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# Synthesis of new xanthone analogues and their biological activity test—Cytotoxicity, topoisomerase II inhibition, and DNA cross-linking study

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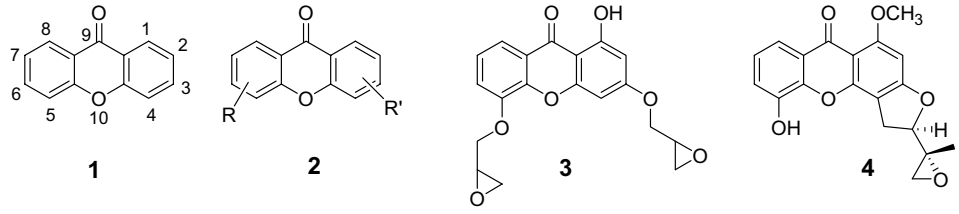
**Abstract**—In this report, we prepared some 3-(2′,3′-epoxypropoxy)xanthones and their epoxide ring opened halohydrin analogues, and evaluated their cytotoxicity and topoisomerase II inhibition activity using doxorubicin and etoposide as references, respectively. Another xanthone compound **9**, 1,3-di(2′,3′-epoxypropoxy)xanthone, was also synthesized and its DNA cross-linking property including other two biological activities investigated. The biological test results showed compound **9** possessed excellent cytotoxic and topoisomerase II inhibitory activity than other compounds tested. It also exhibited significant DNA cross-linking activities.


Xanthone (**1**) compounds, secondary metabolites from higher plants and microorganisms, have very diverse 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 isolate or synthesize these compounds for the development of prospective new drug candidates. Among these, poly-oxygenated xanthones (**2**) either synthesized or isolated from natural resources showed effective inhibitory activity against several cancer cell lines.[2] Especially, 2,3-epoxypropoxy substituted xanthones have efficiently prohibited growth of cancer cells and xanthone (**3**) possessing two 2,3-epoxypropoxy groups at 3 and 5 position showed most active anti-cancer activity in the series prepared.[2a,b] However, the exact action mechanism of these compounds has not been reported yet.

Psorospermin (**4**), an ingredient of African plant *Psorospermum febrifugum*, is another natural compound showing good anti-cancer activity against human and murine cancer cell lines.[3] From the structural viewpoint, **4** also possessed xanthone and 2,3-epoxypropoxy group moieties. According to the literatures, 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]

In this report, first, we tried to synthesize and study anti-cancer activities of some epoxypropoxy xanthones **7**–**9** and their epoxy ring opened halohydrin compounds **10**–**13**. In the literature, epoxide ring opened halohydrin compounds enhanced cytotoxic activities than their parent compounds.[5] We, therefore, expected that

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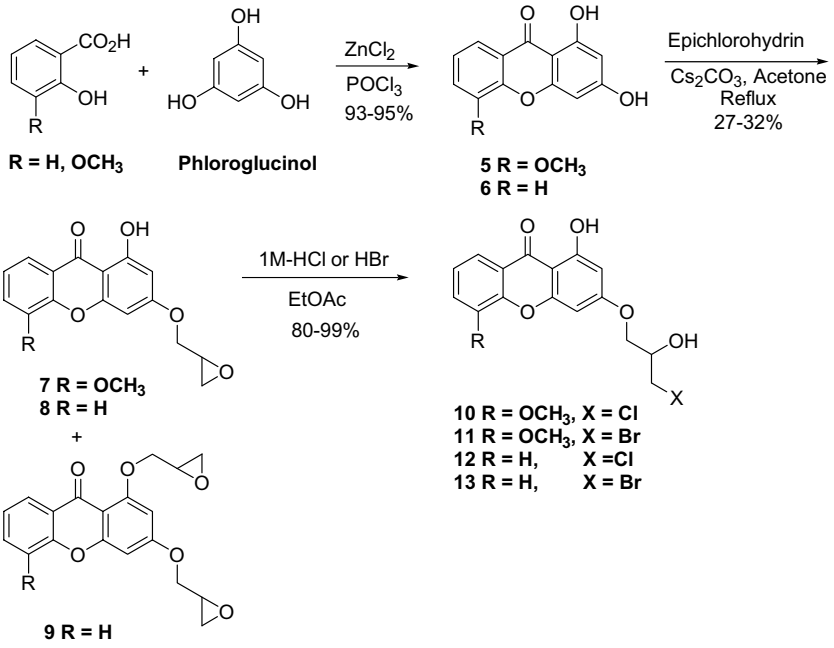
employment of good leaving group (Br or Cl) instead of unstable epoxide group could modulate the biological capacity. Second, topoisomerase II inhibition study was also conducted to check possible action mechanism of the xanthone compounds. Third, we performed DNA cross-linking experiment to see that introduction of two 2,3-epoxypropoxy groups at 1 and 3 position in xanthone core, compound **9**, might generate double alkylated DNA adduct with linearized pBR322 DNA.

The synthetic method employed for the compounds is depicted in Scheme 1. Xanthone core was prepared using phloroglucinol and salicylic acid by the literature method[6]. Introduction of epoxypropoxy group was conducted in the $Cs_2CO_3$ basic acetone condition, subsequently. In this step we could get mono- and bis-epoxypropoxy group substituted xanthone compounds, **8** and **9**, at the same time. Compound **9** was obtained as diastereomeric mixture. Finally, epoxide ring open reaction was conducted in aqueous 1 M-HCl or -HBr in EtOAc to produce halohydrin compounds **10**–**13**.[7]

Compounds **7**–**13** were tested for the cytotoxicity against several human cancer cell lines using adriamycin as a reference. The method applied for the test is typical MTT assay procedure according to the literature.[8] The result is indicated in Table 1. Compound **9** showed most effective cytotoxic activity among the series compounds comparable to the reference. Compound **13** also showed moderate activity but lower than those of compound **9** and adriamycin. In this study, we found two epoxypropoxy group substitutions at 1 and 3 carbon on xanthone core generating better cytotoxic activity than monosubstituted one. This finding is parallel to the previous report of compound **3** though the locations of two epoxypropoxy groups are different.[2a,b] Based on the test result, we asked what pathway might contribute for the activity of xanthone compounds. In order to understand the possible action mechanism of the compounds **7**–**13**, we conducted topoisomerase II inhibition and DNA cross-linking experiment.

Topoisomerase 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. Compound **13** effectively inhibited the topoisomerase II action with 57% inhibition ratio at 100 μM (Fig. 1). But this value is lower than that of etoposide. Furthermore, the inhibitory activity of compound **13** is almost




**Scheme 1.** Synthetic method for target compounds.

**Table 1.** Cytotoxicities of compounds **7**–**13** against various human cancer cells

| Cells (origin)/compound | $IC_{50}$[a] (μM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **7** | **8** | **10** | **11** | **12** | **13** | **9** | Adriamycin |
| LnCap (prostate) | >100 | 93.1 ± 16.9 | >100 | >100 | >100 | 64.4 ± 3.1 | 9.0 ± 0.2 | 4.6 ± 0.6 |
| MCF-7 (breast) | >100 | 68.4 ± 4.8 | >100 | 97.8 ± 0.2 | >100 | 53.5 ± 5.4 | 3.2 ± 0.8 | 4.5 ± 0.3 |
| HCT 116 (colon) | >100 | 80.8 ± 3.1 | 31.4 ± 3.0 | >100 | >100 | 16.9 ± 0.5 | 10.2 ± 0.7 | 7.7 ± 0.2 |
| MDA-MB231 (breast) | >100 | >100 | 81.4 ± 3.5 | 99.8 ± 11.1 | >100 | 76.3 ± 5.0 | 12.8 ± 0.9 | 17.6 ± 0.3 |
| Hela (cervix) | >100 | 68.7 ± 8.7 | 60.3 ± 3.3 | >100 | >100 | 98.1 ± 6.3 | 23.3 ± 1.7 | 3.3 ± 0.4 |

[a] Each value is the average of four experiments.

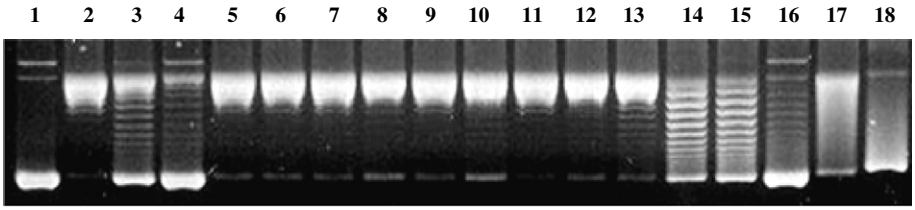



**Figure 1.** Effects of compounds on human topoisomerase II-mediated DNA relaxation. Supercoiled pBR322 plasmid DNA (0.3 μg) was incubated with 0.2 unit of topoisomerase II in the presence of compound (20 and 100 μM) at 37 °C for 30 min. Lane 1: DNA only; lane 2: DNA + topo II; lanes 3 and 4: DNA + topo II + etoposide, 20 and 100 μM; lanes 5–18: DNA + topo II + compounds, **7**, **8**, **10**, **11**, **12**, **13**, **9**, 20 μM and 10 μM, respectively.

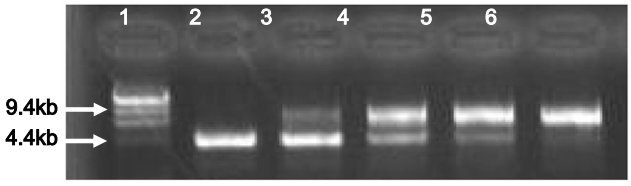



**Figure 2.** DNA cross-linking activity of compound **9** was determined by 1.2 % alkaline agarose gel electrophoresis. 0.5 μg of the linearized pBR322 was incubated with various concentrations of compound **9** (lanes 2–6: 0, 10, 50, 100, 200 μM) for 1 h at room temperature. Lane 1: λ *Hin*dIII DNA Marker (Promega).

half of that of the etoposide at 20 μM. Although compound **9** exhibited strong topoisomearse II inhibition at 100 μM (almost 100% inhibition), we could not make clear decision regarding the inhibition activity because of the smeared band. Other compounds have not showed significant inhibition pattern. This topoisomerase relaxation assay data suggested that the epoxide ring opening of compounds might enhance inhibition activity than epoxide ring possessing ones (compounds **12** and **13** vs **7** and **8**). It also showed that bromohydrin introduced compounds generate higher activity than chlorohydrin ones (compounds **11** and **13** vs **10** and **12**).

Due to its bis-epoxypropoxy groups in the structure, only compound **9** was tested for DNA cross-linking property using linearized pBR322 DNA.[9] In this test, compound **9** showed concentration dependent DNA cross-linking activity (Fig. 2). The effective cross-linking activity below 50 μM implicates the possible application of this compound as a new DNA cross-linking agent.

In conclusion, compound **9** showed best biological activity than other compounds in the test. The test results suggest that compound **9** might exert its cytotoxic activity via topoisomerase II inhibition or DNA cross-linking process. But other mechanisms cannot be excluded. Because of small numbers of compounds, we could not determine structure–activity relationship of compounds tested. We are currently pursuing synthesis and biological study of expanded series of these compounds and results will be reported in the course of time.

## Acknowledgment

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7. *Spectral data for compounds*. Compound **8**: $^{1}$H NMR (250 MHz, $CDCl_3$) $\delta$ 2.77 (dd, $J$ = 2.6, 4.6 Hz, 1H), 2.93 (dd, $J$ = 4.3, 4.6 Hz, 1H), 3.36–3.39 (m, 1H), 3.99 (dd, $J$ = 5.9, 11.1 Hz, 1H), 4.33 (dd, $J$ = 2.8, 11.1 Hz, 1H), 6.34 (d, $J$ = 2.2 Hz, 1H), 6.44 (d, $J$ = 2.2 Hz, 1H), 7.36 (dd, $J$ = 7.8, 8.3 Hz, 1H), 7.40 (d, $J$ = 8.7 Hz, 1H), 7.70 (ddd, $J$ = 1.5, 8.3, 8.7 Hz, 1H), 8.22 (dd, $J$ = 1.5, 7.8 Hz, 1H), 12.85 (s, 1H); $^{13}$C NMR (62.5 MHz, $CDCl_3$) 44.6, 44.7, 69.2, 93.4, 97.4, 104.2, 117.6, 120.6, 124.1, 125.9, 135.1, 156.0, 157.7, 163.6, 165.4, 180.9 ppm; LC–MS (ESI) *m/e* 285.2 $[M+1]^+$.
Compound **9**: $^{1}$H NMR (250 MHz, $CDCl_3$) $\delta$ 2.75–2.78 (m, 1H), 2.91–2.96 (m, 2H), 3.12–3.16 (m, 1H), 3.35–3.38 (m, 1H), 4.43–4.48 (m, 1H), 3.95 (d, $J$ = 6.0, 11.1 Hz, 1H), 4.13 (dd, $J$ = 4.4, 11.1 Hz, 1H), 4.32–4.37 (m, 2H), 6.38 (d, $J$ = 1.9 Hz, 1H), 6.47 (d, $J$ = 1.9 Hz, 1H), 7.24–7.33 (m, 2H), 7.58 (ddd, $J$ = 1.2, 7.0, 8.2 Hz, 1H), 8.23 (dd, $J$ = 1.2, 7.7 Hz, 1H); (400 MHz, $CDCl_3$) $\delta$ 2.80 (dd, $J$ = 2.8, 4.8 Hz, 1H), 2.96 (dd, $J$ = 4.4, 4.8 Hz, 1H), 2.98 (dd, $J$ = 4.0, 4.8 Hz, 1H), 3.16–3.18 (m, 1H), 3.39–3.41 (m, 1H), 3.48–3.50 (m, 1H), 4.01 (ddd, $J$ = 1.6, 6.0, 11.2 Hz, 1H), 4.19 (dd, $J$ = 4.4, 11.2 Hz, 1H), 4.25–4.41 (m, 2H), 6.44 (d, $J$ = 2.4 Hz, 1H), 6.53 (d, $J$ = 2.4 Hz, 1H), 7.33 (ddd, $J$ = 1.2, 7.0, 8.0 Hz, 1H), 7.36 (d, $J$ = 7.6 Hz, 1H), 7.63 (ddd, $J$ = 1.6, 7.0, 7.6 Hz, 1H), 8.27 (dd, $J$ = 1.6, 8.0 Hz,

1H); $^{13}C$ NMR (62.5 MHz, $CDCl_3$) 44.5, 45.0, 49.7, 50.0, 68.8, 69.2, 94.1, 96.7, 107.7, 117.0, 122.9, 123.8, 126.6, 133.8, 154.9, 159.5, 160.6, 163.4, 175.2 ppm; (100 MHz, $CDCl_3$) 44.8, 45.2, 49.9, 50.3, 69.2(69.1), 69.5, 94.5(94.4), 97.1, 108.1, 117.2, 123.2, 124.1, 126.9, 134.0, 155.2, 159.8, 161.0, 163.7, 175.4 ppm; The values in the parentheses correspond to the peaks of diastereomer. LC–MS (ESI) *m/e* 341.2 $[M+1]^+$.

Compound **12**: $^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 3.70–3.80 (m, 2H), 4.13–4.19 (m, 2H), 4.25 (d, $J = 4.5$ Hz, 1H), 6.34 (d, $J = 1.9$ Hz, 1H), 6.44 (d, $J = 1.9$ Hz, 1H), 7.34 (dd, $J = 8.2$, 7.4 Hz, 1H), 7.41 (d, $J = 8.2$ Hz, 1H) 7.71 (dd, $J = 7.4$, 8.1 Hz, 1H), 8.23 (d, $J = 8.1$ Hz, 1H), 12.85 (s, 1H); $^{13}C$ NMR(62.5 MHz, $CDCl_3$) 45.0, 69.1, 69.6, 93.4, 97.6, 104.4, 117.7, 120.7, 124.2, 126.0, 135.2, 156.1, 157.8, 163.7, 165.2, 180.9 ppm; LC–MS (ESI) *m/e* 321.2 $[M+1]^+$.

Compound **13**: $^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 3.56–3.69 (m, 2H), 4.15–4.21 (m, 3H), 6.34 (d, $J = 1.9$ Hz, 1H), 6.43 (d, $J = 1.9$ Hz, 1H), 7.37 (dd, $J = 7.4$, 8.1 Hz, 1H), 7.41 (d, $J = 8.1$ Hz, 1H), 7.71 (dd, $J = 7.4$, 8.1 Hz, 1H), 8.23 (d, $J = 8.1$ Hz, 1H), 12.85 (s, 1H); $^{13}C$ NMR (62.5 MHz, $CDCl_3$) 35.0, 69.2, 69.8, 93.4, 97.6, 104.2, 117.7, 120.7, 124.2, 126.0, 135.3, 156.1, 157.8, 163.7, 165.1, 180.9 ppm; LC–MS (ESI) *m/e* 365.1 $[M+1]^+$.

8. 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 for overnight in 0.1 mL of media supplemented 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 **7**–**13**. On day 4, each well was added with the MTT (Sigma) solution (final concentration 0.5 mg/mL in media) and 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.
9. Alkaline agarose gel (1.2%) was prepared with the solution (pH 8.0) containing 50 mM NaCl and 2 mM EDTA. 0.5 μg of the linearized pBR322 was incubated with variable concentrations of compounds **9** for 1 h at room temperature under the buffered condition of 10 mM Tris–HCl (pH 7.5). The mixture of DNA and compound was loaded with agarose loading dye into the gel which was soaked in an alkaline running buffer (40 mM NaOH and 1 mM EDTA). The gel was run in fresh alkaline running buffer, then neutralized for 45 min in the solution (pH 7.0) containing 100 mM Tris and 150 mM NaCl refreshing every 15 min. The gel was subsequently stained in an ethidium bromide solution (2.5 μL of a 10 mg/mL ethidium bromide in 50 mL of the solution (pH 7.5) containing 100 mM Tris and 150 mM NaCl). The gel was visualized by UV and photographed using ChemiImager™ Ready (Alpha Innotech Corp.).