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Positioning of the Carboxamide Side Chain in 11-Oxo-11*H*-indeno[1,2-*b*]quinolinecarboxamide Anticancer Agents: Effects on Cytotoxicity

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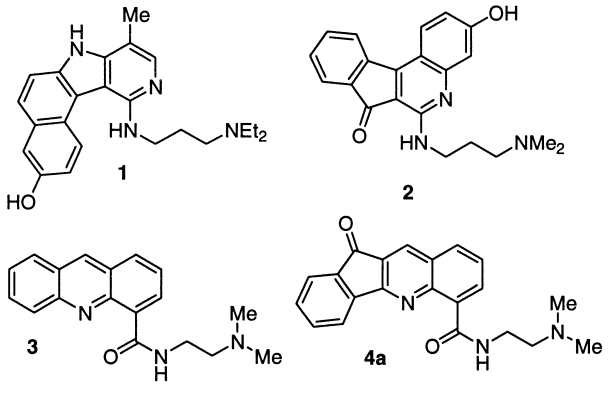
Abstract—A series of 11-oxo-11*H*-indeno[1,2-*b*]quinolines bearing a carboxamide-linked cationic side chain at various positions on the chromophore was studied to determine structure–activity relationships between cytotoxicity and the position of the side chain. The compounds were prepared by Pfitzinger synthesis from an appropriate isatin and 1-indanone, followed by various oxidative steps, to generate the required carboxylic acids. The 4- and 6-carboxamides (with the side chain on a terminal ring, off the short axis of the chromophore) were effective cytotoxins. The dimeric 4- and 6-linked analogues were considerably more cytotoxic than the parent monomers, but had broadly similar activities. In contrast, analogues with side chains at the 8-position (on a terminal ring but off the long axis of the chromophore) or 10-position (off the short axis of the chromophore but in a central ring) were drastically less effective. The 4,10- and 6,10-biscarboxamides had activities between those of the corresponding parent monocarboxamides. The first of these showed good activity against advanced subcutaneous colon 38 tumours in mice.

Introduction

Drugs that kill cells by stimulation of DNA cleavage induced by either topoisomerase I or topoisomerase II constitute one of the most important classes of anticancer drugs.[1,2] There has been recent interest in agents capable of dual inhibition of both topo I and topo II enzymes. This has been stimulated by observations that these enzymes are expressed at different absolute levels in different cell types,[3,4] and that a drop in the level of one enzyme (a mechanism of resistance to either topo I or topo II agents) is often accompanied by a rise in the level of the other.[5,6]

A number of relatively lipophilic, neutral chromophores bearing cationic side chains have been identified as dual inhibitors, including intoplicine[7,8] (**1**), TAS 103[9] (**2**) and DACA[10] (**3**), although recent reports have show that the cytotoxicity of TAS 103 comes primarily through inhibition of topo II.[11] We recently reported[12] that indenoquinoline-6-carboxamides (e.g., **4a**) were also potent cytotoxic agents, with patterns of cell line activity consistent with dual topo I/II inhibition, and with in vivo activity in colon 38 tumours. A later study[13] showed that small lipophilic substituents on the chromophore enhanced activity, in a structure–activity relationship pattern similar to that observed[14,15] with DACA.



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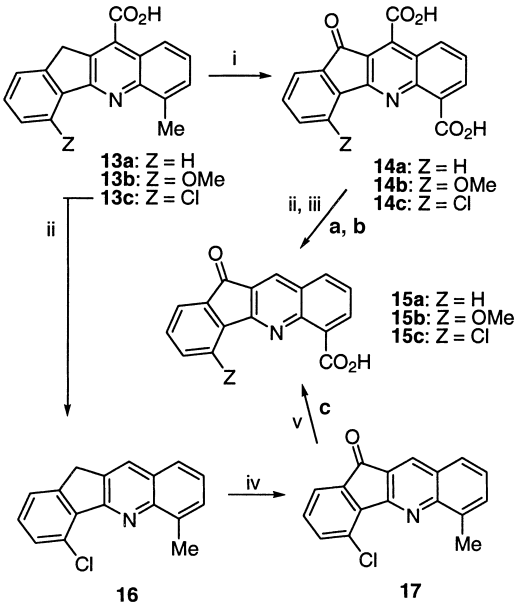
Studies of linear tricyclic DNA-intercalating agents have suggested that the positioning of the cationic side chain off the short axis of the chromophore is critical for good biological activity[16,17] (assumed to be related to the effect on DNA binding). This was strengthened by recent crystal structure studies of acridinecarboxamides bound to oligonucleotides, which showed very specific binding interactions for a correctly-positioned cationic side chain.[18,19]

In this paper we explore this hypothesis with the 11-oxo-11*H*-indeno[1,2-*b*]quinolinecarboxamides, with the synthesis and biological evaluation of a series of analogues in which the position of the cationic carboxamide side chain is varied.

Results and Discussion

Chemistry

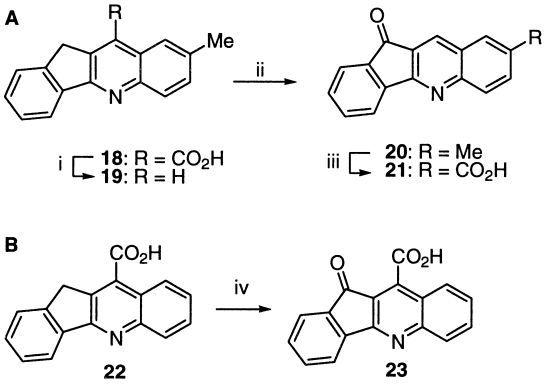
General entry to the tetracyclic system was by Pfitzinger synthesis from an appropriate isatin and 1-indanone. A carboxyl group in position 6 was accessed from isatin-7-carboxylic acid, while one in position 4 or 8 utilised later oxidation of a methyl group provided by 7-methyl-1-indanone and 5-methylisatin, respectively. The Pfitzinger reaction resulted in a 10-carboxyl group and this was subsequently retained for monoamide **7** and the diamides **8**–**11**. For the remaining precursor acids, thermal decarboxylation removed this function, though the detailed conditions depended on the particular compound (Schemes 1 and 2). Heating the high melting solids with a Bunsen flame in a cold finger sublimation apparatus was the method of choice, at a pressure which gave preferential sublimation of the decarboxylated product. The lower melting point of **14b** allowed for successful decarboxylation in boiling diphenyl ether.



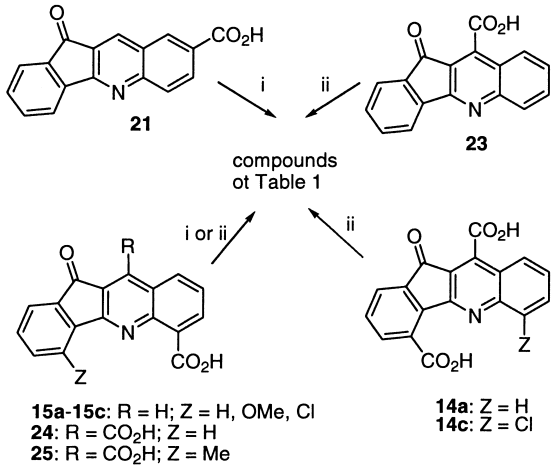
Scheme 1. (i) NiO_2; (ii) Heat ca. 300 °C; (iii) Ph_2O/260 °C; (iv) Na_2-Cr_2O_7/H_2SO_4; (v) Br_2/AcOH/hν.

Oxidation of the bridging CH_2 to carbonyl and, for the 4- and 8-acids, oxidation of a methyl group, was an integral part of the overall scheme. Many sets of conditions were tried, but no one set was generally applicable. Where the presence of a 10-CO_2H made the compound soluble in aqueous base, nickel peroxide was the reagent of choice, oxidising both the CH_2 and CH_3 groups simultaneously under mild conditions in good yield (e.g., **13c** to **14c**; Scheme 1). This latter compound, however, could not be satisfactorily decarboxylated and so a different route was required to access the mono acid **15c** (Scheme 3).

Compound **13c** could be decarboxylated (to **16**) and the CH_2 function was then oxidised with chromic acid. Attempts to also oxidise the CH_3 group with this and other strong oxidants destroyed the compound. Success was eventually achieved by photochemical bromination in acetic acid, presumably by way of an intermediate CBr_3 compound, which spontaneously hydrolysed under the conditions. In some runs, there was a suggestion of minor ring bromination also occurring. In contrast to the behaviour of **16** with chromic acid, sequential oxidation of CH_2 and CH_3 groups in **19** with variants of this reagent did give the oxoacid **21** in modest yield (Scheme 2A). The remaining example of CH_2 oxidation, **22** to **23** (Scheme 2B), was carried out by the permanganate method used previously.[13]



Scheme 2. (i) Heat ca. 300 °C; (ii) $Na_2Cr_2O_7$/3 M H_2SO_4; (iii) $Na_2Cr_2O_7$/concd H_2SO_4; (iv) $KMnO_4/Na_2CO_3$.



Scheme 3. (i) CDI, then amine; (ii) $SOCl_2$, then amine.

Amide formation from all compounds containing a 10-acid was carried out by reacting the appropriate acid chloride with *N*,*N*-dimethylethylenediamine. The other monoamides (and the bis amide **12**) were made by generating an intermediate imidazolide in situ from the precursor acid and 1,1-carbonyldiimidazole, and reacting this with the appropriate amine.

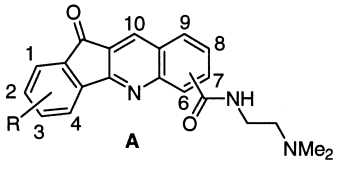
Structure–activity relationships for growth inhibition

Table 1 shows IC_{50} values for the compounds in a panel of cell lines in culture. As discussed previously,[12,20] P388 is a murine leukaemia, LLTC is a late-passage murine Lewis lung carcinoma, and the Jurkat lines are human leukaemias. JL_C is the wild-type (sensitive) line, while JL_A is resistant to the DNA intercalator amsacrine and similar agents (85-fold resistant to amsacrine) by virtue of a reduced level of topo II enzyme, and JL_D is a doxorubicin-resistant line, primarily by virtue of altered levels of topo II, but probably also by additional mechanisms.[21,22] IC_{50} values are given for the P388, LLTC and JL_C lines, together with ratios of IC_{50} values against JL_C and the other two Jurkat lines (JL_A/JL_C and JL_D/JL_C). All of the compounds showed values of these ratios of less than 2.2-fold; previous work[12] has shown this suggests a mechanism of action that involves both topo I and topo II.

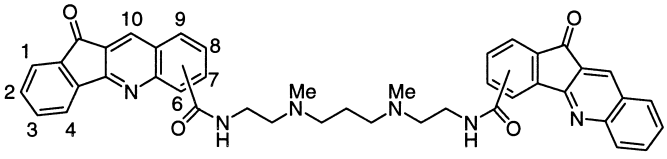
As reported previously,[13,23] the 6-carboxamide **4a** is a moderately potent cytotoxin, with IC_{50}s in the 90–180 nM range across the cell line panel, and its potency is markedly increased by small lipophilic substituents in the pseudo-*peri* 4-position to the carboxamide (**4b**–**d**). The corresponding unsubstituted 4-carboxamide (**5a**) showed similar cytotoxicity to **4a** (IC_{50} range 90–220 nM), and the 6-chloro analogue **5b**, substituted in the pseudo-*peri* 6-position, had moderately enhanced cytotoxicity. In this series the 6-OMe analogue (**5c**) proved slightly less potent. Thus positioning of the side chain on either terminal ring, off the short axis of the chromophore, made little difference to cytotoxicity.

In contrast, placement of the side chain at the 8-position, on a terminal ring but off the long axis of the chromophore (compound **6**), led to a drastic loss of potency (IC_{50} range 2500–7000 nM). Placement at the 10-position, off the short axis of the chromophore but in a central ring, was even less effective (compound **7**). Such a positioning effect has been observed previously with tricyclic chromophores (e.g., acridines[14] and phenazines.[24])

Table 1. Inhibition of cell growth by indeno[1,2-*b*]quinolinecarboxamides



A



B

No.	Fm	Side chain	R	IC_{50} (nM)[a]			Ratios	
				P388[b]	LL[c]	JL_C[d]	JL_A/JL_C	JL_D/JL_C
4a[e]	A	6	H	110	91	180	1.2	0.9
4b[e]	A	6	4-Cl	21	8.2	55	1.8	0.4
4c[e]	A	6	4-OMe	23	23	71	2.2	0.8
4d[e]	A	6	4-Me	14	15	35	2.1	0.9
5a	A	4	H	150	89	223	1.2	0.6
5b	A	4	6-Cl	26	63	213	1.4	0.6
5c	A	4	6-OMe	890	100	450	0.3	0.8
6	A	8	H	2500	2760	7020	0.9	1.1
7	A	10	H	10^4	> 5000	> 5000	NA	NA
8	A	4,10	H	4500	862	229	0.9	1.6
9	A	4,10	6-Cl	27	15	35	0.5	0.8
10	A	6,10	H	1010	254	159	0.7	1.0
11	A	6,10	4-Me	450	75	28	0.6	1.4
12	B	4	H	7.6	1.1	0.36	0.6	0.4
13[f]	B	6	H	22	2.7	0.35	0.3	0.4

[a]IC_{50}, concentration of drug to reduce cell number to 50% of control cultures (see text).
[b]Murine P388 leukaemia.
[c]Murine Lewis lung carcinoma.
[d]Human Jurkat leukaemia.
[e]Data from ref 13.
[f]Data from ref 26.

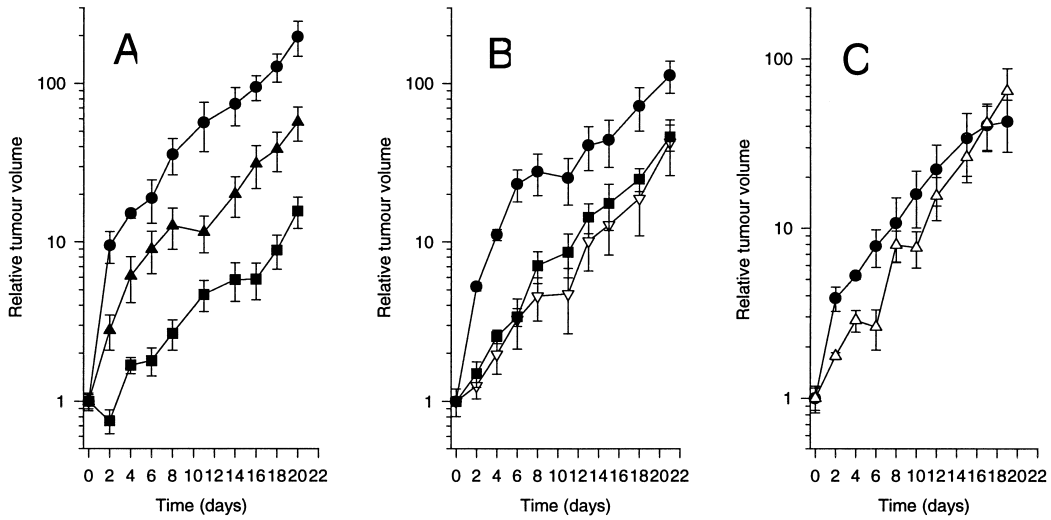


Figure 1. (A) Effect of **4b** on the growth of colon 38 tumours. Mice (with subcutaneously-implanted tumours of approximately 4 mm diameter) were treated with either solvent as a control (●), or with **4b** at doses of 45 mg/kg (■) or 30 mg/kg (▲) on an intermittent schedule (every 3 days×4). (B) Effect of **9** on the growth of colon 38 tumours under the same conditions. Control (●), **9** at doses of 20 mg/kg (■) and **9** at 30 mg/kg (▽). (C) Effect of **11** on the growth of colon 38 tumours under the same conditions. Control (●) and **11** at a dose of 30 mg/kg (△).

The drastic change in cytotoxicity with side chain positioning is most likely due to changes in the binding modes of these compounds to DNA, although other changes (e.g., in cellular uptake and distribution) cannot be ruled out. To investigate the competing effects of different side chains, the 4,10- and 6,10-biscarboxamides (**8** and **10**) were prepared. The potencies of these fell in between those of the parent monocarboxamides, suggesting some competion between the two side chains in orienting the chromophore (although other changes such as increased hydrophobicity may also be involved). Analogues of these (**9** and **11**) with small lipophilic groups at the pseudo-*peri* positions to the 4- or 6-carboxamides showed increased potency (remarkably so in the case of **9**). This further increase in potency may be due to further stabilisation of the "desired" chromophore orientation by the 4- or 6-carboxamide. In support of this, a recent crystal structure[25] of a 5-substituted acridinecarboxamide complexed with an oligonucleotide shows stabilising hydrophobic contacts.

A final comparison of the 4- and 6-positions was made by preparing the dimeric 4-linked analogue **12** and comparing it with the previously-reported[26] 6-linked analogue **13**. Both bis compounds were considerably more cytotoxic than the parent monomers (especially in the JL_C line), but had broadly similar absolute IC_{50}s.

In vivo studies

The parent 6-carboxamide **4a** has been evaluated previously[12] in the colon 38 tumour model in vivo, showing a 7-day growth delay on a single-dose schedule, while the 4-Me analogue **4d** showed a substantial 20-day growth delay using an intermittent 4-day×3 schedule.[23] Further proof of the utility of this configuration was shown by the 4-Cl analogue **4b**, that produced a growth delay of 12 days on the same schedule (Fig. 1). For further comparison of the two subseries, the most cytotoxic 4,10- and 6,10-dicarboxamides **9** and **11** were also evaluated in this model using the 4-day×3 schedule. Compound **9** proved more potent, but gave only moderate growth delay of 6 days at the maximum tolerated dose of 30 mg/kg (Fig. 1). Compound **11** had only minimal activity (3-day growth delay) at the maximum tolerated dose of 20 mg/kg.

Conclusions

The cytotoxicity of the tetracyclic compounds studied here depends critically on the positioning of the cationic side chain on the chromophore. Analogues with the side chain on a terminal ring, off the short axis of the chromophore, were effective, but those with the side chain in a central ring, or off the long axis of the chromophore, were not. This pattern has been noted previously with tricyclic but not tetracyclic compounds. It is consistent with the compounds binding to DNA as shown recently for acridinecarboxamides,[18,19] with the chromophore long axis parallel to the base pair long axis for maximum overlap, and the carboxamide side chain lying in the major groove.

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. In addition to the peaks listed, all monocarboxamides had a common pattern for the side chain: δ 2.4 (s, 6H, $N(CH_3)_2$), 2.7 (t, $J = 6$ Hz, 2H, CH_2N), 3.75 (q, $J = 6$ Hz, 2H, $NHCH_2$). Electro-

spray mass spectra were recorded on a VG Bio-Q triple quadrupole mass spectrometer, with water:MeOH:AcOH (50:50:1) as the mobile phase. Microanalyses were performed at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. 7-Methyl-1-indanone, 11-oxo-11*H*-indeno[1,2-*b*]quinoline-6,10-dicarboxylic acid (**24**) and 4-methyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6,10-dicarboxylic acid (**25**) were available from previous work.[13] 5-Methylisatin[27] was prepared as reported.

7-Chloroisatin. The intermediate oxime, prepared from *o*-chloroaniline by the standard method,[27] was added during 15 min to four times its weight of concentrated H_2SO_4 at 70 °C and the mixture was heated at 100 °C for 0.5 h. The solution was added to ice, extracted with $CHCl_3$, and the extract was dried and concentrated to give the isatin as an orange solid (70%), mp 183–185 °C (lit.[28] mp 190 °C).

7-Methoxyisatin. The intermediate oxime, prepared from *o*-anisidine by the standard method,[27] mp 118–120 °C, was added, with stirring, to seven times its weight of 85% H_2SO_4 maintained at 85 °C. After 15 min, the solution was poured onto brine and the resulting solid was filtered off and extracted with $CHCl_3$. This extract, and additional $CHCl_3$, were used to extract the aqueous filtrate (×3). The combined extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure to give the isatin (18%), mp 240–242 °C (lit.[29] mp 242–243 °C).

4-Methyl-11*H*-indeno[1,2-*b*]quinoline-10-carboxylic acid (13a). Isatin and 7-methyl-1-indanone were reacted in a Pfitzinger reaction for 7 h under the conditions reported previously (Method A[13]). The sodium salt separated from the cold reaction mixture. This was filtered off, dissolved in hot water, and the pH taken to 2 with concd HCl to give the free acid **13a** as a fawn solid (65%), mp 270–272 °C (dec). ^{1}H NMR δ 2.98 (s, 3H, CH_3), 4.22 (s, 2H, CH_2), 7.28 (d), 7.42 (t), 7.52 (d), 7.65 (t), 7.78 (t), 8.13 (d), 8.42 (d).

8-Methyl-11*H*-indeno[1,2-*b*]quinoline-10-carboxylic acid (18). 5-Methylisatin and 1-indanone were reacted as for **13a** to give **18** (95%) as a pale-yellow solid, mp >300 °C. ^{1}H NMR δ 2.53 (s, 3H, CH_3), 4.24 (s, 2H, CH_2), 7.49–7.56 (m, 2H), 7.63 (d), 7.70 (d), 8.05 (d), 8.12 (d), 8.19 (s).

11*H*-Indeno[1,2-*b*]quinoline-10-carboxylic acid (22). This was prepared as for **13a**, from isatin and 1-indanone, in 81% yield, as a pale-yellow solid, mp >300 °C. ^{1}H NMR δ 4.26 (s, 2H, CH_2), 7.5–7.6 (m, 2H), 7.65 (t), 7.71 (d), 7.79 (t), 8.15 (m, 2H), 8.44 (d).

6-Methoxy-4-methyl-11*H*-indeno[1,2-*b*]quinoline-10-carboxylic acid (13b). This was prepared as for **13a**, from reaction of 7-methoxyisatin and 7-methyl-1-indanone for 48 h, in 59% yield, as an orange solid, mp 202–204 °C. ^{1}H NMR δ 3.01 (s, 3H, CH_3), 4.04 (s, 3H, OCH_3), 4.20 (s, 2H, CH_2), 7.24–7.31 (m, 2H), 7.41 (t), 7.52 (d), 7.56 (d), 7.91 (d).

6-Chloro-4-methyl-11*H*-indeno[1,2-*b*]quinoline-10-carboxylic acid (13c). This was prepared from 7-chloroisatin and 7-methyl-1-indanone, as for **13a**, but the basic reaction mixture also contained 20% ethanol, reflux was continued for 48 h, and the solution was concentrated to half volume before being cooled to allow precipitation of the sodium salt. The free acid was subsequently obtained as an orange solid. This was twice extracted with chloroform and the insoluble material gave **13c** as a pale-yellow solid (62%), mp >300 °C. ^{1}H NMR δ 3.01 (s, 3H, CH_3), 4.24 (s, 2H, CH_2), 7.31 (d), 7.44 (t), 7.51 (d), 7.61 (t), 7.99 (d), 8.40 (d).

11-Oxo-11*H*-indeno[1,2-*b*]quinoline-4,10-dicarboxylic acid (14a). Compound **13a** (0.5 g) was added to a solution of NaOH (0.5 g) in water (40 mL) with stirring, at room temperature until the acid was dissolved. Nickel peroxide[30] (4.2 g) was then added and the mixture was stirred at room temperature for ca. 16 h. This was filtered through Celite, washed with warm 10% Na_2CO_3 and the combined filtrates acidified to pH 2 with concentrated HCl. The solid which formed was filtered to give **14a** as a fawn solid (0.19 g, 41%), mp 284–286 °C (with decarboxylation). ^{1}H NMR δ 7.75–7.85 (m, 2H), 7.95–8.10 (m, 3H), 8.13 (d), 8.30 (d).

6-Methoxy-11-oxo-11*H*-indeno[1,2-*b*]quinoline-4,10-dicarboxylic acid (14b). This was prepared from **13b** as for **14a,** as a yellow solid (43%), mp 272–274 °C. ^{1}H NMR δ 4.07 (s, 3H, OCH_3), 7.46 (d), 7.53 (d), 7.71 (t), 7.82 (t), 8.05 (d), 8.45 (d), 17.5 (br s, 1H, CO2H).

6-Chloro-11-oxo-11*H*-indeno[1,2-*b*]quinoline-4,10-dicarboxylic acid (14c). The oxidation of **13c** was carried out as for **14a**, but at 100 °C for 16 h, and the nickel peroxide was removed by filtration. The nickel filter cake was twice extracted with boiling dimethyl sulfoxide and the solvent was removed under reduced pressure. The residue was stirred with the filtrate from the nickel peroxide removal, and acidification gave **14c** as a yellow solid (61%), mp >300 °C. ^{1}H NMR δ 7.36 (t), 7.66 (t), 7.74–7.78 (m, 2H), 8.01 (d), 8.11 (d).

11-Oxo-11*H*-indeno[1,2-*b*]quinoline-10-carboxylic acid (23). Compound **22** (0.5 g) was added to a solution of Na_2CO_3 (0.5 g) in water (20 mL) with stirring, at 55 °C, until the acid was dissolved. Potassium permanganate (0.6 g) was then added and the mixture was heated and stirred for ca. 10 min (until a spot of reaction mixture on filter paper gave no pink colour), then filtered through Celite, 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 to give **23** as a pale-yellow solid (0.32 g, 62%), mp >300 °C (lit.[31] mp 340 °C). ^{1}H NMR δ 7.64 (t), 7.71 (t), 7.79–7.94 (m, 4H), 8.05 (d), 8.13 (d).

11-Oxo-11*H*-indeno[1,2-*b*]quinoline-8-carboxylic acid (21). To a mixture of **19** (0.6 g) and 3 M H_2SO_4 (12 mL) was added $K_2Cr_2O_7$ (0.9 g) and the whole was heated at 105 °C for 30 min, then poured onto ice and the crude solid **20** was filtered off. To this solid and 50% H_2SO_4

(12 mL) at 105 °C was added a solution of $K_2Cr_2O_7$ (2.3 g) in 3 M H_2SO_4 (6 mL). The mixture was heated for 20 min, then poured onto ice–water (150 mL) to give **21** as a yellow solid (0.33 g), mp >300 °C. ^{1}H NMR δ 7.66 (t) 7.79–7.84 (m, 2H), 8.04 (d), 8.11 (d), 8.25 (d), 8.75 (d), 8.80 (s).

6-Chloro-4-methyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline (17). To a stirred solution of **16** (0.8 g) in concentrated H_2SO_4 (15 g) was added, in small portions over 15 min, a solution of $Na_2Cr_2O_7$ (1.6 g) in 3 M H_2SO_4 (18 mL). Stirring was continued for a further 15 min and the whole was poured onto ice–water. The resulting precipitate was collected by filtration and washed thoroughly with hot water to give **17** as a pale-yellow solid (0.66 g, 78%), mp 176–180 °C. ^{1}H NMR δ 2.84 (s, 3H, CH_3), 7.44–7.57 (m, 4H), 7.93 (d), 8.00 (d), 8.55 (s).

6-Chloro-11-oxo-11*H*-indeno[1,2-*b*]quinoline-4-carboxylic acid (15c). To a mixture of **17** (0.25 g) and sodium acetate (2 g) in glacial acetic acid (25 mL) was added bromine (1.3 mL) and the whole was heated under reflux for 7 h using a 150 W tungsten lamp. The solvent was removed from the cooled solution under reduced pressure, water was added to the residue, and the solid was filtered off. This was dissolved in 5% NaOH, filtered to remove a minor amount of insoluble material, and the filtrate was acidified with concentrated HCl to give **15c** as an off-white solid (0.12 g, 44%), mp >300 °C. ^{1}H NMR δ 7.70–7.87 (m, 2H), 8.03–8.17 (m, 2H), 8.24 (d), 8.42 (d), 9.00 (s), 16.55 (br s, 1H, CO_2H).

11-Oxo-11*H*-indeno[1,2-*b*]quinoline-4-carboxylic acid (15a). The finely ground diacid **14a** (0.5 g) was placed in a cold finger sublimation apparatus at 0.5 mmHg and gently heated with a Bunsen flame until decarboxylation was complete (ca. 5 min). The sublimate which formed was collected to give **15a** as a pale-yellow solid (65%), mp >300 °C. ^{1}H NMR δ 7.68–7.85 (m, 2H), 7.90–8.05 (m, 3H), 8.18 (d), 8.30 (d), 8.83 (s).

6-Chloro-4-methyl-11*H*-indeno[1,2-*b*]quinoline (16). Compound **13c** at 160 mmHg was heated with a Bunsen flame. The sublimate (minor) and residue were extracted with hot ethanol/chloroform, a small amount of insoluble material was removed by filtration, and the solvent was removed in vacuo to give **16** as a yellow solid (96%), mp 176–178 °C. ^{1}H NMR δ 3.00 (s, 3H, CH_3), 4.06 (s, 2H, CH_2), 7.28 (d), 7.40 (t), 7.48–7.54 (m, 2H), 7.88 (d), 7.93 (d), 8.43 (s).

6-Methoxy-11-oxo-11*H*-indeno[1,2-*b*]quinoline-4-carboxylic acid (15b). A mixture of **14b** (0.2 g) in diphenyl ether (6 mL) was heated under gentle reflux for 5 h, then cooled, and light petroleum (bp 40–70 °C) was added. The solid which separated was filtered off and washed with hot light petroleum (bp 40–70 °C) to give **15b** as a brown solid (0.1 g, 57%), mp 269–271 °C. ^{1}H NMR δ 4.04 (s, 3H, OCH_3), 7.49 (d), 7.6–7.9 (m, 3H), 8.06 (d), 8.42 (d), 8.87 (s), 17.5 (br s, 1H, CO_2H).

8-Methyl-11*H*-indeno[1,2-*b*]quinoline (19). Compound **18** (0.3 g) was heated above its melting point at 760 mm Hg until decarboxylation ceased. The residue was extracted with chloroform, filtered and the solvent was removed under reduced pressure to give **19** as a brown solid (0.22 g, 88%), mp 127–129 °C. ^{1}H NMR δ 2.53 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 7.50–7.57 (m, 3H), 7.68 (d), 7.74 (s), 7.99 (d), 8.11 (d), 8.32 (s).

***N*-[2-(Dimethylamino)ethyl]-11-oxo-11*H*-indeno[1,2-*b*]-quinoline-4-carboxamide (5a).** Acid **15a** was reacted with 1,1-carbonyldiimidazole in dioxan, by the general method reported previously.[13] The intermediate imidazolide was not isolated but was reacted with *N,N*-dimethylethylenediamine and, after work up, the amide **5a** was obtained as a fawn solid (57%), mp 211–213 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ side chain plus 7.55–7.70 (m, 2H), 7.82 (t), 7.92 (d), 7.98 (d), 8.16 (d), 8.47 (s), 8.77 (d), 12.4 (s, 1H, NH). Anal. calcd for $C_{21}H_{19}N_3O_2$: C, 73.0; H, 5.6; N, 12.2. Found: C, 72.9; H, 5.6; N, 12.2%.

***N*-[2-(Dimethylamino)ethyl]-6-chloro-11-oxo-11*H*-indeno-[1,2-*b*]quinoline-4-carboxamide (5b).** This was prepared from acid **15c** as for **5a**, and column chromatography (alumina; chloroform:diethylamine, 99:1) of the crude product gave the amide **5b**, R_f 0.24, mp 172–174 °C (from toluene/light petroleum (bp 90–110 °C)), in 24% yield. ^{1}H NMR ($CDCl_3$) δ side chain plus 7.56 (t), 7.69 (t), 7.88 (d), 7.95 (d), 8.02 (d), 8.51 (s), 8.85 (d), 12.3 (br s, 1H, NH). Anal. calcd for $C_{21}H_{18}ClN_3O_2 \cdot 2.5H_2O$: C, 59.4; H, 5.5; N, 9.9. Found: C, 59.3; H, 5.0; N, 9.9%.

***N*-[2-(Dimethylamino)ethyl]-6-methoxy-11-oxo-11*H*-indeno[1,2-*b*]quinoline-4-carboxamide (5c).** This was prepared from acid **15b** as for **5a**, as a dark yellow solid (56%), mp 202–204 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ side chain plus 4.14 (s, 3H, OCH_3), 7.45–7.6 (m, 3H), 7.66 (t), 7.98 (d), 8.46 (s), 8.82 (d), 13.2 (br s, 1H, NH). Anal. calcd for $C_{22}H_{21}N_3O_3 \cdot 0.5H_2O$: C, 68.7; H, 5.8; N, 10.9. Found: C, 68.7; H, 5.5; N, 10.8%.

***N*-[2-(Dimethylamino)ethyl]-11-oxo-11*H*-indeno[1,2-*b*]-quinoline-8-carboxamide (6).** Reaction of *N,N*-dimethylethylenediamine with the crude imidazolide from **21** was carried out in dioxan at 80 °C overnight. Some of the product **6** separated during this time as a white solid, which was filtered off. The filtrate was evaporated at reduced pressure, and the residue was dissolved in chloroform and treated as for **5a** to give a second crop of product (75% combined), mp 231–233 °C (from MeCN/$CHCl_3$). ^{1}H NMR δ 7.66 (t), 7.80–7.85 (m, 2H), 8.06 (d), 8.13 (d), 8.21 (d), 8.58 (d), 8.65–8.70 (m, 2H). Anal. calcd for $C_{21}H_{19}N_3O_2$: C, 73.0; H, 5.5; N, 12.2. Found: C, 72.8; H, 5.7; N, 12.2%.

***N,N'*-Bis[2-(Dimethylamino)ethyl]-11-oxo-11*H*-indeno-[1,2-*b*]quinoline-6,10-dicarboxamide (10).** Diacid **24** (0.27 g) in thionyl chloride (3 mL) was heated at 80 °C for 1 h and the excess thionyl chloride was removed at 20 mmHg. Residual thionyl chloride was removed by azeotropic distillation with benzene and the residue was dissolved in dry CH_2Cl_2 (3 mL). The mixture was cooled to 0 °C and stirred, and *N,N*-dimethylethylenediamine (0.20 g) was added. After being stirred at room

temperature for 1 h, the solution was filtered and the filtrate was washed with 10% Na_2CO_3 solution, water, dried ($MgSO_4$) and the solvent removed to give **10** as a yellow solid (0.24 g, 63%), mp 194-196 °C (recrystallised twice from MeCN and once from EtOH). ^{1}H NMR ($CDCl_3$) δ 2.27–2.30 (m, 12H, NCH_3), 2.58 (t, 2H, CH_2N), 2.65 (t, 2H, CH_2N), 3.65 (q, 2H, CH_2NH), 3.74 (q, 2H, CH_2NH), 7.08 (br s, 1H, NH), 7.55–7.65 (m, 2H), 7.65 (t), 7.81 (d), 8.10 (d), 8.34 (d), 8.78 (d), 11.03 (br s, NH). Anal. calcd for $C_{26}H_{29}N_5O_3 \cdot H_2O$: C, 65.4; H, 6.4; N, 14.7. Found: C, 65.7; H, 6.8; N, 14.8%.

***N,N′*-Bis[2-(Dimethylamino)ethyl]-4-methyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6,10-dicarboxamide (11).** The dicarbonyl chloride from diacid **25** was treated with 3 mol equivalents of *N,N*-dimethylethylenediamine, as for **10**, to give diamide **11** as a tan solid (66%), mp 209–210 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 2.25–2.30 (m, 12H, NCH_3), 2.54 (t, 2H, CH_2N), 2.67 (t, 2H, CH_2N), 2.82 (s, 3H, CH_3), 3.60 (q, 2H, CH_2NH), 3.72 (q, 2H, CH_2NH), 7.1 (br s, 1H, NH), 7.38–7.50 (m, 2H), 7.61 (t), 7.68 (d), 8.10 (d), 8.76 (d), 10.5 (s, 1H, NH). Anal. calcd for $C_{27}H_{31}N_5O_3$: C, 68.5; H, 6.6; N, 14.8. Found: C, 68.0; H, 6.8; N, 14.8%.

***N*-[2-(Dimethylamino)ethyl]-11-oxo-11*H*-indeno[1,2-*b*]-quinoline-10-carboxamide (7).** This was prepared from **23** as for **10**. Some of the product separated as its hydrochloride, which was filtered off. Treatment of the filtrate as for **10** gave the rest as the free base (combined yield 74%), mp 180–182 °C. ^{1}H NMR ($CDCl_3$) δ side chain plus 7.46–7.51 (m, 3H), 7.63–7.75 (m, 3H), 7.92 (d), 8.03–8.08 (m, 2H). For analysis, a sample of the hydrochloride was recrystallised from ethanol, and had mp 163–165 °C. Anal. calcd for $C_{21}H_{19}N_3O_2 \cdot HCl \cdot H_2O$: C, 63.1; H, 5.5; N, 10.5. Found: C, 62.9; H, 5.7; N, 10.5%.

***N,N′*-Bis[2-(Dimethylamino)ethyl]-6-chloro-11-oxo-11*H*-indeno[1,2-*b*]quinoline-4,10-dicarboxamide (9).** This was prepared from **14c** as for **11**, in 43% yield, mp 182–184 °C (from MeCN). ^{1}H NMR δ 2.33 (s, 6H), 2.38 (s, 6H), 2.62–2.70 (m, 4H), 3.78 (q, 2H), 3.92 (q, 2H), 7.32 (t), 7.54 (t), 7.77–7.83 (m, 2H), 8.13 (d), 8.70 (d), 8.90 (br s, 1H, NH), 12.52 (br t, 1H, NH). Anal. calcd for $C_{26}H_{28}ClN_5O_3$: C, 63.2; H, 5.7; N, 14.2. Found: C, 63.1; H, 6.0; N, 14.5%.

***N,N′*-Bis[2-(Dimethylamino)ethyl]-11-oxo-11*H*-indeno-[1,2-*b*]quinoline-4,10-dicarboxamide (8).** This was prepared from **14a** as for **11**, in 71% yield, mp 195–198 °C (from MeCN). ^{1}H NMR δ 2.36 (s, 6H), 2.49 (s, 6H), 2.70 (t, 2H), 2.93 (t, 2H), 3.63 (q, 2H), 3.83 (q, 2H), 7.43 (t), 7.67 (t), 7.80–7.90 (m, 2H), 8.0 (br s, 1H, NH), 8.06 (d), 8.13 (d), 8.49 (d), 12.3 (br s, 1H, NH). Anal. calcd for $C_{26}H_{29}N_5O_3$: C, 68.0; H, 6.4; N, 15.2. Found: C, 67.7; H, 6.4; N, 15.2%.

***N,N′*-[(2-Aminoethyl)methylimino]di-3,1-propanediyl]bis-[11-oxo-11*H*-indeno[1,2-*b*]quinoline-4-carboxamide] (12).** This was prepared as for **5a** from acid **15a** and *N,N′*-bis(2-aminoethyl)-*N,N′*-dimethyl-1,3-propanediamine[26] (2:1 mol ratio). Column chromatography of the product (alumina; $CHCl_3$) gave the bisamide **12** (31%), R_f 0.11, mp 184–186 °C (from MeCN). ^{1}H NMR ($CDCl_3$) δ 1.7 (m, 2H, CH_2), 2.29 (s, 6H, NCH_3), 2.46 (m, 4H, NCH_2), 2.69 (m, 4H, NCH_2), 3.65 (m, 4H, CH_2NH), 7.48 (t, 2H), 7.59 (t, 2H), 7.70–7.80 (m, 4H), 7.90 (d, 2H), 8.00 (d, 2H), 8.32 (s, 2H), 8.72 (d, 2H), 12.2 (br s, 2H, NH). ESMS: *m/z* 703.1 (M + 1). Anal. calcd for $C_{43}H_{38}N_6O_4 \cdot 0.5H_2O$: C, 72.6; H, 5.5; N, 11.8. Found: C, 72.3; H, 5.4; N, 11.8%.

In vitro growth delay assays

Murine P388 leukaemia cells, Lewis lung carcinoma cells (LLTC), and human Jurkat leukaemia cells (JL_C), together with their amsacrine- and doxorubicin-resistant derivatives (JL_A and JL_D respectively), were obtained and cultured as described.[21,22] 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)[32] or the sulforhodamine assay.[33] Independent assays were performed in duplicate, and coefficients of variation for all assays were between 18 and 25%.

In vivo colon 38 tumour assay

All animal experiments were approved by the University of Auckland Animal Ethics Committee. Colon 38 tumours were grown subcutaneously from 1 mm^3 fragments implanted in one flank of mice (anaesthetised with pentobarbitone, 90 mg/kg). When tumours 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 tumour 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 tumour diameters were measured with callipers three times a week. Tumour volumes were calculated as $0.52\times a^2\times b$, where *a* and *b* are the minor and major tumour axes and data plotted on a semilogarithmic plot as mean tumour volumes (±SEM) versus time after treatment. The growth delay was calculated as the time taken for tumours to reach a mean volume 4-fold higher than their pre-treatment volume.

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