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Synthesis and Antitumor Activity of Some Indeno[1,2-*b*]quinoline-based bis Carboxamides

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Abstract—A series of bis(11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamides) linked through the 6-carboxamides were prepared by coupling the requisite acid imidazolides with various diamines. Compounds with mono-cationic linker chains were more potent cytotoxins than the corresponding monomer in a panel of rodent and human cell lines, while those with the dicationic linker chains $(CH_2)_2NR(CH_2)_2NR(CH_2)_2$ and $(CH_2)_2NR(CH_2)_3NR(CH_2)_2$ showed extraordinarily high potencies (for example, IC_{50}s of 0.18–1.4 nM against human Jurkat leukemia; up to 1000-fold more potent than the parent monomer). As seen previously in the monomeric series, small, lipophilic 4-substituents significantly increased potency in cell culture. The dimeric compounds were all slightly to significantly more potent in the mutant JL_A and JL_D cell lines that under-express topo II, suggesting that this enzyme is not their primary target. An 11-imino-linked dimer was much less active, and an asymmetric indeno[1,2-*b*]quinoline-6-carboxamide/naphthalimide dimer was less active than the comparable symmetric bis(indeno[1,2-*b*]quinoline-6-carboxamide). Selected analogues were active against sub-cutaneously implanted colon 38 tumors in mice, giving growth delays comparable to that of the clinical topo I inhibitor irinotecan at up to 10-fold lower doses. These compounds form an interesting new class of putative topo I inhibitors.

Introduction

A number of recent reports have delineated a new class of anti-cancer drugs, comprised of dimeric forms of lipophilic, neutral, DNA-intercalating chromophores joined by a flexible cationic or dicationic linker chain. The chromophores employed include naphthalimides,[1–4] benzonaphthalimides,[5] imidazoacridinones,[6,7] anthracyclines,[8] acridines[9] and phenazines.[10] In all of the above cases, while the corresponding monomeric compounds are active cytotoxins and topoisomerase inhibitors, the best dimeric compounds are considerably more cytotoxic, and many show excellent activity against a variety of human solid tumor cell lines, both in culture and as xenografts in nude mice. Two compounds of the bis-(naphthalimide) series; DMP 840 and LU 79553 (**2**) are reported to be in clinical trial.[3,4] A series of substituted bis(acridine-4-carboxamides linked by a $(CH_2)_3N(Me)(CH_2)_3$ chain were significantly more cytotoxic than their monomeric analogues,[11] with structure–activity relationships for ring substitution broadly similar to those for the monomers. Small lipophilic 5-substituents provided compounds of the highest potency (e.g. **3**; IC_{50} of 2 nM against the Lewis lung carcinoma).[9]

We recently reported the development of a new class of tetracyclic 11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamides (**4**) as potent cytotoxins and potential dual topo I/II inhibitors.[12,13] We now report the synthesis and initial biological evaluation of a series of symmetrical dimeric analogues of this chromophore (**5a**–**5l**), together with three miscellaneous analogues (**6**–**8**).

Results

Preparation of the required precursor acids **9**–**11** has been described.[13] The isomeric acid **15** has been prepared by an improvement of the original procedure[12] (Scheme 1). The bis(amides) **5** and **8** were prepared by activation of the appropriate acid to the corresponding imidazolide, and coupling this with half an equivalent of the required diamine[14] (Scheme 2). The intermediate imidazolide (**12**: R=H) from acid **9** was isolated, but

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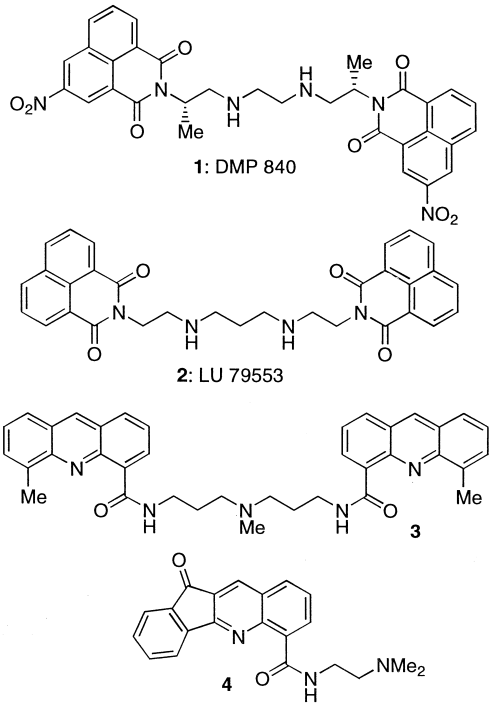
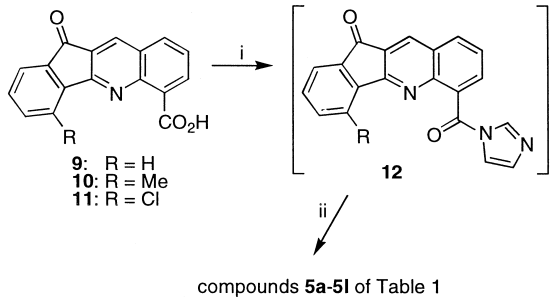
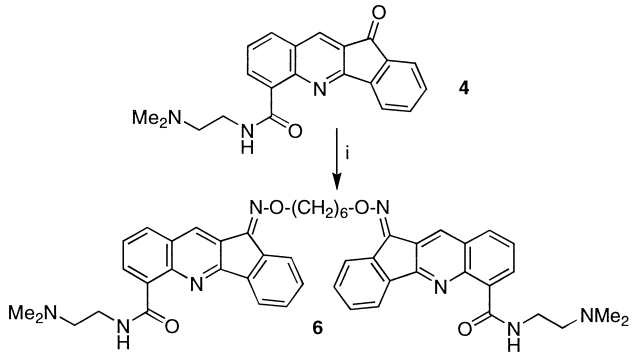




Scheme 2. (i) CDI/dioxan/reflux/3 h; (ii) diamine/CH_2Cl_2/20 °C/24 h.

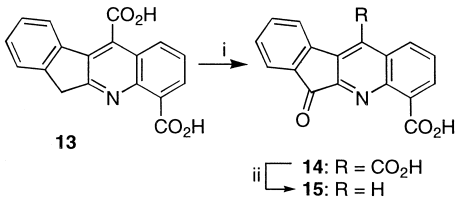


Scheme 3. (i) $H_2NO(CH_2)_6ONH_2$/5% HCl/reflux/3 h.

others were reacted in situ with the required diamine. The bisoxime (**6**) was prepared from the known[12] amide **4** and O,O′-1,6-hexanediylbishydroxylamine in acid conditions (Scheme 3). Most of the polyamine linkers were commercially available. *N,N′*-bis(2-Aminoethyl)-*N,N′*-dimethyl-1,2-ethane- (and 1,3-propane) diamines, though both known,[5,15] were prepared by a common procedure, slightly different to the existing literature. *N,N′*-dimethylethane- and propane-diamines were reacted with chloroacetonitrile, then with borane by a literature procedure[16] for nitrile reduction. An unsymmetrical bis compound **7**, which combined both the above indenoquinoline subunit and the naphthalimide subunit of LU 79553 (**2**) was also prepared, by reacting *N*-[3-[4-(3-aminopropyl)piperazin-1-yl]propyl]naphthalimide (prepared from 1,8-naphthalic anhydride) with the imidazolide (**12**: R=H).

Discussion

Table 1 shows IC_{50} values for the compounds in a panel of cell lines in culture. As discussed previously,[11,12] P388 is a murine leukemia, 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 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.[17,18] 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). Values of these ratios of less than about 2-fold suggest a non-topo II mediated mechanism of action.

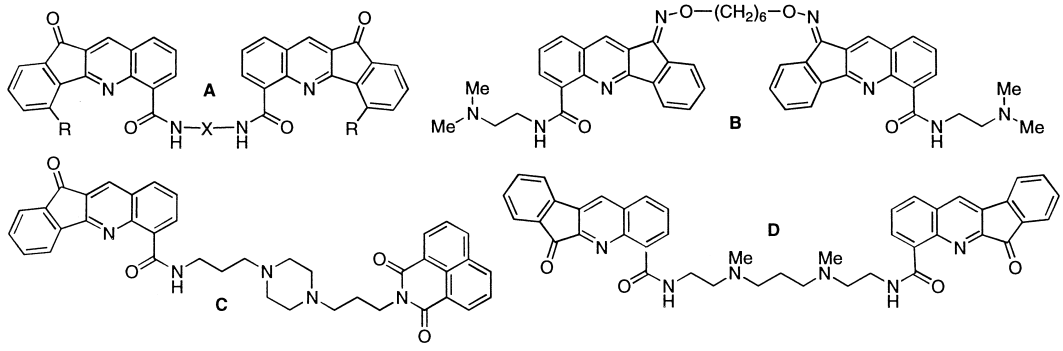


Scheme 1. (i) $KMnO_4$; (ii) heat ca. 300 °C.

Compounds **5a**, **5b** and **5l** have mono-cationic linker chains. The one (**5a**) with the shortest (5-atom) linker, was less effective than the monomer **4**, but those with the longer linkers (**5b**; 7 atoms) and (**5l**; 8 atoms) were significantly more potent. The pairs of dicationic compounds (**5c**/**5d** and **5e**/**5g**) are of particular interest. In the first pair of compounds, linked by a $(CH_2)_2NR(CH_2)_2NR(CH_2)_2$ chain, the R=H analogue **5c** showed activity comparable to **5b**, with the R=Me compound **5d** being about 2–20-fold less cytotoxic. The second pair of compounds, with $(CH_2)_2NR(CH_2)_3NR(CH_2)_2$ linkers (an extra atom in the chain), had significantly higher cytotoxicities, but in this case the R–Me compound **5g** was overall about 2-fold more cytotoxic than the R=H example. While the interpair differences are not large, they show the similar trends to those seen earlier with related pairs of bis(naphthalimides).[4] In particular, **5e** and **5g** exhibit very high potency (sub-nM) against the human leukemia line JL_C.

Compounds **5e**/**5f** and **5g**–**5i** explore the consequences of substitutions in the 4-position of the chromophore.

Table 1. Growth inhibitory data for the indeno[1,2-*b*]quinoline-based bis carboxamides



No.	Fm	R	X	IC_{50}[a] P388[c]	IC_{50}[a] LL[d]	IC_{50}[a] JL_C[e]	Ratios[b] JL_A[f]/JL_C	Ratios[b] JL_D[g]/JL_C
4[h]				130	91	180	1.2	0.9
5a	A	H	$(CH_2)_2NH(CH_2)_2$	1188	240	165	0.4	0.7
5b	A	H	$(CH_2)_3NMe(CH_2)_3$	34	12	23	0.3	0.5
5c	A	H	$(CH_2)_2NH(CH_2)_2NH(CH_2)_2$	34	14	2.5	0.2	0.5
5d	A	H	$(CH_2)_2NMe(CH_2)_2NMe(CH_2)_2$	545	33	21	0.3	0.5
5e	A	H	$(CH_2)_2NH(CH_2)_3NH(CH_2)_2$	26	4.7	0.75	0.3	0.5
5f	A	Me	$(CH_2)_2NH(CH_2)_3NH(CH_2)_2$	88	5.0	1.4	0.55	0.65
5g	A	H	$(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2$	22	2.7	0.35	0.3	0.4
5h	A	Me	$(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2$	7.9	0.69	0.18	0.4	0.6
5i	A	Cl	$(CH_2)_2NMe(CH_2)_3NMe(CH_2)_2$	23	1.1	0.36	0.6	0.4
5j	A	H	$(CH_2)_3NH(CH_2)_2NH(CH_2)_3$	7200	550	155	0.2	1.0
5k	A	H	$(CH_2)_3NpipN(CH_2)_3$	40	2.4	2.3	0.3	0.4
5l	A	H	$(CH_2)_3NH(CH_2)_4$	55	27	84	0.1	1.0
6	B			925	680	1100	0.8	0.6
7	C			130	11	21	1.0	0.5
8	D			23	6.5	0.8	0.45	1.1
2 (LU 79553)				57	11	0.7	0.3	0.6

[a]IC_{50}, concentration of drug (nM) to reduce cell number to 50% of control cultures (see text). The value is the average of at least two independent determinations; the coefficient of variation was ca. 25%.
[b]Ratios of IC_{50}s in the cell lines shown.
[c]Murine P388 leukemia.
[d]Murine Lewis lung carcinoma.
[e]JL_C, wild-type human Jurkat leukemia.
[f]JL_A, amsacrine-resistant Jurkat.
[g]JL_D, doxorubicin-resistant Jurkat.
[h]Data from ref 12.

In previous structure–activity studies[13] on the monomeric compounds, we showed that small, lipophilic 4-substituents substantially increased cytotoxicity (up to 8-fold, with the 4-Me and 4-Cl analogues showing IC_{50}s of 35 and 55 nM, respectively, in the JL_C line, compared with 180 nM for **4** itself). In the dimeric series the 4-substituents had lesser but still positive effects on absolute potencies in the $(CH_2)_2NR(CH_2)_3NR(CH_2)_2$ series, with **5h** and **5i** showing IC_{50}s in JL_C of 0.18 nM (an increase of 1000-fold over **4**). Extending the linker chain further, in the $(CH_2)_3NH(CH_2)_3NH(CH_2)_3$ analogue **5j**, led to a substantial loss of cytotoxicity (e.g. IC_{50} in JL_C 155 nM compared with 0.75 nM for the $(CH_2)_2NH(CH_2)_3NH(CH_2)_2$ analogue **5e**). As seen previously with bis(DACA) derivatives,[9] these dimeric compounds all proved to be slightly to significantly (up to 10-fold) more potent in the mutant JL_A and JL_D cell lines that under-express topo II. In contrast, the corresponding monomeric compounds show essentially equivalent activity in the wild-type and mutant cell lines.[12] These results suggest that the dimeric analogues do not act through topo II, and are likely to be primarily topo I inhibitors.

The 11-imino-linked dimer **6** was prepared to compare the possible utility of linking the two chromophores in a different geometry, but proved much less active. The asymmetric dimer **7** is a hybrid of the 11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide and naphthalimide chromophores. While symmetric bis(naphthalimides) (e.g. **2**) are potent cytotoxins, the hybrid dimer **7** was less active than the similarly-linked bis(11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide) **5k**. Finally, the isomeric bis(6-oxo-6*H*-indeno[2,1-*b*]quinoline-4-carboxamide) (**8**) was 2–4-fold less potent in the cell line panel than the corresponding 11-oxo analogue **5g**. In the initial study of the monomers,[12] the 6-oxo isomer also proved somewhat less active.

Four of the most potent compounds in cell culture (**5e**, **5f**, **5g** and **5h**) were evaluated in vivo against transplanted sub-cutaneous colon 38 tumors in mice, and

Table 2. Comparative in vivo activity of selected bis(indeno[1,2-*b*]-quinolinecarboxamides) in colon 38 tumors (optimal doses using a q4D×3 schedule)

No.	Dose (mg/kg/day)	Growth delay (days)
5e	13.3	8.0
5f	7.5	3.0
5g	65	3.0
5h	3.3	7.5
Irinotecan	30	7.0
LU 79553	5.9	6.0

compared to the clinical topo I inhibitor irinotecan and the experimental bis(naphthalimide) LU 79533 (Table 2). For the bis(indeno[1,2-*b*]quinoline-6-carboxamides), there were little obvious structure–activity relationships. There were large improvements in activity and potency between the H and 4-Me substituted compounds when the linker chain was methylated (**5g** and **5h**), but the opposite when it was not (**5e** and **5f**). There was also no consistent correlation between in vivo activity and methylated/unmethylated linker chains. Overall, the best activities were shown by the least (**5e**) and most (**5h**) lipophilic compounds. Both had activity comparable to that of irinotecan and LU 79553, with **5h** in particular being more potent than both.

Conclusions

The bis(indeno[1,2-*b*]quinoline-6-carboxamides) are an interesting new class of putative topo I inhibitors, some exhibiting very high cytotoxicity in cell culture, and activity comparable to irinotecan against sub-cutaneous colon 38 tumors in vivo.

Experimental

Chemistry

Microanalyses were performed at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. ^{1}H NMR spectra were obtained at 300 MHz, in $CDCl_3$ 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.

Acids

Known acids **9**–**11** were prepared as described.[13]

6-Oxo-6*H*-indeno[2,1-*b*]quinoline-4-carboxylic acid (15). Isatin-7-carboxylic acid and 2-indanone were reacted by the general Pfitzinger reaction (Method A) reported previously.[13] The resulting crude **13** was oxidized[13] with alkaline $KMnO_4$ to give 6-oxo-6*H*-indeno[2,1-*b*]quinoline-4,11-dicarboxylic acid (**14**) (63% yield from 2-indanone): mp > 300 °C; ^{1}H NMR δ 7.60 (t), 7.77–7.82 (m, 2 H), 7.86 (d), 7.91 (t), 8.14 (d), 8.32 (d). This was decarboxylated at reduced pressure,[13] and the sublimate was recrystallized from DMSO to give **15** (51%): mp (DMSO) > 300 °C; ^{1}H NMR (75 °C) δ 7.55 (t), 7.77–7.90 (m, 3H), 8.03 (d), 8.29 (d), 8.40 (d), 8.87 (s).

Linker diamines

Diethylenetriamine, *N,N′*-bis(2-aminoethyl)-1,3-propanediamine,1,4-bis(3-aminopropyl)piperazine, 3,3′-diamino-*N*-methyldipropylamine and spermidine were used without further purification. The free base of triethylenetetramine was prepared from its hydrochloride.[19] O,O′-1,6-hexanediylbishydroxylamine dihydrochloride was prepared as reported.[20]

***N,N′*-bis(2-Aminoethyl)-*N,N′*-dimethyl-1,2-ethanediamine.** A mixture of chloroacetonitrile (4.4 g, 58.2 mmol), *N,N′*-dimethylethylenediamine (2.5 g, 28.4 mmol) and anhydrous K_2CO_3 (14 g) in dry acetone (25 mL) was stirred and refluxed for 24 h. The solid was filtered off, washed with CH_2Cl_2 and the solvent removed from the filtrate under reduced pressure to give the intermediate bisacetonitrile (3.95 g, 84%) as a golden oil, which was used without further purification; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, NCH_3), 2.61 (s, 2H, NCH_2), 3.60 (s, 2H, CH_2CN).

Diborane was prepared in situ[16] and, with a nitrogen carrier gas, was bubbled through a solution of the above bisacetonitrile (2.6 g) in THF at room temperature over ca. 1 h (exothermic). This mixture was allowed to stir for a further 1 h, then EtOH was added cautiously to destroy the excess diborane, before HCl gas was passed into the solution. The salt which formed was filtered off, dissolved in water, the pH was taken to 12 with 10% NaOH, and the solvent was removed under reduced pressure. The residue was extracted with hot toluene, and the solvent was removed under reduced pressure to give the title compound (1.4 g, 53%) (lit.[5] bp 115 °C/0.3 mm Hg), which was sufficiently pure to be used in amide formation. ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3H, NCH_3), 2.36 (t, $J = 6$ Hz, 2H, CH_2), 2.42 (s, 2H, CH_2), 2.68 (t, $J = 6$ Hz, 2H, CH_2). ESMS: m/z 175.1 (M + 1).

***N,N′*-bis(2-Aminoethyl)-*N,N′*-dimethyl-1,3-propanediamine.** The same two-step sequence with *N,N′*-dimethyl-1,3-propanediamine gave the intermediate bisacetonitrile (91%); (^{1}H NMR ($CDCl_3$) δ 1.59 (m, 2H, C-CH_2), 2.33 (s, 6H, NCH_3), 2.48 (t, $J = 7$ Hz, 4H, NCH_2), 3.50 (s, 4H, CH_2CN) and final tetraamine (55%) (lit.[15] bp 32–33 °C/0.5 mmHg). ^{1}H NMR ($CDCl_3$) δ 1.54 (m, 2H, C-CH_2), 2.12 (s, 6H, NCH_3), 2.25–2.35 (m, 8H, CH_2), 2.67 (t, $J = 6$ Hz, 4H, CH_2). ESMS: m/z 189.3 (M + 1).

Preparation of *N,N′*-(iminodi-2,1-ethanediyl)bis-[11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5a): example of the amidation reaction. Oxo acid **9** (0.3 g) and 1,1′-carbonyldiimidazole (0.5 g) in dry dioxan (20 mL) were heated under reflux until dissolution was complete

(ca. 3 h). 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 $MgSO_4$. The solvent was removed to give 6-(1*H*-imidazol-1-ylcarbonyl)-11*H*-indeno[1,2-*b*]quinolin-11-one (**12**; R=H) as an orange/red solid (0.31 g, 87%): mp 190–196 °C (dec.); ^{1}H NMR δ 7.11 (s, 1H, H-4′), 7.48–7.53 (m, 2H, H-2,5′), 7.60 (t, 1H, J = 6.7 Hz, H-3), 7.66 (t, 1H, J = 7.7 Hz, H-8), 7.75 (d, 1H, J = 7.5 Hz, H-1), 7.80 (d, 1H, J = 7.3 Hz, H-4), 7.88 (s, 1H, H-2′), 7.98 (d, 1H, J = 7.0 Hz, H-9), 8.14 (d, 1H, J = 8.0 Hz, H-7), 8.42 (s, 1H, H-10).

Diethylenetriamine (0.063 g, 0.61 mmol) was added to a solution of **12** (R=H) (0.4 g, 1.24 mmol) in dry CH_2Cl_2 (30 mL), and the mixture was stirred at room temperature for 24 h, then washed with 10% Na_2CO_3 solution (2×20 mL), warm water (2×20 mL) and dried ($MgSO_4$). The solvent was removed to give **5a** as a red solid (0.26 g, 69%): mp (MeCN) 245–247 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 3.06 (s, CH_2NHCO), 3.69 (s, CH_2NH), 7.13–7.15 (m, 2H), 7.36–7.46 (m, 2H), 7.82 (d), 7.95 (d), 8.16 (d), 8.22 (s), 10.72 (br s, NH). ESMS: m/z 618 (M + 1). Anal. calcd for $C_{38}H_{27}N_5O_4{\cdot}H_2O$: C, 71.8; H, 4.6; N, 11.0. Found: C, 72.0: H, 4.5: N, 11.2%.

The following amides were prepared in this manner (from imidazolide (**12**: R=H) unless stated otherwise):

***N,N′*-(Methyliminodi-3,1-propanediyl)bis-[11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5b).** As a pale orange semi-solid (85%); ^{1}H NMR ($CDCl_3$) δ 2.02 (m, 4H, CH_2), 2.39 (s, 3H, N-CH_3), 2.76 (t, 4H, J = 7.1 Hz, CH_2N), 3.62 (q, 4H, J = 6.1 Hz, CH_2NHCO), 7.08–7.18 (m, 4H), 7.42 (t, 2H), 7.51 (t, 2H), 7.59 (d, 2H), 7.81 (d, 2H), 8.08 (s, 2H), 8.63 (d, 2H), 10.58 (t, 2H, J = 4.9 Hz, NH). ESMS: m/z 660 (M + 1), 330.7 [(M + 2)/2]. A hygroscopic perchlorate salt was prepared in 2-propanol and had mp 173–176 °C, but could not be freed of trace impurity.

***N,N′*-[(2-Aminoethyl)imino]di-2,1-ethanediyl]bis-[11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5c).** After 72 h reaction time, as an orange solid (73%): mp (DMSO) 181–184 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 2.80–2.88 (m, 6H, CH_2NH), 7.37–7.41 (m, 2H), 7.55–7.65 (m, 2H), 8.05–8.15 (m, 2H), 8.32 (s), 8.42 (d), 10.61 (br s, NH). ESMS: m/z 661 (M + 1). Anal. calcd for $C_{40}H_{32}N_6O_4{\cdot}2.5H_2O$: C, 68.1; H, 5.3; N, 11.9. Found: C, 68.1: H, 5.3: N, 12.1%.

***N,N′*-[(2-Aminoethyl)methylimino]di-2,1-ethanediyl]bis-[11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5d).** The first material obtained was dissolved in CH_2Cl_2 and addition of hexane gave the product in 70% yield. Column chromatography (alumina/$CHCl_3$) followed by recrystallization from MeCN gave a sample with mp 192–193 °C; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3H), 2.69 (t, 2H), 2.77 (s, 2H), 3.59 (q, 2H), 7.30 (t), 7.47–7.51 (m, 2H), 7.60 (t), 7.88 (d), 8.09 (d), 8.15 (s), 8.72 (d), 10.88 (s, 1H, NH). ESMS: m/z 689 (M + 1). Anal. calcd. for $C_{42}H_{36}N_6O_4{\cdot}0.5H_2O$: C, 72.3; H, 5.3; N, 12.0. Found: C, 72.3: H, 5.3: N, 12.3%.

***N,N′*-[(2-Aminoethyl)imino]di-3,1-propanediyl]bis-[11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5e).** As a pale orange solid: mp (MeCN/$CHCl_3$) 130–131 °C; ^{1}H NMR ($CDCl_3$) δ 1.83 (m, 1H), 2.88 (t, 2H), 2.93 (t, 2H), 3.67 (q, 2H), 7.27 (t), 7.45 (d), 7.51 (t), 7.60 (t), 7.90 (d), 8.01 (d), 8.22 (s), 8.80 (d), 10.98 (s, 1H, NH). Anal. calcd. for $C_{42}H_{34}N_6O_4{\cdot}H_2O$: C, 71.1; H, 5.2; N, 12.4. Found: C, 71.2: H, 5.1: N, 12.1%.

***N,N′*-[(2-Aminoethyl)imino]di-3,1-propanediyl]bis-[4-methyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5f).** The imidazolide (**12**: R=Me) was prepared in situ from acid **10**, and was reacted with *N,N′*-bis(2-aminoethyl)-1,3-propanediamine. The crude product (69%) was subjected to column chromatography (alumina; $CHCl_3$: MeOH, 96:4) and the fraction with R_f = 0.55 was collected. The solvent was removed under reduced pressure to give **5f**: mp (EtOH) 138–140 °C; ^{1}H NMR ($CDCl_3$) δ 1.60–1.8 (m, 1H, C-CH_2), 2.7–2.8 (m, 5H, CH_3 + NCH_2), 2.85–2.95 (m, 2H, NCH_2), 3.60–3.70 (m, 2H, CH_2NHCO), 7.25–7.40 (m, 2H), 7.50–7.55 (m, 2H), 7.85 (d), 8.27 (s), 8.75 (d), 10.69 (br s, 1H, NH). ESMS: m/z 703 (M + 1), 352 [(M + 2)/2]. Anal. calcd. for $C_{43}H_{38}N_6O_4{\cdot}3H_2O$: C, 68.2; H, 5.9; N, 11.1. Found: C, 68.6; H, 5.9; N, 11.0%.

***N,N′*-[(2-Aminoethyl)methylimino]di-3,1-propanediyl]bis-[11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5g).** The first obtained material was extracted with hot light petroleum (bp 90–110 °C). The insoluble material was recrystallized twice from $CHCl_3$/MeCN and had mp 185–186 °C; ^{1}H NMR ($CDCl_3$) δ 1.80–2.0 (m, 2H, C-CH_2), 2.34 (s, 6H, NCH_3), 2.50–2.60 (m, 8H, CH_2), 3.55–3.65 (m, 4H, CH_2NHCO), 7.18–7.24 (m, 4H), 7.38–7.46 (m, 2H), 7.62 (t, 2H), 7.89 (dd, J = 7.7, 1.5 Hz, 2H), 8.11 (s, 2H), 8.81 (dd, J = 7.7, 1.5 Hz, 2H), 10.78 (br s, 2H, NH). ESMS: m/z 703.3 (M + 1), 352.2 [(M + 2)/2]. Anal. Calcd. for $C_{43}H_{38}N_6O_4$: C, 73.5; H, 5.5; N, 12.0. Found: C, 73.3: H, 5.3: N, 12.1%.

***N,N′*-[(2-Aminoethyl)methylimino]di-3,1-propanediyl]bis-[4-methyl-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5h).** The imidazolide from acid **10** was prepared in situ and then reacted with *N,N′*bis(2-aminoethyl)-*N,N′*-dimethyl-1,3-propanediamine. Column chromatography of the crude product (57%) (alumina; $CHCl_3$: MeOH, 99:1) gave **5h**: mp 103–105 °C; ^{1}H NMR ($CDCl_3$) δ 1.55–1.7 (m, 2H, CCH_2), 2.24 (s, 6H, NCH_3), 2.40–2.45 (m, 4H, NCH_2), 2.54–2.60 (m, 4H, NCH_2), 2.76 (s, 6H, CH_3), 3.54–3.65 (m, 4H, CH_2NHCO), 7.25–7.35 (m, 4H), 7.48–7.60 (m, 4H), 7.86 (d, 2H), 8.24 (s, 2H), 8.75 (d, 2H), 10.58 (br s, 2H, NH). ESMS: m/z 731.2 (M + 1), 366.4 [(M + 2)/2]. The perchlorate salt had mp (water) 204–206 °C. Anal. calcd for $C_{45}H_{42}N_6O_4{\cdot}2HClO_4.H_2O$: C, 56.9; H, 4.9; N, 8.8. Found: C, 56.9; H, 4.9; N, 8.8%.

***N,N′*-[(2-Aminoethyl)methylimino]di-3,1-propanediyl]bis-[4-chloro-11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5i).** The imidazolide from acid **11** was prepared in situ and then reacted with *N,N′*-bis(2-aminoethyl)-*N,N′*-dimethyl-1,3-propanediamine. Column chromatography of the crude product (23%) (alumina; $CHCl_3$:

MeOH, 98:2) gave **5i**, mp (MeCN) 193–195 °C; ^{1}H NMR ($CDCl_3$) δ 1.6–1.8 (m, 2H, CH_2), 2.32 (s, 6H, NCH_3), 2.46 (m, 4H, NCH_2), 2.68 (m, 4H, NCH_2), 3.64 (m, 4H, CH_2NH), 7.19 (t, 2H), 7.40–7.50 (m, 4H), 7.64 (t, 2H), 7.91 (d, 2H), 8.29 (s, 2H), 8.85 (d, 2H), 10.9 (br s, 2H, NH). ESMS: *m/z* 771 (100%), 772 (50), 773 (83), 774 (43), 775 (15), all (M + 1) for $C_{43}H_{36}Cl_2N_6O_4$. Anal. calcd for $C_{43}H_{36}Cl_2N_6O_4{\cdot}1.5H_2O$: C, 64.7; H, 4.9; N, 10.5. Found: C, 64.9; H, 4.6; N, 10.8%.

***N,N′*-[(3-Aminopropyl)imino]di-3,1-propanediyl]bis-[11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5j).** The crude solid was extracted with hot light petroleum (bp 90–110 °C), then with hot MeCN to give the insoluble pale orange product (78%), mp >300 °C. This could not be recrystallized and no suitable crystalline salt was obtained. ^{1}H NMR (DMSO/TFA) δ aliphatic region poorly resolved, 7.58 (t), 7.70–7.75 (m, 2H), 7.92 (d), 8.04 (d), 8.48 (d), 8.53 (s), 8.62 (d), 10.59 (br s, NH). ESMS: 703 (M + 1).

***N,N′*-(1,4-Piperazinediyldi-3,1-propanediyl)bis-[*11*-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide] (5k).** As a cream solid (47%): mp (DMSO) 280–282 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 1.85–1.95 (m, 2H, CH_2), 2.45–2.53 (m, 6H, pipCH_2N), 3.59 (q, J = 5.6 Hz, $CONHCH_2$), 7.65 (t), 7.71 (t), 7.78–7.84 (m, 2H), 8.06 (d), 8.24 (d), 8.52 (d), 8.68 (s). ESMS: *m/z* 715 (M + 1), 358 [(M + 2)/2]. Anal. calcd for $C_{44}H_{38}N_6O_4{\cdot}H_2O$: C, 72.1; H, 5.5; N, 11.4. Found: C, 72.2: H, 5.3: N, 11.2%.

***N*-[3-[4-(11-oxo-11*H*-indeno[1,2-*b*]quinoline-6-carbonyl-amino)butyl]amino]propyl]-11-oxo-11*H*-indeno[1,2-*b*]-quinoline-6-carboxamide (5l).** Obtained in 69% yield, mp (MeOH) 240–242 °C (dec., after darkening >200 °C); ^{1}H NMR ($CDCl_3$) δ 1.5–2.1 (m, 6H, C-CH_2), 2.80–3.0 (m, 4H, CH_2NH), 3.6–3.8 (m, 4H, CH_2NHCO), 7.35–7.46 (m, 2H), 7.55–7.70 (m, 6H), 7.80–8.0 (m, 4H), 8.30–8.35 (m, 2H), 8.75–8.85 (m, 2H), 10.8–11.0 (br s, 2H, NH). ESMS: *m/z* 660.2 (M + 1). Anal. (after vacuum drying at 50 °C/3 days) calcd for $C_{41}H_{33}N_5O_4.3H_2O$: C, 69.0; H, 5.5; N, 9.8. Found: C, 68.7; H, 5.1; N, 10.2%.

11,11′-[(O,O′-Hexane-1,6-diyl)bisisonitroso]bis-[*N*-[2-(dimethylamino)ethyl]-11*H*-indeno[1,2-*b*]quinoline-6-carboxamide (6). A solution of the known[12] amide **4** (0.4 g) and O,O′-1,6-hexanediylbishydroxylamine dihydrochloride (0.12 g) in 5% HCl (10 mL) was heated under reflux for 3 h. The solution was cooled to room temperature and basified to pH 10 with 10% NaOH. The oily residue which formed was extracted into $CHCl_3$, washed with water (2×10 mL) and dried over $MgSO_4$. Removal of the solvent gave **6** as an orange oil (0.39 g, 84%). The ^{1}H NMR was complex and poorly resolved. ESMS: *m/z* 803 (M + 1), 402 ((M + 2)/2). A hygroscopic perchlorate salt was prepared in isopropanol and dried in vacuo, and had mp 167–169 °C. Anal. calcd for $C_{48}H_{50}N_8O_4{\cdot}2HClO_4{\cdot}4H_2O$: C, 53.6; H, 5.6; N, 10.4. Found: C, 54.0: H, 5.3: N, 10.1%.

***N*-[3-[4-[3-(1,3-Dioxo-1*H*-benz[*de*]isoquinolin-2(3*H*)-yl)-propyl]piperazin-1-yl]propyl]-11*H*-11-oxoindeno[1,2-*b*]-quinoline-6-carboxamide (7).** A solution of 1,8-naphthalic anhydride (0.2 g, 1.0 mmol) in CH_2Cl_2 (20 mL) was added dropwise with stirring over 1 h to a solution of 1,4-bis(3-aminopropyl)piperazine (1 g, 5 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at rt for 16 h, then the solvent was removed under reduced pressure and the excess of diamine was distilled at 150–152 °C/2 mmHg to leave crude *N*-[3-[4-(3-aminopropyl)piperazin-1-yl]propylnaphthalimide as a golden oil. This crude product was reacted directly with imidazolide (**12**: R=H) as above, to give **7** (0.16 g, 25%): mp (MeCN) 189–191 °C; ^{1}H NMR ($CDCl_3$) δ 1.85 (m, 4H), 2.40–2.50 (m, 12H), 3.67 (q, J = 6.2 Hz, 2H), 4.22 (t, J = 7.3 Hz, 2H), 7.60 (t, 1H), 7.65 (t, 1H), 7.73 (t, 2H), 7.86 (d, 1H), 7.93 (d, 1H), 7.99 (d, 1H), 8.18 (d, 2H), 8.45 (s, 1H), 8.57 (d, 2H), 8.87 (d, 1H), 11.0 (s, 1H, NH). ESMS: *m/z* 638.3 (100%) (M + 1); 319.7 (50%) [(M + 2)/2]. Anal. calcd. for $C_{39}H_{35}N_5O_4{\cdot}1{\cdot}5H_2O$: C, 70.5; H, 5.8; N, 10.5. Found: C, 70.2; H, 5.7; N, 10.8%.

***N,N′*-[(2-Aminoethyl)methylimino]di-2,1-propanediyl]bis-[6-oxo-6*H*-indeno[2,1-*b*]quinoline-4-carboxamide] (8).** The imidazolide from acid **15** was prepared in situ and then was reacted with *N,N′*-bis(2-aminoethyl)-*N,N′*-dimethyl-1,3-propanediamine. The crude product was extracted with hot light petroleum (bp 90–110 °C) (2×), then with hot MeCN, to leave the insoluble yellow solid **8** (33%): mp ($EtOH/CHCl_3$) 207–209 °C; ^{1}H NMR δ 1.79 (m, 2H, CH_2), 2.35 (s, 6H, NCH_3), 2.57 (t, 4H, NCH_2), 2.70 (t, 4H, NCH_2), 3.64 (m, 4H, CH_2NHCO), 7.24 (t, 2H), 7.44–7.58 (m, 8H), 7.73 (d, 2H), 7.97 (s, 2H), 8.58 (d, 2H), 10.60 (br s, 2H, NH). ESMS: 703 (M + 1), 352 [(M + 2)/2]. 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, 72.5; H, 5.4; N, 11.9%.

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.[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 mL per well) for 3 (P388) or 4 days in the presence of drug. Cell growth was determined by [^{3}H]TdR uptake (P388)[23] or the sulforhodamine assay.[24] Independent assays were performed in duplicate, and coefficients of variation were ca. 25%.

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 mea-

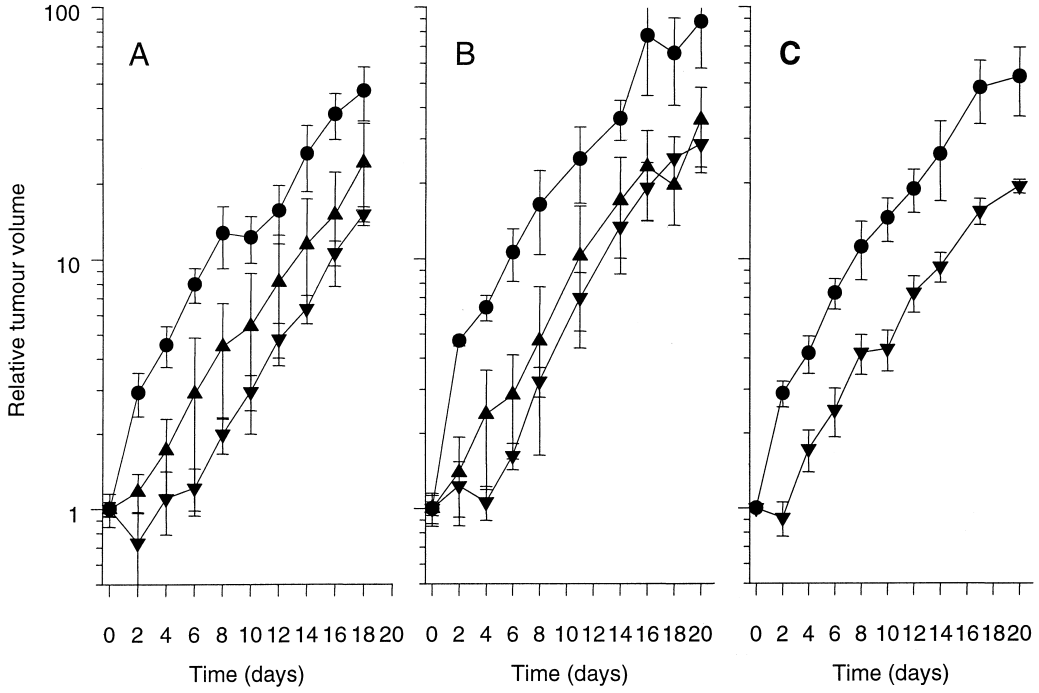


Figure 1. Averaged growth delay data for control (●) and treated mice bearing advanced subcutaneous colon 38 tumors. A: **5e** at doses of 20 mg/Kg (▲) and 13.3 mg/Kg (▼). B: **5h** at doses of 5.0 mg/Kg (▲) and 3.3 mg/Kg (▼). C: LU79553 at a dose of 5.9 mg/Kg (▼). The indicated drug dose was administered intraperitoneally three times at four-day intervals to groups of five mice. Higher doses than those indicated were toxic to at least one animal per group.

sured 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 (Figure 1).

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References

1. McRipley, R. J.; Burns-Horwitz, P. E.; Czerniak, P. M.; Diamond, R. J.; Diamond, M. A.; Miller, J. L. D.; Page, F. J.; Dexter, D. L.; Chen, S. F. *Cancer Res.* **1994**, *54*, 159.
2. Houghton, P. J.; Cheshire, P. J.; Hallman, J. C.; Gross, J. L.; McRipley, R. J.; Sun, J. H.; Behrens, C. H.; Dexter, D. L.; Houghton, J. A. *Cancer Chemother. Pharmacol.* **1994**, *33*, 265.
3. (a) LoRusso, P.; Demchik, L.; Dan, M.; Polin, L.; Gross, J. L; Corbett, T. H. *Inv. New Drugs* **1995**, *13*, 195. (b) Robinson, C. P.; Robinson, K. A.; Castaner, J. *Drugs Future* **1996**, *21*, 239.
4. Brana, M. F.; Castellano, J. M.; Moran, M.; Perez de Vega, M. J.; Perron, D.; Conlon, D.; Bousquet, P. F.; Romerdahl, C. A.; Robinson, S. P. *Anti-Cancer Drug Design* **1996**, *11*, 297.
5. Brana, M. F.; Castellano, J. M.; Perron, D.; Maher, C.; Conlon, D.; Bosquet, P. F.; George, J.; Qian, X.-D.; Robinson, S. P. *J. Med. Chem.* **1997**, *40*, 449.
6. Hernandez, L.; Cholody, W. M.; Hudson, E. A.; Resau, J. H.; Pauly, G.; Michejda, C. *J. Cancer Res.* **1995**, *55*, 2338.
7. Cholody, W. M.; Hernandez, L.; Hassner, L.; Scudiero, D. A.; Djurickovic, D. B.; Michejda, C. J. *J. Med. Chem.* **1995**, *38*, 3043.
8. (a) Chaires, J. B.; Leng, F. F.; Przewloka, T.; Fokt, I.; Ling, Y. H.; Perez-Soler, R.; Priebe, W. *J. Med. Chem.* **1997**, *40*, 261. (b) Leng, F. F.; Priebe, W.; Chaires, J. B. *Biochemistry*, **1998**, *37*, 1743.
9. Gamage, S. A.; Spicer, J. A.; Atwell, G. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1999**, *42*, 2383.
10. Spicer, J. A.; Gamage, S. A.; Atwell, G. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *Anti-Cancer Drug Design* **1999**, *14*, 281.
11. Spicer, J. A.; Gamage, S. A.; Atwell, G. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 1919.
12. Deady, L. W.; Kaye, A. J.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 2040.
13. Deady, L. W.; Desneves, J.; Kaye, A. J.; Thompson, M.; Finlay, G. J.; Baguley, B. C.; Denny, W. A. *Bioorg. Med. Chem.* **1999**, *7*, 2801.
14. Joshua, A. V.; Scott, J. R. *Tetrahedron Lett.* **1984**, *25*, 5725.
15. Braña, M. F.; Pérez de Vega, M. J.; Conlon, D.; Bousquet, P. F.; Robinson, S. P. *J. Het. Chem.* **1997**, *34*, 807.
16. Brown, H. C.; Subba Rao, B. C. *J. Am. Chem. Soc.* **1960**, *82*, 681.
17. Finlay, G. J.; Baguley, B. C.; Snow, K.; Judd, W. *J. Natl. Cancer Inst.* **1990**, *82*, 662.
18. Finlay, G. J.; Holdaway, K. M.; Baguley, B. C. *Oncol. Res.* **1994**, *6*, 33.
19. Glerrup, J.; Josephsen, J.; Michelsen, K.; Pedersen, E.; Schaffer, C. E. *Acta Chem. Scand.* **1970**, *24*, 247.

20. Fuller, A.T.; King, H. *J. Chem. Soc*. **1947**, 963.
21. Finlay, G. J.; Riou, J.-F.; Baguley, B. C. *Eur. J. Cancer* **1996**, *32A*, 708.
22. Wilkoff, L. J.; Dulmadge, E.; Chopra, D. P. *Proc. Soc. Exp. Biol. Med*. **1980**, *163*, 233.
23. Marshall, E. S.; Finlay, G. J.; Matthews, J. H. L.; Shaw, J. H. F.; Nixon, J.; Baguley, B. C. *J. Natl. Cancer Inst*. **1992**, *84*, 340.
24. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst*. **1990**, *82*, 1107.