Naphthofuroquinone Derivatives: DNA Topoisomerase-I Inhibition and Cytotoxicity

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The naphthoquinone skeleton is found in many natural products and has been employed as a synthetic intermediate for the preparation of numerous heterocyclic compounds with interesting biological properties such as antitumor, antibacterial, antifungal and antiinflammatory agents.[1] The quinone core of streptonigrin and lavendamycin has been proposed to be a determining factor in their antitumor activity.[2] Recently, the linearly substituted quinolinediones have revealed the importance of the quinone framework for potent antitumor activity against the cell lines.[3] Furthermore, Cheng *et al.* designed the fused benzofuroquinone that possesses a characteristic "2-phenylnaphthalene-type" structural framework in which the two rings are coplanar.[4] They found that the designed benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione derivatives exhibited strong inhibitory activity throughout the entire series of cancer cell lines.

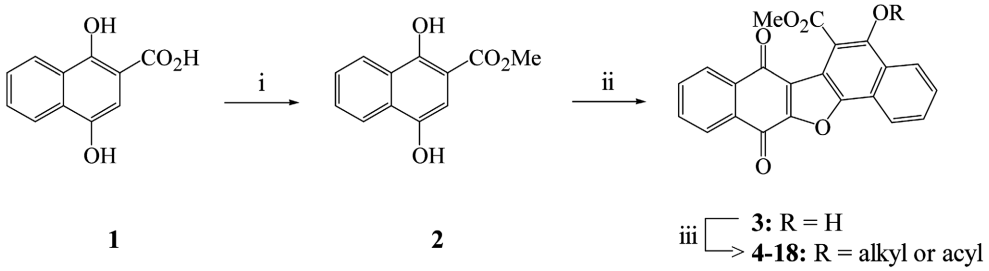
A decade ago, dinaphtho[1,2-*b*;2',3'-*d*]furan-7,12-dione **3** was isolated from stems of *Paulownia tomentos*, a perennial tree widely distributed in China, Japan, and Korea. The naphthofuroquinone **3** significantly reduced viral cytopathic effect in a standard *in vitro* antiviral assay with HeLa cells.[5a] It is also interesting that this structural pattern is commonly observed in other biologically active compounds including camptothecin, ellipticine, and mappicine, a class of DNA Topoisomerase-I (Topo-I) inhibitors that exhibit efficious antitumor activity.[6] Here, we would like to report the synthesis of naphthofuroquinone derivatives, and their DNA Topo-I inhibition and cytotoxicity against various cancer cell lines.

Results and Discussion

The parent compound, dinaphtho[1,2-*b*;2',3'-*d*]furan-7,12-dione **3** was easily synthesized by the base-promoted condensation of 2,3-dichloro-1,4-naphthoquinone with methyl 1,4-dihydroxy-2-naphthoate **2**, as shown in Scheme 1.[5b] Selective esterfication of the 1-naphthol **1** with diazomethane provided the methyl naphthoate **2** in 86% yield, after recrystallization. The base-promoted condensation of 2,3-dichloro-1,4-naphthoquinone with **2** in the presence of K_2CO_3 in refluxing pyridine provided **3** in 64% yield. The known compounds **4-6**[5c] were prepared according to the literature and the other naphthofuroquinone derivatives **7-18** were readily synthesized by the *O*-alkylation of **3** with various halides (R-X, K_2CO_3, DMF) in fair to good yield. Thus, starting from **3**, wide ranges of compounds with random alkyl or benzyl group at the C(5)-position, in principle, possessing a hydrophobic or hydrophilic character were prepared and the structures are presented in Table 1.

The topoisomerase-I (Topo-I) inhibitory activity of the synthesized compounds was carried out by the relexation assay of supercoiled DNA. Briefly, supercoiled DNA and Topo-I was incubated with each compound at the initial concentration of 100 μM and the topology was determined by the relative mobility during electrophoresis. The density of DNA with each topology was measured by a densitometer and the results are shown in Figure 1. A thicker band of supercoiled form implies more potent inhibitory activity of the compound.

Table 1 lists naphthofuroquinone compounds **3-18** with random alkyl or benzyl groups at the C(5)-position, still commonly possess a characteristic "2-phenylnaphthalene-type" structural motif. Also, the table shows a semi-quantitative comparison of the inhibitory activities of them against Topo-I. The prepared compounds have moderate activity, while the alkyl (compounds **4** and **5**) and benzyl derivatives (compounds **12** and **16-18**) are less potent than



1 **2**

Scheme 1. *Reagents and conditions:* i, CH_2N_2-Et_2O, 86%; ii, 2,3-dichloro-1,4-naphthoquinone, pyridine, K_2CO_3, 90 °C, 64%; iii, Ac_2O, pyridine, DMAP, 94% (for **6**); R-X (X = Cl or Br), K_2CO_3, DMF, 58-87% (for **4-5** and **7-18**).

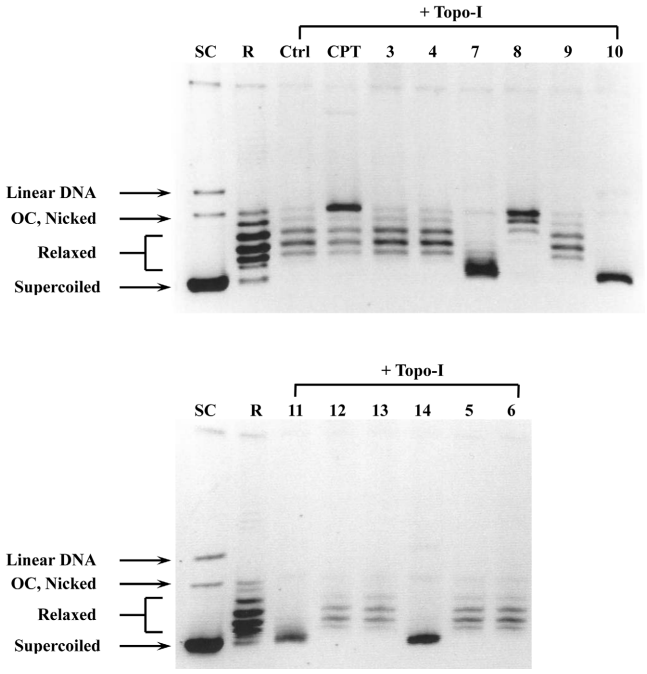


Figure 1. Topoisomerase-I assay. Supercoiled DNA and Topo-I was incubated with each compound (100 μM) for 30 min and topology was determined by the relative mobility during electrophoresis. SC, supercoiled; R, relaxed; OC, open circular; Ctrl, control; CPT, camptothecin.

the derivatives with aminoethyl or -propyl group (compounds **7**-**11** and **13**-**15**). Especially, compounds **7**, **8**, **10**, **11**, and **14**, commonly featuring dialkylaminoethyl functionality at the 5-position, potentially inhibited DNA relaxation induced by Topo-I and the inhibitory activities were much more potent than that of positive control camptothecin at 100 μM. The compound **14** (IC_{50} = 3.4 μM) was proven to be the most potent against Topo-I, comparing to camptothecin of 51.4 μM.

The anti-proliferative potential was screened *in vitro* against several human tumor cell lines such as A431 (epidermoid carcinoma), HELA (human cervix adenocarcinoma), MCF7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), and PC-3 (human prostate cancer cell line) by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) assay. The cytotoxicity was obtained as IC_{50} value at mM and the values are summarized in Table 2. Naphthofuroquinone compounds with lipophilic benzyl group at 5-position did not elicit significant cytotoxicity up to 50 μM. Meanwhile derivatives with aminoethyl or aminopropyl group at the 5-position manifested strong cytotoxicity, as shown in Table 2. The *in vitro* cytotoxicity is obviously correlated with the observed Topo-I mediated DNA cleavage activity. The amino-alcohol derivatives were proven to be less or more active than camptothecin depending on the cell lines, while exhibiting very potent cytotoxicity against HELA. It is interesting to note that camptothecin and naphthofuroquinone have similar overall structure. Both compounds have multiple fused unsaturated rings and a five membered-ring is located in the middle of the two planes.[7] The fused ring of naphthofuroquinone probably intercalates into DNA:Topo-I complex

Table 1. Structures and *in vitro* Topoisomerase-I activity of dinaphtho[1,2-*b*;2',3'-*d*]furan-7,12-dione derivatives

Compd	R	Topo-I activity[a,b]
3	—H	+
4[c]	—Me	+
5[c]	—Pr	+
6[c]	—Ac	+
7	—$(CH_2)_2$—NEt_2	+++
8	—$(CH_2)_2$—N⟨ ⟩	+++
9	—$(CH_2)_3$—N⟨ ⟩N—⟨ ⟩(Cl)	++
10	—$(CH_2)_2$—NMe_2	+++
11	—$(CH_2)_3$—NMe_2	+++
12	—CH_2—⟨ ⟩—CO_2Bu^t	+
13	—$(CH_2)_2$—N⟨ ⟩O	++
14	—$(CH_2)_2$—N⟨ ⟩	+++
15	—$(CH_2)_2$—N⟨ ⟩N—CH_2—⟨ ⟩—CF_3	+
16	—CH_2—⟨ ⟩—OMe	+
17	—CH_2—⟨ ⟩—CF_3	+
18	—CH_2—⟨ ⟩N	+

[a]Activity is expressed qualitatively: +, weak; ++, similar; +++, greater activity than camptothecin. [b]IC_{50} values for the selected compounds: **4** (>100); **5** (>100); **7** (30.0); **8** (35.9); **10** (7.4); **11** (14.7); **14** (3.4); and camptothecin (51.4 μM). [c]See reference 5c.

Table 2. IC_{50} (μM) values of *in vitro* cytotoxicity for the selected compounds

Compd	A431	HELA	MCF7	HT-29	PC-3
7	1.7	1.1	2.2	1.5	2.6
8	4.3	1.8	3.2	3.0	4.5
10	1.1	0.6	1.4	0.8	6.0
11	nd[a]	2.4	2.5	5.7	2.5
14	1.1	1.3	2.2	1.9	2.4
Camptothecin	0.4	>100	5.1	1.5	6.1

[a] Not determined.

like as camptothecin and the amino-alcohol functionality seems to interact with polar group of nucleotides, which seems be the central mechanism of its cytotoxicity because the naphthofuroquinones containing no side-chian did not block the activity of Topo-I.

In summary, a series of dinaphtho[1,2-*b*;2',3'-*d*]furan-7,12-dione derivatives were efficiently synthesized and evaluated for their inhibitory action against DNA Topo-I and various human cancer cell lines. The observed cytotoxicities are obviously correlated with Topo-I mediated DNA cleavage activities. The naphthofuroquinone compounds with dialkylamino-alcohol functionality at the C(5)-position manifested strong cytotoxicity and Topo-I inhibition, while exhibiting very potent cytotoxicity against HELA, comparing to camptothecin. Further studies for more potent cytotoxic naphthoquinones, featuring a characteristic "2-phenylnaphthalene-type" structural motif, are in progress.

Experimental Section

Synthesis of methyl 1,4-dihydroxy-2-naphthoate (2). To a prepared ethereal solution of diazomethane (*ca*. 15-16 mmol, 25 mL of Et_2O) was added **1** (2.04 g, 10 mmol). The reaction mixture was allowed to stir for 30 min and evaporated under reduced pressure to give the residue. The residue was recrystallized from ethyl acetate to give **2** (1.88 g, 86%) as a solid: mp 192-193.5 °C; ^{1}H NMR (DMSO-d_6) δ 11.44 (s, 1H), 9.19 (s, 1H), 8.32 (d, 1H, J = 8.1 Hz), 8.18 (d, 1H, J = 8.1 Hz), 7.63-7.50 (m, 2H), 7.14 (s, 1H), 3.98 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 170.87, 153.04, 145.48, 129.37, 129.06, 126.75, 125.19, 123.54, 122.52, 105.08, 104.13, 52.90; EIMS (70 eV) *m/z* (rel intensity) 218 (40, M^+), 186 (100), 158 (14), 130 (63), 102 (79), 76 (24), 66 (9), 53 (17); Anal. Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 65.72; H, 4.78%.

Synthesis of 5-hydroxydinaphtho[1,2-*b*;2',3'-*d*]furan-7,12-dione-6-carboxylic acid (3). A mixture of 2,3-dichloro-1,4-naphthoquinone (1.04 g, 4.6 mmol), **2** (1.5 g, 6.9 mmol), and K_2CO_3 (7.1 g, 51 mmol) in pyridine (50 mL) was heated to 90 °C for overnight. The reaction mixture was poured into an ice-water. The precipitated solid was filtered and washed with H_2O, and then recrystallized from chloroform to give **3** (1.11 g, 64%) as a solid: mp 248-250 °C; ^{1}H NMR (DMSO-d_6) δ 10.45 (s, 1H), 8.41 (d, 2H, J = 8.1 Hz), 8.17-8.11 (m, 2H), 7.94-7.89 (m, 2H), 7.86-7.78 (m, 2H); EIMS (70 eV) *m/z* (rel intensity) 372 (13, M^+), 340 (100), 256 (12), 228 (22), 200 (33), 156 (6), 100 (13), 76 (18), 40 (17); Anal. Calcd for $C_{22}H_{12}O_6$: C, 70.97; H, 3.25. Found: C, 70.09; H, 3.13%.

General procedure for the synthesis of naphthofuroquinone derivatives (7-18). Methyl 5-(2-diethylaminoethoxy)dinaphtho[1,2-*b*;2',3'-*d*]furan-7,12-dione-6-carboxylate (7): To a solution of **3** (200 mg, 0.54 mmol) and 2-diethylaminoethyl chloride hydrochloride (275 mg, 1.6 mmol) in DMF (50 mL) was added K_2CO_3 (2.0 g, 14.5 mmol) and then stirred for 8 h at room temperature. The reaction mixture was poured into an ice-water. The precipitated solid was collected by filtration and washed with water and dried to give **7** (213 mg, 84%) as a yellow soild: mp 151-152 °C; ^{1}H NMR ($CDCl_3$) δ 8.53 (d, 1H, J = 8.9 Hz), 8.43 (d, 1H, J = 8.8 Hz), 8.28-8.21 (m, 2H), 7.79-7.75 (m, 4H), 4.27 (t, 2H, J = 6.2 Hz), 4.19 (s, 3H), 3.01 (t, 2H, J = 6.2 Hz), 2.69 (q, 4H, J = 7.1, 14.3 Hz), 1.12 (t, 6H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 179.7, 173.9, 166.2, 152.5, 151.2, 149.5, 133.5, 133.2, 132.6, 131.6, 128.2, 128.0, 127.8, 126.6, 126.2, 124.5, 123.8, 121.5, 121.0, 117.8, 115.2, 74.7, 52.3, 52.2, 47.0, 11.3; EIMS (70 eV) *m/z* (rel intensity) 471 (6, M^+), 456 (17), 372 (5), 340 (27), 228 (7), 200 (9), 99 (40), 86 (100), 58 (8).

Compound 8: 87% (yield); mp 177-179 °C; ^{1}H NMR ($CDCl_3$) δ 8.54 (d, 1H, J = 7.2 Hz), 8.47 (d, 1H, J = 7.5 Hz), 8.30-8.23 (m, 2H), 7.81-7.74 (m, 4H), 4.34 (t, 2H, J = 5.7 Hz), 4.18 (s, 3H), 2.88 (s, 2H), 2.58 (s, 4H), 1.68-1.66 (m, 6H); EIMS (70 eV) *m/z* (rel intensity) 483 (1, M^+), 424 (1), 340 (2), 312 (1), 228 (1), 200 (1), 111 (23), 98 (100), 83 (4).

Compound 10: 58% (yield); mp 164-167 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (d, 1H, J = 6.9 Hz), 8.37 (d, 1H, J = 7.5 Hz), 8.30-8.23 (m, 2H), 7.81-7.74 (m, 4H), 4.31 (t, 2H, J = 5.7 Hz), 4.18 (s, 3H), 2.86 (t, 2H, J = 5.7 Hz), 2.42 (s, 6H); EIMS (70 eV) *m/z* (rel intensity) 443 (1, M^+), 372 (2), 340 (20), 256 (4), 213 (13), 200 (28), 71 (100), 57 (68).

Compound 11: 65% (yield); mp 165-166 °C; ^{1}H NMR ($CDCl_3$) δ 8.54 (d, 1H, J = 8.9 Hz), 8.30-8.22 (m, 3H), 7.80-7.73 (m, 4H), 4.26 (t, 2H, J = 6.4 Hz), 4.19 (s, 3H), 2.60 (t, 2H, J = 7.1 Hz), 2.32 (s, 6H), 2.15-2.06 (m, 2H); EIMS (70 eV) *m/z* (rel intensity) 457(1, M^+), 398 (1), 340 (1), 200 (1), 101 (1), 84 (9), 71 (8), 58 (100).

Compound 14: 79% (yield); mp 167-168 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (d, 1H, J = 7.8 Hz), 8.38 (d, 1H, J = 7.2 Hz), 8.30-8.23 (m, 2H), 7.81-7.73 (m, 4H), 4.35 (t, 2H, J = 5.7 Hz), 4.18 (s, 3H), 3.05 (s, 2H), 2.70 (s, 4H), 1.88 (s, 4H); EIMS (70 eV) *m/z* (rel intensity) 469 (3, M^+), 410 (9), 340 (37), 228 (25), 200 (31), 96 (93), 84 (100), 69 (68).

Relaxation assay by Topoisomerase-I. Topoisomerase-I (Topo-I) activity was measured using Topo-I Drug screening kit manufactured by TopoGEN (Columbus, OH, USA). The reaction was fulfilled according to the manufacturer's protocol. Briefly, 0.25 μg of supercoiled DNA (form I) was used as a substrate and incubated with Topo-I (5 U), test material (final 100 μM) in assay buffer (10 mM Tris-HCl, pH 7.9, 1 mM EDTA, 0.15 M NaCl, 0.1% BSA, 0.1 mM Spermidine, 5% glycerol) at 37 °C for 30 min. Reaction was stopped by addition of 1/10 volume of 10% SDS. Then it was treated with proteinase K and extracted once with chloroform:isoamyl alcohol (CIA) prior to loading the gel. Agarose gel (1%) was electrophoresised in TAE buffer without ethidium bromide for 3-4 hr at 30-40 V. The gel was stained with ethidium bromide (0.5 μg/mL) for 30 min and destained in distilled water for more than 30 min.

Cytotoxicity assay. Cytotoxicity was determined by MTT assay to evaluate antineoplastic effect of naphthofuroquinone derivatives. Cells were seeded into 96-well plate in a density of 1×10^4 cells/well and treated with 10, 2, 0.8,

0.16 and 0.032 μM of compounds for 48 hrs. The 1/20 volume of MTT stock (5 mg/mL in propanol) was added and further incubated for 3 hrs. The media were aspirated and 100 μL of DMSO were added to each well. After 10 min incubation, the absorbance at 570 nm was measured. All the experiments were triplicated and IC_{50} value was obtained from the nonlinear regression using GraphPad program Prism®.

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