European Journal of Medicinal Chemistry 45 (2010) 4221–4228

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and pharmacological evaluation of new methyloxiranylmethoxyxanthone analogues

Sangwook Woo [a,1], Da-hye Kang [b,1], Jung Min Nam [b], Chong Soon Lee [c], Eun-Mi Ha [a], Eung-Seok Lee [d], Youngjoo Kwon [b,**], Younghwa Na [a,*]

[a] College of Pharmacy, Catholic University of Daegu, Gyeongsan, Gyeongbuk 712-702, Republic of Korea
[b] College of Pharmacy, Ewha Womans University, Seoul 120-750, Republic of Korea
[c] Department of Biochemistry, College of Natural Sciences, Yeungnam University, Gyeongsan 712-749, Republic of Korea
[d] College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Republic of Korea

ARTICLE INFO

Article history:
Received 17 April 2010
Received in revised form
6 June 2010
Accepted 9 June 2010
Available online 17 June 2010

Keywords:
Anticancer activities
Topoisomerase II inhibition
DNA cross-linking
Oxiranylmethoxyxanthones
Methyloxiranylmethoxyxanthones

ABSTRACT

In order to develop potential anti-cancer agents that act on topoisomerase II and DNA, we have synthesized 12 new xanthone derivatives. In the cytotoxicity test, compounds **17** and **31** exhibited 2- to 7-fold stronger inhibitory activity than adriamycin against most cancer cell lines tested. Halohydrin group-tethered compounds **19**, **21** and **27** showed comparable topoisomerase II inhibitory activity to etoposide at 100 μM concentration. In the DNA cross-linking test, compounds **20**, **30** and **31** produced DNA cross-linked adducts and compound **30** was the strongest DNA cross-linker. Based on the combined pharmacological results, we suspected that the strong anti-cancer activity of compounds **16**, **17**, **20**, **30** and **31** originated from the DNA mono-alkylation or cross-linking properties of the compounds.

1. Introduction

Xanthones (**1**) are mainly identified and isolated as secondary metabolites from higher plants and microorganisms. They have diverse biological activities including anti-hypertensive, anti-oxidative, anti-thrombotic, and anti-cancer activities according to their diverse structures [1]. Because of the simple and interesting structural scaffolds and wide biological profiles of xanthones, researchers have endeavored to isolate or synthesize numerous derivatives of xanthone compounds as potential drug candidates. Among the xanthones reported to date, oxygenated xanthones synthesized or isolated from natural resources inhibit the proliferation of several cancer cell lines [2]. Especially, 2′,3′-epoxypropoxy-substituted xanthones efficiently prohibit the growth of cancer cells, and xanthone (**2**) possessing two 2′,3′-epoxypropoxy groups at the 3 and 5 positions show the most active anti-cancer activity in the series prepared [2a,b]. We previously reported that the 2′,3′-epoxypropoxyxanthones (**3**) and their ring-opened halohydrin (**4**) analogues showed significant cytotoxic and topoisomerase II relaxation inhibitory activities [3]. The 1,3-bis-oxiranylmethyloxy-substituted compound (**5**) was particularly efficient among the compounds tested. Compound **5** also showed strong DNA cross-linking activity. The findings suggested that the epoxide ring coupling and its ring-opened compounds might modulate the biological efficacy of xanthone compounds.

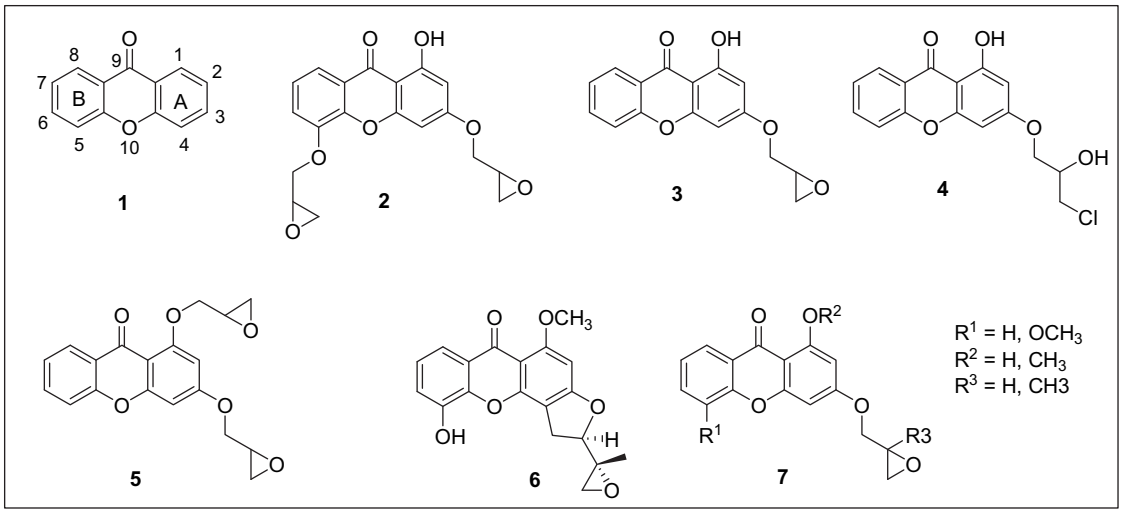
Topoisomerases are critical cellular enzymes necessary for cell proliferation that remove topological hurdles in the process of DNA replication [4]. Among them, topoisomerase II relaxes supercoiled DNA double helices by mediating the cleavage of double-stranded DNA and the process of religation. Because of the importance of these enzymes in the cell proliferative process, topoisomerases have been one of the major targets in anti-cancer drug development.

Interstrand DNA cross-linking of two strands of DNA inhibits the cascade of cell proliferation, such as replication and transcription, and finally causes cell death [5,6]. Some anti-tumor compounds that possess two electrophilic functions, such as mitomycin C and nitrogen mustard, can act as DNA cross-linking agents, which are an important class of anti-tumor drugs targeting DNA [7,8].

Psorospermin (**6**) isolated from an African plant is a natural compound that shows good anti-cancer activity against human and murine cancer cell lines [9]. In its structure, **6** possesses

* Corresponding authors. Tel.: +82 53 850 3616; fax: +82 53 850 3602.
** Corresponding authors. Tel.: +82 2 3277 4653; fax: +82 2 3277 3051.
E-mail addresses: ykwon@ewha.ac.kr (Y. Kwon), yna7315@cu.ac.kr (Y. Na).
[1] These authors equally contributed to this work.

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.06.017



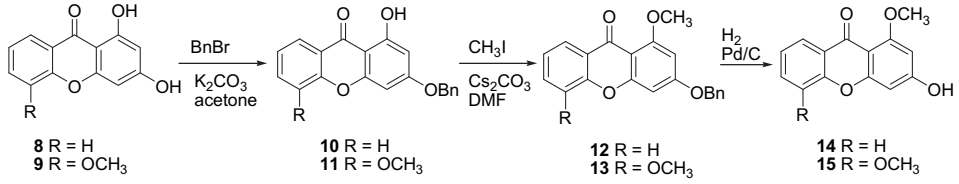
a 2′,3′-epoxypropoxy-xanthone skeleton. Its biological activities are known to be expressed via intercalation of the xanthone group into a DNA base pair and alkylation of the epoxide by N7-guanine in the presence of topoisomerase II [10].

In our continued effort to develop anti-cancer drug candidates that target topoisomerases and DNA, we designed a series of new xanthone analogues possessing methyloxyranyl-methoxy groups. For this purpose, we synthesized three different groups of xanthones (7) based on the addition of 1) a methoxyl group at the C5-position on the B ring, 2) a methoxyl group at the C1-position instead of a hydroxyl group on the A ring, and 3) a [2-(methyl) oxiranyl]methoxy group at the C3-position in the xanthone core. We suspected that the introduction of methoxyl groups in the xanthone core would affect their mode of interaction such as intercalation and/or binding to DNA and enzymes. On the other hand, the addition of a methyl group into the oxiranyl part would change the DNA alkylation pattern compared to the parent compound. Here, we report the synthesis of new xanthone analogues and their pharmacological activities, including cytotoxicity, topoisomerase II inhibition and DNA cross-linking.

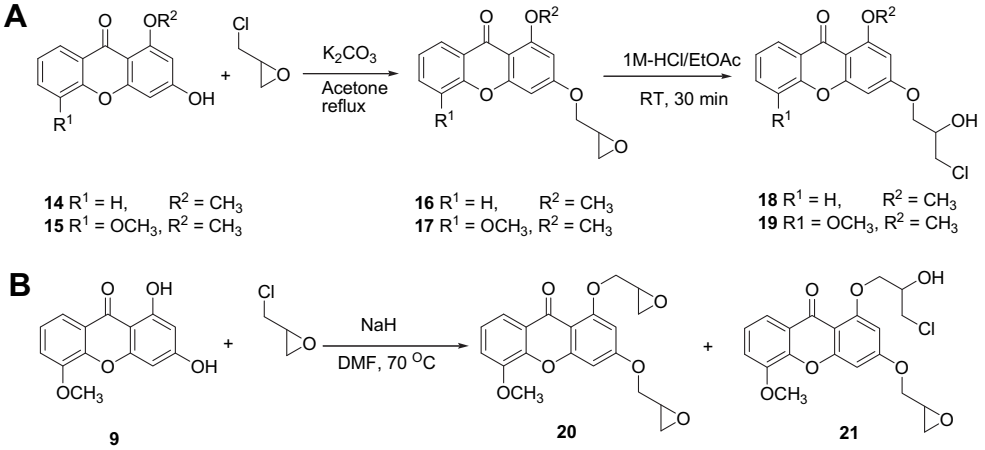
2. Chemistry

2.1. Synthesis of 3-(oxiran-2-ylmethoxy)-9H-xanthen-9-one analogues

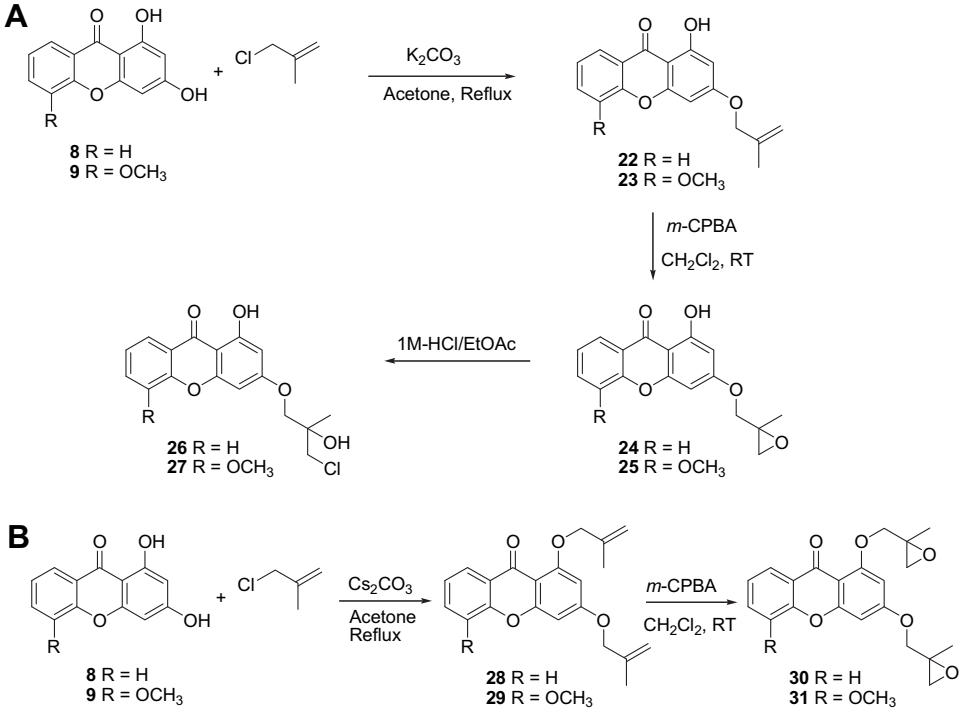
First, 3-hydroxy-1-methoxyxanthone (**14**) and 3-hydroxy-1,5-dimethoxyxanthone (**15**) were used as starting compounds for preparation of 1-methoxy-3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one analogues and were obtained from the corresponding 1,3-dihydroxyxanthone (**8**) [3] and 1,3-dihydroxy-5-methoxyxanthone (**9**). The 3-hydroxyl groups of compounds **8** and **9** were protected



Scheme 1. Synthesis of 3-Hydroxy-1-methoxyxanthone (**14**) and 3-Hydroxy-1,5-dimethoxyxanthone (**15**).



Scheme 2. Synthesis of 3-(oxiran-2-ylmethoxy)-9*H*-xanthen-9-one analogues.



Scheme 3. Synthesis of 3-[(2-methyloxiran-2-yl)methoxy]-9*H*-xanthen-9-one analogues.

with benzyl bromide, and subsequent methylation with methyl iodide under Cs_2CO_3 basic conditions gave compounds **12** and **13**. Final deprotection of the benzyl group was accomplished under hydrogenolysis, and the compounds obtained were applied to the next step without purification (Scheme 1).

The coupling reaction of the prepared xanthones and epichlorohydrin (4–5 equiv.) was conducted in basic K_2CO_3 (2–2.5 equiv.) in acetone. In the ^{1}H NMR spectrum, compound **16** showed two doublet of doublet peaks at δ 2.81 and 2.97 and one multiplet at δ 3.36–3.39 corresponding to the two methylene protons and the methine proton of the epoxide ring. We confirmed the correlation of the proton peaks by gCOSY experiment. In the ^{13}C NMR spectrum, we observed three signals at 69.4 (C-1′), 50.0 (C-2′), and 44.8 (C-3′) ppm. Based on the observation of the molecular ion peak (*m*/*e* 299.1 $[M+1]^+$) in the mass spectrum, we identified the structure of compound **16**. Other compounds also showed similar spectroscopic patterns (Scheme 2A).

Conversion of epoxide ring to halohydrin was accomplished in 1 M HCl aqueous ethyl acetate. For example, in the ^{1}H NMR spectrum, the C-2′ methine proton of compound **16** near δ 3.40 was down-field shifted to around δ 4.20 due to the halohydrin formation in compound **18**. C-3′ methylene also down-field shifted from δ 2.81 and 2.97 to 3.76–3.8. In the ^{13}C NMR spectrum, we observed a down-field shift of C-2′ signal from 50.0 to 69.1 ppm by halohydrin formation. The molecular ion peak (*m*/*e* 335.2 $[M+1]^+$) in the mass spectrum confirmed the identity of compound **18**. Other halohydrin compounds showed similar spectroscopic patterns (Scheme 2A).

Bis-epoxy compound **20** was prepared with 11 equiv. of epichlorohydrin in DMF under basic NaH (3 equiv.) conditions. In the ^{1}H and ^{13}C NMR spectra, two sets of peaks derived from two oxiranylmethoxy groups at the C1 and C3 positions were observed. All spectral data (^{1}H and ^{13}C NMR, COSY, and Mass spectra) were consistent with the proposed structures. Compound **21** was isolated from the same reaction. In the ^{1}H NMR spectrum, two methine protons of C-2′ and C-2″ appeared at δ 3.33–3.37 and 4.12–4.18; one of them was down-field shifted by hydroxyl group introduction at C2″. Two correlation sets for protons of halohydrin and oxyranylmethoxy groups were observed in the gCOSY experiment. In the ^{13}C NMR spectrum, we observed a signal at 71.6 ppm corresponding to C2″, but peak separation between diastereomers was not recorded. The molecular ion peak (*m*/*e* 407.1 $[M+1]^+$) supported the formation of one halohydrin (Scheme 2B).

2.2. Synthesis of 3-[(2-methyloxiran-2-yl)methoxy]-9H-xanthen-9-one analogues

In order to prepare mono 3-[(2-methyloxiran-2-yl)methoxy]-9*H*-xanthen-9-one analogues, we tried a two-step method. First, compounds **22** and **23** were synthesized with 3-chloro-2-methylpropene (1.1 equiv.) and **8** or **9** under basic K_2CO_3 (3.5 equiv.) acetone. In the ^{1}H NMR spectrum, the appearance of a singlet peak at δ 12.88, corresponding to C1–OH, supported one methylpropene

Table 1
Cytotoxicity of prepared compounds.

Compounds	Cell lines/IC_{50}[a] (μM)				
	MDA-MB231	MCF-7	HCT-116	DU-145	HeLa
Adriamycin	4.4 ± 0.2	18.9 ± 0.8	4.0 ± 0.8	1.4 ± 0.2	10.1 ± 0.8
Etoposide	16.3 ± 1.8	17.5 ± 1.2	11.9 ± 2.1	13.8 ± 0.8	18.2 ± 1.2
Camptothecin	4.2 ± 0.3	14.2 ± 2.1	2.3 ± 0.2	3.2 ± 0.2	1.1 ± 0.6
16	10.9 ± 0.4	14.0 ± 0.8	6.6 ± 2.3	1.5 ± 0.3	10.5 ± 0.3
17	3.8 ± 1.4	8.0 ± 0.2	1.5 ± 0.1	0.8 ± 0.1	6.2 ± 0.3
18	>100	>100	21.6 ± 3.4	40.1 ± 2.3	>100
19	56.0 ± 2.1	51.7 ± 5.0	16.4 ± 0.4	15.3 ± 1.1	59.5 ± 3.8
20	5.4 ± 0.7	9.9 ± 0.6	2.0 ± 0.4	2.4 ± 0.6	4.7 ± 0.7
21	18.9 ± 2.4	69.0 ± 1.9	9.9 ± 0.7	3.9 ± 0.5	41.7 ± 2.0
24	41.7 ± 2.0	15.0 ± 0.8	12.1 ± 1.3	18.9 ± 1.5	14.3 ± 0.3
25	>100	>100	69.1 ± 3.3	>100	>100
26	>100	>100	47.1 ± 4.3	>100	>100
27	15.4 ± 1.2	88.4 ± 5.4	15.8 ± 1.0	7.2 ± 0.6	68.2 ± 5.4
30	68.2 ± 5.4	15.2 ± 1.7	2.6 ± 0.9	3.9 ± 0.4	15.8 ± 2.3
31	5.1 ± 0.1	2.5 ± 0.4	0.8 ± 0.0	0.9 ± 0.1	1.5 ± 0.5

[a] Each data point represents mean ± standard deviation, from three different experiments performed in triplicate. Cell lines used are described in the text.

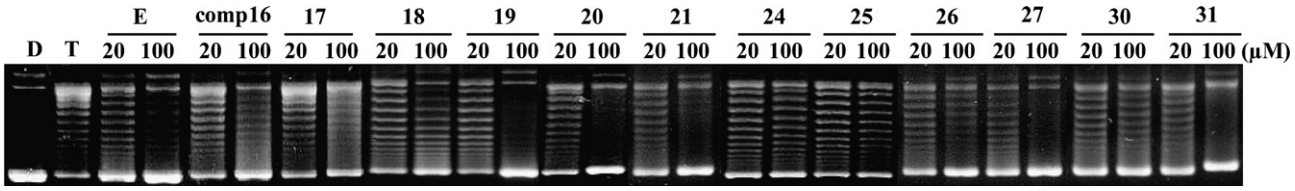


Fig. 1. Topoisomerase IIα inhibitory activities of compounds. Compounds were examined at a final concentration of 20 and 100 μM as indicated. Lane D: pBR322 only, Lane T: pBR322 + Topoisomerase IIα, Lane E: pBR322 + Topoisomerase IIα + etoposide at indicated concentrations, Lanes with compounds, pBR322 + Topoisomerase IIα + compounds at indicated concentrations.

group substitution on C3—OH in compound **22**. Subsequent epoxidation with *m*CPBA afforded desired compounds **24** and **25** (Scheme 3A). In the ^{1}H NMR spectrum of compound **24**, two singlet sp^2 protons at δ 5.07 and 5.10 of compound **22** were up-field shifted to δ 2.71 and 2.82 as two doublet peaks indicating complete epoxidation. In the ^{13}C NMR spectrum, two epoxide ring carbons were detected at 52.1 and 55.4 ppm, respectively.

1,3-Bis[(2-methyloxiran-2-yl)methoxy]-9*H*-xanthen-9-one analogues were also synthesized by the same method as the mono analogues. But, Cs_2CO_3 (4 equiv.) was used as a base instead of K_2CO_3, and 10 equiv. of 3-chloro-2-methylpropene were used. Compounds **28** and **29** did not show C1—OH proton in the ^{1}H NMR spectrum, which supported introduction of two methylpropene group on C1— and C3—OH. Subsequent epoxidation with *m*CPBA afforded desired compounds **30** and **31** (Scheme 3B). All spectral data were consistent with the desired structures.

3. Results and discussion

Synthetic procedures are quite straight-forward and we have prepared 12 new xanthone derivatives. For the pharmacological evaluation as potential anti-cancer agents, we have tested cytotoxicty, topoisomerase II inhibitory activity and DNA cross-linking ability of prepared compounds.

For the evaluation of cytotoxicity, five different cancer cell lines were used: human breast adenocarcinoma cell line (MDA-MB231), human cervix tumor cell line (HeLa), human prostate tumor cell line (DU-145), human colorectal carcinoma cell line (HCT-116), and human breast cancer cell line (MCF-7). The inhibitory activities of the compounds are shown in Table 1. Among these, compounds **16**, **17**, **20**, **30**, and **31** showed excellent suppression of the cancer cell line tested. In particular, compounds **17** and **31** exhibited at least 2- to 7-fold stronger inhibitory activity than Adriamycin (used as a reference), except in the MDA-MB231 cell line. From a structural viewpoint, all five compounds possessing one or two epoxide rings at C1 and C3 position on the xanthone showed strong cytotoxicity in the cancer cell lines tested, which indicates that epoxide rings play an important role in the cell proliferation inhibition. Compounds **24** and **25**, however, which possessed one (2-methyloxiran-2-yl)methoxy group at the C3 position, showed lower activity than **16** and **17**. Compound **25** lost activity almost completely.

Inhibition of topoisomerase relaxation was evaluated using human topoisomerase II (Topogen) with etoposide as a positive control. Data were analyzed and evaluated with LabWork 4.5 Software to calculate the inhibition ratio. Test results are indicated in Fig. 1 and Table 2. Compounds **19**, **21** and **27** showed comparable inhibitory activity to etoposide at 100 μM concentration. Interestingly, all of these compounds hold a halohydrin group at the C3 position and a methoxy group at the C5 position. In contrast, compounds possessing one epoxide ring at the C3 position were not good topoisomerase II inhibitors. However, the bis-epoxy substituted compounds, **30** and **31**, showed moderate inhibitory activity at 100 μM concentration.

Because compound **5** previously showed excellent DNA cross-linking activity, compounds possessing an epoxide ring were tested for DNA cross-linking property. In the test, compounds **20**, **30** and **31** produced DNA cross-linked adducts (Fig. 2). In further experiments with various concentrations, the compounds generated DNA cross-linked adducts in concentration-dependent way (Fig. 3). Compound **30**, which lacks the C5-methoxy group, was the strongest DNA cross-

Table 2
The % inhibition of prepared compounds against topoisomerase IIα.

Compds/concentration	20 μM	100 μM
Etoposide	40	71
16	5	51
17	0	4
18	1	33
19	2	83
20	9	71
21	27	65
24	11	25
25	3	23
26	20	52
27	41	85
30	24	41
31	15	48

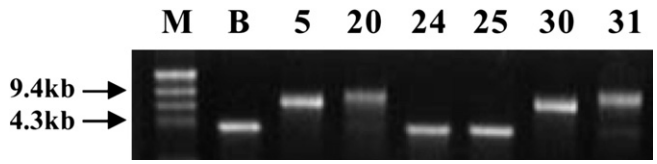


Fig. 2. DNA cross-linking activities of prepared compounds were tested at a concentration of 100 μM. M: λ DNA/Hind III Marker (Promega) B; only DNA.

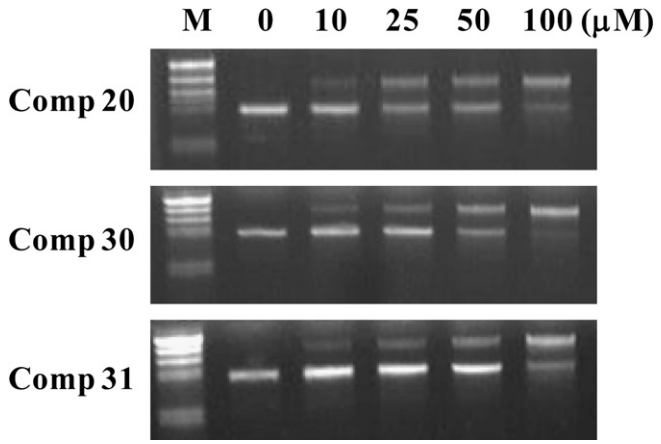


Fig. 3. DNA cross-linking activities of compounds **20**, **30** and **31** at various concentrations. M: λ Hind III DNA Marker (Promega). Lanes 2–6: 0, 10, 25, 50, 100 μM concentrations of compounds.

linker among the compounds tested. Based on our results, the C5-methoxy group and the methyl group in the epoxide ring (**20** *vs*. **31**) reduced the DNA cross-linking ability of the xanthone compounds. The findings suggest that the C5-methoxy group hinders the intercalation of the planar xanthone ring into the DNA base pair and finally positions the compound unfavorably for electrophilic interaction with DNA. The methyl group in the epoxide ring might also sterically affect the position of the epoxide ring or the hydrophobic interaction during compound-DNA interaction.

4. Conclusions

We have synthesized 12 new xanthone derivatives. In the cytotoxicity test, compounds **16**, **17**, **20**, **30**, and **31** showed excellent anti-cancer activities. In particular, compounds **17** and **31** exhibited 2- to 7-fold stronger inhibitory activity than Adriamycin against most cancer cell lines tested. Compounds **19**, **21** and **27**, which possess a halohydrin group at the C3 position and a methoxy group at the C5 position, showed comparable topoisomerase II inhibitory activities to etoposide at 100 μM concentration. The test results revealed that epoxide ring-tethered compounds have strong cytotoxic activity and halohydrin compounds were efficient topoisomerase II inhibitors. In the DNA cross-linking test, compounds **20**, **30** and **31** yielded DNA cross-linked adduct and compound **30** was the strongest DNA cross-linker. These observations suggest that DNA cross-linking occurs after intercalation of the tricyclic xanthone ring into the DNA base pairs, and the substituents on the xanthone control the intercalation and DNA alkylation properties of the xanthone analogues. Although the correlation between DNA cross-linking and topoisomerase II inhibitory activities is not clear, the topoisomerase II inhibition might occur through formation of compound–enzyme binary complex or compound–enzyme–DNA ternary complex by the Vander–Waals interaction and/or hydrogen bond, not by DNA alkylation. However, the DNA alkylation can inhibit topoisomerase II function indirectly and the cross-linked double helix DNA can be hardly relaxed by topoisomerase II. From the combined pharmacological results, we suspect that the strong anti-cancer activity of compounds **16**, **17**, **20**, **30** and **31** originated from the DNA mono-alkylation or cross-linking properties of the compounds. In summary, with elaborate calibration, oxiranylmethoxy- or halohydrin-tethered xanthone derivatives show potential for development as new anti-cancer agents.

5. Experimental

5.1. General

The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. TLC plates were Kieselgel 60 F_{254} (Art A715, Merck) and silica gel for column chromatography was Silica gel 60 (0.040–0.063 mm ASTM, Merck). ^{1}H and ^{13}C NMR spectra were taken on 250 MHz (Bruker) or 400 MHz (Varian NMR AS) instruments. Chemical shifts (δ) are in parts per million (ppm) relative to tetramethylsilane as an internal standard, and coupling constants (J values) are in hertz. Mass spectral investigations were run on a LCQ advantage-trap mass spectrometer equipped with an electrospray ionization (ESI) source (Thermo Finnigan, San Jose, CA, USA). Melting points were measured on a Barnstead International MEL-TEMP® 1202D instrument without correction.

5.2. 3-Benzyloxy-1-hydroxy-9H-xanthen-9-one (*10*)

Compound **8** (1.77 g, 7.75 mmol), benzyl bromide (1.38 mL, 11.63 mmol), K_2CO_3 (2.76 g, 0.02 mol), and anhydrous acetone (200 mL) were added to a dry round-bottom flask. The reaction mixture was refluxed overnight and cooled to room temperature (RT). After addition of H_2O (200 mL), the reaction mixture was acidified with 3 M HCl. The mixture was extracted with ethyl acetate (2 times) and the combined organic layer was washed with H_2O and brine and then dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluant: CH_2Cl_2) to give compound **10** (1.05 g, 42.6%) as a pale yellow solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.14 (s, 2H), 6.42 (d, $J = 2.3$ Hz, 1H), 6.49 (d, $J = 2.3$ Hz, 1H), 7.24–7.44 (m, 7H), 7.70 (ddd, $J = 1.6$, 7.2, 8.3 Hz, 1H), 8.23 (dd, $J = 1.6$, 7.9 Hz, 1H), 12.85 (s, 1H).

5.3. 3-Benzyloxy-1-hydroxy-5-methoxy-9H-xanthen-9-one (*11*)

Compound **9** (2.0 g, 7.75 mmol), benzyl bromide (1.38 mL, 11.63 mmol), K_2CO_3 (2.76 g, 0.02 mol), and anhydrous acetone (200 mL) were added to a dry round-bottom flask. Reaction mixture was refluxed overnight and cooled to RT. After the addition of H_2O (200 mL), the reaction mixture was acidified with 3 M HCl. The mixture was extracted with ethyl acetate (2 times), and the combined organic layer was washed with H_2O and brine and then dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluant: CH_2Cl_2) to give compound **11** (1.29 g, 47.4%) as a pale yellow solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.05 (s, 3H), 5.18 (s, 2H), 6.46 (d, $J = 2.2$ Hz, 1H), 6.66 (d, $J = 2.2$ Hz, 1H), 7.23–7.48 (m, 7H), 7.84 (dd, $J = 1.8$, 7.8 Hz, 1H), 12.86 (s, 1H).

5.4. 3-Benzyloxy-1-methoxy-9H-xanthen-9-one (*12*)

A DMF (10 mL) solution of compound **10** (0.40 g, 1.26 mmol) and Cs_2CO_3 (0.82 g, 2.52 mmol) was added to methyl iodide (535.68 mg, 3.77 mmol). The mixture was stirred at 50 °C (3 h), transferred to an Erlenmeyer flask and then acidified with 2 M HCl in an ice bath. The mixture was extracted with CH_2Cl_2 and H_2O, and the combined organic layer was washed with H_2O and brine and then dried over Na_2SO_4. Solvent was evaporated under reduced pressure and sludge residue was triturated with ethanol. The solid formed was filtered, collected and dried to give compound **12** (0.41 g, 97.3%) as a white solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.95 (s, 3H), 5.15 (s, 2H), 6.42 (d, $J = 2.2$ Hz, 1H), 6.56 (d, $J = 2.2$ Hz, 1H), 7.24–7.47 (m, 7H), 7.61 (ddd, $J = 1.4$, 6.9, 7.0 Hz, 1H), 8.27 (dd, $J = 1.4$, 7.9 Hz, 1H).

5.5. 3-Benzyloxy-1,5-dimethoxy-9H-xanthen-9-one (*13*)

To a DMF (10 mL) solution of compound **11** (0.5 g, 1.45 mmol) and Cs_2CO_3 (0.94 g, 2.86 mmol) was added methyl iodide (0.62 g, 4.35 mmol). The mixture was stirred at 50 °C (3 h), transferred to an Erlenmeyer flask and then acidified with 2 M HCl in an ice bath. The mixture was extracted with CH_2Cl_2 and H_2O, and the combined organic layer was washed with H_2O and brine and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the sludge residue was triturated with ethanol. The solid formed was filtered, collected and dried to give compound **13** (0.29 g, 55.9%) as a white solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.00 (s, 3H), 3.95 (s, 3H), 5.13 (s, 2H), 6.42 (d, $J = 2.2$ Hz, 1H), 6.70 (d, $J = 2.2$ Hz, 1H), 7.12–7.26 (m, 2H), 7.35–7.45 (m, 5H), 7.85 (dd, $J = 1.6$, 7.9 Hz, 1H).

5.6. 3-Hydroxy-1-methoxy-9H-xanthen-9-one (*14*)

A bottle containing compound **12** (120 mg, 0.36 mmol), Pd/C (60 mg), and absolute ethanol (30 mL) was shaken under hydrogen (50 psi) for 12 h. The reaction mixture was filtered with celite and

washed with DMF (20 mL). The combined filtrate was evaporated under reduced pressure to remove the ethanol and the remaining solution was directly used for the next reaction.

5.7. 3-Hydroxy-1,5-dimethoxy-9H-xanthen-9-one (**15**)

A bottle containing compound **13** (100 mg, 0.28 mmol), Pd/C (50 mg), and absolute ethanol (30 mL) was shaken under hydrogen (50 psi) for 12 h. The reaction mixture was filtered with celite and washed with DMF (20 mL). The combined filtrate was evaporated under reduced pressure to remove ethanol and the remaining solution was directly used for the next reaction.

5.8. 1-Methoxy-3-(oxiran-2-ylmethoxy)-9H-xanthen-9-one (**16**)

A mixture of compound **14** and K_2CO_3 (99.8 mg, 0.72 mmol) in DMF (20 mL) was added to epichlorohydrin (0.11 mL, 1.44 mmol). The reaction mixture was stirred at 70 °C (15 h) and H_2O (20 mL) was added. After extraction of the mixture with ethyl acetate (2 times), the organic layer was washed with brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 3:1) to give compound **16** (28 mg, 26%) as an orange solid. Mp 122 °C; R_f 0.09 (ethyl acetate/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (dd, J = 2.6, 4.8 Hz, 1H), 2.97 (dd, J = 4.6, 4.8 Hz, 1H), 3.39–3.42 (m, 1H), 3.98 (s, 3H), 4.02 (dd, J = 5.6, 11.4 Hz, 1H), 4.38 (dd, J = 3.0, 11.4 Hz, 1H), 6.40 (d, J = 2.2 Hz, 1H), 6.50 (d, J = 2.2 Hz, 1H), 7.31–7.37 (m, 2H), 7.63 (ddd, J = 1.6, 7.2, 8.4 Hz, 1H), 8.28 (dd, J = 1.6, 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 44.8, 50.0, 56.6, 69.4, 93.7, 95.7, 107.8, 117.1, 123.3, 124.1, 127.0, 134.0, 155.1, 159.9, 162.3, 163.8, 175.7 ppm; LC-ESI: *m*/*e* 299.1 $[M+1]^+$.

5.9. 1,5-Dimethoxy-3-(oxiran-2-ylmethoxy)-9H-xanthen-9-one (**17**)

A mixture of compound **15** and K_2CO_3 (76.29 mg, 0.55 mmol) in DMF (20 mL) was added to epichlorohydrin (0.09 mL, 1.10 mmol). The reaction mixture was stirred at 70 °C (15 h) and H_2O (20 mL) was added. After extraction of the mixture with ethyl acetate (2 times), the organic layer was washed with brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 3:1) to give compound **17** (29 mg, 32%) as a white solid. Mp 186 °C; R_f 0.09 (ethyl acetate/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.79 (dd, J = 2.6, 4.6 Hz, 1H), 2.96 (dd, J = 4.6, 4.6 Hz, 1H), 3.40–3.42 (m, 1H), 3.98 (s, 3H), 4.01 (s, 3H), 4.02 (dd, J = 5.5, 11.2 Hz, 1H), 4.37 (dd, J = 2.8, 11.2 Hz, 1H), 6.42 (d, J = 2.0 Hz, 1H), 6.61 (d, J = 2.0 Hz, 1H), 7.16 (dd, J = 0.4, 7.6 Hz, 1H), 7.25 (dd, J = 7.6, 8.0 Hz, 1H), 7.86 (dd, J = 0.4, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 44.8, 49.9, 56.6, 56.6, 69.4, 93.7, 96.0, 107.8, 114.8, 118.0, 123.6, 124.2, 145.4, 148.1, 159.7, 162.2, 163.8, 175.5 ppm; LC-ESI: *m*/*e* 329.2 $[M+1]^+$.

5.10. 3-(3-Chloro-2-hydroxypropoxy)-1-methoxy-9H-xanthen-9-one (**18**)

A mixture of compound **16** (25 mg, 0.08 mmol) in 1 M HCl in aqueous ethyl acetate (3 mL) was stirred at RT (30 min). Solvent was removed under reduced pressure and the residue was dried under vacuum to give compound **18** (20 mg, 71.3%) as a brown solid. Mp 107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.76–3.85 (m, 2H), 3.99 (s, 3H), 4.20–4.22 (m, 2H), 4.26–4.34 (m, 1H), 6.38 (d, J = 2.4 Hz, 1H), 6.53 (d, J = 2.4 Hz, 1H), 7.30–7.35 (m, 2H), 7.63 (ddd, J = 1.6, 7.2, 8.3 Hz, 1H), 8.29 (dd, J = 1.6, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 46.1, 56.6, 69.1, 69.8, 93.7, 95.5, 108.0, 117.2, 123.3, 124.1, 127.0, 134.0, 155.2, 159.9, 162.4, 163.6, 175.6 ppm; LC-ESI: *m*/*e* 335.2 $[M+1]^+$.

5.11. 3-(3-Chloro-2-hydroxypropoxy)-1,5-dimethoxy-9H-xanthen-9-one (**19**)

A mixture of compound **17** (10 mg, 0.03 mmol) in 1 M HCl in aqueous ethyl acetate (3 mL) was stirred at RT (30 min). Solvent was removed under reduced pressure and residue was dried under vacuum to give compound **19** (10 mg, 90%) as a brown jelly. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75–3.82 (m, 2H), 3.99 (s, 3H), 4.01 (s, 3H), 4.20 (m, 2H), 4.28–4.31 (m, 1H), 6.37 (d, J = 2.0 Hz, 1H), 6.53 (d, J = 2.0 Hz, 1H), 7.17 (dd, J = 1.2, 7.6 Hz, 1H), 7.25 (dd, J = 7.6, 8.0 Hz, 1H), 7.86 (dd, J = 1.2, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 46.0, 69.3, 69.8, 56.6, 56.7, 93.8, 95.9, 107.9, 114.8, 118.0, 123.6, 124.2, 145.4, 148.2, 159.7, 162.2, 163.6, 175.5 ppm; LC-ESI: *m*/*e* 365.1 $[M+1]^+$.

5.12. 5-Methoxy-1,3-bis(oxiran-2-ylmethoxy)-9H-xanthen-9-one (**20**) and 1-(3-chloro-2-hydroxypropoxy)-5-methoxy-3-(oxiran-2-ylmethoxy)-9H-xanthen-9-one (**21**)

A mixture of compound **9** (100 mg, 0.39 mmol) and NaH (27.9 mg, 1.16 mmol) in DMF (15 mL) was added to epichlorohydrin (0.3 mL, 4.09 mmol) under nitrogen. The reaction mixture was stirred at 70 °C (15 h) and then quenched by slow addition of H_2O. The mixture was repeatedly extracted with CH_2Cl_2, and the combined organic layer was washed with brine and dried over Na_2SO_4. Solvent was evaporated and the residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 2:1) to afford compound **20** (35 mg, 24.4%) as a brown jelly and compound 2**1** (19 mg, 12.1%) as a white solid. Compound **20**: R_f 0.19 (ethyl acetate/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.77 (dd, J = 2.6, 4.6 Hz, 1H), 2.93–2.97 (m, 2H), 3.16–3.18 (m, 1H), 3.37–3.41 (m, 1H), 3.45–3.49 (m, 1H), 3.97–4.01 (m, 1H), 4.00 (s, 3H), 4.17 (dd, J = 4.6, 11.0 Hz, 1H), 4.33–4.37 (m, 2H), 6.44 (d, J = 2.0 Hz, 1H), 6.63 (d, J = 2.0 Hz, 1H), 7.15 (dd, J = 1.2, 8.4 Hz, 1H), 7.24 (dd, J = 7.8, 8.4 Hz, 1H), 7.84 (dd, J = 1.4, 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 44.8, 45.3, 49.9, 50.3, 56.6, 69.2 (69.1), 69.5, 94.4, 97.51, 108.0, 114.8, 117.9, 123.6, 124.2, 145.4, 148.2, 159.6, 160.9, 163.6, 175.3 ppm; LC-ESI: *m*/*e* 371.1 $[M+1]^+$. Compound **21**: Mp 180 °C; R_f 0.09 (ethyl acetate/*n*-hexane = 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71–2.74 (m, 1H), 2.89 (dd, J = 4.4, 4.4 Hz, 1H), 3.33–3.37 (m, 1H), 3.72–3.74 (m, 1H), 3.92–3.97 (m, 1H), 3.95 (s, 3H), 4.12–4.18 (m, 1H), 4.20 (dd, J = 3.2, 8.8 Hz, 1H), 4.29 (dd, J = 3.2, 8.8 Hz, 1H), 4.31 (dd, J = 2.8, 11.2 Hz, 1H), 6.41 (d, J = 2.0 Hz, 1H), 6.62 (d, J = 2.0 Hz, 1H), 7.12 (dd, J = 1.4, 8.0 Hz, 1H), 7.20 (dd, J = 8.0, 8.0 Hz, 1H), 7.76 (dd, J = 1.2, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 44.5, 44.7, 49.9, 56.6, 69.8, 69.6 (69.5), 71.6, 95.2 (95.1), 99.5 (99.4), 108.3, 115.1, 117.9, 123.8, 123.9, 145.6, 148.3, 159.4, 161.3, 164.0, 176.2 ppm; LC-ESI: *m*/*e* 407.1 $[M+1]^+$.

5.13. 1-Hydroxy-3-(2-methylallyloxy)-9H-xanthen-9-one (**22**)

A mixture of compound **8** (0.75 g, 3.29 mmol) and K_2CO_3 (1.63 g, 11.8 mmol) in anhydrous acetone (40 mL) was added to 3-chloro-2-methylpropene (0.36 mL, 3.72 mmol) in acetone (10 mL). The reaction mixture was refluxed overnight and the solvent was removed under reduced pressure. H_2O (50 mL) was added and acidified with 1 M HCl, and then the mixture was extracted with CH_2Cl_2 (2 times). The combined organic layer was washed with brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 1:1) to afford compound **22** (0.62 g, 66.8%) as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.87 (s, 3H), 4.55 (s, 2H), 5.07 (s, 1H), 5.14 (s, 1H), 6.40 (d, J = 2.0 Hz, 1H), 6.48 (d, J = 2.0 Hz, 1H), 7.36–7.47 (m, 2H), 7.74 (ddd, J = 1.4, 7.0, 8.4 Hz, 1H), 8.27 (dd, J = 1.4, 8.0 Hz, 1H), 12.88 (s, 1H).

5.14. 1-Hydroxy-5-methoxy-3-(2-methylallyloxy)-9H-xanthen-9-one (**23**)

A mixture of compound **9** (848.8 mg, 3.29 mmol) and K_2CO_3 (1.63 g, 11.8 mmol) in anhydrous acetone (50 mL) was added 3-chloro-2-methylpropene (0.36 mL, 3.72 mmol) in acetone (10 mL). The reaction mixture was refluxed overnight and solvent was removed under reduced pressure. H_2O (50 mL) was added and acidified with 1 M HCl and then the mixture was extracted with CH_2Cl_2 (2 times). The combined organic layer was washed with brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 1:1) to afford compound **23** (0.61 g, 59.6%) as a pale yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.86 (s, 3H), 4.05 (s, 3H), 4.54 (s, 2H), 5.05 (s, 1H), 5.13 (s, 1H), 6.40 (d, J = 2.4 Hz, 1H), 6.59 (d, J = 2.4 Hz, 1H), 7.26–7.36 (m, 2H), 7.84 (dd, J = 2.2, 7.6 Hz, 1H), 12.84 (s, 1H).

5.15. 1-Hydroxy-3-[(2-methyloxiran-2-yl)methoxy]-9H-xanthen-9-one (**24**)

A CH_2Cl_2 (10 mL) solution of compound **22** (30 mg, 0.11 mmol) was added to *m*CPBA (55 mg, 0.32 mmol). The mixture was stirred at RT (6 h) and *m*CPBA (60 mg, 0.35 mmol) was added one more time. After stirring for 3 h, solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 1:1) to afford compound **24** (12 mg, 38.0%) as a pale yellow solid. Mp 124 °C; R_f 0.71 (ethyl acetate/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 3H), 2.71 (d, J = 4.6 Hz, 1H), 2.82 (d, J = 4.6 Hz, 1H), 3.95 (d, J = 10.8 Hz, 1H), 4.09 (d, J = 10.8 Hz, 1H), 6.30 (d, J = 2.0 Hz, 1H), 6.41 (d, J = 2.0 Hz, 1H), 7.32 (dd, J = 7.5, 7.8 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.65 (ddd, J = 1.5, 7.8, 8.4 Hz, 1H), 8.19 (dd, J = 1.5, 7.5 Hz, 1H), 12.79 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 18.6, 52.1, 55.4, 72.1, 93.6, 97.8, 104.4, 117.8, 120.8, 124.3, 126.1, 135.3, 156.2, 157.9, 163.3, 165.8, 181.1 ppm; LC-ESI: *m*/*e* 299.2 $[M+1]^+$.

5.16. 1-Hydroxy-5-methoxy-3-[(2-methyloxiran-2-yl)methoxy]-9H-xanthen-9-one (**25**)

A CH_2Cl_2 (15 mL) solution of compound **23** (70 mg, 0.22 mmol) was added to *m*CPBA (120 mg, 0.70 mmol). The mixture was stirred at RT (6 h) and *m*CPBA (60 mg, 0.35 mmol) was added one more time. After stirring for 3 h, solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 1:1) to afford compound **25** (32 mg, 43.5%) as a yellow jelly. R_f 0.65 (ethyl acetate/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 3H), 2.79 (d, J = 4.6 Hz, 1H), 2.91 (d, J = 4.6 Hz, 1H), 4.02 (d, J = 10.8 Hz, 1H), 4.05 (s, 3H), 4.16 (d, J = 10.8 Hz, 1H), 6.40 (d, J = 2.1 Hz, 1H), 6.60 (d, J = 2.1 Hz, 1H), 7.16–7.26 (m, 2H), 7.84 (dd, J = 7.6, 1.8 Hz, 1H), 12.84 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) 18.6, 52.1, 55.4, 56.7, 72.1, 93.6, 98.3, 104.4, 116.0, 117.0, 121.9, 123.9, 147.6, 148.5, 157.7, 163.3, 165.8, 181.1 ppm; LC-ESI: *m*/*e* 329.2 $[M+1]^+$.

5.17. 3-(3-Chloro-2-hydroxy-2-methylpropoxy)-1-hydroxy-9H-xanthen-9-one (**26**)

A mixture of compound **24** (5 mg, 0.02 mmol) in 1 M HCl in aqueous ethyl acetate (3 mL) was stirred at RT (30 min). Solvent was removed under reduced pressure and the residue was dried under vacuum to give compound **26** (5 mg, 89.1%) as a pale yellow solid. Mp 142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 3H), 2.48 (s, 1H), 3.71 (d, J = 2.8 Hz, 2H), 4.01–4.08 (m, 2H), 6.38 (d, J = 2.2 Hz, 1H), 6.47 (d, J = 2.2 Hz, 1H), 7.39 (ddd, J = 0.8, 7.1, 8.0 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.73 (ddd, J = 1.6, 7.1, 8.4 Hz, 1H), 8.26 (dd, J = 1.6, 8.0 Hz, 1H), 12.86 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 22.5, 50.3, 71.9, 72.0, 93.5, 97.8, 104.6, 117.8, 120.8, 124.1, 126.1, 135.4, 156.2, 157.9, 163.8, 165.4, 181.1 ppm; LC-ESI: *m*/*e* 335.2 $[M+1]^+$.

5.18. 3-(3-Chloro-2-hydroxy-2-methylpropoxy)-1-hydroxy-5-methoxy-9H-xanthen-9-one (**27**)

A mixture of compound **25** (20 mg, 0.06 mmol) in 1 M HCl in aqueous ethyl acetate (3 mL) was stirred at RT (30 min). Solvent was removed under reduced pressure and the residue was dried under vacuum to give compound **27** (19 mg, 85.5%) as a pale yellow solid. Mp 142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 3H), 2.48 (s, 1H), 3.70 (d, J = 1.6 Hz, 2H), 4.00–4.07 (m, 2H), 4.03 (s, 3H), 6.38 (d, J = 1.8 Hz, 1H), 6.59 (d, J = 1.8 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.31 (dd, J = 7.2, 8.0 Hz, 1H), 7.82 (d, J = 7.2 Hz, 1H), 12.81 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 22.5, 50.3, 56.7, 71.9, 72.1, 93.4, 98.4, 104.6, 116.0, 117.0, 121.7, 123.9, 146.5, 148.5, 157.8, 163.6, 165.4, 181.1 ppm; LC-ESI: *m*/*e* 365.2 $[M+1]^+$.

5.19. 1,3-Bis(2-methylallyloxy)-9H-xanthen-9-one (**28**)

A mixture of compound **8** (532 mg, 2.33 mmol) and Cs_2CO_3 (3.04 g, 9.32 mmol) in anhydrous acetone (30 mL) was added to 3-chloro-2-methylpropene (2.11 g, 23.3 mmol). The solid was removed through filtration and solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluant: CH_2Cl_2) to afford compound **28** (285 mg, 36.4%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86 (s, 3H), 1.93 (s, 3H), 4.52 (s, 2H), 4.56 (s, 2H), 5.06 (s, 1H), 5.08 (s, 1H), 5.14 (s, 1H), 5.43 (s, 1H), 6.37 (d, J = 2.4 Hz, 1H), 6.49 (d, J = 2.4 Hz, 1H), 7.26–7.36 (m, 2H), 7.60 (ddd, J = 1.6, 6.8, 7.0 Hz, 1H), 8.29 (dd, J = 1.6, 8.0 Hz, 1H).

5.20. 5-Methoxy-1,3-bis(2-methylallyloxy)-9H-xanthen-9-one (**29**)

A mixture of compound **9** (458 mg, 1.77 mmol) and Cs_2CO_3 (2.31 g, 7.09 mmol) in anhydrous acetone (25 mL) was added to 3-chloro-2-methylpropene (1.6 g, 17.74 mmol). The solid was removed through filtration and solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluant: CH_2Cl_2) to afford compound **29** (176 mg, 27.1%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86 (s, 3H), 1.93 (s, 3H), 4.01 (s, 3H), 4.51 (s, 2H), 4.56 (s, 2H), 5.04 (s, 1H), 5.08 (s, 1H), 5.13 (s, 1H), 5.42 (s, 1H), 6.39 (d, J = 2.2 Hz, 1H), 6.61 (d, J = 2.2 Hz, 1H), 7.16 (dd, J = 1.4, 7.8 Hz, 1H), 7.60 (dd, J = 7.8, 8.0 Hz, 1H), 8.29 (dd, J = 1.6, 8.0 Hz, 1H).

5.21. 1,3-Bis[(2-methyloxiran-2-yl)methoxy]-9H-xanthen-9-one (**30**)

A CH_2Cl_2 (15 mL) solution of compound **28** (200 mg, 0.60 mmol) was added to *m*CPBA (537 mg, 2.97 mmol). The mixture was stirred at RT (6 h) and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 2:1) to afford compound **30** (78 mg, 38.6%) as a yellow jelly. R_f 0.29 (ethyl acetate/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 3H), 1.61 (s, 3H), 2.78 (d, J = 4.6 Hz, 1H), 2.82 (d, J = 4.6 Hz, 1H), 2.91 (d, J = 4.6 Hz, 1H), 3.28 (d, J = 4.6 Hz, 1H), 4.01 (dd, J = 2.4, 10.4 Hz, 1H), 4.10–4.19 (m, 3H), 6.41 (d, J = 2.2 Hz, 1H), 6.52 (d, J = 2.2 Hz, 1H), 7.32 (ddd, J = 1.0, 7.2, 8.0 Hz, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.63 (ddd, J = 1.6, 7.2, 8.3 Hz, 1H), 8.28 (dd, J = 1.6, 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 18.6, 18.9, 52.1, 52.5, 55.4, 55.8, 72.0, 72.2(72.1), 94.4, 96.8, 108.3, 117.2, 123.3,

124.1, 126.9, 134.0, 155.2, 159.8, 161.0, 163.8, 175.4 ppm; LC-ESI: *m*/*e* 369.2[M+1]$^+$.

5.22. 5-Methoxy-1,3-bis[(2-methyloxiran-2-yl)methoxy]-9H-xanthen-9-one (**31**)

A CH_2Cl_2 (15 mL) solution of compound **29** (120 mg, 0.33 mmol) was added to *m*CPBA (283 mg, 1.64 mmol). The mixture was stirred at RT (6 h) and solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (eluant: ethyl acetate/*n*-hexane = 2:1) to afford compound **31** (42 mg, 32.1%) as a pale yellow jelly. R_f 0.22 (ethyl acetate/*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 3H), 1.61 (s, 3H), 2.78 (d, J = 4.6 Hz, 1H), 2.82 (d, J = 4.8 Hz, 1H), 2.90 (d, J = 4.6 Hz, 1H), 3.29 (d, J = 4.8 Hz, 1H), 3.99–4.01 (m, 1H), 4.01 (s, 3H), 4.10–4.18 (m, 3H), 6.41 (d, J = 2.4 Hz, 1H), 6.63 (d, J = 2.4 Hz, 1H), 7.17 (dd, J = 1.4, 7.8 Hz, 1H), 7.25 (dd, J = 7.8, 7.8 Hz, 1H), 7.86 (dd, J = 1.4, 7.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 18.6, 18.9, 52.0, 52.6, 55.4, 55.8, 56.6, 71.8(71.9), 72.1(72.0), 94.3, 97.1, 107.9, 114.7, 117.9, 123.6, 124.2, 145.4, 148.2, 159.6, 160.8, 163.8, 175.3 ppm; LC-ESI: *m*/*e* 399.1 [M+1]$^+$.

5.23. Cytotoxicity test [3]

For the evaluation of cytotoxicity, five different cancer cell lines were used: human breast adenocarcinoma cell line (MDA-MB231), human cervix tumor cell line (HeLa), human prostate tumor cell line (DU-145), human colorectal carcinoma cell line (HCT-116), and human breast cancer cell line (MCF-7). Cancer cells were cultured according to the supplier's instructions. 2–4 $\times$ 10^4 cells per well in 96-well microplates were attached overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Welgene, Korea) under 5% CO_2 in a humidified atmosphere at 37 °C. On day 1, the culture medium in each well was exchanged with 0.1-mL aliquots of medium containing graded concentrations of compounds. On day 4, each well was treated with MTT (Sigma) solution (final concentration 0.5 mg/mL in media) then incubated for an additional 4 h under the same conditions. The culture medium in each well was discarded and replaced with 0.1 mL of dissolving solution (DMSO). The absorbance of each well was determined by an Automatic ELISA Reader System (Bio-Rad 3550) at 570 nm wavelength. For determination of the IC_{50} values, the absorbance readings at 570 nm were fitted to the four-parameter logistic equation.

5.24. Assay for DNA Topoisomerase II Inhibition in vitro [11]

DNA topoisomerase II inhibition was measured by assessing relaxation of supercoiled pBR322 plasmid DNA. The reaction mixture contained 50 mM Tris–HCl (pH 8.0), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol, 30 μg/ml bovine serum albumin, 0.3 μg pBR322 plasmid DNA, 0.3 U human DNA topoisomerase IIα (TopoGEN) in a final volume of 20 μL. The reactions were incubated for 30 min at 37 °C and terminated by the addition of 3 μL of 0.77% sodium dodecyl sulfate, 77 mM EDTA. Samples were mixed with 2 μL of 30% sucrose, 0.5% bromophenol blue and 0.5% xylene cyanol, and subjected to electrophoresis on a 1% agarose gel at 1.5 V/cm for 10 h. 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 quantitated by an image analyzer and LabWork 4.5 software (UVP).

5.25. DNA cross-linking assay [12]

The DNA cross-linking property of each compound was tested using linearized pBR322 plasmid DNA and denaturing 1.2% alkaline agarose gel electrophoresis. Linear plasmid DNA was obtained by treating circular pBR322 plasmid DNA with EcoRI (Invitrogen, USA) and purified by ethanol precipitation. Alkaline agarose gel (1.2%) was prepared with solution (pH 8.0) containing 50 mM NaCl and 1 mM EDTA. 0.5 μg of the linearized pBR322 was incubated with designated concentrations of compounds for 2 h at RT in buffer containing 10 mM Tris–HCl and 1 mM EDTA adjusted to pH 8.0. After the mixture of DNA and compound was loaded with agarose loading dye, the gel was soaked in an alkaline running buffer containing 50 mM NaOH and 1 mM EDTA. The gel was run in fresh alkaline running buffer and then neutralized for 1 h in neutralizing buffer solution containing 100 mM Tris and 150 mM NaCl adjusted to pH 7.6 with refreshing every 20 min. The gel was subsequently stained with ethidium bromide solution (2.5 μL of 10 mg/mL ethidium bromide in 50 mL of neutralizing buffer solution). The gel was visualized by UV transillumination and photographed using ChemiImager™ Ready (Alpha Innotech Corp.).

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2006-521-E00159). J.M.N. was partially supported by the Brain Korea 21 project.

References

[1] (a) M.M.M. Pinto, M.E. Sousa, M.S.J. Nascimento, Curr. Med. Chem. 12 (2005) 2517–2538;
(b) V. Peres, T.J. Nagem, F.F. de Oliveira, Phytochemistry 55 (2000) 683–710;
(c) L.-W. Wang, J.-J. Kang, I.-J. Chen, C.-M. Teng, C.-N. Lin, Bioorg. Med. Chem. 10 (2002) 567–572;
(d) C.-N. Lin, S.-S. Liou, F.-N. Ko, C.-M. Teng, J. Pharm. Sci. 81 (1992) 1109–1112;
(e) C.-N. Lin, S.-S. Liou, F.-N. Ko, C.-M. Teng, J. Pharm. Sci. 82 (1993) 11–16.
[2] (a) S.-S. Liou, W.-L. Shieh, T.-H. Cheng, S.-J. Won, C.-N. Lin, J. Pharm. Pharmacol. 45 (1993) 791–794;
(b) C.-N. Lin, S.-S. Liou, T.-H. Lee, Y.-C. Chuang, S.-J. Won, J. Pharm. Pharmacol. 48 (1996) 539–544;
(c) M. Pedro, F. Cerqueira, M.E. Sousa, M.S.J. Nascimento, M. Pinto, Bioorg. Med. Chem. 10 (2002) 3725–3730;
(d) L. Saraiva, P. Fresco, E. Pinto, E. Sousa, M. Pinto, J. Goncalves, Bioorg. Med. Chem. 11 (2003) 1215–1225;
(e) Y.-S. Zhou, A.-J. Hou, G.-F. Zhu, Y.-F. Chen, H.-D. Sun, Q.-S. Zhao, Bioorg. Med. Chem. 12 (2004) 1947–1953.
[3] S. Woo, J. Jung, C. Lee, Y. Kwon, Y. Na, Bioorg. Med. Chem. Lett. 17 (2007) 1163–1166.
[4] (a) P. Forterre, S. Gribaldo, D. Gadelle, M.C. Serre, Biochimie 89 (2007) 427–446;
(b) J.B. Schvartzman, A. Stasiak, EMBO 5 (2004) 256–261;
(c) J.A. Holden, Curr. Med. Chem. Anticancer Agents 1 (2001) 1–25.
[5] J. Grillari, H. Katinger, R. Voglauer, Nucleic Acids Res. 35 (2007) 7566–7576.
[6] S.R. Rajski, R.M. Williams, Chem. Rev. 98 (1998) 2723–2796.
[7] X. Weng, L. Ren, L. Weng, J. Huang, S. Zhu, X. Zhou, L. Weng, Angew. Chem. Int. Ed. 46 (2007) 8020–8023.
[8] M. Tercel, S.M. Stribbling, H. Sheppard, B.G. Siim, K. Wu, S.M. Pullen, K. J. Botting, W.R. Wilson, W.A. Denny, J. Med. Chem. 46 (2003) 2132–2151.
[9] (a) S.M. Kupchan, D.R. Streelman, A.T. Sneden, J. Nat. Prod. 43 (1980) 296–301;
(b) J.M. Cassady, W.M. Baird, C.-J. Chang, J. Nat. Prod. 53 (1990) 23–41.
[10] (a) M. Hansen, S.-J. Lee, J.M. Cassady, L.H. Hurley, J. Am. Chem. Soc. 118 (1996) 5553–5561;
(b) Y. Kwok, L.H. Hurley, J. Biol. Chem. 273 (1998) 33020–33026.
[11] P. Thapa, R. Karki, A. Basnet, U. Thapa, H. Choi, Y. Na, Y. Jahng, C.-S. Lee, Y. Kwon, B.-S. Jeong, E.-S. Lee, Bull. Korean Chem. Soc. 29 (2008) 1605–1608.
[12] (a) J.J. Tepe, R.M. Williams, J. Am. Chem. Soc. 121 (1999) 2951–2955;
(b) Y. Na, V.-S. Li, Y. Nakanishi, K.F. Bastow, H. Kohn, J. Med. Chem. 44 (2001) 3453–3462.