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Studies on quinones. Part 43: Synthesis and cytotoxic evaluation of polyoxyethylene-containing 1,4-naphthoquinones☆

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Abstract—A series of naphthoquinones 2,3-disubstituted with chlorine and oxyethylene groups have been prepared from 2,3-dichloro- and 2,3-dimethoxy-1,4-naphthoquinone. The members of these series were tested on normal human fibroblasts and on a panel of four human cancer cell lines. Antitumor activities, which were in the range of IC_{50} 1.3–89.5 μM, discussed in terms of LUMO energy, lipophilicity and size of the polyoxyethylene moiety.

1. Introduction

Quinone moieties are present in many drugs such as anthracyclines, daunorubicin, doxorubicin, mitomycin, mitoxantrone, and saintopin, which are used clinically in the therapy of solid cancers. Anticancer quinones are currently the focus of intensive research because of their biological activity and complex modes of action, which differ depending on their particular structure, and they can act as DNA intercalators, bioreductive alkylators of biomolecules, and/or generators of reactive oxygen species (ROS) (superoxide radical anions, hydrogen peroxide, and hydroxyl radicals) in a process known as redox cycling.[2–6] There are numerous reports on cytotoxic evaluation of substituted 1,4-naphthoquinones that show the biological relevance of this system, in particular when they contain oxyalkyl substituents.[7–17] With respect to 2,3-substituted 1,4-naphthoquinones, there is evidence of the dependence of the cytotoxic activity on their half-wave potential and lipophilicity.[18] Both the antitumor activity and the toxicity of these compounds depend on the same variables, particularly the redox potentials, so that increasing the antitumor activity also increases the toxicity. However, analysis of the therapeutic index of the active compounds indicates that substituents with greater lipophilicity will give larger therapeutic indexes.

As part of our research program on the synthesis of biologically active quinones,[19–26] we became interested in the synthesis and antitumor evaluation of polyoxyethylene-containing 1,4-naphthoquinones, having a range of similar redox potentials. These compounds were designed to explore the effect of the size of polyoxyethylene moiety in podands and coronands on the antitumor activity of the 2,3-disubstituted 1,4-naphthoquinone chromophore. To the authors' best knowledge, there is only one report concerning the synthesis and antitumor activity of 1,4-naphthoquinones fused through the quinone nucleus to cyclic polyoxy- and polythioxyethylene fragments (thiol-crown ethers).[27] We now report our results on the synthesis of 1,4-naphthoquinones linked to chlorine atoms and podand and coronand moieties from commercially available compounds. All of the 2,3-disubstituted 1,4-naphthoquinones exhibited cytotoxic activity against normal fibroblasts and a panel of four cancer cell lines.

2. Results and discussion

It is well known that 2,3-dichloro-1,4-naphthoquinone **1** reacts with nucleophiles and, depending on their capa-

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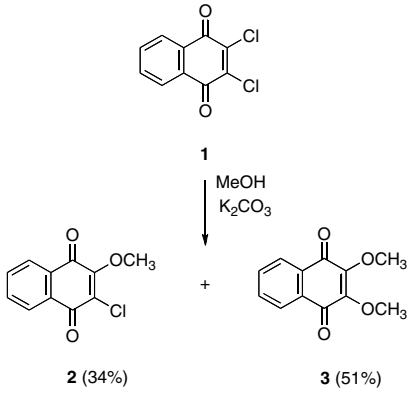
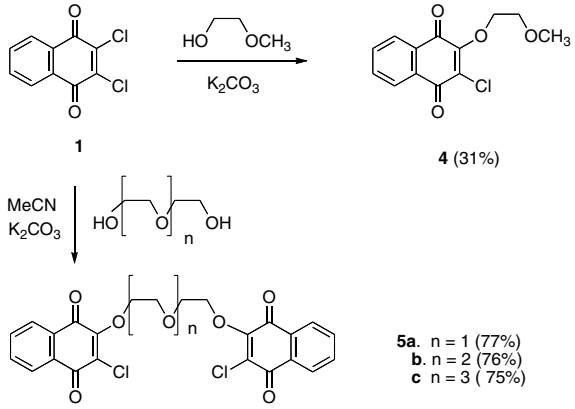
bility, it may undergo substitution of one or both chlorine atoms.[28] Based on the reactivity of **1** we studied its reaction with methanol in the presence of potassium carbonate as a model reaction to be used for the preparation of naphthoquinones containing podand and coronand fragments. Treatment of quinone **1** with methanol at room temperature gave a mixture of the substitution products **2** and **3** in 34% and 51% yield, respectively (Scheme 1). These compounds were easily separated and purified by column chromatography.

We then studied the reaction of quinone **1** with 2-methoxyethanol under the same conditions. The treatment produced the monosubstituted product **4** in 31% yield, and no disubstituted product was detected by ^{1}H NMR (Scheme 2). The inertness of compound **4** to undergo a second substitution reaction is probably due to the steric hindrance caused by the O–CH_2–CH_2–OCH_3 group to the attack of the nucleophile on the vicinal position.

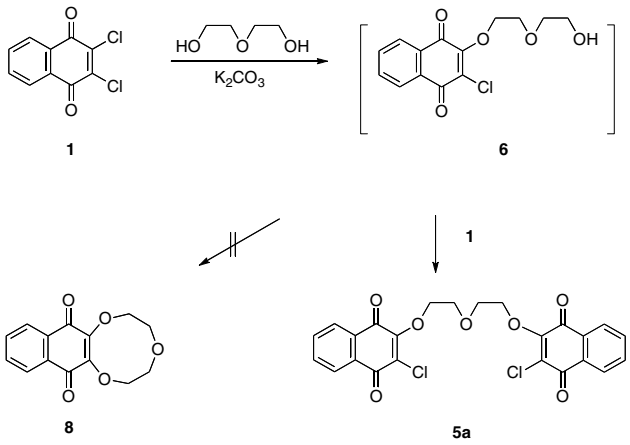
On the basis of these results we studied the preparation of podands linked to the 1,4-naphthoquinone system by displacement of one chlorine atom with glycols. Attempts to prepare substitution products derived from quinone **1** and excess glycol were unsuccessful, and complex mixtures were obtained in all the trials. However, when the treatment was made in acetonitrile using



Scheme 1. Preparation of compounds **2** and **3**.



Scheme 2. Preparation of compounds **4** and **5**.



Scheme 3. Probable formation of podand **5a**.

2.2 equiv of diethylene glycol and potassium carbonate, podand **5a** was isolated in 77% yield (Scheme 2).

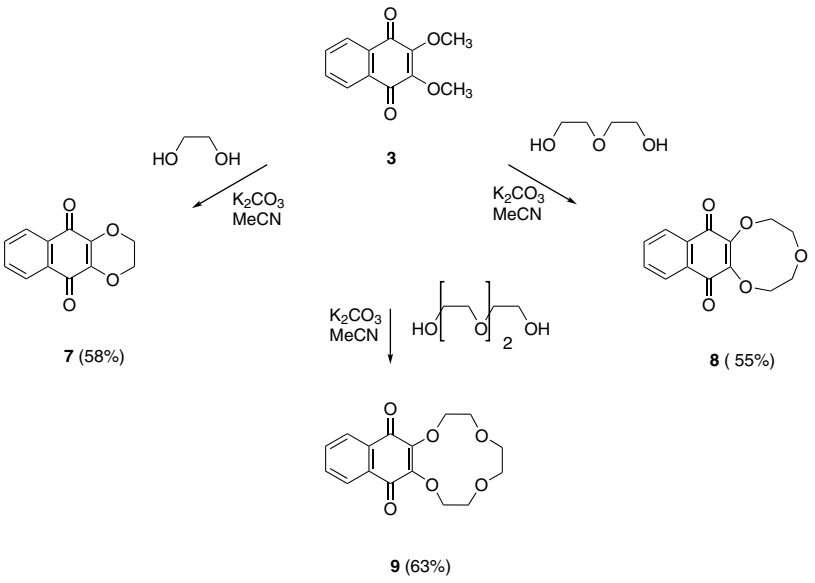
This result suggests that formation of **5a**, which probably occurs by reaction of intermediate **6** with **1**, is more favorable than a cyclization reaction of **6** to give compound **8** (Scheme 3).

In separate experiments, quinone **1** was reacted with 2.2 equivalents of tri- and tetraethylene glycol under the conditions used in the preparation of podand **5a**, giving podands **5b** and **5c** (Scheme 2). The structure of compound **5** was established by NMR and high resolution MS.

Previous results from our laboratory on the substitution behavior of 2,3-dimethoxy-1,4-naphthoquinone **3** with nucleophiles[29] suggest the possibility of preparing 1,4-naphthoquinones 2,3-fused to coronand moieties by double substitution reactions of quinone **3** with glycols in basic media. The reaction was first explored with ethylene glycol and potassium carbonate in acetonitrile at room temperature. The treatment yielded the expected dioxine **7** in 58% yield (Scheme 4). Under similar conditions, coronands **8** and **9** were isolated in 55% and 63% yield, respectively. The structures of these new compounds were established mainly by NMR and high resolution MS.

The in vitro cytotoxic evaluation of the tested 1,4-naphthoquinones derivatives **2**–**4**,**5a**–**c**, and **7**–**9** against normal human MRC-5 lung fibroblasts and human AGS gastric adenocarcinoma cell lines, SK-MES-1 lung cancer cells, J82 bladder carcinoma cells, and HL-60 leukemia cells, are shown in Table 1. Data for the anticancer agent etoposide are included for comparison. The results are expressed as IC_{50}-values, that is, as the micromolar concentration of a compound that achieves 50% cellular growth reduction after 72 h of drug exposure.

Since the screening indicates that podands **4**, **5** and their precursor **2** exhibited more potency than the coronands **7**–**9** and their precursor **3** we considered to analyze the results by separating the tested substituted naphthoqui-



Scheme 4. Preparation of quinones **7**–**9**.

nones into two distinct groups. The first group contains the chlorine derivatives **2**, **4**, and **5a**–**c** and the second group the dialkoxy derivatives **3**, **7**–**9**.

In order to analyse the antitumor capacity of the quinonoid compounds of Table 1, in particular those of podand **3a**–**c** and coronands **7**–**9** on the cell lines, their lowest unoccupied molecular orbital energies (E_{LUMO}) and lipophilicity (C log *P*) were estimated by MOPAC using the AM1 semiempirical method (Table 2). These parameters were considered by taking into account the precedents reported by Hodgnett[18] on the influence of the half-wave potential ($E_{1/2}$) and lipophilicity on the cytotoxic activity of substituted 1,4-naphthoquinones and those on the correlation of $E_{1/2}$ with the E_{LUMO} in 1,4-quinones.[30]

No correlation between the cytotoxic and antitumor potencies with the E_{LUMO} and the lipophilicity for the compounds of Table 2 was observed. However, examination of the data showed that the E_{LUMO} and the lipophilicity of quinonoids derivatives of the first group (**2**, **4**, and **5**) were parallel to their cytotoxicities and antitumoral potencies, except for compound **5c**. Comparison of the IC_{50} values of podands **5b** and **5c** indicates that the increase of the length of the $(OCH_2CH_2)_n$ podand spacer causes a significant decrease of the toxicity and antitumor activity. It should be noted that since there are no significant differences of the antitumor activity for compounds **2** and **4** with respect to podands **5a** and **5b**, it seems reasonable to assume that a second quinone nucleus into podands **5a** and **5b** does not make any influence in increasing the antitumor activity.

Concerning the data of quinonoids compounds of the second group (**3**,**7**–**9**), there is a correlation between the IC_{50} values and the E_{LUMO} and lipophilicity, except for the leukemia cell line. Comparison of the IC_{50} values of coronands **7**–**9** indicates that the decrease of the 9-membered heterocyclic ring (compound **8**) to a 6-membered one (compound **7**) causes an increase of the antitumor activity. Similarly, the increase of the size of the 9-membered ring to a 12-membered ring (compound **9**) causes an increase of the antitumor activity.

It is noteworthy that among the tested quinones, compounds **2**, **4**, **5a** **5b**, and **6** exhibited the most antitumor activities against leukemia cells with IC_{50} within the value range 1.3–2.6 μM.

The results reported here demonstrate that in addition to redox capacity and lipophilicity of the tested 2,3-disubstituted-1,4-naphthoquinone, other molecular factors such as polyoxyethylene spacer length and ring size are probably involved in the supramolecular recognition[31] by the enzymatic system associated with cytotoxic activity.

3. Conclusions

In summary, we have synthesized podands containing one and two 3-chloro-1,4-naphthoquinone moieties (**4** and **5**) by selective displacement of one chlorine atom of 2,3-dichloronaphthoquinone (**1**) with 2-methoxyethanol and polyethylene glycols. Through a sequential substitution reaction of 2,3-dimethoxy-1,4-naphthoquinone **3** with polyethylene glycols, coronands **7**–**9** were prepared.

The 1,4-naphthoquinone derivatives prepared in this study expressed in vitro cytotoxic and antitumor activity against normal human fibroblasts MRC-5 and the human cancer cell lines AGS, HL-60, SK-MES-1, and J82. Podands **5a**–**c** displayed higher cytotoxic and antitumor potencies than those exhibited by coronands **7**–**9**. In both groups of quinonoids compounds, the electron transfer capacity of the quinonic nucleus, the lipophilicity and the size of the polyoxyethylene moiety are involved in the cell inhibitory growth properties.

Table 1. Cytotoxic activity of quinones against MRC-5 fibroblasts and a panel of four tumor cell lines

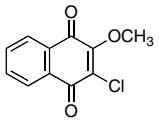
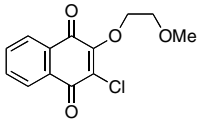
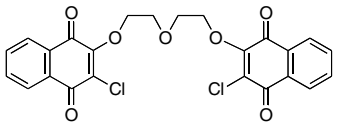
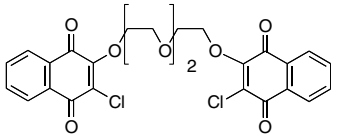
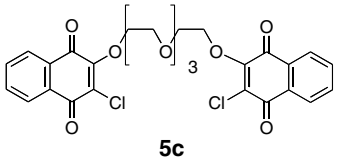
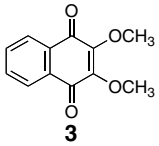
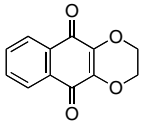
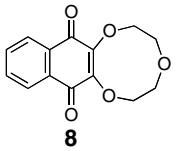
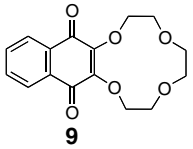
Compound	IC_{50} (μM)				
	MRC-5 fibroblasts	AGS gastric	SK-MES-1 lung	J82 bladder	HL-60 leukemia
 **2**	3.5 ± 0.2	9.1 ± 0.5	11.5 ± 0.5	10.1 ± 0.3	1.8 ± 0.1
 **4**	3.5 ± 0.1	9.9 ± 0.4	16.7 ± 0.7	19.4 ± 0.7	1.7 ± 0.08
 **5a**	7.3 ± 0.3	11.0 ± 0.6	16.1 ± 0.8	26.6 ± 1.1	1.9 ± 0.1
 **5b**	4.0 ± 0.2	5.2 ± 0.3	7.1 ± 0.3	8.6 ± 0.4	1.3 ± 0.06
 **5c**	55.8 ± 2.3	54.8 ± 2.8	12.2 ± 0.5	33.9 ± 1.6	89.5 ± 3.5
 **3**	7.2 ± 0.4	9.1 ± 0.5	5.3 ± 0.2	16.1 ± 0.7	8.9 ± 0.4
 **7**	22.9 ± 1.2	19.5 ± 1.1	8.3 ± 0.4	22.3 ± 1.2	20.1 ± 1.1
 **8**	95.2 ± 4.7	63.2 ± 3.2	16.4 ± 0.8	39.6 ± 1.9	21.4 ± 1.1
 **9**	10.1 ± 0.5	17.5 ± 0.9	7.9 ± 0.4	19.8 ± 0.9	2.6 ± 0.2
Etoposide	3.9 ± 0.2	0.36 ± 0.02	2.5 ± 0.2	2.8 ± 0.1	0.8 ± 0.04

Table 2. LUMO energy and lipophilicity of the substituted 1,4-naphthoquinones

Quinone	$-E_{LUMO}$ (eV)	Clog *P*	MRC-5 fibroblasts	AGS gastric	SK-MES-1 lung	J82 bladder	HL-60 leukemia
2	1.57211	5.3687	3.5 ± 0.2	9.1 ± 0.5	11.5 ± 0.5	10.1 ± 0.3	1.8 ± 0.1
4	1.60333	6.4494	3.5 ± 0.1	9.9 ± 0.4	16.7 ± 0.7	19.4 ± 0.7	1.7 ± 0.08
5a	1.62222	11.6407	7.3 ± 0.3	11.0 ± 0.6	16.1 ± 0.8	26.6 ± 1.1	1.9 ± 0.1
5b	1.43756	12.7214	4.0 ± 0.2	5.2 ± 0.3	7.1 ± 0.3	8.6 ± 0.4	1.3 ± 0.06
5c	1.76121	14.8828	55.8 ± 2.3	54.8 ± 2.8	12.2 ± 0.5	33.9 ± 1.6	89.5 ± 3.5
3	1.48041	5.4942	7.2 ± 0.4	9.1 ± 0.5	5.3 ± 0.2	16.1 ± 0.7	8.9 ± 0.4
7	1.49107	5.23385	22.9 ± 1.2	19.5 ± 1.1	8.3 ± 0.4	22.3 ± 1.2	20.1 ± 1.1
8	1.59605	6.3145	95.2 ± 4.7	63.2 ± 3.2	16.4 ± 0.8	39.6 ± 1.9	21.4 ± 1.1
9	1.56948	8.4759	10.1 ± 0.5	17.5 ± 0.9	7.9 ± 0.4	19.8 ± 0.9	2.6 ± 0.2

4. Experimental

4.1. Chemical synthesis

All reagents were commercially available reagent grade and were used without further purification. Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. The IR spectra were recorded on an FT Bruker spectrophotometer using KBr disks, and the wave numbers are given in cm^{-1}. ^1H NMR spectra were run on Bruker AM-200 and AM-400 instruments in deuterochloroform ($CDCl_3$). Chemical shifts are expressed in ppm downfield relative to tetramethylsilane (TMS, δ scale), and the coupling constants (*J*) are reported in Hz. ^{13}C NMR spectra were obtained in $CDCl_3$ at 50 and 100 MHz. 2D NMR techniques (COSY, HMBC) and DEPT were used for signal assignment. Chemical shifts are reported in δ ppm downfield from TMS, and *J*-values are given in Hz. HRMS were obtained on a trisector WG AutoSpec spectrometer. Silica gel Merck 60 (70–230 mesh) was used for preparative column chromatography, and TLC aluminum foil 60 F254 for analytical TLC.

4.1.1. 2-Methoxy-3-chloro- and 2,3-dimethoxy-1,4-naphthoquinone (2 and 3). A suspension of 2,3-dichloro-1,4-naphthoquinone **1** (500 mg, 2.2 mmol), methanol (20 mL), and dry potassium carbonate (2 g) was stirred for 6 h at room temperature. The mixture was filtered and the filtrate evaporated under reduced pressure. The residue was extracted with dichloromethane and the extract was evaporated under reduced pressure. Column chromatography on silica gel of the residue produced pure quinone **2** (dichloromethane; 166 mg; 34%) as a yellow solid, mp 151–153 °C (lit.[32] mp 153 °C). The ^1H NMR spectrum of compound **2** was identical with that reported in the literature.[33] Further elution yielded pure quinone **3** (98:2 dichloromethane/ethyl acetate; 244 mg; 51%) as a pale yellow solid, mp 118–120 °C (lit.[34] mp 115–117 °C). The ^1H NMR spectrum of compound **3** was identical with that reported in the literature.[34]

4.1.2. 2-Chloro-3-(2-methoxyethoxy)naphthalene-1,4-dione (4). A suspension of **1** (500 mg, 2.2 mmol), dry potassium carbonate (2 g), and 2-methoxyethanol (5 mL) in acetonitrile (10 mL) was stirred for 2 h at room temperature. The mixture was filtered, the filtrate was evaporated under reduced pressure, and the residue was partitioned between water/dichloromethane. The organic extract was washed with water and dried over magnesium sulfate. Evaporation of the solvent followed by chromatography of the residue yielded compound **4** (181 mg, 31%) as a yellow solid, mp 153–155 °C. IR ν_{max}: 1670 (C=O); 1264, 1206 (C–O). ^1H NMR: δ 3.34 (s, 3H, CH_3), 3.72 (t, $J = 5$, 2H, CH_2), 4.71 (t, $J = 5$, 2H, CH_2); 7.72 (m, 2H, 6- and 7-H); 8.10 (m, 2H, 5- and 8-H); ^{13}C NMR: δ 59.1, 72.1, 73.2, 126.6, 126.9, 129.1, 130.8, 131.0, 133.6, 134.2, 157.0, 178.6, 179.6. HRMS (FAD^+) found 289.0242 (calcd for $C_{13}H_{11}O_4ClNa$, $[M+Na]^+$ 289.0244).

4.1.3. 3,3′-(2,2′-Oxy(bis))(ethane-2,1-diylbis(oxy))bis(2-chloronaphthalene-1,4-dione) (5a). A suspension of **1**(250 mg, 1.10 mmol), 2,2-oxydiethanol (270 mg, 2.54 mmol), potassium carbonate (2 g) in acetonitrile (10 mL) was stirred for 18 h at room temperature. The mixture was evaporated under reduced pressure and the residue was partitioned between water and dichloromethane. The organic extract was washed with water, dried over magnesium sulfate, and evaporated under reduced pressure to yield crude compound **5a** (206 mg, 77%). Column chromatography of the residue, eluting with dichloromethane, yielded pure podand **5a** (100 mg, 39%) as a yellow solid mp, 182–185 °C. IR ν_{max}: 1676 (C=O), 1256 and 1200 (C–O). ^1H NMR: δ 3.84 (t, 2H, $J = 5$, CH_2); 4.63 (t, 2H, $J = 5$, CH_2); 8.07 (m, 2H, Ar); ^{13}C NMR: δ 61.4, 126.2,130.6, 133.7, 147.5, 161.9. 176.4, 179.3. HRMS (FAD^+) found 509.016658 (calcd for $C_{24}H_{16}O_7Cl_2Na$, $[M+Na]^+$ 509.017078).

4.1.4. 3,3′-(2,2′-(Ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-chloronaphthalene-1,4-dione) (5b). A suspension of **1** (160 mg, 0.70 mmol), 2,2′-(ethane-1,2-diylbis(oxy))diethanol (55 mg, 0.37 mmol), dry potassium carbonate (2 g) in acetonitrile (10 mL) was stirred for 18 h at room temperature. Work-up afforded crude podand **5b** (140 mg, 76%) as a yellow solid. Column chromatography (dichloromethane) of the crude yielded a pure sample of **5b** as a yellow solid, mp 101–103 °C. ^1H NMR: δ 3.46 (t, 4H, $J = 5$, CH_2); 3.65 (t, 4H, $J = 4$, CH_2); 4.61 (t, 4H, $J = 4$, CH_2); 7.70 (m, 4H, Ar); 8.06 (m, 4H, Ar); ^{13}C NMR: δ: 70.7, 70.6, 73.2, 126.6, 126.6, 126.6,130.6, 131.0, 133.0, 134.0, 157.0, 176.5, 179.5. HRMS (FAD^+) found 553.0433 (calcd for $C_{26}H_{20}O_8Cl_2Na$, $[M+Na]^+$ 553.0433).

4.1.5. 3,3′-(2,2′-(2,2′-Oxybis(ethane-2,1-diyl)bis(oxy))bis-(ethane-2,1-diyl))bis(oxy)-bis(2-chloronaphthalene-1,4-dione) (5c). A suspension of **1** (250 mg, 1.10 mmol), 2,2′-(2,2′-oxybis(ethane-2,1-diyl)bis(oxy))diethanol (2 mL) and potassium carbonate (250 mg, 2.12 mmol) in THF (10 mL) was stirred for 24 h at room temperature.

Work-up yielded crude podand **5c** (237 mg, 75%) as a yellow solid. Column chromatography (dichloromethane) of the crude yielded a pure sample of **6** as a brown-yellow solid, mp 208–210 °C. ^{1}H NMR: δ 3.68 (m broad, 16H, 8× CH_2), 7.78 (m, 4H, Ar), 8.11 (m, 4H, Ar); ^{13}C NMR: δ 61.6, 69.9, 70.5, 72.9, 126.9, 127.5, 133.7, 135.4,176.5, 179.5.

4.1.6. 2,3-Dihydronaphtho[2,3-*b*]-1,4-dioxin-5,10-dione (7). A suspension of **3** (160 mg, 0.73 mmol), ethane-1,2-diol (220 mg, 3.5 mmol), and dry potassium carbonate (2 g) in acetonitrile (10 mL) was stirred for 18 h at room temperature. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was poured in dichloromethane and the solution was washed with water (2× 20 mL), and dried over magnesium sulfate. Removal of the solvent yielded compound **7** (91 mg, 58%), which by further chromatography (dichloromethane) yielded pure **7**, mp 267–268 °C (lit.,[35] 268–270 °C). ^{1}H NMR: 4.42 (s, 4H, 2- and 3-H), 7.71 (m, 2H, 7- and 8-H), 8.10 (m, 2H, 5- and 8-H). HRMS (FAD^+) found 239.03203 (calcd for $C_{12}H_8O_4Na$, $[M+Na]^+$ 239.03218).

4.1.7. 2,3,5,6-Tetrahydronaphtho[2,3-*b*]-1,4,7-trioxonin-8,13-dione (8). A suspension of **3** (50 mg, 0.23 mmol), 2,2′-oxydiethanol (122 mg, 1.15 mmol), and dry potassium carbonate (2 g) in acetonitrile (10 mL) was stirred for 18 h at room temperature. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was poured in dichloromethane and the solution was washed with water (2× 20 mL) and dried over magnesium sulfate. Evaporation of the solvent yielded crude compound **7** (33 mg, 55%). Further chromatography of the crude (dichloromethane) yielded pure **8** as a yellow viscous oil. ^{1}H NMR: 3.93 (t, 4H, $J = 5$, 3- and 5-H), 4,57 (t, 4H, $J = 5$, 2- and 6-H), 7.71 (m, 2H, 10- and 11-H), 8.07 (m, 2H, 9- and 12-H); ^{13}C NMR δ 71.7 (2C), 72.0 (2C), 126.2 (2C), 130.7 (2C), 133.7 (2C), 146.2 (2C), 181.8 (2C). HRMS (FAD^+) found 283.0589 (calcd for $C_{14}H_{12}O_5Na$, $[M+Na]^+$ 283.0582).

4.1.8. 2,3,5,6,8,9-Hexahydronaphtho[2,3-*b*]-1,4,7,10-tetraoxacyclododecin-11,16-dione (9). A suspension of **3** (54 mg, 0.25 mmol), 2,2′-(ethane-1,2-diylbis(oxy))diethanol (250 mg, 1.7 mmol), and dry potassium carbonate (2 g) in acetonitrile (8 mL) was stirred for 4.5 h at room temperature. Work-up yielded crude compound **9**(48 mg, 63%). Further chromatography (7:3 petroleum ether/ethyl acetate) of the crude yielded pure **9**as a viscous yellow oil. ^{1}H NMR: 3.70 (s, 4H, 5- and 6-H), 3.82 (t, 4H, $J = 4$, 3- and 8-H), 4.55 (t, 4H, $J = 4$, 2- and 9-H), 7.67 (m, 2H, 13- and 14-H), 8.02 (m, 2H, 12- and 15-H); ^{13}C NMR δ 70.4 (2C), 70.6 (2C), 73.6 (2C), 126.2 (2C), 131.0 (2C), 133.7 (2C), 148.7 (2C), 182.4 (2C). HRMS (FAD^+) found 327.0845 (calcd for $C_{16}H_{16}O_6Na$, $[M+Na]^+$ 327.0846).

4.2. Anticancer assay[36]

The cell lines used in this work were obtained from the American Type Culture Collection (ATCC, Manasas, VA, USA). They included MRC-5 normal human lung fibroblasts (CCL-171), AGS human gastric adenocarcinoma cells (CRL-1739), HL-60 human leukemia cells (CCL-240), SK-MES-1 human lung cancer cells (HTB-58), and J82 human bladder carcinoma cells (HTB-1). Cells were grown in the following media: MRC-5, SK-MES-1, and J82 in MEM, AGS cells in Ham F-12, and HL-60 in RPMI. The MEM medium contained 2 mM L-glutamine, 1 mM sodium pyruvate, and 1.5 g/L sodium bicarbonate. Ham F-12 was supplemented with 2 mM L-glutamine and 1.5 g/L sodium bicarbonate. RPMI contained 1 mM sodium pyruvate and 2 g/L sodium bicarbonate. All media were supplemented with 10% heat-inactivated FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 in air at 37 °C. For the experiments, cells were plated at a density of 50,000 cells/mL in 96-well plates. One day after seeding, the cells were treated with the medium containing the compounds at concentrations ranging from 0 up to 100 μM for 3 days, and finally the MTT reduction assay was carried out. The compounds were dissolved in DMSO (1% final concentration) and complete medium. Untreated cells were used as controls. Each experiment was carried out in sextuplicate.

Etoposide, used as a positive control, was tested in the same way.

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