamide (5b).** Imidazolide formation was given 26 h and subsequent amination 18 h to afford the amide in 25% yield, mp 226–228 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 6H, $N(CH_3)_2$), 2.77 (s, 3H, CH_3), 2.98 (t, J = 5.8 Hz, 2H, CH_2), 3.89 (q, J = 5.8 Hz, 2H, CH_2), 6.46 (m, 1H), 6.77–6.79 (m, 2H), 7.31 (t), 7.54 (s), 7.57 (d), 8.57 (d), 11.12 (s, NH), 11.65 (br t, NH). Anal. calcd for $C_{21}H_{22}N_4O \cdot 0.25H_2O$: C, 71.9; H, 6.5; N, 16.1. Found: C, 71.6; H, 6.3; N, 16.1.

In vitro growth delay assays

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), were obtained and cultured as described.[16,17] Growth inhibition assays were performed by culturing cells at 4.5×10^3

(P388), 10^3 (LLTC), and 3.75×10^3 (Jurkat lines) per well in microculture plates (150 μL per well) for 3 (P388) or 4 days in the presence of drug. Cell growth was determined by [^{3}H]TdR uptake (P388)[28] or the sulforhodamine assay.[29] Independent assays were performed in duplicate, and coefficients of variation for all assays were $<20\%$.

In vivo colon 38 tumor assay

Colon 38 tumors were grown subcutaneously from 1 mm^3 fragments implanted in one flank of C57/Bl mice (anesthetized with pentobarbitone, 90 mg/kg). When tumors reached a diameter of approximately 4 mm (7–8 days), mice were divided into control and drug treatment groups (5 mice/group), with similar average tumor volumes in each group. Drugs were administered as solutions of the hydrochloride salts in distilled water, and were injected in a volume of 0.01 mL/g body weight in two equal injections administered 1 h apart. The mice were monitored closely and tumor diameters were measured with calipers three times a week. Tumor volumes were calculated as $0.52\times a^2\times b$, where a and b are the minor and major tumor axes, and data plotted on a semilogarithmic plot 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 four-fold higher than their pre-treatment volume.

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