Synthesis, Cytotoxicity and Topoisomerase II Inhibition Study of New Thioxanthone Analogues

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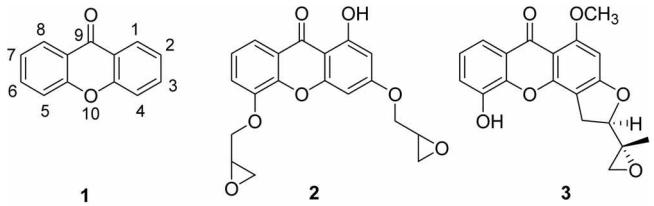
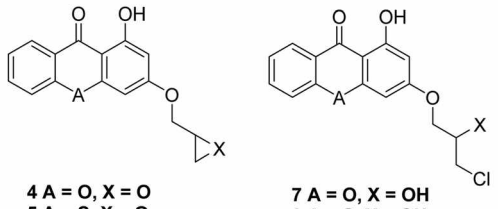
Xanthone (**1**) compounds found as secondary metabolites from higher plants and microorganisms have wide biological profiles including anti-hypertensive, anti-oxidative, anti-thrombotic, and anti-cancer activity, based on their diverse structures.[1] The interesting structural scaffold and biological efficacy of xanthones enforced many scientists to synthesize these compounds for the development of prospective new drug candidates. Among these xanthones, oxygenated xanthones synthesized or isolated from natural sources revealed effective inhibitory activity against several cancer cell lines.[2] Especially, 2',3'-epoxypropoxy substituted xanthones have efficiently prohibited growth of cancer cells and xanthone (**2**) possessing two 2',3'-epoxypropoxy groups at 3 and 5 position showed most active anticancer activity in the series prepared.[2a,b]



1 **2** **3**



4 A = O, X = O
5 A = S, X = O
6 A = S, X = S

7 A = O, X = OH
8 A = S, X = OH
9 A = S, X = SH

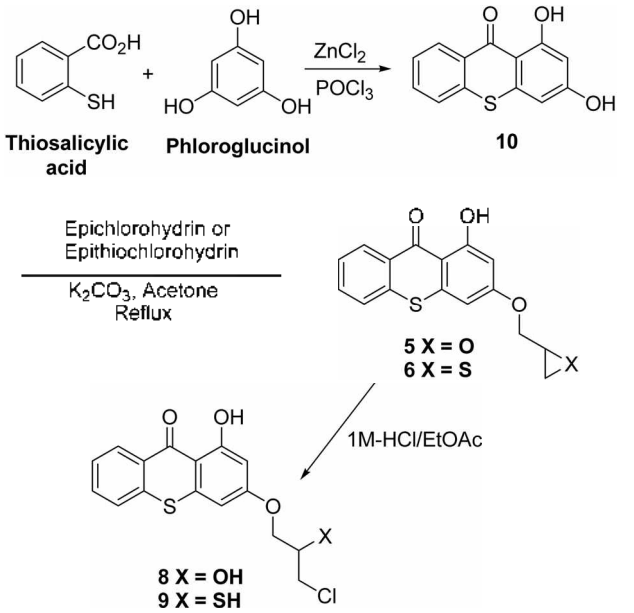
Psorospermin (**3**) isolated from African plant is a natural compound showing good anti-cancer activity against human and murine cancer cell lines.[3] In the structural viewpoint, **3** also possessed xanthone and 2',3'-epoxypropoxy group moieties. Psorospermin has been known to show biological activities via intercalation of xanthone group into DNA base pair and alkylation of epoxide by N7-guanine in the presence of topoisomerase II.[4] Topoisomerase II is critical enzyme for cell propagation pathway by mediating DNA double strand cleavage and religation process to relax supercoiled DNA.[5]

Previously we reported that the 2',3'-epoxypropoxyxanthones and their ring opened halohydrin analogues showed significant cytotoxic and topoisomerase II relaxation inhibitory activities.[6] These findings suggested that the epoxide ring opened compounds might modulate the biological efficacy of epoxide compounds. According to the literature, replacement of oxygen with sulfur in the xanthone core also changed the pharmacological profiles of parent compounds.[7]

In this report, we have synthesized and examined anti-cancer activities of four new thioxanthone analogues including 2',3'-epoxypropoxy coupled thioxanthones **5** and **6** and their epoxy ring opened halohydrin compounds **8** and **9**.

The synthetic method employed for the compounds was depicted in Scheme 1. Thioxanthone core was prepared using phloroglucinol and thiosalicylic acid according to the literature.[7c] Introduction of epoxypropoxy or thioepoxypropoxy group at C3 oxygen position in 1,3-dihydroxythioxanthone[7c] (**10**) was accomplished in the K_2CO_3 basic acetone solution. In this step, interestingly, we could not get any bis-1,3-(2',3'-epoxypropoxy) substituted compound but separated only C3-monoepoxypropoxy substituted thioxanthone compounds, **5** and **6**. These structures are confirmed by comparison of the chemical shift values of the corresponding xanthone (**4**)[6] in the ^{1}H and ^{13}C-NMR spectra. We also observed that two methylene protons of epoxide ring of compound **5** shifted 0.31-0.45 ppm more down field than those values of compound **6** in the ^{1}H-NMR spectra. This is the typical pattern between epoxide and thioepoxide ring. Finally, epoxide ring open reactions of **5** and **6** were conducted in aqueous 1 M or 3M-HCl in EtOAc to produce chlorohydrin or chlorothiohydrin compounds **8** and **9**. Reaction of compound **6** took much longer time than **5** (overnight *vs* 30 min), which indicates that thioepoxide is more resistant to the nucleophile than epoxide itself under acidic conditions. All the spectral data (^{1}H and ^{13}C NMR, COSY, and mass spectra) were consistent with the proposed structures.

Compounds **5**, **6**, **8**, and **9** were tested for the cytotoxicity against several human cancer cell lines using adriamycin as



Scheme 1. Synthetic method for target compounds.

a reference. The method applied for the test is typical MTT assay method. The result is indicated in Table 1. Most compounds tested showed significant cytotoxic activity against certain cancer cell lines and compound **5**, especially, was the most active one which is comparable to the reference. From this result we suspected that the epoxide ring is important for the cytotoxic activity for the compounds tested. When we compared the activities between ring opened compounds **8** and **9**, there were not significant activity variations. These cytotoxic results showed that thioxanthone analogues are more active in the anticancer activities than the corresponding xanthone analogues, especially, compound **5** *vs* compound **4**.[6]

With these cytotoxicity test results, compounds are screened for the topoisomerase II inhibition function. Topoisomerase II relaxation assay was conducted using human topoisomerase II (Topogen) with etoposide as a positive control. The data were analyzed and calculated with LabWork 4.5 Software for the inhibition ratio. The result is described in the Table 2 and Figure 1. Among the compounds, compound **8** showed 77% inhibition rate against topoisomerase II action at 20 μM and 84% at 100 μM. The topoisomerase II inhibition of compound **8** was superior to etoposide used as reference, almost two times more active at 20 μM concentration. This compound was also more potent topoisomerase II inhibitor than the corresponding xanthone analogue **7**.[6] But other compounds are not active at all. The outcome of topoisomerase II inhibition test revealed that epoxide ring tethered to thioxanthone core did not mediate the topoisomerase II inhibition pathways. But the epoxide ring opened chlorohydrin not chlorothiohydrin enhanced the topoisomerase II inhibition activity of thioxanthone structure.

Table 1. Cytotoxic activities of prepared compounds against various human cancer cell lines (unit: μM)

	Adriamycin	5	6	8	9
MCF-7	18.9 ± 0.8	50.2 ± 0.1	> 100	32.7 ± 3.3	> 100
HCT-116	4.0 ± 0.8	5.0 ± 0.1	22.7 ± 2.4	> 100	31.1 ± 1.8
DU-145	1.4 ± 0.2	1.0 ± 0.2	3.9 ± 0.3	22.3 ± 2.9	11.1 ± 2.4
HeLa	10.1 ± 0.8	42.3 ± 2.2	> 100	> 100	> 100

[a]Each data point represents mean ± S.D. from three different experiments performed in triplicate. Cell lines used are MCF-7, human breast tumor; HCT 116, human colon tumor; DU 145, human prostate tumor; HeLa, human cervix tumor.

Table 2. Topoisomerase II inhibition rate (%) of prepared compounds

Comp. conc.	Etoposide	5	6	8	9
20 μM	40	0(40[a])	0	77	0
100 μM	70	21(81[a])	0	84	16

[a]These values are the result of etoposide from different batch of test.

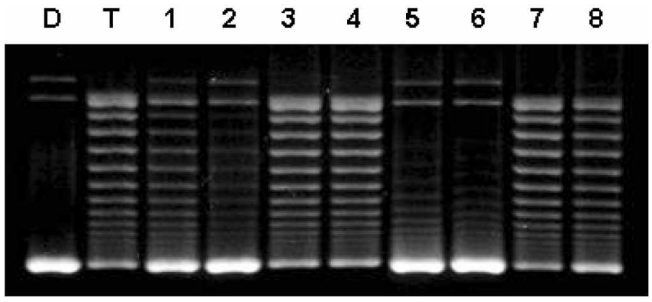


Figure 1. Topoisomerase II inhibition test of prepared compounds. Compounds were examined in a final concentration of 20 μM and 100 μM, respectively. Lane D: pBR322 only, Lane T: Topo II only, Lane 1-2: etoposide, 20 μM and 100 μM, Lane 3-4: **6**, 20 μM and 100 μM; Lane 5-6: **8**, 20 μM and 100 μM; Lane 7-8: **9**, 20 μM and 100 μM.

In conclusion, four thioxanthone analogues were synthesized and tested their biological capacities, cytotoxicity and topoisomerase II inhibition. In the cytotoxicity test compound **5** showed best cytotoxic activity than other compounds tested. But topoisomerase II inhibition test generated different results. Compound **8** exerted highly efficient topoisomerase II inhibition activity, 77% inhibition rate against topoisomerase II action at 20 μM and 84% at 100 μM. Nonetheless, cytotoxicity and topoisomerase II inhibition test result have not been correlated with each other *in vitro*. More works on the SAR for the xanthone and thioxanthone analogues are on going to elucidate the optimized structure and the result will be reported in the near future.

Experimental Section

The solvents and reactants were of the best commercial grade available and were used without further purification unless noted. TLC plate was 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 Varian NMR AS 400 MHz instrument. Chemical shifts (δ) are in parts per million (ppm)

relative to tetramethylsilane as internal standard. and coupling constants (J values) are in Hertz. Mass spectral investigations were run on a LCQ advantage-trap mass spectrometer equipped with electrospray ionization (ESI) source at Yeungnam University. Gyeongsan, Korea. Melting points were measured on a Barnstead International MEL-TEMP® 1202D instrument without correction.

1-Hydroxy-3-(2',3'-epoxypropoxy)thioxanthone (5). To the reaction mixture of compound **10** (137 mg, 0.61 mmol) and K_2CO_3 (169 mg, 1.22 mmol) in anhydrous acetone (15 mL) was added epichlorohydrin (0.24 mL, 3.05 mmol) under nitrogen condition. The reaction mixture was refluxed overnight and solvent was removed under reduced pressure. The residue was diluted with ethyl acetate and organic layer was washed with water and brine. successively. After evaporation of solvent, the residue was purified by silica gel column chromatography (eluent: CH_2Cl_2) to afford yellow solid compound. (34 mg, 18.6%): m.p. 160-162 °C; R_f 0.71 (CH_2Cl_2); ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.71 (dd, J = 2.6. 4.6 Hz, 1H. C3'-H_a). 2.87 (dd, J = 3.6. 4.6 Hz, 1H, C3'-H_b). 3.30-3.32 (m, 1H. C-2'H), 3.93 (dd, J = 5.6. 11.2 Hz, 1H. C1'-H_a). 4.26 (dd, J = 2.8, 11.2 Hz. 1H. C1'-H_b). 6.38 (d, J = 2.6 Hz. 1H. C4-H). 6.53 (d, J = 2.6 Hz. 1H, C2-H). 7.37-7.43 (m, 2H. C5. 7-H). 7.53 (ddd, J = 1.4, 7.6. 11.3 Hz, 1H. C6-H), 8.47 (dd. J = 1.4, 8.2 Hz, 1H, C8-H): ^{13}C-NMR (100 MHz. $CDCl_3$) 43.6, 48.7, 68.0. 99.0. 101.2. 108.6, 124.4. 127.2. 128.2, 131.6, 131.6, 136.1, 139.4, 162.4, 166.4. 183.2 ppm: LC-ESI: m/e 301.1 $[M+1]^-$.

1-Hydroxy-3-(2',3'-thioepoxypropoxy)thioxanthone (6). To the reaction mixture of compound **10** (100 mg, 0.41 mmol) and K_2CO_3 (113 mg. 0.82 mmol) in anhydrous acetone (15 mL) was added epithiochlorohydrin (0.22 g. 2.05 mmol) under nitrogen condition. The reaction mixture was refluxed overnight and solvent was removed under reduced pressure. The residue was diluted with ethyl acetate and organic layer was washed with water and brine, successively. After evaporation of solvent. the residue was purified by silica gel column chromatography (eluent:ethyl acetate/ n-hexane = 1:1) to afford yellow green solid compound. (43 mg. 33.3%): m.p. 148-150 °C; R_f 0.89 (ethyl acetate/ n-hexane = 1:1); ^{1}H-NMR (400 MHz. $CDCl_3$) δ 2.27 (dd, J = 1.4. 5.7 Hz. 1H. C3'-H_a), 2.56 (d. J = 5.7 Hz. 1H. C3'-H_b). 3.16-3.21 (m, 1H, C-2'H). 3.92 (dd, J = 7.2. 10.0 Hz. 1H. C1'-H_a). 4.13 (dd, J = 5.6. 10.0 Hz, 1H, C1'-H_b), 6.12 (d, J = 2.4 Hz, 1H. C4-H). 6.50 (d, J = 2.4 Hz, 1H. C2-H), 7.36 (ddd. J = 1.4. 6.8. 7.8 Hz. 1H. C7-H). 7.37 (d. J = 8.2 Hz. 1H. C5-H). 7.49 (ddd. J = 1.6. 8.0. 8.2 Hz. 1H. C6-H). 8.43 (dd. J = 1.4. 7.8 Hz. 1H. C8-H); ^{13}C-NMR (100 MHz. $CDCl_3$) 24.0. 31.0. 72.9, 100.2, 102.2, 109.8. 125.6. 126.5. 128.4. 129.4, 132.8. 137.3. 140.6. 163.4. 167.5. 184.3 ppm: LC-ESI: m/e 317.1 $[M+1]^-$.

1-Hydroxy-3-(3'-chloro-2'-hydroxy-1'-propoxy)thioxanthone (8). Compound **5** (13 mg, 0.04 mmol) was dissolved in aqueous ethyl acetate 1 M-HCl (3 mL) and this solution was stirred for 30 min at room temperature. The solvent was removed under reduced pressure and the residue was dried under vacuum to give yellow solid compound. (14 mg. 96.0%): m.p. 150-152 °C: ^{1}H-NMR (400 MHz. $CDCl_3$) δ 3.68-3.75 (m, 2H, C3'-H), 4.11 (m, 2H, C1'-H), 4.18-4.22 (m,1 H. C-2'H), 6.39 (d. J = 2.4 Hz, 1H, C4-H), 6.53 (d. J = 2.4 Hz. 1H. C2-H), 7.39-7.45 (m, 2H. C5, 7-H), 7.54 (ddd. J = 1.2, 7.5. 7.5 Hz, 1H, C6-H), 8.49 (dd, J = 1.2. 8.4 Hz. 1H, C8-H): ^{13}C-NMR (100 MHz. $CDCl_3$) 46.0 (C3'), 69.0 (C1'), 69.7 (C2'). 100.3 (C4). 102.2 (C2), 110.0 (C9a). 125.6 (C5), 126.6 (C7). 128.4 (C8a), 129.5 (C8), 132.9 (C6). 137.3 (C10a). 140.8 (C4a). 163.3 (C1). 167.6 (C3), 184.4 (C9) ppm: LC-ESI: m/e 337.3 $[M+1]^+$.

1-Hydroxy-3-(3'-chloro-2'-thioyl-1'-propoxy)thioxanthone (9). Compound **5** (30 mg, 0.09 mmol) was dissolved in aqueous ethyl acetate 3 M-HCl (3 mL) and this solution was stirred at room temperature overnight. The solvent was removed under reduced pressure and residue was dried under vacuum to give yellow solid compound. (30 mg, 89.9%): m.p. 133-134 °C: ^{1}H-NMR (400 MHz. $CDCl_3$) δ 2.12 (d. J = 10.0 Hz, 1H. C2'-SH). 3.37-3.42 (m, 1H, C-2'H), 3.84 (dd. J = 6.8. 11.3 Hz. 1H. C3'-H_a). 3.97 (dd. J = 4.2, 11.3 Hz, 1H, C3'-H_b), 4.20 (dd. J = 6.0, 9.6 Hz, 1H. C1'-H_a), 4.35 (dd. J = 4.4, 9.6 Hz. 1H. C1'-H_b). 6.45 (d. J = 2.2 Hz, 1H. C4-H), 6.59 (d. J = 2.2 Hz. 1H. C2-H), 7.26-7.51 (m, 2H. C5. 7-H). 7.61 (ddd, J = 1.2, 7.4. 7.5 Hz. 1H, C6-H), 8.55 (d. J = 7.6 Hz. 1H, C8-H); ^{13}C-NMR (100 MHz, $CDCl_3$) 40.0. 46.9. 69.0, 100.3, 102.3, 110.0, 125.6, 126.6, 128.4, 129.4, 132.9. 137.3 , 140.7. 163.2, 167.6, 184.4 ppm: LC-ESI: m/e 353.1 $[M+1]^+$.

Cytotoxicity Test. Cytotoxicity was determined by MTT assay. Cancer cells were purchased from the American Tissue Culture Collection (Rockville, MD) and 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, culture medium in each well was exchanged with 0.1 mL aliquots of medium containing graded concentrations of compounds **5**. **6**. **8**, and **9**. On day 4, each well was added by the MTT (Sigma) solution (final concentration 0.5 mg/mL in media) then incubated for additional 4 h under the same condition. 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) with a 570 nm wavelength. For determination of the IC_{50} values, the absorbance readings at 570 nm were fitted to the four-parameter logistic equation.

Assay for DNA Topoisomerase II Inhibition *in vitro*. 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, 0.2 μg pBR322 plasmid DNA. 0.3 U human DNA topoisomerase IIα (TopoGEN), and test compounds 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 solution containing 0.77% sodium dodecyl sulfate, and 77 mM EDTA. Samples

were mixed with 2 μL of solution containing 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 with a running buffer of Tris-borate-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 quantitated by an image analyzer and LabWork 4.5 software (UVP).

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