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Synthesis and Cytotoxic Activity of *N*-(2-Diethylamino)ethylcarboxamide and Other Derivatives of 10*H*-Quindoline

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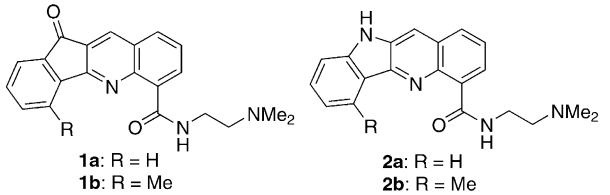
Abstract—A series of mono- and dimeric *N*-methylquindoline carboxamides were prepared by Friedlander condensation between methyl 2-amino-3-formyl benzoate and 3-acetoxy-1-acetylindoles, followed by exhaustive methylation with methyl iodide to give *N*-methylquindoline esters. Direct amination of these, or hydrolysis to the acids and amine coupling via intermediate imidazolides gave the desired carboxamides. The compounds were evaluated in a panel of cell lines in culture. The monomeric compounds showed similar structure–activity relationships to the known indeno[1,2-*b*]quinolines, with a 4-methyl group increasing potency several-fold. Bis analogues linked through the carboxamide were more cytotoxic than the corresponding monomers in the human leukemia lines, but N–N linked dimers were generally less potent, except for a tetracationic derivative. The most potent monomeric analogue showed moderate growth delay (ca. 5 days) against sub-cutaneously implanted colon 38 tumors in mice.

Introduction

In a study[1] of tetracyclic quinoline carboxamides as potential dual topoisomerase I/II inhibitors, we found that the 11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide **1a** was a moderately potent cytotoxin (IC_{50} 180 nM in the Jurkat human leukemia cell line). In later studies[2] we explored structure–activity relationships for ring substitution, and showed that small lipophilic groups at the 4-position (especially Me) significantly improved potency (IC_{50} 35 nM for the 4-Me analogue **1b** in the same leukemia line). Such substituents also confer a potency advantage on related analogues, particularly the quindoline-4-carboxamides (e.g., **2a** and **2b**).[3] Later work[4] on dimeric 11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamides, linked through the carboxamide groups, showed that further dramatic increases in potency could be achieved, with the piperazine-linked analogue **4a** showing an IC_{50} of 2.3 nM in the same line. Similar potency improvements have been seen with dimeric analogues of other neutral DNA-binding chromophores, including naphthalimides,[5] imidazoacridones,[6] acridines[7] and phenazines.[8]



The significantly greater cell line activity of **2b** (IC_{50} 70 nM in the same line)[3] when compared to **2a**, and the possibility of also linking through the indoline nitrogen to make dimers of different topology to the carboxamide-linked dimers, prompted us to extend the quindoline

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series to include *N*-methyl compounds and dimeric examples of both types of chromophore.

Results and Discussion

Chemistry

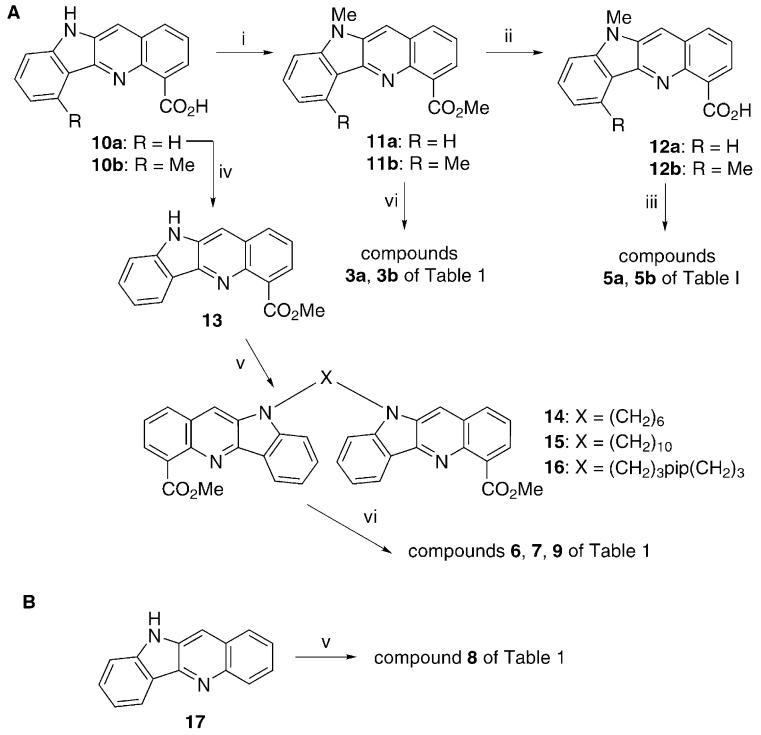
Access to the quindolinecarboxylic acids **10a** and **10b** was by Friedlander condensation between methyl 2-amino-3-formyl benzoate[9] and the appropriate 3-acetoxy-1-acetylindole, as reported elsewhere.[3] *N*-Methylation of the indole nitrogen in **10a** and **10b** was attempted by reaction of the preformed potassium salts with methyl iodide in DMSO. However, it was found that the carboxylate function was the more nucleophilic. Therefore, a double methylation was carried out (to **11a** and **11b**) and direct aminolysis of the esters with *N,N*-dimethylethylenediamine gave monoamides **3a** and **3b** (Scheme 1). This reaction was considerably slower for the 6-methyl compound **11b**.

Hydrolysis of the above esters gave acids **12a** and **12b**. The bis-amides **5a** and **5b** were made by generating an intermediate imidazolide in situ from these precursor acids and 1,1′-carbonyldiimidazole, and reacting this with a one-half equivalent of 1,4-bis(3-aminopropyl)piperazine. Both imidazolide formation and amination reaction were considerably slower with the 6-methyl compound **12b**. Conventional esterification of **10** gave **13**, and two of these moieties were linked through *N*-10 by reaction with the appropriate α,ω-dihalo compounds to give the diesters **14**–**16** (Scheme 1). Ester amination then gave the bis amides **6**, **8**, and **9**. The same linking through *N*-10 of 10*H*-quindoline **17** gave the example of a non-amide bis compound **7**. Throughout, electrospray mass spectra provided evidence for formation of the bis compounds.

Structure–activity relationships

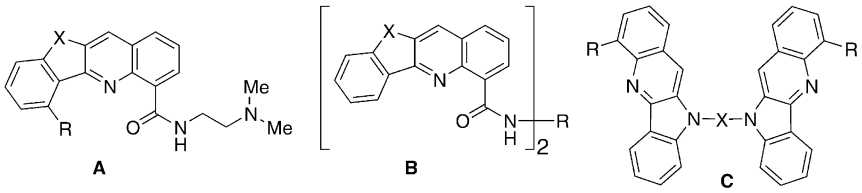
The compounds were evaluated in a panel of cell lines in culture, and the results are given in Table 1. P388 is a murine leukemia,[1,10] LLTC is a late-passage murine Lewis lung carcinoma, and the Jurkat lines are human leukemias. JL_C is the wild-type (sensitive) line, while JL_A is resistant (85-fold) to the DNA intercalator amsacrine and to similar agents by virtue of a reduced level of topo II enzyme. The JL_D line is doxorubicin-resistant line, primarily by virtue of altered levels of topo II, but probably also by additional mechanisms.[11,12] IC_{50} values are given for the P388, LLTC and JL_A 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). Values of these ratios of about unity (no resistance) suggest a primarily non-topo II mediated mechanism of action.

As noted above, 10*H*-quindolines (e.g., **2a** and **2b**) are somewhat less potent cytotoxins than the indeno[1,2-*b*]-quinolines (e.g., **1a** and **1b**), although they show similar potency-enhancing effects (ca. 5- to 10-fold) of a methyl substituent in a pseudo-*peri* position to the carboxamide. The *N*-methylquindoline (**3a**) has similar potency to **1a**, with a 4-Me group (in **3b**) having an equivalent potency-enhancing effect. These results suggest that the *N*-methyl-quindolines bind in a similar fashion to the indeno[1,2-*b*]quinolines, with some bulk tolerance at the indoline nitrogen position, consistent with a previous proposal for indeno[1,2-*b*]quinoline-6-carboxamides.[2]



Scheme 1. (i) MeI, KOH, DMSO; (ii) aq NaOH, reflux; (iii) CDI, dioxan, then $H_2N(CH_2)_3pip(CH_2)_3NH_2$; (iv) H_2SO_4, MeOH, reflux; (v) α,ω-dihalide, NaOH, dioxan; (vi) $H_2N(CH_2)_2NMe_2$.

Table 1. Inhibition of cell growth by quindoline-4-carboxamides



Compd	Fm	X	R	IC_{50} (nM)[a]			IC_{50} ratios	
				P388[b]	LL[c]	JL_C[d]	JL_A/JL_C	JL_D/JL_C
1a[e]	A	CO	H	109	91	180	1.2	0.9
1b[f]	A	CO	Me	13.5	15	35	2.1	0.9
2a[f]	A	NH	H	370	290	450	1.0	1.1
2b[g]	A	NH	Me	59	33	77	1.1	1.2
3a	A	NMe	H	77	140	230	1.4	1.4
3b	A	NMe	Me	20	18	55	1.2	1.4
4a[h]	B	CO	$(CH_2)_3NpipN(CH_2)_3$	40	2.4	2.3	0.3	0.4
5a	B	NMe	$(CH_2)_3NpipN(CH_2)_3$	4100	5.8	24	0.8	0.8
5b	B	NMe (6-Me)	$(CH_2)_3NpipN(CH_2)_3$	4000	14	21	0.34	0.29
6	C	$(CH_2)_6$	$CONH(CH_2)_2NMe_2$	$>2\times10^4$	360	840	0.7	1.2
7	C	$(CH_2)_{10}$	$CONH(CH_2)_2NMe_2$	$>2\times10^4$	750	4.5	0.8	1.3
8	C	$(CH_2)_3Npip(CH_2)_3$	H	1.6×10^4	2100	9900	0.4	1.1
9	C	$(CH_2)_3Npip(CH_2)_3$	$CONH(CH_2)_2NMe_2$	140	4.5	13.9	0.14	0.32
DACA[i]				98	189	580	1.9	2.3

[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 1.
[f]Data from ref 2.
[g]Data from ref 3.
[h]Data from ref 4.
[i]Data from ref 10.

Many monomeric agents, such as DACA, show JL_A/JL_C and JL_D/JL_C ratios of ca. 2 (Table 1), suggesting that topo II poisoning is at least partially responsible for cytotoxicity[10] (type I agents). However, all of the monomeric quindolines showed very low discrimination between both P388 (high topo II level) and LLTC (moderate topo II level), and also between JL_C (moderate topo II) and JL_A or JL_D (low topo II). The independence of the cytotoxicity of these compounds (type 2 agents) on topo II levels suggests this may not be a target.

This observation prompted us to consider bis analogues linked through this position. As noted above,[5–7] large increases in potencies have been observed with dimeric analogues of a number of neutral DNA-binding chromophores, including indeno[1,2-*b*]quinoline-6-carboxamides (**4a**). The 'conventional' (carboxamide-linked) bis(quindoline) **5a** was prepared and was less potent than **3a** in P388, but more potent than **3a** in the human leukemia lines. The corresponding bis(4-methylquindoline) **5b** had a broadly similar pattern of cytotoxicity. Linking of the quindoline-4-carboxamide chromophore through the indoline nitrogen with C6 and C10 polymethylene chains (N–N distances 7.9 and 12.5 Å, respectively, for the fully extended forms), gave the bis analogues **6** and **7**. Both compounds were poorly active in the P388 and Lewis lung lines, but the C10 was unexpectedly more active in the human leukemia lines. In contrast to the monomers, the cytotoxicities of the dimeric quindolines are inversely proportional to the level of topo II in the cells, with the P388 (high topo II) being the most resistant, and JL_A and JL_D (low topo II) being the most sensitive (compounds **4a** and **5a**). Other dimeric compounds have been shown to also have such 'type 3' behavior,[7,8] which remains unexplained.

Compounds **8** and **9** employ the cationic bis[$(CH_2)_3$]piperazine linker. This has an extended length of 12.6 Å (equivalent to the C10 polymethylene). Compound **8**, lacking the 6-carboxamide side chains, was non-toxic, demonstrating the importance of the latter, presumably in making DNA binding contacts. In contrast, the analogue (**9**), with these side chains, was among the most active analogues.

In vivo studies

The 4-methyl derivative **3b**, the most cytotoxic monomer across the cell line panel, was evaluated in vivo against sub-cutaneously implanted colon 38 tumors in mice, using an intermittent dose schedule (three doses, 4 days apart; q4d×3). At the maximum tolerated dose of 65 mg/kg/day (determined by testing at a range of doses), **3b** showed a modest growth delay of about 5 days. This is comparable to that reported for DACA (5.5 days at 65 mg/kg) using the same schedule.[13]

Conclusions

In this series of tetracyclic quindoline carboxamides, methylation of the quindoline NH is acceptable, not markedly affecting either the potencies or the pattern cytotoxcity of the compounds. This modification may thus be useful for improving the bioavailability of these compounds. The monomeric quindolines discriminate poorly between cells with varying levels of topo II, but the dimers have cytotoxicities inversely proportional to topo II levels.

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. Electrospray 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. 10*H*-Quindoline[14] **17**, 10*H*-quindoline-4-carboxylic acid[3] **10a**, and 6-methyl-10*H*-quindoline-4-carboxylic acid[3] **10b** were prepared as reported.

Methyl 10-methyl-10*H*-quindoline-4-carboxylate (11a). Powdered KOH (0.37 g) was added to a solution of **10a** (0.45 g) in dry DMSO (5 mL). To this was added MeI (1.0 g) and the whole was stirred at room temperature for 48 h. Water (40 mL) was added and the mixture was extracted with benzene (3×10 mL). The combined extracts were washed with water, and the solvent was removed to give a yellow residue (0.5 g). Crystallization from toluene gave **11a** as a yellow solid (0.23 g, 46%), mp 165–166 °C. ^{1}H NMR δ 3.87 (s, 3H), 4.13 (s, 3H), 7.31 (t), 7.38 (d), 7.51 (t), 7.64 (t), 7.93–8.08 (m, 3H) 8.06 (d), 8.51 (d).

Methyl 6,10-dimethyl-10*H*-quindoline-4-carboxylate (11b). This was prepared from **10b**, as for **11a**, in 55% yield, mp 183–185 °C. ^{1}H NMR ($CDCl_3$) δ 3.16 (s, 3H, 6-CH_3), 3.83 (s, 3H, NCH_3), 4.11 (s, 3H, OCH_3), 7.08 (d), 7.21 (d), 7.48–7.53 (m, 2H), 7.84 (s), 7.90 (d), 8.02 (d).

Methyl 10*H*-quindoline-4-carboxylate (13). A solution of **10a** (1.05 g, 4.0 mmol) in methanol (60 mL) and sulfuric acid (4 mL) was heated under reflux for 48 h. Most of the solvent was removed at reduced pressure, water was added and the pH was taken to 12 with 10% sodium hydroxide. The solution was extracted with chloroform, and the extract was dried and the solvent was removed at reduced pressure to give **13** as a yellow solid (0.74 g, 67%), mp 184–186 °C (from ethyl acetate). ^{1}H NMR ($CDCl_3$) δ 4.1 (s, 3H, CH_3), 7.25 (t), 7.33 (d), 7.44–7.55 (m, 2H), 7.86 (s), 7.92–7.95 (m, 2H), 8.26 (br s, 1H, NH), 8.44 (d).

10-Methyl-10*H*-quindoline-4-carboxylic acid (12a). Ester **11a** (0.44 g) in 5% sodium hydroxide solution (19 mL) and ethanol (1 mL) was heated under reflux for 3 h. The solution was cooled, acidified with concentrated HCl and the solid which separated was collected by filtration to give **12a** as a yellow solid (0.35 g, 83%) mp > 300 °C. Its low solubility in DMSO resulted in a very poorly resolved ^{1}H NMR spectrum.

6,10-Dimethyl-10*H*-quindoline-4-carboxylic acid (12b). This was prepared from **11b** as for **12a**, in 90% yield, mp > 300 °C (from ethanol). ^{1}H NMR (DMSO-d_6) δ 2.95 (s, 3H, 6-CH_3), 3.96 (s, 3H, 10-CH_3), 7.17 (d), 7.59 (d), 7.67 (t), 7.77 (t), 8.43 (d), 8.51 (d), 8.77 (s), 17.10 (br s, OH).

Dimethyl 10,10′-[hexane-1,6-diyl]bis[10*H*-quindoline-4-carboxylate] (14). A mixture of **13** (0.28 g, 1.0 mmol) and sodium hydride (0.09 g, 60% dispersion in mineral oil, 2.25 mmol) in dry dioxan was heated under reflux for 1 h. 1,6-Dibromohexane (0.12 g, 0.5 mmol) was added and the whole was heated under reflux for a further 20 h. The dioxan was removed at reduced pressure, water was added to the residue and the solution was extracted with chloroform. The extract was dried and the solvent was removed to give **14** as a yellow solid (0.2 g, 63%), mp 229–231 °C (from ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$) δ 1.33 (br s, 2H), 1.80 (br s, 2H), 4.11 (s, 3H, OCH_3), 4.23 (t, $J = 7.0$ Hz, 2H, NCH_2), 7.26–7.31 (m, 2H), 7.47–7.59 (m, 2H), 7.80 (s), 7.91–7.97 (m, 2H), 8.51 (d).

Dimethyl 10,10′-[decane-1,10-diyl]bis[10*H*-quindoline-4-carboxylate] (15). This was obtained from **13** and 1,10-dibromodecane, as for **14**, as a yellow oil (87%). Further extraction with hot light petroleum (bp 90–110 °C) left the insoluble product as a yellow gum sufficiently pure for further reaction.

Dimethyl 10,10′-[piperazine-1,4-diylbis(propane-3,1-diyl)]bis[10*H*-quindoline-4-carboxylate] (16). This was obtained from **13** and 1,4-bis(3-chloropropyl)piperazine dihydrochloride[15] as for **14**, but with 7.5 mol equivalents of sodium hydride and a reflux period of 7 days, as an oil (80%), and used without further purification.

10,10′-[Piperazine-1,4-diylbis(propane-3,1-diyl)]bis[10*H*-quindoline] (8). This was obtained from **17** and 1,4-bis(3-chloropropyl)piperazine dihydrochloride, as for **16**, as an oil (33%) which was further purified by column chromatography (alumina, chloroform, R_f 0.6). ^{1}H NMR ($CDCl_3$) δ 2.01–2.10 (m, 2H), 2.28–2.33 (m, 2H), 2.38–2.51 (br s, 4H), 4.40 (t, $J = 6.3$ Hz, 2H), 7.31 (t), 7.44–7.52 (m, 2H), 7.57–7.65 (m, 2H), 7.89 (d), 8.03 (s), 8.31 (d), 8.54 (d); ^{13}C NMR ($CDCl_3$) δ 25.5 (CH_2), 40.4 (CH_2), 53.0 (CH_2), 54.6 (CH_2), 108.9 (CH), 111.3 (CH), 119.6 (CH), 121.7 (C), 122.1 (CH), 125.3 (CH), 126.2 (CH), 126.8 (C), 127.1 (CH), 129.2 (CH), 129.6 (CH), 133.8 (C), 144.0 (C), 144.4 (C), 146.0 (C). ESMS: *m*/*z* 603.6 (28%) (M + 1), 302.4 (100%) [(M + 2)/2]. A yellow perchlorate salt had mp 188–190 °C (from water). Anal. calcd for $C_{40}H_{38}N_6 \cdot 3HClO_4 \cdot 2H_2O$: C, 51.1; H, 4.8; N, 8.9. Found: C, 51.4; H, 4.9; N, 9.1%.

***N*-[2-(Dimethylamino)ethyl]-10-methyl-10*H*-quindoline-4-carboxamide (3a).** A solution of ester **11a** (0.21 g) and *N,N*-dimethylethylenediamine (0.67 g) in dry 1-propanol (10 mL) was heated under reflux in a nitrogen atmosphere for 48 h. The propanol was removed at reduced pressure and the residue was dissolved in dichloromethane, washed with 10% Na_2CO_3 (×2), H_2O (×2), dried, and the solvent removed at reduced pressure to give **3a** as a yellow solid (0.24 g, 95%), mp 151–153 °C [from toluene/light petroleum (bp 90–110 °C)]. ^{1}H NMR δ 2.59 (s, 6H, $N(CH_3)_2$), 2.88 (m, 2H), 3.89 (s, 3H, CH_3), 3.88–3.98 (m, 2H), 7.36 (t), 7.44 (d), 7.62 (t), 7.69 (t), 8.00 (s), 8.07 (d), 8.63 (d), 8.80 (d), 12.03 (br s, NH). Anal. calcd for $C_{21}H_{22}N_4O$: C, 72.8; H, 6.4; N, 16.2. Found: C, 72.8; H, 6.6; N, 16.2%.

***N*-[2-(Dimethylamino)ethyl]-6,10-dimethyl-10*H*-quindoline-4-carboxamide (3b).** This was prepared from **11b**, as for **3a**, except that dioxan was used as solvent and reflux was for 7 days, in 44% yield, mp 144–146 °C (from acetonitrile). ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H, $N(CH_3)_2$), 2.64 (t, J = 6.7 Hz, 2H, CH_2), 2.92 (s, 3H, CH_3), 3.67 (s, 3H, CH_3), 3.72 (q, J = 6.7 Hz, 2H, CH_2), 7.03 (d), 7.17 (d), 7.48–7.55 (m, 2H), 7.68 (s), 7.90 (d), 8.72 (d), 11.52 (br t, NH). Anal. calcd for $C_{22}H_{24}N_4O{\cdot}0.25H_2O$: C, 72.4; H, 6.7; N, 15.3. Found: C, 72.8; H, 6.4; N, 15.4%.

10,10′-[Hexane-1,6-diyl]bis[*N*-[2-(dimethylamino)ethyl]-10*H*-quindoline-4-carboxamide] (6). A solution of bis ester **14** (0.1 g, 0.16 mmol) and *N,N*-dimethylethylenediamine (0.6 g, 6.8 mmol) in dry dioxan (10 mL) was heated under reflux for 4 days in a nitrogen atmosphere. The solvent was removed at reduced pressure and the residue was dissolved in chloroform. The organic solution was washed with water, dried and the solvent was removed to give **6** (0.09 g, 76%), mp 217–219 °C (from acetonitrile). ^{1}H NMR ($CDCl_3$) δ 1.37 (br s, 2H, CH_2), 1.85 (br s, 2H, CH_2), 2.43 (s, 6H, NCH_3), 2.73 (t, J = 6.0 Hz, 2H, NCH_2), 3.82 (q, J = 6.0 Hz, 2H, NCH_2), 4.26 (t, J = 6.8 Hz, 2H, NCH_2), 7.28–7.33 (m, 2H), 7.56–7.62 (m, 2H), 7.90 (s), 7.96 (dd, J = 8.2, 1.2 Hz, 1H), 8.62 (d), 8.80 (dd, J = 7.3, 1.2 Hz, 1H), 11.92 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.1 (CH_2), 28.6 (CH_2), 37.9 (NCH_2), 43.0 (NCH_2), 45.5 (NCH_3), 58.5 (NCH_2), 108.8 (CH), 112.3 (CH), 119.8 (CH), 121.4 (C), 122.6 (CH), 124.9 (CH), 126.9 (C), 128.5 (C), 130.2 (CH), 131.0 (CH), 131.2 (CH), 132.8 (C), 141.2 (C), 144.6 (C), 166.6 (C). ESMS: m/z 747 (20%) (M + 1), 374.4 (100%) [(M + 2)/2]. Anal. calcd for $C_{46}H_{50}N_8O_2{\cdot}0.5H_2O$: C, 73.1; H, 6.8; N, 14.8; Found: C, 72.8; H, 6.5; N, 14.8%.

10,10′-[Decane-1,10-diyl]bis[*N*-[2-(dimethylamino)ethyl]-10*H*-quindoline-4-carboxamide] (7). This was obtained from **15**, as for **6**, in 67% yield as a yellow solid, mp 187–190 °C (from acetonitrile). ^{1}H NMR ($CDCl_3$) δ 1.15–1.35 (m, 6H, CH_2), 1.83 (m, 2H, CH_2), 2.44 (s, 6H, NCH_3), 2.75 (t, J = 6.0 Hz, 2H, NCH_2), 3.83 (q, J = 6.0 Hz, 2H, NCH_2), 4.29 (t, J = 7.0 Hz, 2H, NCH_2), 7.32 (t), 7.41 (d), 7.58–7.67 (m, 2H), 7.96 (s), 8.03 (dd, J = 8.4, 1.0 Hz, 1H), 8.64 (d), 8.81 (dd, J = 7.2, 1.0 Hz, 1H), 11.96 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.2 (CH_2), 28.6 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 37.9 (NCH_2), 43.2 (NCH_2), 45.5 (NCH_3), 58.6 (NCH_2), 108.9 (CH), 112.4 (CH), 119.7 (CH), 121.4 (C), 122.6 (CH), 124.9 (CH), 126.9 (C), 128.5 (C), 130.2 (CH), 131.0 (CH), 131.3 (CH), 132.9 (C), 141.1 (C), 144.7 (C), 166.6 (C). ESMS: m/z 803.5 (20%) (M + 1), 402.4 (100%) [(M + 2)/2]. Anal. calcd for $C_{50}H_{58}N_8O_2{\cdot}H_2O$. C, 73.1; H, 7.4; N, 13.6; Found: C, 73.3; H, 7.4; N, 12.9%.

10,10′-[Piperazine-1,4-diylbis(propane-3,1-diyl)]bis[*N*-[2-(dimethylamino)ethyl]-10*H*-quindoline-4-carboxamide] (9). This was obtained from **16**, as for **6**, as an oil (36%) which was purified by column chromatography (alumina, chloroform, R_f 0.6). ^{1}H NMR ($CDCl_3$) δ 2.07 (m, 2H, CH_2), 2.25 (m, 2H, NCH_2), 2.35–2.39 (m, 4H, NCH_2), 2.44 (s, 6H, NCH_3), 2.75 (t, J = 6.0 Hz, 2H, NCH_2), 3.83 (q, J = 6.0 Hz, 2H, NCH_2), 4.42 (t, J = 5.8 Hz, 2H, NCH_2), 7.32 (t), 7.49 (d), 7.55–7.64 (m, 2H), 7.99 (d), 8.10 (s), 8.62 (d), 8.78 (d), 11.98 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 25.4 (CH_2), 37.9 (CH_2), 40.4 (CH_2), 45.5 (NCH_3), 53.1 (CH_2), 54.5 (CH_2), 58.6 (CH_2), 109.2 (CH), 112.9 (CH), 119.8 (CH), 121.4 (C), 122.5 (CH), 124.8 (CH), 126.9 (C), 128.4 (C), 130.2 (CH), 130.9 (CH), 131.2 (CH), 133.1 (C), 141.1 (C), 144.6 (C), 166.7 (C). ESMS m/z 831.6 (18%) (M + 1), 416.4 (100%) [(M + 2)/2]. A hydrated pentaperchlorate salt was made in ethanol and obtained as a yellow solid, mp 228–230 °C (from water). Acceptable microanalytical figures could not be obtained.

***N,N′*-[Piperazine-1,4-diylbis(propane-3,1-diyl)]bis-10-methyl-10*H*-quindoline-4-carboxamide (5a).** A mixture of **12a** (0.34 g, 1.23 mmol) and 1,1′-carbonyldiimidazole (0.52 g, 3.2 mmol) in dry dioxan (25 mL) was heated under reflux for 16 h and filtered from a small amount of insoluble material. The solvent was removed from the filtrate at reduced pressure. The residue was taken up in CH_2Cl_2 (25 mL) and the solution was washed with water and dried. To this solution of intermediate imidazolide was added 1,4-bis(3-aminopropyl)piperazine (0.12 g, 0.6 mmol) and the solution was kept at room temperature for 16 h. A first crop of yellow **5a** (0.09 g) was filtered off. The filtrate was washed with 10% Na_2CO_3 solution (×2), water, dried and the solvent was evaporated. The residue was extracted with hot light petroleum (bp 90–110 °C), then with hot acetonitrile and the remaining yellow solid was further product (0.1 g) (total 44%), which had mp 239–240 °C after recrystallization from DMSO. ^{1}H NMR δ 1.90 (m, 2H), 3.28–3.42 (m, 6H), 3.58 (br q, 2H), 3.93 (s, 3H), 7.32 (t), 7.66–7.70 (m, 3H),8.26 (d), 8.36 (d), 8.52 (d), 8.57 (s), 11.36 (br t, 1H). ESMS m/z 717.3 (80%) (M + 1), 359.2 (100%) [(M + 2)/2]. Anal. calcd for $C_{44}H_{44}N_8O_2{\cdot}2H_2O$ C, 70.2; H, 6.4; N, 14.9. Found: C, 70.5; H, 5.8; N, 15.2%.

***N,N′*-[Piperazine-1,4-diylbis(propane-3,1-diyl)]bis-6,10-dimethyl-10*H*-quindoline-4-carboxamide (5b).** This was prepared from **12b** and 1,1′-carbonyldiimidazole as for **5a**. Formation of the imidazolide took 2 days of reflux and reaction with 1,4-bis(3-aminopropyl)piperazine was carried out in dioxan solvent, at reflux for 3 days. The solvent was removed under reduced pressure and the

residue was dissolved in chloroform. This solution was washed with water, dried and the solvent was removed to give **5b** (41%), as a yellow solid, mp 230–232 °C (from chloroform/acetonitrile). ^{1}H NMR ($CDCl_3$) δ 1.97 (br s, 2H), 2.58 (br s, 6H), 3.02 (s, 3H), 3.65 (q, $J = 6.4$ Hz, 2H), 3.81 (s, 3H), 7.09 (d), 7.25 (d), 7.51–7.61 (m, 2H), 7.90 (s), 8.00 (d), 8.76 (d), 11.77 (br t, NH); ^{13}C NMR ($CDCl_3$) δ 20.5 (CH_2), 26.8 (CH_2), 29.1 (CH_3), 38.1 (CH_2), 52.0 (CH_2), 56.0 (CH_2), 106.4 (CH), 111.9 (CH), 119.0 (C), 121.7 (CH), 124.8 (CH), 126.3 (C), 128.3 (C), 130.1 (CH), 130.8 (CH), 131.4 (CH), 133.4 (C), 135.4 (C), 141.0 (C), 145.3 (C), 145.4 (C), 166.8 (C). ESMS m/z 745.4 (90%) (M + 1), 373.3 (100%) [(M + 2)/2]. Anal. calcd for $C_{46}H_{48}N_8O_2 \cdot 3.5H_2O$ C, 68.4; H, 6.9; N, 13.9. Found: C, 68.3; H, 6.5; N, 13.9%.

Calculation of linker chain lengths

These were computed from the programme ACD/3-D (www.acdlabs.com).

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.[11,12] 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 mL per well) for 3 (P388) or 4 days in the presence of drug. Cell growth was determined by [^{3}H]TdR uptake (P388)[16] or the sulforhodamine assay.[17] Independent assays were performed in duplicate, and coefficients of variation for all assays were between 7.9 and 8.5%.

In vivo colon 38 tumor assay

Colon 38 tumors were grown subcutaneously from 1 mm^3 fragments implanted in one flank of mice (anaesthetised 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 callipers 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 4-fold higher than their pre-treatment volume.

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