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Original article

Synthesis and evaluation of mansonone F derivatives as topoisomerase inhibitors

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ABSTRACT

A series of mansonone F (MF) derivatives were designed and synthesized. These compounds were found to be strong inhibitors for topoisomerases, with much more significant inhibition for topoisomerase II rather than topoisomerase I. The best inhibitor showed 20 times stronger anti-topoisomerase II activity than a positive control Etoposide. The cytotoxic activity of these MF derivatives was evaluated against human cancer cell lines CNE-2 and Glc-82, which showed that these compounds were potent antitumor agents. The structure–activity relationships (SARs) study revealed that *o*-quinone group and pyran ring are important for their cytotoxic activity.

1. Introduction

Topoisomerases play an essential role in the topological rearrangement of DNA during replication, transcription and recombination [1]. In recent years, they have been proved to be effective chemotherapeutic targets for the treatment of cancer [2]. Although numerous agents have been found to inhibit topoisomerases and possess anticancer activity, few classes of these topoisomerase inhibitors have exhibited high potency without significant toxicity. Besides, most of these inhibitors have large molecular weight and complex structure, which make it difficult and expensive to synthesize these compounds at an industrial scale. Therefore, it is necessary to search for new types of topoisomerase inhibitors that can be easily synthesized with better therapeutic index.

Mansonone F (MF) is a naturally occurring sesquiterpene *o*-quinone that exists at a low level in natural plants such as *Mansonia altissima* and *Ulmus pumila* [3–5]. This compound shows a wide variety of biological activities, such as antibacterial [6,7] and anti-proliferative effects [8,9]. Our previous studies have shown that 9-substituted and 6,9-substituted MF derivatives possess potential cytotoxic activity, and 9-H or 9-halogenated derivatives are more cytotoxic than 9-alkyl derivatives [8]. Recently, we have also found that the activity of thioredoxin reductase, a potential target for anticancer drugs, has been significantly inhibited by MF derivatives [10]. However, the range of their antitumor activity and their exact mechanisms remain unclear. In the present research, a variety of MF derivatives were synthesized and their structure–activity relationships were studied. Many of these compounds were found to be potent topoisomerase inhibitors.

2. Chemistry

In order to further investigate the impact of different substituents on MF, a variety of MF derivatives were designed and synthesized (Fig. 1, Scheme 1–3), including a series of 7,8-dione derivatized compounds **10**–**14** (Fig. 1), 3- and 9-substituted 7,8-dione derivatives **3a**–**f**, **4b**–**f**, **5a**–**c**, **5f**, **6b**, **6c**, **6f**, **7**, and **8** (Scheme 2), and pyran-ring-opened 7,8-dione derivatives **18a**–**18f** (Scheme 3), following the procedures reported previously with some proper modifications [7,11,12].

Compounds **3a**–**f** were prepared through seven steps according to a reported procedure [12]. In this previous procedure, *ortho*-bromination of 1-naphthol had poor selectivity and required further product purification using column chromatography, which would affect subsequent reactions. In our present study, 2-chloro-1-naphthol (**1**) was prepared with high selectivity and yield using

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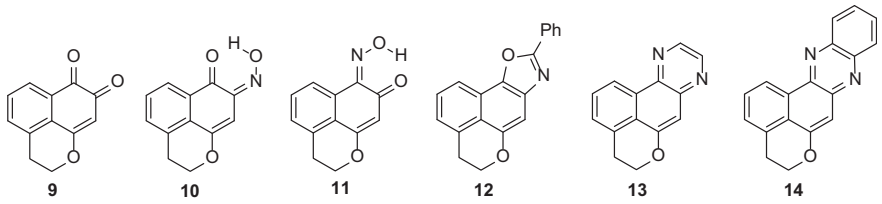
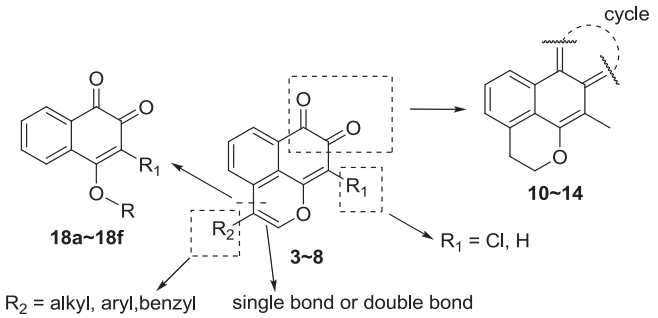


Fig. 1. Chemical structures of 7,8-dione compound **9** and 7,8-dione derivatized compounds (**10**–**14**) [14].

naphthol as starting material and sodium hypochlorite as chlorinating agent. Therefore, we used **1** instead of 2-bromo-1-naphthol for the next step. Upon reduction of the nitro group of compound **2** to amine, sodium hydrosulfite was used as a reducing agent. In comparison, when Pd–C/H_2 was used as a reducing agent and catalytic hydrogenation happened, 9-Cl was removed simultaneously, to give compound **5**. In addition, the reduction of the ester **2g** in the presence of Pd/C catalyst gave compound **7** that had both 9-Cl and 3-ester groups removed (Scheme 2).

Dehydration of compounds **3** and **5** produced compounds **4** and **6** respectively (Scheme 2). A procedure reported earlier has given poor yield (50%–60%) accompanied with by-products when concentrated sulfuric acid has been used as a dehydrating reagent [13]. Recently, some other reagents including Burgess reagent, PTSA/benzene and MsCl/DMAP/Et_3N, have been used as dehydrating reagents [7], however, these reagents had lower efficiency (yield <50%) for our reaction or are expensive. In the present study, the desired products were obtained at high yield (more than 90%, except compound **4e**), at room temperature with reaction for 30 min by using polyphosphoric acid (PPA) instead of concentrated sulfuric acid in the final step. Esterification of compound **3a** with benzoyl chloride gave compound **8** (Scheme 2).

In addition, to investigate whether the *o*-quinone group and the pyran ring of the core MF structure play essential roles for their cytotoxicity, two series of compounds, compounds **10**–**14** (Fig. 1) and compounds **18a**–**f** (Scheme 3) were designed and synthesized. 7,8-Dione derivatized compounds **10**–**14** were obtained with compound **9** as starting material, through 8,9-dicarbonyl derivatization based on our preliminary work [14]. Pyran-ring-opened 7,8-dione derivatives **18a**–**f** were prepared through protection of 1-naphthol with tetrahydropyran (THP), and introduction of the side chain, followed with oxidation of 1-naphthol. Here we used THP instead of benzyl group as the protection group of 1-naphthol, because THP does not produce either positive carbon ions during the deprotection or nucleophilic by-products [15]. We also found that 2-iodoxybenzoic acid (IBX) was highly selective for oxidizing 1-naphthol to *o*-quinone, while *p*-quinone products were obtained instead of *o*-quinone when SeO_2 was used as the oxidant.



Scheme 1. Design of compounds derived from mansonone F for SARs study.

3. Results and discussion

3.1. Cytotoxic activity

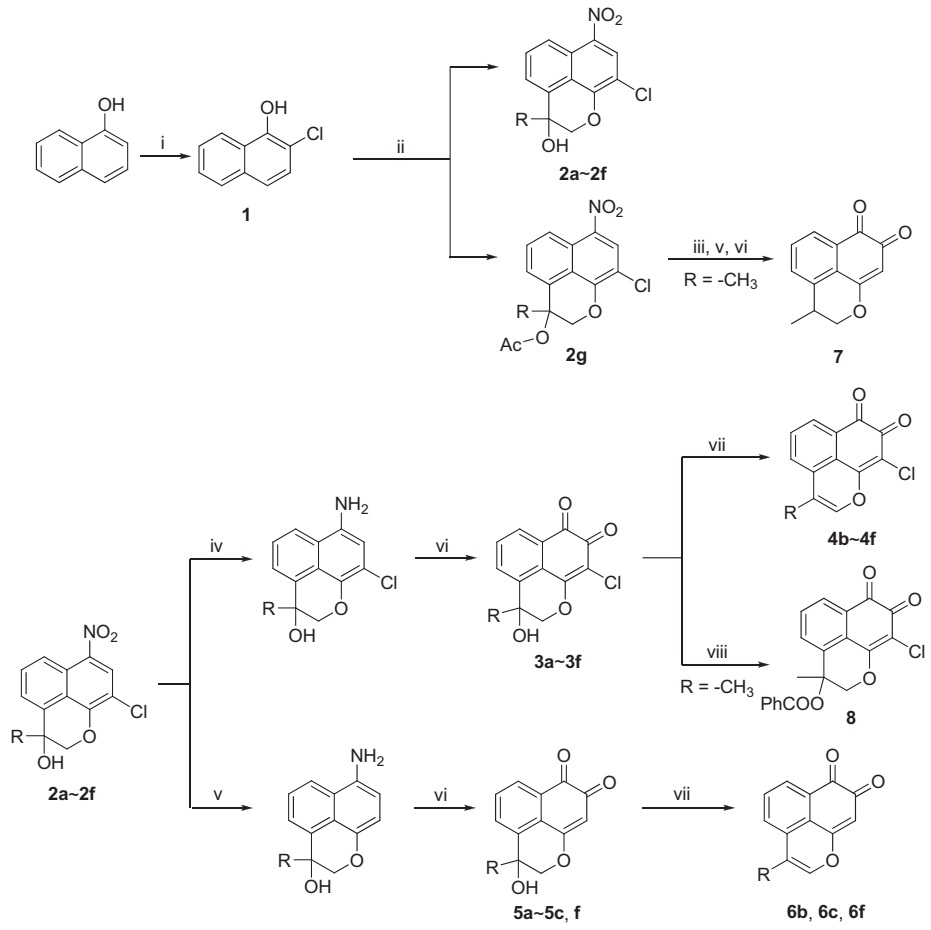
Previous studies have shown that 9-H and 9-Cl groups provide higher cytotoxicity than 9-alkyl and aryl alkyl groups do. In the present study, we evaluated cytotoxic activity of our synthesized 3-substituted derivatives with 9-H or 9-Cl group, and 7,8-dione derivatives, as well as pyran-ring-opened derivatives. The growth inhibitory effect (IC_{50}) of the MF derivatives toward human nasopharyngeal carcinoma cell line (CNE-2) and human lung adenocarcinoma cell line (Glc-82) was evaluated by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay as described by Mosmann with modifications [16].

As shown in Table 1, our new 9-H and 9-chloro derivatives had more potent cytotoxicity than previous 9-alkyl or 9-aryl alkyl derivatives [8]. On the other hand, the 2,3-position saturated derivatives were generally more potent than the related unsaturated derivatives. Especially, compounds **3b** and **3f**, substituted with phenyl and 4-F phenyl group respectively, were much more effective than compounds **4b** and **4f**. This may be due to the conjugation between their substituents and the parent ring, which restricted their conformations from fitting into their targets. The benzyl-substituted compound **4c** was 3.4/7.8 times (CNE2/Glc-82) less potent than the corresponding saturated compound **3c**. This may be due to the planar conformation of its parent ring, which put the benzyl group to an unfavored position. For the allyl- and propyl-substituted compounds, their cytotoxic activity was not susceptible to the saturation of the 2,3-double bond, since they can freely rotate without constrains. In addition, 9-H and 9-Cl groups of the 2,3-position saturated derivatives made little difference in their cytotoxic activity. However, for the 2,3-position unsaturated derivatives, the chloride-substituted compounds showed lower activity than the other ones.

Two series of compounds, **9**–**14** and **18a**–**f**, were also evaluated for their cytotoxic activity (Table 2). It was found that the *o*-quinone group derivatives had significant loss of the cytotoxic activity except compound **11**. These compounds had IC_{50} values over 50 μM, compared with the IC_{50} values of 4.83/5.32 μM (CNE2/Glc-82) for compound **9**. This may be due to the loss of hydrogen bond interaction between the *o*-quinone group and the target. The cytotoxic activity was also susceptible to the opening of pyran ring, suggesting that the rigid conformation of the pyran ring and its oxygen atom made great contributions to the interaction. These results indicated that both the *o*-quinone group and the pyran ring were essential for cytotoxic activity and tolerated little modification.

3.2. In vitro mode of action

A preliminary study of the synthesized compounds was also conducted to determine the mechanism of their antitumor activity. To identify major targets of these compounds, they were screened for inhibitory activity against several antitumor related targets



a: R=-CH_3; **b**: R=-Ph; **c**: R=-CH_2Ph; **d**: R=-$CH_2CH_2CH_3$; **e**: R=-$CH_2CH{=}CH_2$; **f**: R=-4-F-Ph

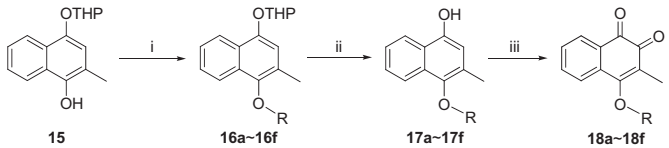
Scheme 2. Syntheses of 3- and 9-substituted 7,8-dione derivatives (**3a**–**f**, **4b**–**f**, **5a**–**c**, **5f**, **6b**, **6c**, **6f**, **7**, and **8**) of mansonone F. Reagents and conditions: (i) NaClO, 2 N NaOH, 5 °C, addition for 2 h followed with stirring for 30 min, 88%; (ii) six steps following Ref. [10]; (iii) $Cu(NO_3)_2 3H_2O$, Ac_2O, r.t., 30 min; (iv) $Na_2S_2O_4$, MeOH/H_2O = 3/1, 40 °C, 2 h; (v) 10% Pd/C, MeOH, $HCOONH_4$, 40 °C, 1–1.5 h; (vi) Fremy's salt, 0.06 NaH_2PO_4, r.t., 30–50 min; (vii) PPA, 40 °C, 30 min. (viii) Py, CH_2Cl_2, PhCOCl, r.t., overnight, 97%.

including topoisomerase II, topoisomerase I, telomerase and primase. Results showed that the tested compounds could strongly inhibit topoisomerase II at micromolar concentrations and compound **7**, which possessed potent topoisomerase II inhibitory activity, showed very slightly effect on telomerase and primase activities (22% inhibition at 20 μM and 29% inhibition at 100 μM respectively).

3.2.1. Topoisomerase II inhibitory activity

Topoisomerase II is a cell cycle-dependent nuclear enzyme in eukaryotic cells that interconvert topological isomers of DNA by breaking and rejoining double strands. It modifies the DNA linking in two steps and is able to relax the supercoiled form of double-stranded DNA in an ATP dependent manner. A couple of o-quinones, such as salvicine [17] and β-lapachone [18], have been found to be potent topoisomerase II inhibitors.

Based on the above cytotoxicity test results, our synthesized *o*-quinones were screened for their possible topoisomerase II inhibition. Topoisomerase II relaxation assay was conducted using human topoisomerase II (TopoGEN) with Etoposide as a positive control, and the results are shown in Fig. 2. The experiments were carried out at five different concentrations of 100, 50, 25, 5, 1 μM. Compounds **7**, **4e** and **5c** showed high inhibitory activity (IC_{50} = 1.88, 1.54, 4.88 μM respectively), and their IC_{50} values were 10–40 times of that for the positive control Etoposide (IC_{50} = 60.3 μM).



a: R = -CH_2COCH_3; **b**: R = -CH_2CN; **c**: R = -$CH_2CH{=}CH_2$; **d**: R = -$CH_2COC_6H_5$-4-OCH_3
e: R = -$CH_2COC_6H_5$-3,4-di-Me; **f**: R = -$CH_2COC_6H_5$-4-Cl

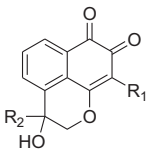
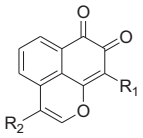
Scheme 3. Syntheses of pyran-ring-opened 7,8-dione derivatives (**18a**–**f**) of mansonone F [13]. Reagents and conditions: (i) RX, K_2CO_3/acetone, r.t., overnight; (ii) PPTS, MeOH/H_2O, r.t., 3 h; (iii) IBX, DMF, r.t., 1–1.5 h, yield 32%–95%.

3.2.2. Topoisomerase I inhibitory activity

Topoisomerase I is also involved in DNA repair, replication, transcription, and chromosome segregation during mitosis. While unlike topoisomerase II, topoisomerase I acts by making single-stranded breaks in DNA in an ATP independent manner, allowing controlled rotation about the nick. The *o*-quinone β-lapachone was also able to inhibit topoisomerase I efficiently [19]. Thus the inhibitory activity of our synthesized o-quinones against topoisomerase I was evaluated.

The inhibitory activities of the MF derivatives against topoisomerase I are shown in Fig. 3. Some of these compounds had

Table 1
Cytotoxic activity of mansonone F derivatives.

Comp.			IC_{50} (μM)[c]		Comp.			IC_{50} (μM)[c]	
	R_1	R_2	CNE-2	Glc-82		R_1	R_2	CNE-2	Glc-82
10-HCT[a]	–	–	1.04	0.23	**7**	–	–	2.05	1.77
Msn F[b]	–	–	27.34	15.53	**8**	–	–	8.05	10.85
3a	−Cl	$-CH_3$	8.12	11.26	–	–	–	–	–
3b	−Cl	−Ph	2.87	5.63	**4b**	−Cl	−Ph	33.3	40.16
3c	−Cl	$-CH_2Ph$	6.34	2.75	**4c**	−Cl	$-CH_2Ph$	21.5	21.6
3d	−Cl	$-CH_2CH_2CH_3$	3.59	7.93	**4d**	−Cl	$-CH_2CH_2CH_3$	3.93	2.15
3e	−Cl	$-CH_2CH{=}CH_2$	3.54	5.33	**4e**	−Cl	$-CH_2CH{=}CH_2$	5.20	3.77
3f	−Cl	−4-F−Ph	13.45	9.74	**4f**	−Cl	−4-F−Ph	27.48	31.31
5a	−H	$-CH_3$	17.33	19.20	–	–	–	–	–
5b	−H	−Ph	3.18	5.33	**6b**	−H	−Ph	9.19	3.68
5c	−H	$-CH_2Ph$	4.47	3.82	**6c**	−H	$-CH_2Ph$	6.52	2.88
5f	−H	−4-F−Ph	9.76	6.63	**6f**	−H	−4-F−Ph	15.97	3.21

[a] 10-HCT represents 10-hydroxycamptothecin.
[b] Msn F represents mansonone F.
[c] The IC_{50} represents compound concentration giving 50% survival of each cell line.

weak topoisomerase I inhibitory activity at 250 μM. Their inhibitory activity was close to that of camptothecin, however, their inhibitory activity had little linear correlation with their cytotoxic activity. For example, the most active compound, **7**, showed no significant topoisomerase I inhibitory activity. This might indicate that topoisomerase I is a minor target for their antitumor activity.

4. Conclusion

The antitumor structure–activity relationship study was carried out for MF derivatives, including compounds with saturation of 2,3-position, compounds with substituents at 3-position and 9-position, and compounds with *o*-quinone and pyran ring. Compared with previously synthesized 9-substituted compounds [8], 9-H or 9-Cl substituted derivatives were found to have stronger cytotoxic effect, and the derivatives with saturation of 2,3-position had significant antitumor activity. However, substituents conjugated with the parent ring at 3-position were unfavored. It was found that the 7,8-dione and the pyran ring were particularly important for the cytotoxic activity. All of these results indicated that the active domains of antitumor target were located near the 3- and 7,8-positions of compounds. Steric hindrance of 9-substituted group had an adverse effect for the interaction between 3- and 7,8-position of compounds and the corresponding active domains. These results provided important information for further structural modifications of these compounds for better antitumor drugs.

Our studies on the antitumor mechanisms of the synthesized compounds demonstrated that some of these compounds exhibited significant inhibition against topoisomerase II and only slight inhibition against topoisomerase I, telomerase and primase activity. These observations indicated that topoisomerase II may be one of the major targets for their antitumor action. In addition, the molecular weights of these MF derivatives are about half of that of Etoposide, which may help in easy uptake by cell. These encouraged us to further explore the antitumor activity of other MF derivatives, and further studies are currently in progress.

5. Experimental

5.1. Chemistry

Melting points were determined with a WRR or SGW X-4 melting point apparatus and were uncorrected. The NMR spectra (^1H and ^{13}C) were obtained on a Varian INOVA 500NB or Mercury-Plus 300 spectrometer using TMS as an internal standard. Mass spectra were recorded with a VG ZAB-HS(FAB) and Finnigan TSQ QUANTUM (ESI, APCI). High resolution mass spectrometry was carried out using a Shimadzu LCMS-IT-TOF.

5.1.1. 2-Chloro-naphthol (*1*)

To a solution of 1-naphthol (25 g, 170 mmol) in pre-cooled 2 N NaOH (100 ml, 200 mmol), 5% sodium hypochlorite (260 ml, 170 mmol) was added dropwise at 5 °C in an ice bath within 2 h, and the resulting solution was stirred for 30 min. A crystalline precipitate was formed during the addition, and the solution became darker in color. After the starting material was consumed, as monitored with TLC, concentrated hydrochloric acid was added

Table 2
Cytotoxic activity of nonsubstituted 7,8-dione derivatives and opened-pyran ring derivatives of mansonone F.

Product	IC_{50} (μM)		Product	R	IC_{50} (μM)	
	CNE2	Glc-82			CNE2	Glc-82
9	4.83	5.32	**18a**	$-CH_2COCH_3$	>50	>50
10	>50	>50	**18b**	$-CH_2CN$	39.47	39.21
11	5.70	42.83	**18c**	$-CH_2CH{=}CH_2$	>50	>50
12	>50	>50	**18d**	$-CH_2COC_6H_5$-4-OCH_3	30.35	44.26
13	>50	>50	**18e**	$-CH_2COC_6H_5$−3,4-diMe	>50	>50
14	>50	>50	**18f**	$-CH_2COC_6H_5$−4-Cl	30.43	>50

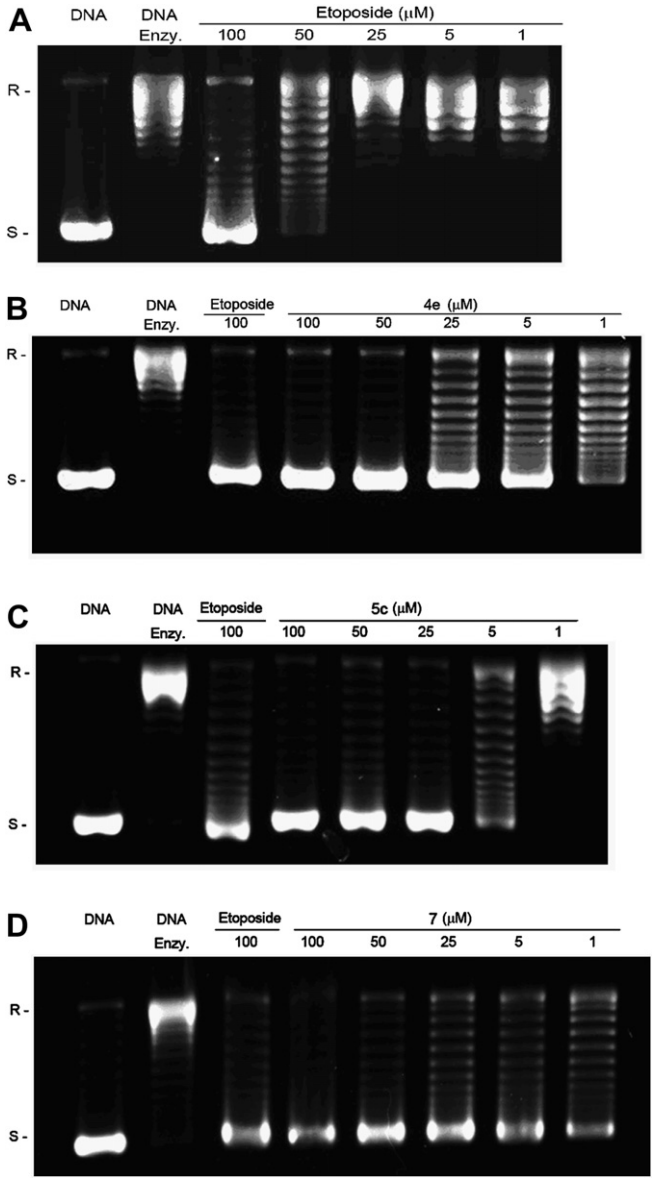


Fig. 2. Topoisomerase II inhibitory activity of mansonone F derivatives. A–D: The inhibitory activity assay of Topo II for compound Etoposide, **4e**, **5c**, and **7**, respectively. The IC_{50} values of 60.3, 1.31, 1.54 and 4.88 μM were obtained for pBR322 DNA relaxing reaction in different concentrations of 100, 50, 25, 5 and 1 μM, respectively. The positions for supercoiled DNA (S) and relaxed DNA (R) are marked on the left.

to adjust pH to 1. A light brown solid was precipitated out, which was filtered, washed to neutral, and dried under vacuum to give 27 g Gy solid with a yield of 88%. The solid became black on exposure to air.

*5.1.2. General procedure for synthesis of 3-substituted-3-hydroxyl-9-chloro-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**3a**–**f**)*

Sodium hyposulfite (4.35 g, 25 mmol) was added to a solution of nitro-substituted products **2** dissolved in MeOH (45 ml) and H_2O (15 ml). The reaction mixture was heated at 40 °C in a water bath with stirring for 2 h. After the solution turned from yellow to nearly colorless, 250 ml ice water was added, and the mixture was extracted with $CHCl_3$. The extract was washed with water, and dried over sodium sulfate. The $CHCl_3$ was removed under vacuo, and the residue was dissolved in 150 ml acetone. A solution of Fremy's salt (3.93 g, 15 mmol) dissolved in 500 ml 0.06 M KH_2PO_4 solution was added under vigorous stirring.

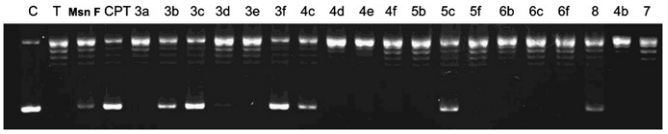


Fig. 3. The topoisomerase I inhibitory activity of mansonone F derivatives. C is DNA control; T is topoisomerase I and DNA; lane 3: 250 μM mansonone F; lane 4: 150 μM camptothecin (CPT); lane 5–23: 250 μM compounds.

*5.1.2.1. 3-Methyl-3-hydroxyl-9-chloro-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**3a**).* The product was purified using flash chromatography (petroleum ether/EtOAc = 1:1) to give a reddish brown solid **3a** with a yield of 56%. m.p. 234–235 °C. IR ν_{max} (KBr)/cm^{-1}: 3483, 1691, 1648, 1602, 1543, 1462, 1310. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (3H, s), 4.31 (1H, d, J = 11 Hz), 4.31 (1H, d, J = 11 Hz), 4.42 (1H, s), 6.00 (1H, br), 7.71 (1H, t, J = 8 Hz), 7.90 (1H, d, J = 8 Hz), 7.96 (1H, d, J = 8 Hz). ^{13}C NMR (100 MHz, d_6-DMSO) δ 25.9, 65.5, 75.6, 112.0, 123.4, 128.1, 128.9, 131.0, 132.4, 141.7, 161.1, 173.2, 177.2. FAB-MS m/z 265 $[M+1]^+$. HRMS (EI) calcd for $C_{13}H_9ClO_4$ $[M + Na]^+$: 287.0087; Found: 287.0076.

*5.1.2.2. 3-Phenyl-3-hydroxyl-9-chloro-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**3b**).* The product was purified using flash chromatography (petroleum ether/EtOAc = 2:1) to give a reddish black solid **3b** with a yield of 40%. m.p. 283–284 °C. IR ν_{max} (KBr)/cm^{-1}: 3459, 1747, 1694, 1648, 1600, 1541, 1307, 1150, 763, 703. ^{1}H NMR (300 MHz, d_6-DMSO) δ 4.48 (1H, d, J = 11 Hz), 4.73 (1H, d, J = 11 Hz), 6.66 (1H, s), 7.33 (5H, m), 7.46 (1H, d, J = 8 Hz), 7.63 (1H, t, J = 8 Hz), 7.92 (1H, dd, J = 8, 1.5 Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 69.75, 75.67, 124.2, 126.3, 127.6, 127.9, 128.1, 128.5, 131.8, 132.6, 139.6, 141.6, 160.5, 172.6, 176.7. FAB-MS m/z 327 $[M+1]^+$. HRMS (EI) calcd for $C_{18}H_{11}ClO_4$ $[M + Na]^+$: 349.0244; Found: 349.0247.

*5.1.2.3. 3-Benzyl-3-hydroxyl-9-chloro-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**3c**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 5:1) to give a salmon pink solid **3c** with a yield of 53%. m.p. 219–220 °C. IR ν_{max} (KBr)/cm^{-1}: 3424, 1697, 1658, 1608, 1555, 1494, 1309, 1143, 1107, 987, 780,720. ^{1}H NMR (300 MHz, d_6-DMSO) δ 3.01 (2H, s), 4.18 (1H, d, J = 11 Hz), 4.44 (1H, d, J = 11 Hz), 6.04 (1H, s), 7.01 (2H, dd, J = 7, 2.5 Hz), 7.17–7.19 (3H, m), 7.44 (1H, dd, J = 8, 1.5 Hz), 7.52 (1H, t, J = 8 Hz), 7.83 (1H, dd, J = 8, 1.5 Hz). ^{13}C NMR (100 MHz, d_6-DMSO) δ 45.0, 68.2, 73.4, 112.1, 123.7, 126.7, 127.8, 128.0, 128.8, 130.9, 131.5, 135.6, 141.0, 160.8, 173.2, 177.2. FAB-MS m/z 341 $[M+1]^+$. HRMS (EI) calcd for $C_{19}H_{13}ClO_4$ $[M + Na]^+$: 363.0400; Found: 363.0391.

*5.1.2.4. 3-n-Propyl-3-hydroxyl-9-chloro-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**3d**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 2:1) to give a reddish brown solid **3d** with a yield of 51%. m.p. 282–283 °C. IR ν_{max} (KBr)/cm^{-1}: 3431, 2959, 1699, 1648, 1602, 1546, 1455, 1385, 1310, 1150, 981, 877, 780. ^{1}H NMR (300 MHz, d_6-DMSO) δ 0.83 (3H, t, J = 6.6 Hz), 1.22 (1H, sextet, J = 7.5 Hz), 1.39 (1H, sextet, J = 7.5 Hz), 1.71 (2H, t, J = 8 Hz), 4.22 (1H, d, J = 11 Hz), 4.48 (1H, d, J = 11 Hz), 5.86 (1H, s), 7.68 (1H, t, J = 8 Hz), 7.86 (2H, d, J = 8 Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 14.1, 16.0, 40.9, 67.3, 73.5, 111.6, 123.2, 127.5, 128.6, 131, 131.5, 141.1, 160.4, 172.6, 176.6. FAB-MS m/z 293 $[M+1]^+$. HRMS (EI) calcd for $C_{15}H_{13}ClO_4$ $[M + Na]^+$: 315.0400; Found: 315.0397.

*5.1.2.5. 3-Allyl-3-hydroxyl-9-chloro-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**3e**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 5:1) to give a reddish brown solid **3e** with a yield of 65%. m.p. 163–165 °C. IR ν_{max} (KBr)/cm^{-1}: 3486, 1690, 1650, 1602, 1548, 1460, 1310, 1137, 1090, 982, 783. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.53 (2H, d, J = 6 Hz), 4.24 (1H, d, J = 11 Hz),

4.47 (1H, d, $J = 11$ Hz), 5.02 (1H, dd, $J = 20$, 2 Hz), 5.07 (1H, dd, $J = 10$, 2 Hz), 5.73–5.87 (1H, m), 6.01 (1H, br), 7.68 (1H, t, $J = 8$ Hz), 7.87 (2H, d, $J = 8$ Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 43.8, 68.1, 74.1, 112.6, 119.9, 120.2, 124.2, 128.5, 129.4, 132.1, 132.4, 133.0, 141.3, 161.3, 173.6. FAB-MS m/z 291 $[M+1]^+$. HRMS (EI) calcd for $C_{15}H_{11}ClO_4$ $[M+1]^+$: 291.0424; Found: 291.0408.

*5.1.2.6. 3-(4-Fluorophenyl)-3-hydroxyl-9-chloro-2,3-dihydrobenzo [de]benzopyran-7,8-dione (**3f**).* The product was purified using flash chromatography ($CHCl_3$/THF = 2:1) to give a orange red solid **3f** with a yield of 38%. m.p. 294–296 °C. IR ν_{max} (KBr)/cm^{-1}: 3443, 1693, 1649, 1602, 1542, 1511, 1307, 1150, 840. ^{1}H NMR (300 MHz, d_6-DMSO) δ 4.48 (1H, d, $J = 11$ Hz), 4.75 (1H, d, $J = 11$ Hz), 6.73 (1H, s), 7.21 (2H, m), 7.42 (2H, m), 7.48 (1H, dd, $J = 8$, 1.5 Hz), 7.65 (1H, t, $J = 8$ Hz), 7.93 (1H, dd, $J = 8$, 1.5 Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 69.4, 75.5, 111.7, 114.5, 114.8, 124.2, 128.4, 128.1128.5, 131.9, 132.5, 137.7, 139.3, 160.3, 172.6, 176.6. FAB-MS m/z 345 $[M+1]^+$. HRMS (EI) calcd for $C_{18}H_{10}ClFO_4$ $[M + Na]^+$: 367.0149; Found: 367.0142.

*5.1.3. General procedure for synthesis of 3-subtituted-9-chlorobenzo[de]benzopyran-7,8-dione (**4b**–**f**)*

A mixture of **3** (2 mmol) and PPA (2 g) was stirred at 40 °C for 30 min. Then 50 ml water was added, and the resulting solution was stirred for 30 min. The mixture was extracted with $CHCl_3$, washed with water, dried over anhydrous sodium sulfate, and concentrated. The residue was purified using flash chromatography to give the desired product.

*5.1.3.1. 3-Phenyl-9-chlorobenzo[de]benzopyran-7,8-dione (**4b**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 15:1) to give a purple black solid **4b** with a yield of 94%. m.p. 197–198 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44–7.47 (2H, m), 7.49–7.58 (4H, m), 7.77 (1H, t, $J = 8$ Hz), 8.06 (1H, dd, $J = 8$, 1.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 110.5, 121.2, 129.0, 129.2, 129.3, 129.5, 129.8, 130.6, 131.4, 131.5, 132.1, 133.2, 142.1, 160.0, 172.7, 177.9. FAB-MS m/z 309 $[M+1]^+$. HRMS (EI) calcd for $C_{18}H_9ClO_3$ $[M + Na]^+$: 309.0318; Found: 309.0309.

*5.1.3.2. 3-Benzyl-9-chlorobenzo[de]benzopyran-7,8-dione (**4c**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 15:1) to give a purple black solid **4c** with a yield of 91%. m.p. 223–225 °C. IR ν_{max} (KBr)/cm^{-1}: 1692, 1648, 1599, 1551, 1493, 1346, 1135, 730. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.96 (2H, s), 7.18 (1H, t, $J = 7$ Hz), 7.26–7.35 (4H, m), 7.70 (2H, t, $J = 7$ Hz), 7.67–7.75 (2H, m), 7.84 (1H, s), 7.96 (1H, d, $J = 7$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.8, 110.5, 117.0, 122.5, 127.48, 128.6, 129.2, 130.4, 130.7, 131.9, 133.4, 136.8, 142.9, 160.4, 172.6, 177.9. FAB-MS m/z 323 $[M+1]^+$. HRMS (EI) calcd for $C_{19}H_{11}ClO_3$ $[M+1]^+$: 323.0475; Found: 323.0470.

*5.1.3.3. 3-Propyl-9-chlorobenzo[de]benzopyran-7,8-dione (**4d**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 15:1) to give a purple black solid **4d** with a yield of 91%. m.p. 220–222 °C. IR ν_{max} (KBr)/cm^{-1}: 1695, 1646, 1602, 1549, 1348, 1270, 1227, 1157, 1141. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (3H, t, $J = 7.5$ Hz), 1.65 (2H, sextet, $J = 7.5$ Hz), 2.55 (2H, t, $J = 7.5$ Hz), 7.23 (1H, s), 7.68 (2H, m), 8.13 (1H, dd, $J = 8$, 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 21.9, 29.2, 109.9, 117.5, 122.1, 128.5129.6, 130.6, 131.5, 133.1, 141.2, 160.3, 172.2, 177.7. FAB-MS m/z 275 $[M+1]^+$. HRMS (EI) calcd for $C_{15}H_{11}ClO_3$ $[M+1]^+$: 275.0475; Found: 275.0475.

*5.1.3.4. 3-Allyl-9-chlorobenzo[de]benzopyran-7,8-dione (**4e**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 15:1) to give an oxford blue solid **4e** with a yield of 50%. m.p. 171–172 °C. IR ν_{max} (KBr)/cm^{-1}: 1698, 1645, 1598, 1545, 1345, 1268, 1181, 1156, 1134, 867, 850, 786. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.32 (1H, q, $J = 1.5$ Hz), 3.34 (1H, q, $J = 1.5$ Hz), 5.20 (1H, dq, $J = 12.5$, 2 Hz), 5.23 (1H, dq, $J = 12.5$, 2 Hz), 5.95 (1H, m), 7.24 (1H, t, $J = 1.5$ Hz), 7.66 (1H, t, $J = 7$ Hz), 7.71 (1H, dd, $J = 7$, 1.5 Hz), 8.13 (1H, dd, $J = 7$, 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.8, 105.7, 111.4, 114.1, 117.6, 123.9, 125.5, 125.9, 127.1, 128.5, 129.0, 137.5, 155.6, 167.8, 173.2. FAB-MS m/z 273 $[M+1]^+$. HRMS (EI) calcd for $C_{15}H_9ClO_3$ $[M+1]^+$: 273.0318; Found: 273.0313.

*5.1.3.5. 3-(4-Fluorobenzyl)-9-chlorobenzo[de]benzopyran-7,8-dione (**4f**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 15:1) to give a purple black solid **4f** with a yield of 94%. m.p. 296–297 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (2H, m), 7.35 (3H, m), 7.52 (1H, dd, $J = 8$, 1.5 Hz), 7.63 (1H, t, $J = 8$ Hz), 8.17 (1H, dd, $J = 8$, 1.5 Hz). ESI-MS m/z 327 $[M+1]^+$. HRMS (EI) calcd for $C_{18}H_8ClFO_3$ $[M + Na]^+$: 349.0044; Found: 349.0015.

*5.1.4. General procedure for synthesis of 3-subtituted-3-hydroxyl-2,3-dihydrobenzo[de]-benzopyran-7,8-dione (**5a**–**c**, **f**)*

To a solution of nitro-substituted products **2** dissolved in MeOH (50 ml), were added ammonium formate (3.15 g, 50 mmol) and 0.4 g 10% Pd/C. The reaction mixture was stirred at 40 °C for 2 h. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was evaporated under vacuum. The residue was washed with water and dissolved in 150 ml acetone. A solution of Fremy's salt (3.93 g, 15 mmol) dissolved in 500 ml 0.06 M KH_2PO_4 solution was added under vigorous stirring, and the solution changed from bluish violet to pale yellowish brown with solid precipitation. After reaction for 30–50 min, the mixture was extracted with chloroform and washed with water. The organic layer was dried over anhydrous sodium sulfate and concentrated. The product was purified using chromatography.

*5.1.4.1. 3-Methyl-3-hydroxyl-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**5a**).* The product was purified with flash chromatography (petroleum ether/EtOAc = 2:1) to give a khaki solid **5a** with a yield of 61%. m.p. 205–209 °C. ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.49 (3H, s), 4.30 (1H, d, $J = 11$ Hz), 4.42 (1H, d, $J = 11$ Hz), 6.06 (1H, br), 7.71 (1H, t, $J = 8$ Hz), 7.88 (1H, dd, $J = 8$, 1.5 Hz), 7.95 (1H, dd, $J = 8$, 1.5 Hz). FAB-MS m/z 231 $[M+1]^+$. HRMS (EI) calcd for $C_{13}H_{10}O_4$ $[M + Na]^+$: 253.0477; Found: 253.0475.

*5.1.4.2. 3-Phenyl-3-hydroxyl-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**5b**).* The product was purified using flash chromatography (petroleum ether/EtOAc = 2:1) to give a flavous solid **5b** with a yield of 48%. m.p. 241–242 °C. IR ν_{max} (KBr)/cm^{-1}: 3447, 1693, 1641, 1613, 1563, 1466, 1388, 1341, 1263, 1206, 856, 722, 649. ^{1}H NMR (300 MHz, d_6-DMSO) δ 4.37 (1H, d, $J = 11$ Hz), 4.63 (1H, d, $J = 11$ Hz), 5.97 (1H, s), 6.60 (1H, br), 7.32 (5H, m), 7.48 (1H, d, $J = 8$ Hz), 7.65 (1H, t, $J = 8$ Hz), 7.93 (1H, d, $J = 8$ Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 69.6, 75.1, 107.4, 124.7, 126.3, 127.5, 127.9, 129.4, 131.9, 132.7, 139.9, 141.8, 165.7, 178.5, 178.8. FAB-MS m/z 293 $[M+1]^+$. HRMS (EI) calcd for $C_{18}H_{12}O_4$ $[M+1]^+$: 293.0814; Found: 293.0818.

*5.1.4.3. 3-Benzyl-3-hydroxyl-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**5c**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 2:1) to give a brown solid **5c** with a yield of 50%. m.p. 238–239 °C. IR ν_{max} (KBr)/cm^{-1}: 3466, 1697, 1641, 1612, 1564, 1458, 1382, 1344, 1263, 1199, 994, 776, 702. ^{1}H NMR (300 MHz, d_6-DMSO) δ 3.01 (2H, s), 4.10 (1H, d, $J = 11$ Hz), 4.25 (1H, d, $J = 11$ Hz), 5.90 (1H, s), 5.97 (1H, br), 7.02 (2H, dd, $J = 7.5$, 2 Hz), 7.15–7.23 (3H, m), 7.51–7.58 (2H, m), 7.84 (1H, dd, $J = 7$, 1.5 Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 44.9, 67.9, 72.4, 107.4, 123.8, 126.3, 127.5, 129.3, 130.5, 131.3, 135.3, 141.1, 165.8, 178.5, 178.7. FAB-MS m/z 307 $[M+1]^+$. HRMS (EI) calcd for $C_{19}H_{14}O_4$ $[M+1]^+$: 307.0970; Found: 307.0970.

*5.1.4.4. 3-(4-Fluorophenyl)-3-hydroxyl-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**5f**).* The product was purified using flash chromatography ($CHCl_3$/THF = 6:1) to give a reddish black solid **5f** with a yield of 45%. m.p. 263–265 °C. IR ν_{max} (KBr)/cm^{-1}: 3436, 1694, 1640, 1613, 1564, 1511, 1468, 1341, 1223, 855, 838, 775. ^{1}H NMR (300 MHz, d_6-DMSO) δ 4.36 (1H, d, J = 11 Hz), 4.62 (1H, d, J = 11 Hz), 5.96 (1H, s), 6.71 (1H, s), 7.17 (2H, t, J = 8 Hz), 7.15–7.21 (2H, m), 7.39–7.44 (2H, m), 7.48 (1H, dd, J = 8, 1.5 Hz), 7.65 (1H, t, J = 8 Hz), 7.93 (1H, dd, J = 8, 1.5 Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 70.2, 75.9, 108.3, 115.4, 115.7, 125.6, 128.9, 129.3, 129.5, 130.4, 132.9, 133.5, 139.0, 140.7, 166.6, 179.4, 179.6. FAB-MS m/z 311 $[M+1]^+$. HRMS (EI) calcd for $C_{18}H_{11}FO_4$ $[M + Na]^+$: 333.0545; Found: 333.0514.

*5.1.5. Synthesis of 3-substituted benzo[de]benzopyran-7,8-dione (**6b**, **6c**, and **6f**)*

3-Substituted benzo[*de*]benzopyran-7,8-dione (**6b**, **6c**, and **6f**) was synthesized with compounds **5** as starting materials following the same method for the preparation of compounds **4b**–**f**.

*5.1.5.1. 3-Phenyl-benzo[de]benzopyran-7,8-dione (**6b**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 15:1) to give a deep magenta solid **6b** with a yield of 90%. m.p. 230–231 °C. IR ν_{max} (KBr)/cm^{-1}: 3089, 1699, 1631, 1595, 1570, 1366, 1251, 1129, 849, 776. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.13 (1H, s), 7.17 (1H, s), 7.46–7.51 (3H, m), 7.54 (1H, dd, J = 8, 1.5 Hz), 7.61 (1H, t, J = 8 Hz), 8.14 (1H, dd, J = 8, 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 105.1, 120.3, 122.2, 128.8, 129.0, 129.6, 130.6, 130.9, 131.2, 131.6, 133.0, 141.9, 165.2, 177.9, 179.5. FAB-MS m/z 274 $[M+1]^+$. HRMS (EI) calcd for $C_{18}H_{10}O_3$ $[M+1]^+$: 275.0708; Found: 275.0703.

*5.1.5.2. 3-Benzyl-benzo[de]benzopyran-7,8-dione (**6c**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 15:1) to give a dark violet solid **6c** with a yield of 80%. m.p. 229–230 °C. IR ν_{max} (KBr)/cm^{-1}: 1690, 1639, 1609, 1559, 1275, 1198,851, 769, 708. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.98 (2H, s), 6.00 (1H, s), 7.18 (1H, t, J = 7 Hz), 7.33 (2H, d, J = 7 Hz), 7.63 (1H, s), 7.69 (2H, d, J = 4 Hz), 7.92 (2H, d, J = 4 Hz). FAB-MS m/z 289 $[M+1]^+$. HRMS (EI) calcd for $C_{19}H_{12}O_3$ $[M+1]^+$: 289.0865; Found: 289.0865.

*5.1.5.3. 3-(4-Fluorophenyl)-benzo[de]benzopyran-7,8-dione (**6f**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc, 15:1) to give a deep magenta solid **6f** with a yield of 83%. m.p. 253–255 °C. IR ν_{max} (KBr)/cm^{-1}: 1630, 1603, 1570, 1508, 1281, 1252, 1221, 1161, 1133, 848, 779. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.14 (1H, s), 7.16–7.25 (3H, m), 7.33–7.37 (2H, m), 7.48 (1H, d, J = 8 Hz), 7.63 (1H, t, J = 8 Hz), 8.16 (1H, d, J = 8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 105.5, 116.3, 116.6, 119.7, 122.6, 127.9, 130.0, 130.9, 131.6, 133.4, 142.3, 161.5, 164.8, 165.3, 178.2, 179.7. FAB-MS m/z 293 $[M+1]^+$. HRMS (EI) calcd for $C_{18}H_9FO_3$ $[M+1]^+$: 293.0614; Found: 293.0615.

*5.1.6. 3-Methyl-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**7**)*

Starting from **2g**, compound **7** was obtained as an orange solid by using the method for the preparation of **5a**–**c** except that the reaction time was extended to 1.5 h (yield 40%). m.p. 159–160 °C. IR ν_{max} (KBr)/cm^{-1}: 2975, 1693, 1641, 1607, 1561, 1347, 1285, 1263, 1198, 846, 772. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (3H, d, J = 7 Hz), 3.20 (1H, sextet, J = 1.5 Hz), 4.26 (1H, dd, J = 11, 5 Hz), 4.47 (1H, dd, J = 11, 5 Hz), 6.03 (1H, s), 7.57 (2H, m), 8.01 (1H, dd, J = 8, 1.5 Hz). FAB-MS m/z 215 $[M+1]^+$. HRMS (EI) calcd for $C_{13}H_{10}O_3$ $[M + Na]^+$: 237.0528; Found: 237.0523.

*5.1.7. -Methyl-3-benzoyloxy-9-chloro-2,3-dihydrobenzo[de]benzopyran-7,8-dione (**8**)*

Compound **3a** (230 mg, 1 mmol) was dissolved in 5 ml pyridine. DMAP (20 mg) and benzoyl chloride (1.4 g, 10 mmol) were added, and the suspension was stirred at room temperature overnight. 20 ml 5% diluted hydrochloric acid was added to make the pH>2, and the mixture was extracted with $CHCl_3$. The organic layer was washed once with diluted hydrochloric acid, and then with water to neutral pH, dried over anhydrous sodium sulfate, and concentrated. The residue was purified using silica gel column eluted with CH_2Cl_2 to give 0.36 g of a salmon pink solid (yield 97%). IR ν_{max} (KBr)/cm^{-1}: m.p. 169–171 °C. 1723, 1699, 1653, 1604, 1557, 1453, 1314, 1261, 777. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (3H, s), 4.72 (1H, d, J = 11 Hz), 5.00 (1H, d, J = 11 Hz), 7.46–7.52 (3H, m), 7.58 (1H, q, J = 7.5 Hz), 7.75 (1H, t, J = 7.5 Hz), 7.89–7.99 (3H, m), 8.17 (1H, t, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 72.6, 74.6, 112.1, 123.8, 128.2, 128.5, 128.7, 129.4, 130.4, 132.5, 133.4, 133.8, 159.5, 164.2, 166.9, 172.5, 176.2. FAB-MS m/z 369 $[M+1]^+$. HRMS (EI) calcd for $C_{20}H_{13}ClO_5$ $[M + Na]^+$: 391.0349; Found: 391.0361.

*5.1.8. General procedure for synthesis of 4-substituted-3-methyl-1,2-naphthoquinone (**18a**–**f**) [13]*

A mixture of **15** (2.6 g, 10 mmol), alkyl halide (11 mmol), anhydrous potassium carbonate (4.1 g, 30 mmol) and acetone (50 ml) was stirred at room temperature overnight, and the acetone was removed in vacuo. Water was added, and the solution was extracted with $CHCl_3$. The organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated. The crude product (**16a**–**f**) was used for the next step without purification.

Compounds **16a**–**f** (20 mmol) and PPTS (0.60 g) were suspended in 120 ml MeOH and 60 ml H_2O, and the solution was stirred at room temperature under N_2 for 3 h. After completion of the reaction (monitored with TLC), MeOH was removed in vacuo and water was added. The above mixture was extracted with $CHCl_3$, washed with water, dried over anhydrous sodium sulfate, and concentrated. The crude product (**17a**–**f**) was susceptible to oxidation in air, and was used for next step without purification.

Compounds **17a**–**f** (0.5 mmol) and IBX (0.15 g, 0.55 mmol) were suspended in 2 ml DMF, and the solution was stirred at room temperature for 1–1.5 h. Water was added, and the mixture was extracted with $CHCl_3$. The organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated. The crude product (**18a**–**f**) was purified using a silica gel column.

*5.1.8.1. 4-(2-Oxyproproxy)-3-methyl-1,2-naphthoquinone (**18a**).* The product was purified using flash chromatography (petroleum ether/EtOAc = 2:1) to give a saffron yellow solid **18a** with a yield of 64%. m.p. 133–135 °C. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.09 (3H, s), 2.17 (3H, s), 4.24 (2H, s), 7.64 (1H, td, J = 7, 1.5 Hz), 7.72 (1H, td, J = 7, 1.5 Hz), 8.05 (1H, dd, J = 7, 1.5 Hz), 8.10 (1H, dd, J = 7, 1.5 Hz). HRMS (EI) calcd for $C_{14}H_{12}O_4$ $[M + Na]^+$: 267.0633; Found: 267.0631.

*5.1.8.2. 2-(1,2-Dihydro-3-methyl-1,2-dioxy-4-naphthoxy) acetonitrile (**18b**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 5:1) to give a yellow solid **18b** with a yield of 94%. m.p. 162–164 °C. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.11 (3H, s), 4.84 (2H, s), 7.53 (1H, t, J = 7, 1.5 Hz), 7.62 (1H, t, J = 7 Hz), 7.73 (1H, t, J = 7 Hz), 8.10 (1H, d, J = 7, 1.5 Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 9.9, 57.5, 114.0, 124.4, 126.1, 129.4, 130.1, 131.0, 131.9, 135.7, 162.8, 177.8, 181.1. HRMS (EI) calcd for $C_{13}H_9NO_3$ $[M + Na]^+$: 250.0480; Found: 250.0477.

*5.1.8.3. 4-Allyl-3-methyl-1,2-naphthoquinone (**18c**).* The product was purified using flash chromatography (petroleum ether/EtOAc = 5:1) to give a saffron yellow solid **18c** with a yield of 52%. m.p. 92–93 °C. ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.51 (3H, s), 2.63 (2H, s), 5.01 (1H, d, J = 17 Hz), 5.06 (1H, d, J = 10 Hz), 5.55 (1H, m), 7.85 (1H, td, J = 7, 1.5 Hz), 7.90 (1H, td, J = 7, 1.5 Hz), 8.16 (1H, d,

J = 7 Hz), 8.23 (1H, d, J = 7 Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 17.4, 41.7, 120.5, 127.0, 128.1, 128.2, 130.4, 133.2, 134.7, 134.9, 135.9, 182.0, 194.1, 194.4. HRMS (EI) calcd for $C_{14}H_{12}O_3$ $[M + Na]^+$: 251.0684; Found: 251.0678.

*5.1.8.4. 4-[2-(Oxyl-1-(4-methoxylphenzyl)proproxy)]-3-methyl-1,2-naphthoquinone (**18d**).* The product was purified using flash chromatography ($CHCl_3$/MeOH, 50:1) to give a flavous solid **18d** with a yield of 95%. m.p. 130–131 °C. ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.99 (3H, s), 3.85 (3H, s), 5.63 (2H, s), 7.05 (2H, d, J = 9 Hz), 7.56 (1H, t, J = 7.5 Hz), 7.77 (1H, t, J = 7.5 Hz), 7.90–7.96 (4H, m). ^{13}C NMR (75 MHz, d_6-DMSO) δ 9.5, 55.5, 74.8, 113.9, 122.6, 125.0, 126.6, 127.9, 129.2, 129.9, 130.0, 133.1, 135.0, 163.3, 163.9, 177.8, 180.6, 191.4. HRMS (EI) calcd for $C_{20}H_{16}O_5$ $[M + Na]^+$: 359.0888; Found: 359.0895.

*5.1.8.5. 4-[2-(Oxyl-1-(2,3-dimethylphenzyl)proproxy)]-3-methyl-1,2-naphthoquinone (**18e**).* The product was purified using flash chromatography (petroleum ether/EtOAc = 1:1) to give a saffron yellow solid **18e** with a yield of 32%. m.p. 172–174 °C. ^{1}H NMR (500 MHz, d_6-DMSO) δ 1.98 (3H, s), 2.28 (3H, s), 2.30 (3H, s), 5.64 (2H, s), 7.31 (1H, d, J = 8 Hz), 7.59 (1H, t, J = 8 Hz), 7.70 (1H, d, J = 8 Hz), 7.76–7.80 (2H, m), 7.92–7.95 (2H, m). ^{13}C NMR (125 MHz, d_6-DMSO) δ 9.3, 19.0, 19.4, 75.1, 122.8, 125.1, 125.3, 128.1, 128.5, 129.3, 129.7, 130.2, 131.7, 133.3, 135.2, 136.8, 143.1, 164.1, 178.1, 180.8, 192.9. HRMS (EI) calcd for $C_{21}H_{18}O_4$ $[M + Na]^+$: 357.1103; Found: 357.1095.

*5.1.8.6. 4-[2-(Oxyl-1-(4-chlorophenzyl)proproxy)]-3-methyl-1,2-naphthoquinone (**18f**).* The product was purified using flash chromatography ($CHCl_3$/EtOAc = 30:1) to give a yellow solid **18f** with a yield of 72%. m.p. 129 °C. ^{1}H NMR (300 MHz, d_6-DMSO) δ 2.09 (3H, s), 5.40 (2H, s), 7.47 (3H, m), 7.68 (1H, t, J = 7 Hz), 7.87 (3H, t, J = 7 Hz), 8.03 (1H, d, J = 7, 1.5 Hz). ^{13}C NMR (75 MHz, d_6-DMSO) δ 10.2, 74.7, 124.1, 125.5, 129.3, 129.6, 129.7, 130.8, 132.3, 133.4, 135.8, 141.1, 165.1, 178.6, 181.6, 191.2. HRMS (EI) calcd for $C_{19}H_{13}O_4Cl$ $[M + Na]^+$: 363.0400; Found: 363.0397.

5.2. Cell growth inhibition assay

The growth inhibitory effect of mansonone F derivatives toward human nasopharyngeal carcinoma cell line (CNE2) and human lung adenocarcinoma cell line (Glc-82) were evaluated by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium-bromide) assay as described by Mosmann [13] with modifications. The cells were plated at a density of 1×10^4 per well in 96-well microplates, and allowed to incubate overnight. MF derivatives were added to the wells at increasing concentrations (0–100 μM). After 48 h, each well was treated with 20 μl 5 mg/ml MTT solution, and the cells were further incubated at 37 °C for 4 h. At the end of the incubation, the untransformed MTT was removed, and 150 μl of DMSO was added. The microplates were well shaken to dissolve the formazan dye, and the absorbance at 570 nm was measured using a microplate-reader (Bio-Tek).

5.3. Topoisomerase II inhibition assay

We used the topoisomerase II assay kit from TopoGEN. Relaxation assays were carried out according to the manufacturer's instructions with minor modifications. The assay was performed in a final volume of 20 μl in topoisomerase II reaction buffer (1 × topoisomerase II buffer = 50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 10 mM $MgCl_2$, 5 mM ATP, 0.5 mM dithiothreitol, and 30 μg BSA/ml) with 0.2 μg pBR322 DNA. Compounds were included in the reactions at a constant solvent volume. Reactions were initiated by addition of 1U human topoisomerase IIα, and incubated for 30 min at 37 °C. Reaction was terminated with 5×stop buffer (5 μl per 20 μl reaction volume). Stop buffer contained 5% sarkosyl, 0.0025% bromophenol blue and 25% glycerol. Reaction products were analyzed on a 1% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/ml). DNA bands were visualized through trans-illumination with UV light and then photographed.

5.4. Topoisomerase I inhibition assay

The activity of DNA topoisomerase I (TaKaRa, Kyoto, Japan) was determined by measuring the relaxation of supercoiled DNA pBR322 using camptothecin as a positive control. The reaction mixture was prepared according to the provided protocol, and incubated at 37 °C for 30 min. The reactions were terminated by the addition of dye solution containing 1% SDS, 0.02% bromophenol blue and 50% glycerol. The mixtures were applied to 1% agarose gel and subjected to electrophoresis for 1 h, in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/ml). DNA bands were visualized by transillumination with UV light and then photographed.

5.5. Telomerase inhibitory activity

Telomerase activity was determined using the Telo TAGGG Telomerase PCR ELISA kit (Roche, Mannheim, Germany), which is based on the Telomeric Repeat Amplification Protocol assay with a nonradioactive ELISA detection [19]. The procedure was performed for compound 7 at 20 μM using K562 human leukemia cells in accordance with the manufacturer's instructions.

5.6. Primase inhibition activity

DNA primase was isolated from Ehrlich ascites carcinoma (EAC) cells grown in cell culture and the assay was carried out for compound **7** at 100 μM according to the method described previously [20].

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References

[1] J.J. Champoux, DNA topoisomerases: structure, function, and mechanism, Annu. Rev. Biochem. 70 (2001) 369–413.
[2] Y. Pommier, E. Leo, H. Zhang, C. Marchand, DNA topoisomerases and their poisoning by anticancer and antibacterial drugs, Chem. Biol. 17 (2010) 421–433.
[3] N. Tanaka, M. Yasue, H. Imamura, Quinonoid pigments *Mansonia Altissima*, Tetrahedron Lett. 24 (1966) 2767–2773.
[4] C. Galeffi, E.M. Delle Monache, C.G. Casinovi, G.B. Marini-Bettolo, New quinone from the heartwood of *Mansonia altissima*, Tetrahedron Lett. 40 (1969) 3583–3584.
[5] Y.-G. Suh, S.N. Kim, D.-Y. Shin, S.-S. Hyun, D.-S. Lee, K.-H. Min, S.M. Han, F. Li, E.-C. Choi, S.-H. Choi, The structure–activity relationships of mansonone F, a potent anti-MRSA sesquiterpenoid quinone: SAR studies on the C6 and C9 analogs, Bioorg. Med. Chem. Lett. 16 (2006) 142–145.
[6] D.-Y. Shin, S.N. Kim, J.-H. Chae, S.-S. Hyun, S.-Y. Seo, Y.-S. Lee, K.-O. Lee, S.-H. Kim, Y.-S. Lee, J.M. Jeong, N.-S. Choi, Y.-G. Suh, Syntheses and anti-MRSA activities of the C3 analogs of mansonone F, a potent anti-bacterial sesquiterpenoid: insights into its structural requirements for anti-MRSA activity, Bioorg. Med. Chem. Lett. 14 (2004) 4519–4523.
[7] S.-L. Huang, Y. Luo, Z.-S. Huang, X.-Y. Wang, X.-Z. Bu, P.-Q. Liu, L. Ma, B.-F. Xie, Z.-C. Liu, Y.-M. Li, A.S.C. Chan, L.-Q. Gu, Synthesis and cytotoxicity of 9-substituted benzo[*de*]chromene-7,8-dione and 5-benzyl-9-substituted benzo[*de*]chromene-7,8-dione, Synth. Commun. 36 (2006) 2667–2684.

[8] D. Wang, M.Y. Xia, Z. Cui, S. Tashiro, S. Onodera, T. Ikejima, Cytotoxic effects of mansonone E and F isolated from *Ulmus pumila*, Biol. Pharm. Bull. 27 (2004) 1025–1030.
[9] Z. Liu, S.L. Huang, M.M. Li, Z.S. Huang, K.S. Lee, L.Q. Gu, Inhibition of thioredoxin reductase by mansonone F analogues: implications for anticancer activity, Chem. Biol. Interact. 177 (2009) 48–57.
[10] H.-Y. Han, Y.-B. Kim, J.-S. Kim, H.-J. Park, M.-J. Noh, Anti-cancer agent containing naphthoquinone compound. WO 01/51004, 2001, pp. 28.
[11] Y.-G. Suh, D.-Y. Shin, K.-H. Min, S.-S. Hyun, J.-K. Jung, S.-Y. Seo, Facile construction of the oxaphenalene skeleton by peri ring closure. formal synthesis mansonone F, Chem. Commun. 13 (2000) 1203–1204 (Cambridge).
[12] W.M. Best, D. Wege, Intramolecular Diels–Alder additions of benzynes to furans. Application to the total synthesis of biflorin, and the mansonones E, I, and F, Aust. J. Chem. 39 (1986) 647–666.
[13] H.P. Jian, X.Y. Wang, Z.S. Huang, S.L. Huang, L.Q. Gu, A facile synthesis of novel heteropolycyclic pyrazines and oxazoles and mechanistic studies of 2,3-dihydrobenzo [*de*]-chromene-7,8-dione with amines, Heterocycles 75 (2008) 1773–1778.
[14] S.L. Huang, Y. Luo, Z.S. Huang, X.Z. Bu, P.Q. Liu, L. Ma, Y.M. Li, A.S.C. Chan, L.Q. Gu, The mechanism on cyclization, debenzylation, and oxidation of 1-[1-(benzyloxy)-3-methyl- naphthalen-4-yloxy]propan-2-one, Chin. Chem. Lett. 17 (2006) 769–772.
[15] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[16] L.H. Meng, J.S. Zhang, J. Ding, Salvicine, a novel DNA topoisomerase II inhibitor, exerting its effects by trapping enzyme-DNA cleavage complexes, Biochem. Pharmacol. 62 (2001) 733–741.
[17] B. Frydman, L.J. Marton, J.S. Sun, K. Neder, D.T. Witiak, A.A. Liu, H.M. Wang, Y. Mao, H.Y. Wu, M.M. Sanders, L.F. Liu, Induction of DNA topoisomerase II-mediated DNA cleavage by beta-lapachone and related naphthoquinones, Cancer Res. 57 (1997) 620–627.
[18] C.J. Li, L. Averboukh, A.B. Pardee, beta-Lapachone, a novel DNA topoisomerase I inhibitor with a mode of action different from camptothecin, J. Biol. Chem. 268 (1993) 22463–22468.
[19] S. Giri, K. Nieber, A. Acikgöz, S. Pavlica, M. Keller, A. Bader, Telomerase activity and hepatic functions of rat embryonic liver progenitor cell in nanoscaffold-coated model bioreactor, Mol. Cell. Biochem. 336 (2009) 137–149.
[20] L.L. Wotring, L.B. Townsend, L.M. Jones, K.Z. Borysko, D.L. Gildersleevea, W.B. Parker, Dual mechanisms of inhibition of DNA synthesis by triciribine, Cancer Res. 50 (1990) 4891–4899.