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Rational Design, Synthesis and Biological Evaluation of 3*H*-Naphtho[1,2,3-*de*]quinoline-2,7-diones: a New Class of Potential Antitumor Agents*

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A series of novel potential DNA-binding antitumor agents, 6-[(ω-aminoalkyl)amino]-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-diones **3a**–**j**, has been prepared by nucleophilic substitution of commercially available 6-bromo-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione with the suitable 1-[ω-(alkylamino)alkyl]amine. *In vitro* cytotoxic potencies of these derivatives toward six tumor cell lines, including human colon adenocarcinoma (HT29) and human ovarian carcinoma (A2780 sensitive, A2780cisR cisplatin-resistant, CH1, CH1cisR cisplatin-resistant, and SKOV-3), are described and compared to that of reference drugs. The 6-[3-(diethylamino)propyl]-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-diones (**3e**), which possesses good cytotoxicity and low or none cross resistance with Cs on resistant cell lines, can be regarded as a new lead in the development of intercalating anticancer derivatives.

Key words: cytotoxicity, antitumor, DNA binding, acridine derivatives, structur-activity relationships

The antitumor drugs that intercalate DNA are of growing interest in the field of anticancer derivatives. Generally, they are characterized by the presence of a planar chromophore, often constituted by three or four condensed rings, which can intercalate into base pairs, and, at least, of one flexible basic side chain, which can improve the DNA binding and/or interact with enzymes such as topoisomerases. Some examples of derivatives undergoing clinical trials endowed with only one side chain are constituted by acridine-4-carboxamide DACA [1], mitonafide [2], azonafide [3] (**1**), imidazoacridones [4]. Among intercalating anticancer agents with two basic chains, it can be cited mitoxantrone [5], anthrapyrazoles [6], aza-anthracendiones [7], aza-anthrapyrazoles [8], clinically used or in clinical trials, and also a number of interesting acridine derivatives synthesized by our research group: the bis functionalized acridone-4-carboxamides [9], the bis functionalized acridine-4-carboxamides [10],

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* Dedicated to Prof. E. Borowski on the occasion of his 75th birthday.

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the pyrazolo[3,4,5-*kl*]acridine-5-carboxamides [11], the pyrazolo[3,4,5-*mn*]pyrimido[5,6,1-*de*]acridines [12], and, in particular, the pyrimido[5,6,1-*de*]acridines [13–16] (**2**).

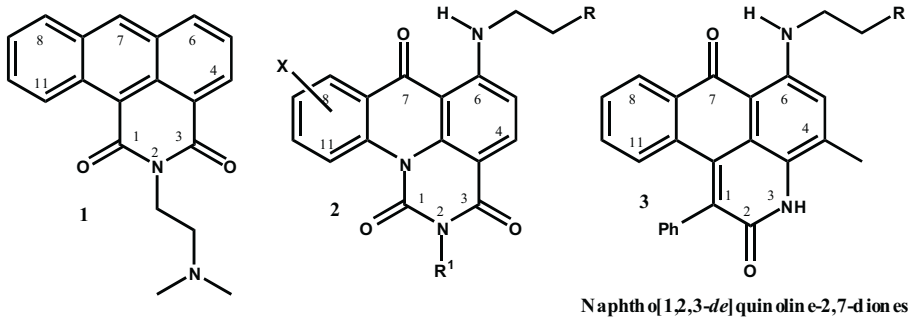
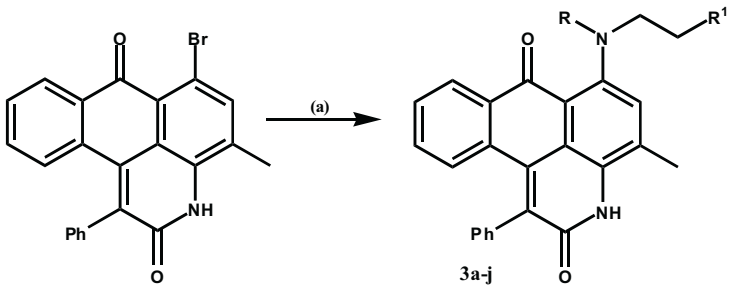


Figure 1. Structures and ring numbering of parent compounds azonafide (**1**) and pyrimido[5,6,1-*de*]acridines (**2**) in comparison with the target naphto[1,2,3-*de*]quinoline derivatives (**3**).

In the continual search for new classes of antitumor agents, we decided to investigate the 6-[(*ω*-aminoalkyl)amino]-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-diones (**3**) as analogues related to the azonafide **1** and, especially, to the pyrimido[5,6,1-*de*]acridines **2**, which chromophores are constituted by similar ring systems, as shown in Figure 1. Prompted by the above rationale, we have synthesized compounds **3a**–**j** and studied their cytotoxic activity on different cell lines.

RESULTS AND DISCUSSION

Scheme 1 shows the synthetic pathways leading to the target derivatives **3a**–**j**. Compounds **3a**–**i** were obtained by nucleophilic substitution of the commercially available 6-bromo-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione with the appropriate (*ω*-aminoalkyl)amine in different experimental conditions. The preparation of **3j** was achieved by nucleophilic substitution of 6-bromo-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione with *tert*-butyl *N*-(2-aminoethyl)carbamate and subsequently Boc (Boc = *tert*-butoxycarbonyl) deprotection in dioxane and hydrochloric acid.



Scheme 1. Reagents (a): $NHR(CH_2)_2R^1$ for **3a**–**i**; $NH_2(CH_2)_2NHC(O)OC(CH_3)_3$, then H^+ for **3j**. Substituents: R = Me for **3a**; R = H for **3b**–**j**. R^1 = $N(CH_3)_2$ for **a** and **b**, $CH_2N(CH_3)_2$ for **c**, $N(C_2H_5)_2$ for **d**, $CH_2N(C_2H_5)_2$ for **e**, $CH_2NH(CH_2)_2OH$ for **f**, piperidino for **g**, 1-pyrrolidinyl for **h**, morpholino for **i**, NH_2 for **j**.

The free base forms of the target derivatives were converted, by usual methods, into their more soluble hydrochlorides, to examine the cytotoxic activity of these agents and, in some case, to allow the dissolution for NMR.

HT29 human colon adenocarcinoma cell line. In vitro cytotoxic potencies of target 6-[(*ω*-aminoalkyl)amino]-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-diones (**3**) in comparison with reference drug doxorubicin (**Dx**) against human colon adenocarcinoma cell line (HT29) are reported in Table 1. Parent 6-[2-(dimethylamino)ethyl]amino-2,3-dihydro-1*H*,7*H*-pyrimido[5,6,1-*de*]acridine-1,3,7-trione (**2a**, Figure 1: R = $N(CH_3)_2$, X = R^1 = H) was also included in Table 1 to hallow a direct comparison between compounds **3** and the unique derivative **2** possessing only a basic side chain in position 6 [14]. The IC_{50} represents the drug concentration (μM) required to inhibit cell growth by 50%.

Table 1. Cytotoxic activity of **3a-j** in comparison with parent pyrimido[5,6,1-*de*]acridine **2a** and with reference drugs doxorubicin (Dx) and cisplatin (Cs) *versus* human colon adenocarcinoma cell line[a] (HT29) and *versus* ovarian carcinoma cell line panel[b] (A2780 and A2780/Cs cisplatin resistant, CH1 and CH1/Cs cisplatin resistant, and SKOV3).

	IC_{50} (μM)[c]					
Comp.	HT29	A2780	A2780/Cs[d]	CH1	CH1/Cs[d]	SKOV-3
3a	3.3	9.0	11 (1.2)	12	12 (1.0)	15
3b	0.27	1.6	3.0 (1.9)	2.3	9.8 (4.3)	2.7
3c	0.32	2.4	3.7 (1.1)	5.4	12 (2.2)	3.5
3d	3.3	2.1	>25 (>12)	>25	>25 (n.c.)	9.0
3e	0.27	1.2	2.5 (2.1)	2.4	2.3 (1.0)	2.4
3f	1.4	0.7	8.6 (12)	3.2	8.0 (2.5)	7.4
3g	>10	>25	>25 (n.c.)	>25	>25 (n.c.)	>25
3h	0.97	1.6	3.2 (2.0)	5.6	18 (3.2)	6.0
3i	>10	>25	>25 (n.c.)	>25	>25 (n.c.)	>25
3j	3.2	5.6	12 (2.2)	11	9.0 (0.8)	12
2a[e]	0.90	2.1	2.0 (0.95)	1.4	2.45 (1.7)	3.85
Dx	0.026					
Cs		0.89	3.4 (3.8)	0.15	2.4 (16)	3.4

[a]All assays were performed in triplicate. [b]Values represent the mean of a representative head to head experiment using 4 replicate wells per drug concentration and 8 control wells. [c]Drug concentration required to inhibit cell growth by 50%. [d] In parentheses RF (resistance factor) is the ratio of IC_{50} values of cisplatin-resistant cell line to the sensitive cell line; n.c. = not calculable. [e]Data from reference [14].

The results indicate that: (a) **3b,c,e** emerge as the most potent among the new derivatives with IC_{50} values of ~ 0.3 micromolar and with cytotoxicity higher than related **2a**; (b) many compounds **3** possess a moderate antiproliferative activity in the micromolar range; (c) none of the new derivatives posses potency similar to that of reference drug doxorubicin (Dx, IC_{50} = 26 nanomolar), but only **3g,i** are devoid of cytotoxic activity.

The data obtained allow us to formulate some considerations regarding the side chains: (i) the side chain of **3a** has been chosen to check if the intramolecular hydrogen bond between the carbonyl in position 7 and the hydrogen of amine in position 6

for compounds **3** is important as in the case of derivatives **2** [14] and of similar acridine derivatives [9]. In agreement to what previously observed [9,14], the difference in activity between the pair **3a⇔3b**, with **3b** being 12 times more cytotoxic than its the *N*-methyl derivative **3a**, clearly indicates the importance of the cited intramolecular hydrogen bond also for these compounds. (ii) In contrast to what previously noted with derivatives **2** [14], especially for bulky substituents, the optimal distance between the two nitrogen atoms seems to be of three methylene units as indicated by the difference in potency between the pair **3d⇔3e** which substituents at distal nitrogen atom are two ethyls. (iii) A side chain similar to that of the clinically useful drug mitoxantrone, compound **3f**, leads to a fair activity, but not excellent as for the parent compounds **2** [14]. (iv) In the sub series **3g**–**3i**, in which the distal nitrogen atom is part of a cycle, the best results are obtained with **3h** (R^1 = 1-pyrrolidinyl, Scheme 1) endowed of a good activity, while **3g,i** (R^1 = piperidino or morpholino, respectively) are not active. The good activity of **3h** is not surprising in the light of we found with derivatives bearing similar side chain [11,12]; also expected was the inactivity of **3i**, in which the distal nitrogen atom is not so basic to be protonated at physiological conditions [9,10]; instead, it was surprising the inactivity of **3g** bearing a side chain that was found to be efficient in many other derivatives [9–12]. (v) Finally, compound **3j**, bearing a side chain similar to that of BBR 2778 [7], an aza-anthracendione in phase II of clinical trial [17], possesses border line cytotoxic activity, showing that this kind of side chain is not optimal for **3**.

Human ovarian carcinoma cell line panel. *In vitro* cytotoxic potencies of the new 6-[(*ω*-aminoalkyl)amino]-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-diones (**3**), in comparison with the parent compound **2a** and the reference drug Cisplatin (Cs), against five human ovarian carcinoma cell lines, A2780 (sensitive), A2780/Cs (cisplatin resistant), CH1 (sensitive), CH1/Cs (cisplatin resistant), and SKOV-3, are also shown in Table 1. In the resistant cell line columns, besides the IC_{50} values, are reported, in parentheses, the resistance index (RI = IC_{50} ratio of resistant line on sensitive one) values.

Generally, the results parallel what we found for HT29. On sensitive cell lines the best compounds seem to be **3b,e**, which possess activity comparable to that of reference drug Cs; also **3c,f,h** are endowed of fair activity on some cell lines. In addition, some derivatives **3**, especially, **3a,c,e,j** are scarcely or not cross resistant with Cs on resistant cell lines.

CONCLUSIONS

It can be concluded that the target the 6-[(*ω*-aminoalkyl)amino]-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-diones (**3a–j**) constitute a new class of potential intercalating derivatives endowed with good cytotoxic properties. The new chromophore seems to be interesting in comparison with derivatives **2**, since some compounds **3** posses activity profile similar to that of mono functionalized **2a**. Perhaps, the introduction of a second basic side chain in position 3 may increases biological properties

as we found for reference compounds **2** [13,14]. However, the 6-[3-(diethylamino)propyl]-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-diones (**3e**), which possesses good cytotoxicity and low or none cross resistance with Cs on resistant cell lines, can be regarded as a new lead in the development of anticancer derivatives.

EXPERIMENTAL

Chemistry. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Thin-layer chromatography (TLC) was accomplished using plates precoated with silica gel 60 F-254 (Merck). All ^{1}H NMR spectra were recorded on a Varian VXR 300 instrument. Chemical shifts are reported as δ values (ppm) downfield from internal Me_4Si in the solvent shown. The following NMR abbreviations are used: br (broad), vbr (very broad), s (singlet), d (doublet), t (triplet), m (multiplet), ar (aromatic proton), ex (exchangeable with D_2O). Elemental analyses were performed on a Model 1106 elemental analyzer (Carlo Erba Strumentazione).

6-{[(2-Dimethylamino)ethyl](methyl)amino}-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3a). Example of general procedure for the preparation of 3f–i. The commercially available 6-bromo-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (300 mg, 0.72 mmol) was suspended in 2-ethoxyethanol (20 ml), added of N^1,N^1,N^2-trimethyl-1,2-ethanediamine (0.92 ml, 7.2 mmol), and refluxed for 4 h under stirring. A TLC eluted with $CHCl_3$/MeOH (30:1 v/v) reveled that the reaction was terminated. The volatile was evaporated and the residue was refluxed in methanol. After cooling, a solid was obtained which was washed successively with methanol and ether, and dried at room temperature to yield pure **3a** (89%): m.p. 220–221°C; hydrochloride 113–116°C; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 6H, 2× CH_3), 2.54 (s, 3H, 4-CH_3), 2.68–2.83 (m, 2H, CH_2), 2.90 (s, 3H, 6-NCH_3), 3.58 (t, 2H, CH_2), 7.07–7.13 (m, 2H, ar), 7.27 (s, 1H, ar), 7.35–7.45 (m, 5H, ar), 8.16 (d, 1H, ar), 10.78 (br s, 1H, NH ex). Anal. Calcd. for $C_{28}H_{27}N_3O_2$: C, 76.86; H, 6.22; N, 9.60. Found: C, 76.94; H, 6.01; N, 9.94.
Derivatives **3f–i** were prepared in a similar manner.

6-({2-[(2-Hydroxyethyl)amino]ethyl}amino)-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3f). (82%); m.p. 159–161°C; hydrochloride 201–203°C; ^{1}H NMR (DMSO-d_6) δ 2.57–2.77 (m, 5H, CH_2 + CH_3), 2.92 (t, 2H, CH_2), 3.43–3.60 (m, 4H, 2 × CH_2), 4.54 (s, 1H, OH ex), 7.12–7.20 (m, 2H, ar), 7.29–7.58 (m, 9H, 7ar + 2 × NH ex), 8.38 (d, 1H, ar), 10.87 (s, 1H, 6-NH ex). Anal. Calcd. for $C_{27}H_{25}N_3O_3$: C, 73.78; H, 5.73; N, 9.56. Found: C, 73.89; H, 5.51; N, 9.42.

4-Methyl-1-phenyl-6-[(2-piperidinoethyl)amino]-2,7-dihydro-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3g). (51%); m.p. 259–260°C; hydrochloride > 300°C; hydrochloride ^{1}H NMR (DMSO-d_6) δ 1.30–1.92 (m, 6H, 3 × CH_2), 2.70 (s, 3H, CH_3), 2.88–3.10 (m, 2H, CH_2), 3.24–3.41 (m, 2H, CH_2), 3.47–3.60 (m, 2H, CH_2), 4.00 (t, 2H, CH_2), 6.75–7.70 (m, 10H, 9ar + NH ex), 8.36 (d, 1H, ar), 10.58–10.94 (m, 2H, 2 × NH ex). Anal. Calcd. for $C_{30}H_{29}N_3O_2$: C, 77.73; H, 6.31; N, 9.06. Found: C, 77.98; H, 6.53; N, 8.84.

4-Methyl-1-phenyl-6-[(2-pyrrolidin-1-yl-ethyl)amino]-2,7-dihydro-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3h). (77%); m.p. 168–170°C; hydrochloride > 300°C; ^{1}H NMR ($CDCl_3$) δ 1.80–1.95 (m, 4H, 2× CH_2), 2.57 (s, 3H, CH_3), 2.64–2.80 (m, 4H, 2× CH_2), 2.93 (t, 2H, CH_2), 3.59–3.70 (m, 2H, CH_2), 7.07–7.17 (m, 2H, ar), 7.23–7.28 (m, 1H, ar), 7.37–7.50 (m, 6H, ar), 8.42 (d, 1H, ar), 10.08 (vbr s, 1H, NH ex), 10.68 (t, 1H, 6-NH ex). Anal. Calcd. for $C_{29}H_{27}N_3O_2$: C, 77.48; H, 6.05; N, 9.35. Found: C, 76.29; H, 6.33; N, 9.04.

4-Methyl-6-[(2-morpholinoethyl)amino]-1-phenyl-2,7-dihydro-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3i). (69%); m.p. 264–266°C; hydrochloride 186–189°C; hydrochloride ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 3H, CH_3), 3.13–3.28 (m, 2H, CH_2), 3.37–3.47 (m, 2H, CH_2), 3.49–3.60 (m, 2H, CH_2), 3.78–3.92 (m, 2H, CH_2), 3.96–4.09 (m, 4H, 2 × CH_2), 5.05 (vbr s, 1H, NH ex), 7.13–7.25 (m, 2H, ar), 7.30–7.40 (m, 2H, ar), 7.42–7.61 (m, 5H, ar), 8.38 (d, 1H, ar), 10.79 (br s, 1H, NH ex), 11.58 (br s, 1H, NH ex). Anal. Calcd. for $C_{29}H_{27}N_3O_3$: C, 74.82; H, 5.85; N, 9.03. Found: C, 74.98; H, 5.62; N, 9.25.

6-{[(2-Dimethylamino)ethyl]amino}-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3b). **Example of general procedure for the preparation of 3b–e**. The commercially available

6-bromo-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (200 mg, 0.48 mmol) was suspended in N^1,N^1-dimethyl-1,2-ethanediamine (4 ml) and refluxed for 2 h under stirring. A TLC eluted with $CHCl_3$/MeOH (9:1 v/v) reveled that the reaction was terminated. After cooling, a solid was obtained which was washed successively with methanol and ether, and dried at room temperature to yield pure **3b** (69%): m.p. 163–165°C; hydrochloride 263–265°C; hydrochloride ^{1}H NMR (DMSO-d_6) δ 2.70 (s, 3H, CH_3), 2.88 (d, 6H, 2 × CH_3), 3.30–3.46 (m, 2H, CH_2), 3.88–4.06 (m, 2H, CH_2), 7.10–7.27 (m, 2H, ar), 7.29–7.41 (m, 2H, ar), 7.43–7.60 (m, 6H, 5ar + NH ex), 8.37 (d, 1H, ar), 10.70 (br s, 1H, NH ex), 10.90 (br s, 1H, NH ex). Anal. Calcd. for $C_{27}H_{25}N_3O_2$: C, 76.57; H, 5.95; N, 9.92. Found: C, 76.24; H, 6.12; N, 10.14.

Derivatives **3c–e** were prepared in a similar manner.

6-{[(3-Dimethylamino)propyl]amino}-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3c). (78%); m.p. 231–233°C; hydrochloride 170–171°C; ^{1}H NMR ($CDCl_3$) δ 1.90–2.10 (m, 2H, CH_2), 2.31 (s, 6H, 2 × CH_3), 2.50 (t, 2H, CH_2), 2.57 (s, 3H, CH_3), 3.42–3.58 (m, 2H, CH_2), 7.03–7.19 (m, 2H, ar), 7.24–7.32 (m, 2H, ar), 7.36–7.52 (m, 6H, 5ar + NH ex), 8.45 (d, 1H, ar), 10.67 (t, 1H, NH ex). Anal. Calcd. for $C_{28}H_{27}N_3O_2$: C, 76.86; H, 6.22; N, 9.60. Found: C, 76.73; H, 6.31; N, 9.31.

6-{[(2-Diethylamino)ethyl]amino}-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3d). (52%); m.p. 151–153°C; hydrochloride 269–270°C; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, 6H, 2 × CH_3), 2.58 (s, 3H, CH_3), 2.67–2.86 (m, 4H, 2 × CH_2), 2.93 (t, 2H, CH_2), 3.52–3.70 (m, 2H, CH_2), 7.07–7.19 (m, 2H, ar), 7.23–7.31 (m, 2H, ar), 7.38–7.52 (m, 6H, 5ar + NH ex), 8.47 (d, 1H, ar), 10.63 (t, 1H, NH ex). Anal. Calcd. for $C_{29}H_{29}N_3O_2$: C, 77.13; H, 6.47; N, 9.31. Found: C, 76.98; H, 6.53; N, 9.64.

6-{[(3-Diethylamino)propyl]amino}-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3e). (87%); m.p. 193–195°C; hydrochloride 210–211°C; ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 6H, 2 × CH_3), 1.93–2.07 (m, 2H, CH_2), 2.57 (s, 3H, CH_3), 2.60–2.85 (m, 6H, 3 × CH_2), 3.42–3.56 (m, 2H, CH_2), 7.01 (s, 1H, ar), 7.06–7.12 (m, 1H, ar), 7.22–7.30 (m, 2H, ar), 7.38–7.48 (m, 6H, 5ar + NH ex), 8.43 (d, 1H, ar), 10.63 (t, 1H, NH ex). Anal. Calcd. for $C_{30}H_{31}N_3O_2$: C, 77.39; H, 6.71; N, 9.03. Found: C, 77.09; H, 6.54; N, 9.24.

6-{[(2-Amino)ethyl]amino}-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (3j). The commercially available 6-bromo-4-methyl-1-phenyl-3*H*-naphtho[1,2,3-*de*]quinoline-2,7-dione (300 mg, 0.72 mmol) was suspended in 2-ethoxyethanol (20 ml), added of *tert*-butyl *N*-(2-aminoethyl)carbamate (860 mg, 5.4 mmol), and refluxed for 24 h under stirring. A TLC eluted with $CHCl_3$/MeOH (30:1 v/v) reveled that the reaction was terminated. After cooling at room temperature, a solid precipitated, which was washed subsequently with methanol and ether. The solid **(3j** N-Boc protected) was suspended in dioxane (20 ml) and 37% HCl and stirred at room temperature for 7 h. The volatile was evaporated and the residue was refluxed in ethanol. After cooling, a solid was obtained which was washed successively with ethanol and ether, and dried at room temperature to yield pure **3j** (35%): m.p. 259–261°C; hydrochloride 264–267°C; hydrochloride ^{1}H NMR (DMSO-d_6) δ 2.64 (s, 3H, 4-CH_3), 3.03–3.20 (m, 2H, CH_2), 3.65–3.87 (m, 2H, CH_2), 7.09–7.30 (m, 2H, ar), 7.30–7.59 (m, 7H, ar), 7.83–8.03 (m, 3H, NH_3^+), 8.30–8.42 (m, 1H, ar), 10.50 (br s, 1H, 3-H ex), 11.50 (br s, 1H, 6-NH ex). Anal. Calcd. for $C_{25}H_{21}N_3O_2$: C, 75.93; H, 5.35; N, 10.63. Found: C, 76.09; H, 5.53; N, 10.78.

In vitro cytotoxicity

HT29 human colon adenocarcinoma. Details of HT29 human colon adenocarcinoma cell line assay have been previously described [18]. Drug solutions of appropriate concentration were added to a culture containing HT29 cells at 2.5×10^4 cells/ml of medium and the drug exposure was protracted for 144 h. All assays were performed in triplicate, as previously described [18].

Human Ovarian Carcinoma Experimental Protocol. Established details and biological properties of human ovarian carcinoma cell lines (A2780, A2780cisR, CH1, CH1cisR, and SKOV-3) have been described previously [19]. The sulforhodamine B (SRB) experimental protocol used has been described previously [19,20]. Cells were plated (100–5000 cells) in 96-well microtiter plates and left overnight to adhere prior to drug treatment. Aqueous drug solutions at pH 7.0 were then added to the cells at various concentrations following dilution of a stock DMSO solution. After 96 h continuous drug exposure at 37°C, growth inhibition was assessed using SRB protein staining. IC_{50} values, as mean of two independent assays, (drug dose required for 50% growth inhibition compared to drug-free controls) were determined by comparing treated and untreated cells.

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