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Synthesis and cytotoxic activity of *N*-[(alkylamino)alkyl]-carboxamide derivatives of 7-oxo-7*H*-benz[*de*]anthracene, 7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline, and 7-oxo-7*H*-benzo[*e*]perimidine

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Abstract—7-Oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxamides and analogues were prepared and evaluated for in vitro and in vivo antitumor activity. Chromophore variations included 'deaza' (7-oxo-7*H*-benz[*de*]anthracene) and 'diaza' (7-oxo-7*H*-benzo[*e*]perimidine) analogues, and side chain variations included chiral α-methyl compounds. The naphthoquinolines were the most cytotoxic, with IC_{50} values of 5–20 nM, and showed the strongest DNA binding, with high selectivity for G-C rich DNA. The chiral α-methyl analogues were 10–20-fold more cytotoxic than the parent des-methyl compound. Both enantiomers provided substantial growth delays against s.c. colon 38 tumors in mice, with the *R*-enantiomer more active than the *S* (tumor growth delays of >35 and 12 days, respectively).

1. Introduction

We recently reported on the cytotoxic activity of derivatives of 7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (previously called dibenz[*f*,*ij*]isoquinoline), where the 11-carboxamide **1a** in particular showed remarkable curative activity against colon 38 tumors in mice.[1] In this series, the position of the carboxamide appeared to be critical for activity, with the 2-, 4-, and 8-carboxamides not being as effective.[1] We have now extended these studies to include 'deaza' (7-oxo-7*H*-benz[*de*]-anthracene; **2**) and 'diaza' (7-oxo-7*H*-benzo[*e*]perimidine; **3**) analogues of **1a**, as well as varying the nature of the carboxamide linker chain in system **1**. We report here on the synthesis, DNA binding and growth-inhibitory properties of these compounds against a panel of cell lines and, for selected examples, in vivo growth delay data.

2. Results and discussion

2.1. Chemistry

The 'deaza' compound, 7-oxo-7*H*-benz[*de*]anthracene-11-carboxylic acid (**4**) was prepared from methyl 2-iodobenzoate and methyl 8-bromo-1-naphthoate as reported[2,3] (Scheme 1). The starting point for all other compounds was 1-amino-8-chloroanthraquinone **5a** prepared by literature methods, which we outlined previously.[1] Now, in an improved procedure over our previous route to **5c** (Scheme 2), the chloro group was substituted by reaction with CuCN. This gave access to nitrile **5b**, which then afforded acid **5c** on acid hydrolysis, and subsequent reaction with acetone in aqueous sodium hydroxide[1] gave acid **6**, the precursor to the 'monoaza' amides **1a**–**f** of Table 1.

Keywords: Naphthoquinolines; GC-selectivity; Cytotoxicity; Colon 38 tumors.
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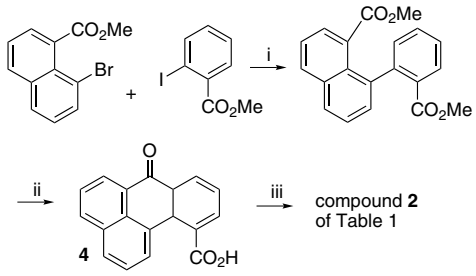
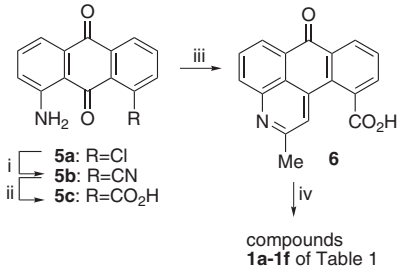
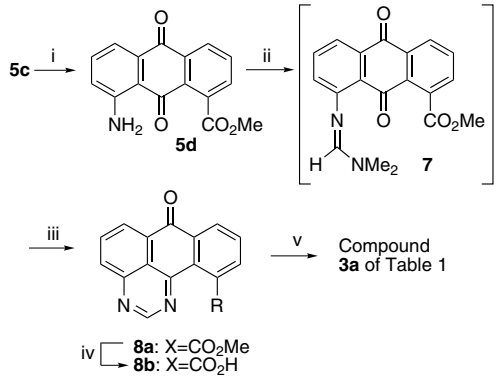


Scheme 1. Reagents and conditions: (i) Cu/180 °C/4 h; (ii) concd H_2SO_4/100 °C; (iii) $SOCl_2$ then $NH_2(CH_2)_2NMe_2$.



Scheme 2. Reagents and conditions: (i) CuCN/DMF/reflux; (ii) 80% H_2SO_4/reflux 3 h; (iii) acetone/4% NaOH/reflux 1 h; (iv) CDI/dioxan/reflux, then amine in dichloromethane or amine·HCl/BOP·Cl/NEt_3/20 °C 1 h.

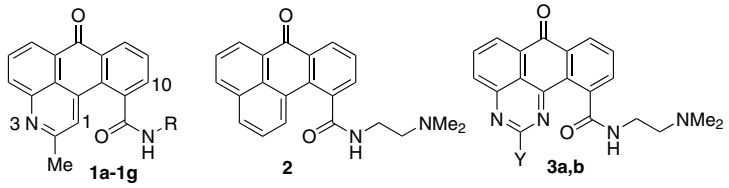
For construction of the diaza ring system, the very insoluble **5c** was converted to the methyl ester **5d**. This was reacted with dimethylformamide dimethyl acetal in refluxing acetonitrile and gave the intermediate amidine **7** (not isolated), which was reacted with ammonium acetate in boiling ethanol, as reported for the parent compound,[4] to give **8a** in 90% yield (Scheme 3). Hydrolysis of the 11-ester functionality in **8a** was best carried out in mildly acid conditions; aqueous trifluoroacetic acid provided a suitable solubilizing and acidic medium.



Scheme 3. Reagents and conditions: (i) MeOH/H_2SO_4/reflux 16 h; (ii) $Me_2NCH(OMe)_2$/MeCN/reflux 3 h; (iii) NH_4OAc/EtOH/reflux 1 h; (iv) TFA–H_2O (1:1.6)/reflux 24 h; (v) $SOCl_2$ then amine.

The dimethylformamide reaction gives a single tetracyclic product, but when the analogous dimethylacetamide dimethyl acetal is used, the resulting intermediate **9** can ring close in two different ways (Scheme 4). Studies with the model compound **5e** established that the course of the reaction was affected by the solvent. In refluxing ethanol, reaction with the acetal went directly to the substituted naphtho[1,2,3-*de*]quinolinone **10a**, as reported previously,[5] and this was isolated in 64% yield. In acetonitrile solvent the reaction was more complex, and **10a** was only a minor component of the initial product. The major product, amidine **9a**, could be separated from **10a** and, on subsequent reaction with ammonium

Table 1. Structural, DNA binding, and growth-inhibitory data for naphtho[1,2,3-*de*]quinoline **1a** and analogues

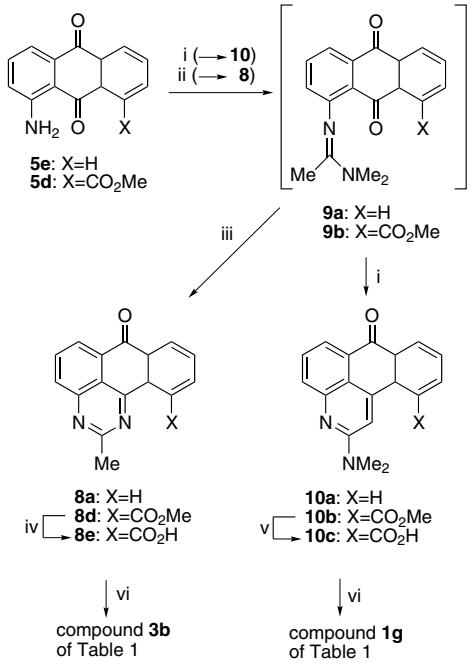


No.	R	Y	DNA binding[a]			Growth delays[b]				
			dAT	dGC	Ratio	P388	LLTC	Jurkat	L_A/L_C[c]	L_D/L_C[c]
1a	$(CH_2)_2NMe_2$	Me	0.37	4.1	11	100	101	424	2.5	2.5
1b	CH(*S*-Me)CH_2NMe_2	Me	0.30	3.9	13	4.1	5.9	42	12	18
1c	CH(*R*-Me)CH_2NMe_2	Me	0.28	8.2	29	9.0	12	53	4.1	5.2
1d	$(CH_2)_3NMe_2$	Me	0.71	2.9	4.0	28	89	858	2	3
1e	$(CH_2)_2NH(CH_2)_2OH$	Me	0.50	6.1	12	550	650	693	3.2	5.1
1f	$(CH_2)_2$Npiperidinyl	Me	0.68	3.8	5.5	180	296	959	1.9	2.2
1g	$(CH_2)_2NMe_2$	NMe_2	0.87	16	19	1500				
2	$(CH_2)_2NMe_2$		1.3	2.4	1.9	1530	1930	2510	0.9	0.9
3a		H	1.6	4.9	3.4	64				
3b		Me	1.3	11	8.5	48				

[a] Association constants ($\times 10^6$ M^{-1}) for binding to poly-(dA-dT)·(dA-dT) [dAT] and poly-(dG-dC)·(dG-dC) [dGC] double-stranded DNA, at 0.01 ionic strength, pH 7.0, and the resulting ratio dGC/dAT.

[b] IC_{50} (nM) for growth inhibition of cultured murine lymphocytic leukemia (P388), a cell line derived from the Lewis lung murine carcinoma (LLTC), and a human acute leukemia (Jurkat) cell line.

[c] L_A/L_C and L_D/L_C; ratios of IC_{50}s for amsacrine-resistant (L_A) and doxorubicin-resistant (L_D) Jurkat cell lines, compared to the parental line (L_C).



Scheme 4. Reagents and conditions: (i) $Me_2NC(Me)(OMe)_2$/EtOH/reflux 3 h; (ii) $Me_2NC(Me)(OMe)_2$/MeCN/reflux 3 h; (iii) NH_4OAc/EtOH/reflux 1 h; (iv) TFA–H_2O (3:4)/reflux 7 days; (v) 10% NaOH–MeOH–H_2O (1:2:2)/reflux 24 h; (vi) $SOCl_2$ then amine.

acetate in ethanol, followed the path seen for dimethylformamide above, giving the alternative perimidine tetracycle **8a** in 57% overall yield.

The same solvent effect was noted with the ester **5d**, but the yields were lower. Thus, reaction with dimethylacetamide dimethyl acetal in methanol went directly to the tetracycle **10b**, which separated from the reaction mixture in 28% yield as a mixture of two components. These were separated by chromatography and, unexpectedly, both were consistent with structure **10b**. The detail of these presumed atropisomers was not established, and the mixture was used directly in further reaction. No solid separated from the analogous reaction mix in acetonitrile and, after evaporation of the volatiles, reaction of the residue with ammonium acetate in ethanol afforded the readily isolable **8d** in 36% yield. In spite of the modest yields, these reactions did allow sufficient **8d** and **10b** to be obtained for the rest of the sequence to be carried out.

Hydrolysis of the 11-ester functionality in **8d** was again carried out in aqueous trifluoroacetic acid, whereas base conditions were preferable for the naphthoquinoline **10b**. Amide formation was achieved in various ways. Compounds **1b–f** were prepared from **6** and the appropriate amine with CDI or BOP·Cl as coupling agents. The synthesis of these amides used the crude acid **6**, which by NMR was ≈50% pure, with a 2:1 ratio of the desired compound to a major impurity (the 4-methyl analogue) and many minor impurities. The crude amides were then purified by preparative reverse-phase HPLC. This procedure gave pure products, but in low yields (8–30%) based on the acid **6** in the crude mixture. Subsequently, a modified purification procedure was developed for **1a** based on reverse-phase C_{18} flash column chromatography in dilute aqueous TFA/MeCN; this gave a sample of improved yield and purity. The remaining amides were obtained from **4**, **8b**, **8e**, and **10c** by way of intermediate acid chlorides (not isolated).

2.2. Structure–activity relationships

The DNA binding properties of the series were determined by competition with ethidium for binding sites on the synthetic double-stranded copolymers poly-d(A-T) and poly-d(G-C) as previously described.[6] All compounds bound selectively to poly-d(G-C), as has been found for a number of anticancer drugs containing chromophores attached to a carboxamide side chain.[7] The degree of GC selectivity was affected by the side chain and the twofold difference in the poly-d(G-C) binding of the chiral compounds **1b** and **1c** was of interest. The in vitro growth-inhibitory activity of the compounds was determined in a panel of tumor cell lines in culture; a murine leukemia (P388), a murine lung carcinoma (Lewis lung)[8] and three human leukemia lines. Of these, JL_C is wild-type, while JL_A and JL_D are resistant to topo II agents (85-fold to amsacrine and 13-fold to doxorubicin, respectively) because of a reduced level of topo II.[9,10] It is considered that IC_{50} ratios (JL_A/JL_C and JL_D/JL_C) of less than about twofold suggest a non-topoisomerase II mediated mechanism of action.[11]

Compounds **1a**, **2**, and **3b** represent three different chromophores, with one, none or two aza atoms, respectively. Of these, **2** was the least cytotoxic, while the compounds with aza atoms were broadly equivalent and more potent. The ratio of their binding to AT/GC DNA roughly paralleled cytotoxicity, with **2** also being the least selective. The $CONH(CH_2)_2NRR$ side chain was previously shown[1] to be the best of several explored in this series, and compounds **1a–g** study additional aspects of this. The enantiomers **1b** and **1c** show DNA binding broadly similar to that of the parent **1a**, and both were much more cytotoxic than **1a**, with the *S*-enantiomer being slightly better than the *R*-enantiomer. Lengthening the carboxamide chain (**1d**) retained reasonable activity, but weakening the pendant base (**1e**) was not favorable. In the 'diaza' series (**3a** and **3b**), the presence of the 2-methyl group had only a minor effect on activity. In the aza series, the corresponding 'desmethyl' derivative of **1a** was not accessible, but the 2-NMe_2 analogue **1g** was much less active. Of the compound studied here, the *S*-methyl enantiomer **1b** stood out in the Jurkat assays, with its high ratios (12 and 18) suggesting it is primarily a topo II inhibitor.

2.3. In vivo studies

Compound **1a** has been previously evaluated in vivo against subcutaneously implanted colon 38 tumors in mice, where it provided cures when administered by a repeated dose schedule and a growth delay of 17 days when administered as a single dose of 65 mg/kg.[1] Selected derivatives were therefore compared using a single dose administration schedule. Compounds **1b** and **1c** were found to be considerably more dose-potent than

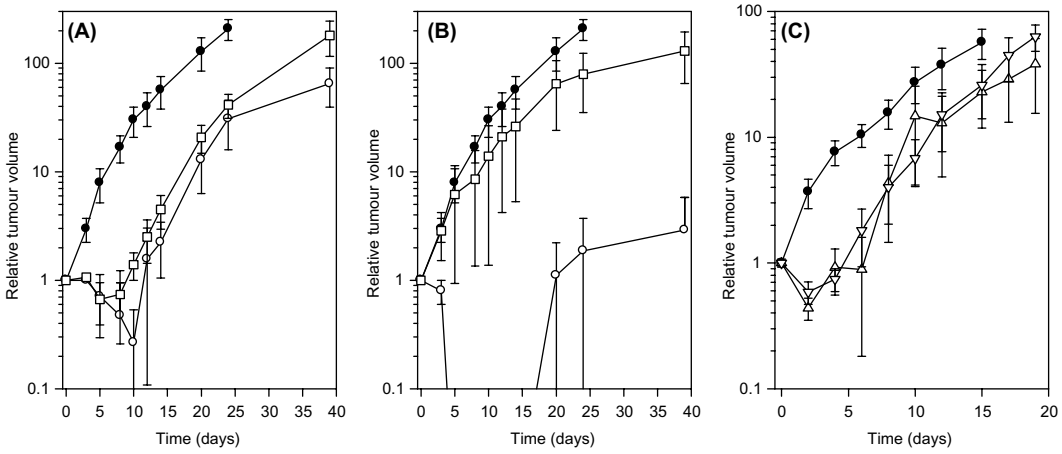
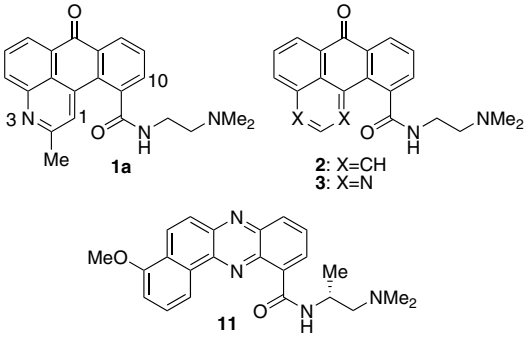


Figure 1. Growth curves of subcutaneously implanted Colon 38 tumors. (A) Untreated mice (●), **1b** at 8.9 mg/kg (○) and 5.9 mg/kg (□). (B) Untreated mice (●), **1c** at 8.9 mg/kg (○) and 5.9 mg/kg (□). (C) Untreated mice (●), **3a** at doses of 45 mg/kg (▽) and 65 mg/kg (△).

1a, consistent with their in vitro properties. At a dose of 5.9 mg/kg, both **1b** and **1c** provided growth delays (12 and >35 days, respectively), with some complete tumor regressions, with the *R*-enantiomer **1c** appearing the more active. Previous studies with benzophenazinecarboxamides[12] also showed a preference for the *R*-enantiomer **11** (XR-11576), which is currently in clinical trial.[13] Compound **3a** provided growth delays of 6 days when tested at doses of 45 and 65 mg/kg (Fig. 1). Both chromophore series therefore display in vivo antitumor activity.



3. Conclusions

N-[(Alkylamino)alkyl]carboxamide derivatives of 7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline (**1**) are shown to have greater antitumor activity than analogues with an extra (**3**), or zero (**2**) aza functions in the chromophore. The potency is enhanced by the presence of a chiral α-methyl group in the side chain, and compounds **1** remain an interesting series for further development.

4. Experimental

Melting points are uncorrected. NMR spectra were recorded on a Bruker AM-300 spectrometer operating at 300.13 MHz, (^{1}H) and 75.47 MHz, (^{13}C) and a Bruker DRX-400 spectrometer operating at 400.13 MHz, (^{1}H) and 100.62 MHz, (^{13}C), with chemical shifts are reported as δ values (ppm) relative to Me_4Si. Various standard techniques were used to identify proton-bound carbons in ^{13}C NMR spectra. EI and LSI (3-nitrobenzyl alcohol as liquid matrix) mode high-resolution mass spectra were obtained by Dr. N. Davies, University of Tasmania, Australia, or on a Varian VG-70SE spectrometer at nominal 5000 resolution at Auckland, New Zealand. Microanalyses were carried out at the Campbell Microanalytical Laboratory, University of Otago, New Zealand.

4.1. 7-Oxo-7*H*-benzo[*de*]anthracene-11-carboxylic acid (4)

This was prepared from methyl 2-iodobenzoate and methyl 8-bromo-1-naphthoate as reported[2,3] (Scheme 1): mp 272–273 °C. ^{1}H NMR [$(CD_3)_2SO$] δ 7.71 (t, 1H, J = 7.6 Hz), 7.79 (t, 1H, J = 7.9 Hz), 7.89–7.96 (m, 2H), 8.27 (d, 1H, J = 8.1 Hz), 8.40 (d, 1H, J = 7.5 Hz), 8.46–8.53 (m, 2H), 8.65 (d, 1H, J = 7.4 Hz).

4.2. 8-Aminoanthraquinone-1-carbonitrile (5b)

CuCN (1.8 g, 20 mmol) was added in one portion to a warm solution of 1-amino-8-chloroanthraquinone[1] (**5a**) (2.6 g, 10 mmol) in dimethyl formamide (25 mL), and the mixture was stirred under reflux for 4 h, then cooled. Water (200 mL) was added and the solid was filtered off, refluxed with HCl (70 mL from concd HCl and water 2:3) for 1 h, and filtered while warm. The solid was washed with water and dried, to give **5b** as a red-brown solid (2.0 g, 80%). This was used directly for the hydrolysis reaction, but could be recrystallized from acetic acid: mp 263–265 °C. ^{1}H NMR ($CDCl_3$) δ 7.10 (d, 1H, J = 8.1 Hz), 7.60 (t, 1H, J = 8.0 Hz), 7.78 (t, 1H, J = 9.6 Hz), 7.88 (d, 1H, J = 8.3 Hz), 8.05 (d, 1H, J = 8.4 Hz), 8.46 (d, 1H, J = 6.8 Hz).

4.3. 8-Aminoanthraquinone-1-carboxylic acid (5c)

Nitrile **5b** (1.8 g) in a mixture of concd H_2SO_4 (16 mL) and water (8 mL) was heated under reflux for 3 h, then cooled and poured onto ice/water. The solid that separated was filtered off, washed with water, then stirred with 4% aqueous NaOH and filtered. The filtrate was

taken to pH 3–4 with concd HCl, and the solid that separated was filtered off, washed with water and dried to give the acid **5c** (1.1 g, 57%), with the ^{1}H NMR spectrum of an authentic sample.[1]

4.4. Methyl 8-aminoanthraquinone-1-carboxylate (5d)

A mixture of acid **5c** (1.71 g, 6.4 mmol), absolute MeOH (50 mL), and concd H_2SO_4 (0.5 mL) was refluxed for 16 h, then cooled and a little unreacted acid was filtered off. The filtrate was concentrated to *c* 20 mL, water was added and the solid which separated was filtered off and dried to give the red-brown ester **5d** (1.40 g, 78%): mp 149–151 °C. ^{1}H NMR ($CDCl_3$) δ 4.02 (s, OCH_3), 6.95 (dd, 1H, J = 8.4, 1.0 Hz), 7.45 (t, 1H, J = 7.9 Hz), 7.61–7.66 (m, 2H), 7.74 (t, 1H, J = 7.7 Hz), 8.34 (d, 1H, J = 7.7, 1.4 Hz).

4.5. 2-Methyl-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxylic acid (6)

This was prepared from **5d** as reported, previously,[1] and used directly as the crude product from the cyclization reaction (ca. 50% pure).

4.6. Methyl 7-oxo-7*H*-benzo[*e*]perimidine-11-carboxylate (8a)

A mixture of methyl 8-aminoanthraquinone-1-carboxylate (**5d**) (0.14 g, 0.5 mmol) and dimethylformamide dimethyl acetal (0.3 g, 2.5 mmol) in MeCN (5 mL) was heated under reflux for 3 h. The solvent was removed at reduced pressure and to the dark residue was added ammonium acetate (0.24 g, 3.1 mmol) and dry EtOH (4 mL). The mixture was heated under reflux for 1 h, cooled to room temperature and the precipitate that formed was collected by filtration, to give **8a** as a light tan solid (0.13 g, 90%): mp (MeCN) 229–230 °C. ^{1}H NMR ($CDCl_3$) δ 4.04 (s, OCH_3), 7.77–7.81 (m, 2H), 8.12 (t, 1H, J = 8.8 Hz), 8.39 (d, 1H, J = 8.6 Hz), 8.54 (dd, J = 7.1, 1.5 Hz), 8.63 (d, 1H, J = 7.1 Hz), 9.47 (s). ^{13}C NMR ($CDCl_3$) δ 52.5 (CH_3), 119.5 (C), 127.8 (C), 129.0 (CH), 129.4 (CH), 130.7 (C), 130.8 (C), 131.8 (CH), 132.5 (CH), 133.3 (C), 133.8 (CH), 135.0 (CH), 149 0 (C), 154.9 (CH), 170.0 (C), 180.7 (C). Anal. Calcd for $C_{17}H_{10}N_2O_3$: C, 70.3; H, 3.5; N, 9.7. Found: C, 70.4; H, 3.4; N, 9.9.

4.7. 7-Oxo-7*H*-benzo[*e*]perimidine-11-carboxylic acid (8b)

A solution of ester **8a** (0.32 g) in water (4 mL) and trifluoroacetic acid (2.5 mL) was heated under reflux for 24 h, then cooled and added, with stirring, to water (25 mL). The solid which separated was filtered and washed with water to yield **8b** as a red solid (0.27 g, 89%). ^{1}H NMR [$(CD_3)_2SO$] δ 7.83–7.94 (m, 2H), 8.29 (t, 1H, J = 8.0 Hz), 8.40–8.45 (m, 2H), 8.57 (d, 1H, J = 7.2 Hz), 9.47 (s, 1H).

4.8. 2-Dimethylamino-7*H*-naphtho[1,2,3-*de*]quinolin-7-one (10a)

Reaction of 1-aminoanthraquinone (**5e**) with dimethylacetamide dimethyl acetal under reflux for 3 h in EtOH as reported[5] gave **10a**, mp (EtOH) 178–180 °C (lit.[5] mp 189 °C) in 64% yield. ^{1}H NMR ($CDCl_3$) δ 3.34 (s, 6H, $N(CH_3)_2$), 7.60–7.77 (m, 4H), 8.1 (br s, 1H), 8.25 (d, 1H, J = 8.0 Hz), 8.29 (d, 1H, J = 7.4 Hz), 8.47 (d, 1H, J = 7.7 Hz). ^{13}C NMR ($CDCl_3$) δ 37.8 ($N(CH_3)_2$), 104.2 (CH), 118.0 (C), 122.4 (CH), 123.0 (CH), 127.7 (CH), 127.8 (C) 129.1 (CH), 129.3 (CH), 131.6 (C), 132.3 (CH), 132.6 (CH), 133.7 (C), 134.3 (C), 147.4 (C), 157.2 (C), 182.7 (C).

4.9. 2-Methyl-7*H*-benzo[*e*]perimidin-7-one (8a)

A solution of **5e** (0.40 g, 1.80 mmol) and dimethylacetamide dimethyl acetal (1.20 g, 8.96 mmol) in dry MeCN (20 mL) was heated under reflux for 5 h under an atmosphere of nitrogen, then cooled to −15 °C and **10a** (0.05 g) was removed by filtration. The filtrate was evaporated to dryness at reduced pressure to leave the intermediate **9a** as a brown solid (0.40 g, 77%), which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 1.81 (s, 3H, CH_3), 3.31 (s, 6H, $N(CH_3)_2$), 7.07 (d, 1H, J = 8.0 Hz), 7.58 (t, 1H, J = 7.8 Hz), 7.68–7.73 (m, 2H), 7.94 (d, 1H, J = 6.5 Hz), 8.20–8.24 (m, 2H).

A mixture of **9a** (0.40 g) and ammonium acetate (0.52 g, 6.75 mmol) in dry EtOH (8 mL) was heated under reflux for 2 h then cooled to room temperature. The precipitate that formed was collected by filtration to yield **8a** as a brown solid (0.25 g, 74%), mp 201–203 °C (lit.[1] mp 211–212 °C; lit.[14] mp 201–203 °C), with NMR data as reported.[1]

4.10. Methyl 2-dimethylamino-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxylate (10b)

A solution of methyl 8-aminoanthraquinone-1-carboxylate **(5d)** (1.0 g) and dimethylacetamide dimethyl acetal (2.0 g), in MeOH (15 mL) was heated under reflux for 3 h, then stored in the freezer overnight. The dark solid which separated was filtered off and washed with a little cold MeOH to give 0.33 g (28%) of predominantly a mixture of two compounds (*c* 2:1). PTLC of a sample (0.1 g) (silica:chloroform) gave the two components, R_f 0.2 (major) and R_f 0.1 (minor), both of which were consistent with structure **10b**:

Major: Orange needles: mp (MeOH) 178–180 °C ^{1}H NMR ($CDCl_3$) δ 3.27 (s, 6H, $N(CH_3)_2$), 4.03 (s, 3H, OCH_3), 7.44 (s, 1H), 7.66 (t, 1H, J = 7.7 Hz), 7.70–7.77 (m, 2H), 8.03 (dd, 1H, J = 8.3, 1.1 Hz), 8.28 (dd, 1H, J = 7.3, 1.1 Hz), 8.61 (dd, 1H, J = 7.8, 1.6 Hz). ^{13}C NMR ($CDCl_3$) δ 37.8 ($N(CH_3)_2$), 52.6 (OCH_3), 108.9 (CH), 118.0 (C), 123.4 (CH), 127.2 (C), 128.8 (CH), 129.3 (CH), 129.9 (CH), 131.3 (C), 132.1 (C), 132.94 (CH), 132.99 (CH), 133.2 (C), 133.3 (C), 147.6 (C), 157.3 (C), 171.1 (C), 182.2 (C). EIMS: *m/z* 332 (65%), 317 (75), 303 (65), 285 (30), 273 (25), 258 (20), 245 (100), 231 (55). HRMS (EI): Calcd for $C_{20}H_{16}N_2O_3$: 332.1162 (M^+). Found: 332.1150.

Minor: Orange solid: mp (MeCN) 261–264 °C. ^{1}H NMR ($CDCl_3$) δ 3.34 (s, 6H, $N(CH_3)_2$), 4.05 (s, 3H,

OCH_3), 7.55 (d, 1H, $J = 7.0$ Hz), 7.69 (s, 1H), 7.71–7.79 (m, 2H), 8.01 (dd, 1H, $J = 8.4$, 1.1 Hz), 8.24 (d, 1H, $J = 7.4$ Hz), 8.38 (d, 1H, $J = 8.1$ Hz). ^{13}C NMR ($CDCl_3$) δ 39.0 ($N(CH_3)_2$), 52.6 (OCH_3), 105.8 (CH), 117.0 (C), 123.9 (CH), 124.5 (CH), 127.0 (C), 128.5 (C), 128.6 (CH), 130.3 (CH), 132.5 (CH), 133.2 (C), 135.3 (CH), 170.5 (C), 181.0 (C). EIMS: *m*/*z* 332 (65%), 317 (65), 303 (100), 289 (20), 272 (12), 258 (30), 245 (10), 230 (15). HRMS (EI): Calcd for $C_{20}H_{16}N_2O_3$: 332.1162 (M^+). Found: 332.1161.

Ammonium acetate (0.4 g, 5.2 mmol) was added to the dark filtrate, and the mixture was heated under reflux for 2.5 h, then cooled in the freezer. The dark solid which separated was filtered off to give **8d** (0.06 g). Dilution of the filtrate with much water provided a solid (0.05 g) shown by NMR to be largely starting material **5d**.

4.11. 2-Dimethylamino-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxylic acid (10c)

Ester **10b** (0.3 g) in MeOH (6 mL), water (6 mL), and 10% NaOH (3 mL) was heated under reflux for 24 h and filtered. The filtrate was concentrated to a small volume, acidified with hydrochloric acid and a first crop of **10c** was filtered off as a black solid (0.1 g). The filtrate was evaporated and the residue was extracted with hot EtOH. The extract was filtered while hot, and the yellow-brown filtrate was evaporated to leave a second crop (0.1 g). ^{1}H NMR [$(CD_3)_2SO$] δ 3.26 (s, 6H, $N(CH_3)_2$), 7.7–7.9 (m, 4H), 8.0–8.2 (m, 2H), 8.40 (d, 1H, $J = 7.2$ Hz).

4.12. Methyl 2-methyl-7-oxo-7*H*-benzo[*e*]perimidine-11-carboxylate (8d)

Dimethylacetamide dimethyl acetal (0.7 g, 5 mmol) was added to a solution of methyl 8-aminoanthraquinone-1-carboxylate (**5d**) (0.28 g, 1 mmol) in dry MeCN (5 mL), and the solution was heated under reflux for 3 h. No solid separated when this solution was then stored in the freezer. The solvent was removed at reduced pressure, ammonium acetate (0.5 g), and EtOH (5 mL) were added to the dark residue and the mixture was heated under reflux for 1.5 h. After cooling, the brown solid was filtered off to give **8d** (0.11 g, 36%): mp 211–213 °C. ^{1}H NMR ($CDCl_3$) δ 2.95 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 7.72–7.81 (m, 2H), 8.05 (t, 1H, $J = 8.1$ Hz), 8.26 (d, 1H, $J = 8.3$ Hz), 8.53–8.55 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 26.2 (CH_3), 52.3 (OCH_3), 117.3 (C), 127.7 (C), 128.3 (CH), 128.9 (CH), 130.7 (C), 131.6 (CH), 132.4 (CH), 133.1 (C), 133.7 (CH), 133.9 (C), 134.3 (CH), 149.5 (C), 154.7 (C), 164.3 (C), 170.1 (C), 181.0 (C). EIMS (*m*/*z*): 304 (40%), 273 (100), 246 (80). HRMS (EI): Calcd for $C_{18}H_{12}N_2O_3$: 304.0848 (M^+). Found: 304.0841.

4.13. 2-Methyl-7-oxo-7*H*-benzo[*e*]perimidine-11-carboxylic acid (8e)

A solution of ester **8d** (0.64 g) in water (12 mL) and trifluoroacetic acid (9 mL) was heated under reflux for 7 days, then cooled and added, with stirring, to water (25 mL). The black solid which separated was filtered and washed with water to give a first crop of **8e** (0.28 g, 46%). ^{1}H NMR [$(CD_3)_2SO$] δ 2.82 (s, 3H, CH_3), 7.8–7.9 (m, 2H), 8.15 (t, 1H, $J = 7.3$ Hz), 8.28 (d, 1H, $J = 8.0$ Hz), 8.35 (d, 1H, $J = 7.5$ Hz), 8.41 (d, 1H, $J = 7.1$ Hz). The filtrate was evaporated to dryness, the dark residue was stirred with 4% sodium hydroxide (5 mL), the mixture was filtered and the filtrate was acidified with hydrochloric acid. The light brown solid which separated was filtered and washed with water to give a second crop (0.16 g, 26%), with an identical ^{1}H NMR spectrum.

4.14. *N*-[2-(Dimethylamino)ethyl]-7-oxo-7*H*-benz[*de*]anthracene-11-carboxamide (2)

A suspension of **4** (0.14 g, 0.51 mmol) in dry benzene (20 mL) was treated with $SOCl_2$ (0.07 g, 0.59 mmol), and the mixture was heated under reflux for 2 h, then cooled to room temperature. *N*,*N*-Dimethylethylenediamine (0.11 g, 1.25 mmol) was added over 10 min and the resulting mixture was stirred for 1 h, then heated under reflux for 1 h. Volatiles were evaporated at reduced pressure and the residue was dissolved in chloroform (30 mL), washed with water (2×10 mL), and dried (Na_2SO_4). The solvent was removed to give **2** as a pale yellow solid (0.16 g, 91%): mp (benzene/light petroleum) 163–165 °C. ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6H, NMe_2), 2.48 (t, 2H, $J = 6.0$ Hz, CH_2), 3.59 (q, 2H, $J = 5.8$ Hz, CH_2), 6.57 (br t, 1H, CONH), 7.51 (t, 1H, $J = 7.6$ Hz), 7.68 (t, 1H, $J = 7.9$ Hz), 7.69–7.75 (m, 2H), 7.96 (d, 1H, $J = 8.0$ Hz), 8.18 (d, 1H, $J = 8.0$ Hz), 8.47 (d, 1H, $J = 7.6$ Hz), 8.52 (d, 1H, $J = 7.6$ Hz), 8.68 (d, 1H, $J = 7.6$ Hz).^{13}C NMR ($CDCl_3$) δ 37.6 (CH_2), 45.0 (CH_3), 57.3 (CH_2), 125.4 (C), 126.27 (CH), 126.34 (CH), 17.7 (CH), 128.2 (C), 128.6 (CH), 129.3 (CH), 129.8 (CH), 130.6 (CH), 132.3 (C), 132.8 (C), 133.3 (C), 133.7 (CH), 135.5 (CH), 135.8 (C), 171.8 (C), 183.3 (C). Anal. Calcd for $C_{22}H_{20}N_2O_2 \cdot 0.75H_2O$: C, 73.8; H, 6.05; N, 7.8. Found: C, 74.0; H, 5.6; N, 7.9.

4.15. Preparation of amides 1a–f: general

4.15.1. Method A. A solution of the crude acid **6** (0.5 g, 1.73 mmol) in 50 mL of 1,4-dioxane was heated under reflux with CDI (0.45 g, 2.8 mmol) for 4 h, cooled to room temperature, the solvent was removed at reduced pressure and the residue was dissolved in CH_2Cl_2 (50 mL), washed with 10% Na_2CO_3 aqueous solution, water, and dried. The solvent was evaporated under reduced pressure to give the crude imidazolide (0.5 g, 85%) as a pale brown solid, which was used for next step without further purification. The imidazolide (0.5 g, 1.47 mmol) and the amine (0.5 g, 5 equiv) was dissolved in dichloromethane (10 mL) and stirred at room temperature for 24 h, and the reaction mixture was then filtered through a short alumina column with EtOAc/CH_2Cl_2 to give the crude amide. This was purified by preparative HPLC to remove closely running impurities.

4.15.2. Method B. A solution of crude **6** (0.5 g, 1.73 mmol) and the amine hydrochloride in Et_3N

(2.0 g) was cooled in an ice-bath and treated with BOP·Cl (0.54 g, 2.1 mmol) added in one portion. The reaction was stirred at room temperature for 1 h, then diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried, and concentrated under reduced pressure. The residue was filtered through a short alumina column with EtOAc/CH_2Cl_2 and then purified by preparative HPLC to remove closely running impurities.

4.16. Preparative HPLC

These were performed using a Synergi Polar-RP (250 × 21.2 mm) column with detection at 254 nM. The column was eluted by washing for 10 min with a linear gradient of 35–100% of MeCN (contained 0.1% of TFA) in water (acidified to pH 3 with TFA). Aliquots were basified with ammonia, extracted with dichloromethane (3 × 50 mL), dried, and concentrated under reduced pressure to give the pure products.

4.17. *N*-[(1*R*)-2-(Dimethylamino)-1-methylethyl]-2-methyl-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxamide (1b)

Reaction of **6** (0.5 g, 1.73 mmol) with (1*R*)-2-(dimethylamino)-1-methylethylamine hydrochloride (0.5 g, 2.4 mmol) by method B gave **1b** as a solid (53 mg, 8%); ^{1}H NMR [$(CD_3)_2SO$] δ 8.41 (dd, J = 7.3, 0.9 Hz), 8.37 (dd, J = 7.7, 1.5 Hz), 8.26 (dd, J = 8.2, 0.9 Hz), 8.19 (s), 7.79 (t), 7.65 (dd, J = 7.4, 1.5 Hz), 7.51 (t), 6.96 (br s, NH), 4.31 (m, 1H), 2.78 (s, 3H), 2.54 (m, 1H), 2.37 (m, 1H), 2.29 (s, 6H), 1.47 (d, J = 6.3 Hz, 3H). HRMS(EI) Calcd for $C_{23}H_{24}N_3O_2$: 374.1869 (M^+). Found: 374.1871.

4.18. *N*-[(1*S*)-2-(Dimethylamino)-1-methylethyl]-2-methyl-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxamide (1c)

Reaction of **6** (0.5 g, 1.73 mmol) with (1*S*)-2-(dimethylamino)-1-methylethylamine hydrochloride (0.5 g, 2.4 mmol) by method B gave **1c** as a solid (25 mg, 8% based on available **6**); ^{1}H NMR [$(CD_3)_2SO$] δ 8.41 (dd, J = 7.3, 0.9 Hz), 8.37 (dd, J = 7.7, 1.5 Hz), 8.26 (dd, J = 8.2, 0.9 Hz), 8.19 (s), 7.79 (t), 7.65 (dd, J = 7.4, 1.5 Hz), 7.51 (t), 6.96 (br s, NH), 4.31 (m, 1H), 2.78 (s, 3H), 2.54 (m, 1H), 2.37 (m, 1H), 2.29 (s, 6H), 1.47 (d, J = 6.3 Hz, 3H). HRMS (EI) Calcd for $C_{23}H_{24}N_3O_2$: 374.1869 (M^+). Found: 374.1858.

4.19. *N*-[3-(Dimethylamino)propyl]-2-methyl-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxamide (1d)

Reaction of **6** (0.5 g, 1.73 mmol) with 3-(dimethylamino)propylamine (0.5 g, 4.9 mmol) by method A gave **1d** as a solid (100 mg, 32% based on available **6**); ^{1}H NMR [$(CD_3)_2SO$] δ 8.83 (br s, 1H), 8.56 (dd, J = 7.3, 0.9 Hz, 1H), 8.46 (dd, J = 7.7, 1.5 Hz, 1H), 8.41 (dd, J = 8.2, 0.9 Hz, 1H), 8.14 (s, 1H), 7.84 (t, 1H), 7.80 (dd, J = 7.4, 1.5 Hz, 1H), 7.78 (t, 1H), 2.76 (s, 3H), 2.50 (m, 2H), 2.27 (m, 2H), 2.12 (s, 6H), 1.72 (m, 2H). HRMS(EI) Calcd for $C_{23}H_{23}N_3O_2$: 373.1790 (M^+). Found: 373.1782 [M+H].

4.20. *N*-{2-[(2-Hydroxyethyl)amino]ethyl}-2-methyl-7-oxo-7*H*-naphtho[1,2,3-*de*] quinoline-11-carboxamide (1e)

Reaction of **6** (0.5 g, 1.73 mmol) with 2-(2-aminoethylamino)ethanol by method A gave **1e** as a solid (100 mg, 0.27 g, 32% based on available **6**); ^{1}H NMR [$(CD_3)_2SO$] δ 8.85 (br s, 1H), 8.56 (dd, J = 7.3, 0.9 Hz, 1H), 8.46 (dd, J = 7.7, 1.5 Hz, 1H), 8.41 (dd, J = 8.2, 0.9 Hz, 1H), 8.14 (s, 1H), 7.84 (t, 1H), 7.80 (dd, J = 7.4, 1.5 Hz, 1H), 7.78 (t, 1H), 4.45 (br s, 1H), 3.44 (m, 4H), 2.78 (m, 2H), 2.76 (s, 3H), 2.61 (m, 2H). HRMS(EI) Calcd for $C_{22}H_{22}N_3O_3$: 376.1661 (M^+). Found: 376.1662.

4.21. 2-Methyl-7-oxo-*N*-[2-(1-piperidinyl)ethyl]-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxamide (1f)

Reaction of **6** (0.5 g, 1.73 mmol) with 1-(2-aminoethyl)piperidine by method A gave **1f** as a solid (90 mg, 26% based on available **6**); ^{1}H NMR [$(CD_3)_2SO$] δ 8.79 (br s, 1H), 8.56 (dd, J = 7.3, 0.9 Hz, 1H), 8.46 (dd, J = 7.7, 1.5 Hz, 1H), 8.41 (dd, J = 8.2, 0.9 Hz, 1H), 8.14 (s, 1H), 8.05 (t, 1H), 7.80 (dd, J = 7.4, 1.5 Hz, 1H), 7.75 (t, 1H), 3.46 (m, 4H), 2.76 (s, 3H), 2.40 (m, 4H), 1.50 (m, 4H), 1.38 (m, 2H). HRMS(EI) Calcd for $C_{25}H_{26}N_3O_2$: 400.2025 (M^+). Found: 400.2018.

4.22. *N*-[2-(Dimethylamino)ethyl]-2-methyl-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxamide (1a): modified method

A solution of crude **6** (1.45 g, 5.01 mmol, 50% by NMR) in dry dioxan (50 mL) was treated with 1,1′-carbonyldiimidazole (1.22 g, 7.5 mmol), and the mixture was refluxed under N_2 for 4 h. The solvent was removed under reduced pressure, CH_2Cl_2 (150 mL) was added, and organic layer was washed with 10% Na_2CO_3 (80 mL) and brine (80 mL), dried (Na_2SO_4). Evaporation under reduced pressure gave the intermediate imidazolide as a brown solid (1.43 g). This was dissolved in dioxane (15 mL) and *N*,*N*-dimethylethylenediamine (5 mL), and refluxed under N_2 for 3 h. The solvent was removed under reduced pressure, and the residue was diluted with 10% Na_2CO_3 (150 mL) and extracted with CH_2Cl_2 (3 × 80 mL). The combined organic layers were washed with brine (2 × 100 mL) and dried (Na_2SO_4). The resulting solution was washed through an alumina column (50 × 50 mm), eluting with CH_2Cl_2/MeOH (9:1). Reversed-phase C_{18} flash column chromatography (150 × 20 mm) of this product, eluting with 0.05% aqueous TFA/MeCN (90:10, then 40:60) gave **1a** as an orange amorphous solid (516 mg, 58% based on available **6**, 82% pure by HPLC). This product (200 mg) was dissolved in dioxane and saturated HCl in dioxan was added dropwise until the solution reached pH ~2. The solvent was removed under reduced pressure, and the resulting solid was recrystallized from EtOAc/MeOH to give the hydrochloride salt as an amorphous orange solid (170 mg, 45% overall yield based on available **6**, 92.5% pure by HPLC); ^{1}H NMR [$(CD_3)_2SO$] δ 10.36 (br s, 1H), 9.14 (br t, J = 5.5 Hz, 1H), 8.57 (dd, J = 7.3, 1.2 Hz, 1H), 8.50 (dd, J = 7.8, 1.6 Hz, 1H,), 8.45 (dd, J = 8.3, 1.2 Hz, 1H), 8.09 (dd,

J = 8.2, 7.4 Hz, 1H), 8.05 (s, 1H), 7.93 (dd, J = 7.5, 1.6 Hz, 1H), 7.85 (t, J = 7.7 Hz, 1H), 3.75 (td, J = 6.4, 5.7 Hz, 2H), 3.34 (td, J = 6.4, 5.8 Hz, 2H), 2.87 (s, 3H), 2.86 (s, 3H), 2.81 (3H, s); HRMS(EI) Calcd for $C_{22}H_{22}N_3O_2$: 360.1712 (M^+). Found: 360.1707.

4.23. *N*-[2-(Dimethylamino)ethyl]-7-oxo-7*H*-benzo[*e*]perimidine-11-carboxamide (3a)

A mixture of acid **8b** (0.27 g, 1 mmol) in $SOCl_2$ (3 mL) was kept at 50 °C for 30 min. The $SOCl_2$ was evaporated under reduced pressure, and residual traces were removed by azeotroping with benzene. Dichloromethane (10 mL) was added, the mixture was stirred and cooled on ice, a solution of *N*,*N*-dimethylethylenediamine (0.5 mL) in dichloromethane was then added, and the solution was stirred at room temperature for 30 min. It was washed with water, 10% sodium carbonate, dried, and the solvent was evaporated to leave a red residue of the amide (0.22 g, 63%). This was taken up in EtOH, a little insoluble material was filtered and the filtrate was evaporated. The residue was dissolved in acetone and addition of a drop of concentrated hydrochloric acid gave a brown, slightly hygroscopic solid. This was filtered, recrystallized from 2-propanol, and filtered and washed with acetone under nitrogen to give **3a** as the hydrochloride salt (0.12 g), mp indistinct (darkened and shrank >160 °C). ^{1}H NMR [$(CD_3)_2SO$] δ 2.85 (d, J = 4.8 Hz, 6H, $^+NHMe_2$), 3.66 (q, 2H, J = 6.5 Hz, CH_2), 7.74–7.92 (m, 2H), 8.26 (t, 1H, J = 7.4 Hz), 8.35–8.49 (m, 2H), 8.57 (d, 1H, J = 7.3 Hz), 9.52 (s, 1H), 10.03 (br s, NH).

The free base of **3a** was a red-brown flaky solid. ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 6H, NMe_2), 2.65 (t, 2H, J = 6.5 Hz, CH_2), 3.69 (q, 2H, J = 6.7 Hz, CH_2), 6.45 (br s, NH), 7.75–7.77 (m, 2H), 8.09 (t, 1H, J = 7.4 Hz), 8.35 (d, 1H, J = 8.2 Hz), 8.49–8.52 (m, 1H), 8.61 (d, 1H, J = 8.0 Hz), 9.47 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 37.3 (CH_2), 44.7 (CH_3), 57.0 (CH_2), 119.5 (C), 127.7 (C), 128.6 (CH), 129.3 (CH), 131.8 (CH), 133.6 (CH), 133.7 (CH), 133.9 (C), 135.1 (CH), 137.4 (C), 149.0 (C), 155.1 (CH), 155.5 (C), 170.4 (C), 180.9 (C). HRMS(EI): Calcd for $C_{20}H_{18}N_4O_2$: 346.1430 (M^+). Found: 346.1432.

4.24. *N*-[2-(Dimethylamino)ethyl]-2-methyl-7-oxo-7*H*-benzo[*e*]perimidine-11-carboxamide (3b)

Acid **8e** (0.16 g; the light brown second crop) was reacted with $SOCl_2$ (50 °C for 45 min) and then with *N*,*N*-dimethylethylenediamine as above for **3a**, to give the crude amide **3b** as a dark solid (0.10 g, 50%). ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 6H, NMe_2), 2.80 (br t, 2H, CH_2), 2.90 (s, 3H, CH_3), 3.77 (q, 2H, J = 6.7 Hz, CH_2), 6.82 (br s, NH), 7.6–7.75 (m, 2H), 8.00 (t, 1H, J = 8.3 Hz), 8.20 (d, 1H, J = 8.2 Hz), 8.41–8.48 (m, 2H). This was converted in EtOH to an oxalate salt, which separated as a sticky solid upon addition of ether. This product was boiled in EtOH, the liquid was decanted from some sticky residue, and the salt was reprecipitated with ether. This was repeated, then the salt was recrystallized twice from MeCN and filtered in a nitrogen stream (the solid rapidly went sticky in air when wet but remains crystalline when dry) to give pure **3b** as an orange-brown solid: mp 122–123 °C. ^{1}H NMR (D_2O) δ 2.57 (s, 3H, CH_3), 2.96 (s, 6H, NMe_2), 3.45 (t, 2H, J = 6.1 Hz, CH_2), 3.79 (t, 2H, J = 6.1 Hz, CH_2), 7.45–7.75 (m, 5H), 7.88 (br d, 1H). HRMS (LSI): Calcd for $C_{21}H_{21}N_4O_2$: 361.1666 $(M+H)^+$. Found: 361.1613. Anal. Calcd for $C_{21}H_{20}N_4O_2 \cdot C_2H_2O_4 \cdot 1.5H_2O$: C, 57.9; H, 5.3; N, 11.7. Found: C, 57.6; H, 5.0; N, 11.7.

4.25. *N*-[2-(Dimethylamino)ethyl]-2-dimethylamino-7-oxo-7*H*-naphtho[1,2,3-*de*]quinoline-11-carboxamide (1g)

A mixture of acid **10c** (0.1 g) in $SOCl_2$ (3 mL) was kept at 50 °C for 45 min, then treated as for **3a** to give the crude amide (0.040 g, 33%) as a red semisolid. From PTLC [silica:chloroform/diethylamine (10:1)], an orange band (R_f 0.32) was extracted with chloroform to give **1g** as an orange semisolid (0.01 g). ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 6H, $N(CH_3)_2$), 2.49 (t, 2H, J = 5.3 Hz), 3.33 (s, 6H, $N(CH_3)_2$), 3.57 (q, 2H, J = 5.3 Hz), 6.72 (br s 1H, NH), 7.55–7.71 (m, 3H), 7.89 (s, 1H), 7.98 (d, 1H, J = 8.3 Hz), 8.21 (d, 1H, J = 7.6 Hz), 8.50 (d, 1H, J = 7.6 Hz). HRMS (EI): Calcd for $C_{23}H_{24}N_4O_2$: 388.1900 (M^+). Found: 388.1899.

4.26. DNA binding studies

Fluorescence was measured in 0.01 ionic strength buffered sodium chloride (pH 7.0) containing ethidium bromide (1.260 μM) and either poly-(dA-dT)·(dA-dT) and poly-(dG-dC)·(dG-dC) at a concentration of 1 μM in nucleotides. Aqueous solutions of the drugs as chloride salts were added stepwise and fluorescence was measured at 625 nm with excitation at 595 nm. Drug concentrations for 50% reduction in DNA-ethidium fluorescence (with correction for the fluorescence of free ethidium) were calculated and binding constants were calculated from the assumption that ethidium and the drugs competed for the same DNA binding site.[6]

4.27. In vitro cytotoxicity 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.[15] Growth inhibition assays were performed by culturing cells at 4.5×10^3 (P388), and 10^3 (LLTC and Jurkat) per well in microculture plates (150 mL per well) for 3 days (P388) or 4 days (LLTC and Jurkat) in the presence of drug. Cell growth was determined by [^{3}H]-thymidine uptake over the last 2 h of incubation (P388)[16] or by sulforhodamine staining (LLTC and Jurkat).[17] Results represent means of at least two independent assays.

4.28. In vivo tumor assays

Procedures followed institutional ethical guidelines. Colon 38 tumors were grown subcutaneously from 1 mm^3 fragments in one flank of BDF1 mice (anesthetized with pentobarbitone 90 mg/kg). When tumors reached a

diameter of ≈4–6 mm (7–8 days), mice were divided into control and drug treatment groups (5 mice/group), with similar average tumor volumes in each group. The drugs, as solutions of the hydrochloride salts in distilled water, were injected intraperitoneally as a single dose in a volume of 0.01 mL/g body weight. Tumor diameters were measured with calipers three times a week and 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 graph as mean tumor volumes (±SEM) versus time after treatment. Growth delay was calculated as the time taken for a tumor to reach a mean volume four-fold higher than its pre-treatment volume.

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