Design, Synthesis, Antitumor Activity and Mode of Action of Novel Oxiranyl and Thiiranyl Phenol Derivatives

Zunhua Yang,[†,‡,a] Jin-Ah Kang,[†,a] Won Hee Kim,[†] Ah-Young Park,[†] Hyung Sik Kim,[†] Jungsu Kim,[†] Jin-Ah Kim,[†] Ping Gong,[‡] Lak Shin Jeong,[§] and Hyung Ryong Moon[†,*]

[†]*Laboratory of Medicinal Chemistry, College of Pharmacy and Research Institute for Drug Development, Pusan National University, Busan 609-735, Korea. [*]E-mail: mhr108@pusan.ac.kr*
[‡]*School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, Liaoning, China*
[§]*Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea*
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Eleven novel oxiranyl and thiiranyl phenolic compounds were synthesized as potential antitumor agents using epichlorohydrin and epithiohydrin in the presence of K_2CO_3. Cytotoxicities were found in range of IC_{50} values of 2.5-14.8 μM, which was partially attributed to topoisomerase II inhibition. Bis-thiiranyl anthraquinone analog, **19** showed more cytotoxicity against MDA-MB-231 (breast cancer cell) and PC3 (prostate cancer cell) after 24 and/or 48 h and more potent topoisomerase II inhibitory activity than etoposide.

Key Words: Oxiranyl, Thiiranyl, Polyphenol, Antitumor activity, Topoisomerase II inhibition

Introduction

Xanthone[1] (Figure 1) compounds are secondary metabolites found in some higher plant families, fungi and lichens, and showed antioxidant,[2] antidiabetic,[3] anti-inflammatory,[4] and anticancer[5] activities. A study of hydroxyxanthones showed that successive introduction of hydroxyl groups on the aromatic ring increased in vitro antimalarial potency. Polyoxygenated xanthones also showed effective inhibition against several cancer cell lines. Especially, 2,3-epoxypropoxy substituted xanthones have efficiently prohibited growth of cancer cells and exhibited strong topoisomerase II inhibition.[6]

DNA topoisomerases are nuclear enzymes that make transient strand breaks in DNA to allow a cell to manipulate its topology in the sugar-phosphate backbone of the double helix.[7] Type II topoisomerases act by generating a transient double-stranded DNA break, followed by a double stranded DNA passage event. Consequently, these enzymes are able to relax superhelical twists from DNA and resolve knotted or tangled duplex molecules. Type II topoisomerases function in numerous DNA processes and are required for recombination, the separation of daughter chromosomes, and proper chromosome structure, condensation, and decondensation.

Clinically important anticancer drugs have been shown to kill tumor cells by targeting topoisomerase II.[8] Etoposide, doxorubicin, teniposide, idarubicin, epirubicin and mitoxantrone were approved by FDA as topoisomerase II inhibitors.

Psorospermin,[9] (Figure 1) one of the 2,3-epoxypropoxy substituted xanthones is a natural product isolated from the roots and stembark of the African plant *Psorospermum febrifugum*. It has been reported to intercalate into the DNA helix and to covalently modify the N-7 position of guanine bases in the major groove of DNA through an epoxide-mediated electrophilic attack. The covalent modification of guanine

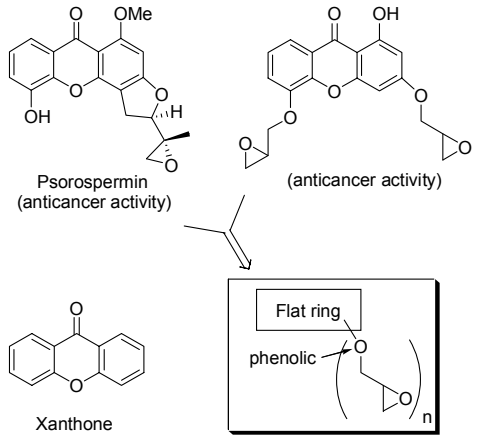


Figure 1

base may lead depurination inside cells, due to the property of its positive charge. It was also reported that psorospermin alkylation at specific sites on DNA was greatly enhanced in the presence of topoisomerases II, indicating that the abasic sites on DNA and antitumor activity of psorospermin might be related to its specific interaction with the topoisomerase II-DNA complex. It has been proposed that psorospermin induces DNA strand break, abasic sites and protein-DNA cross-links.

Psorospermin consists of a flat fused ring, which is able to intercalate into DNA helix and interact with topoisomerases, and an oxiranyl substituent, which is able to act as an electrophile on interaction with N-7 nitrogen of guanine base in DNA.[10] Although psorospermin shows potent anticancer efficacy, it seems to be difficult for psorospermin to be developed as an anticancer drug, due to its synthetic difficulty. If there are compounds having both a flat fused ring and a proper electrophile in their structure, they might show potent anticancer activity. Therefore, we tried to synthesize topoisomerase II-

[a]Zunhua Yang and Jin-Ah Kang contributed equally to this article.

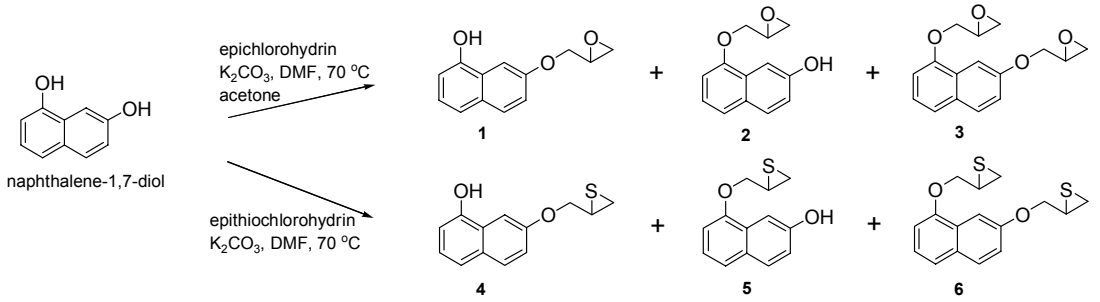
induced alkylators or effective intercalating alkylators by introducing proper electrophiles into a flat phenolic template, as in psorospermin. It was of great interest to introduce electrophiles such as 2,3-epoxypropyl and 2,3-thioepoxypropyl groups into flat (poly)phenolic compounds and to evaluate their cytotoxicity against four human cancer cell lines. Topoisomerase II inhibition[11] study was also conducted to identify the possible mechanism of anti-cancer action of the phenolic compounds bearing epoxypropyl and thioepoxypropyl groups.

Results and Discussion

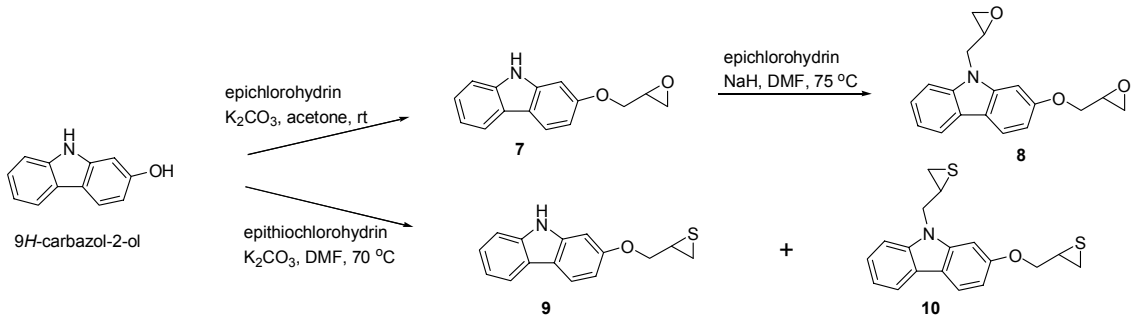
The synthetic method for 2,3-epoxypropoxy and 2,3-thioepoxypropoxy substituted naphthalene derivatives is depicted in Scheme 1. Treatment of 1,7-dihydroxynaphthalene with epichlorohydrin and potassium carbonate in DMF at 70 °C gave monoalkylated and bis-alkylated products **1**, **2** and **3**, but the monoalkylated products **1** and **2** were obtained as an inseparable mixture. Reaction with epithiochlorohydrin also gave bis(2,3-thioepoxypropoxy)naphthalene **6** and inseparable monoalkylated naphthalene products **4** and **5**, respectively, in 23 and 17% yields.

2,3-Epoxypropoxycarbazole **7** was synthesized by treatment of 2-hydroxycarbazole with epichlorohydrin in acetone solvent in the presence of potassium carbonate to afford only *O*-alkylated product, 2,3-epoxypropoxycarbazole **7** without formation of *N*-alkylated product (Scheme 2). *N*,*O*-Bis-alkylated product **8** was obtained from **7** by treatment with epichlorohydrin under more vigorous reaction conditions using NaH in the elevated temperature (75 °C). In contrast to epoxypropanylation of 2-hydroxycarbazole, thioepoxypropanylation with epithiochlorohydrin in DMF at 70 °C in the presence of potassium carbonate produced *N*,*O*-bis-alkylated product **10** as well as *O*-alkylated product **9**, maybe due to higher reactivity of epithiochlorohydrin.

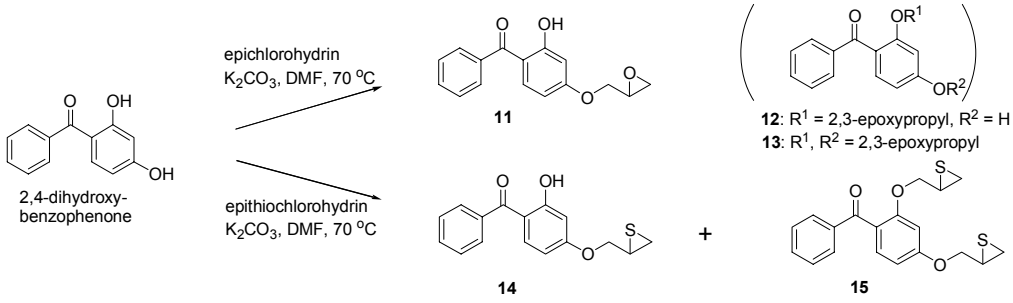
Although benzophenone analog **11** having a 2,3-epoxypro-



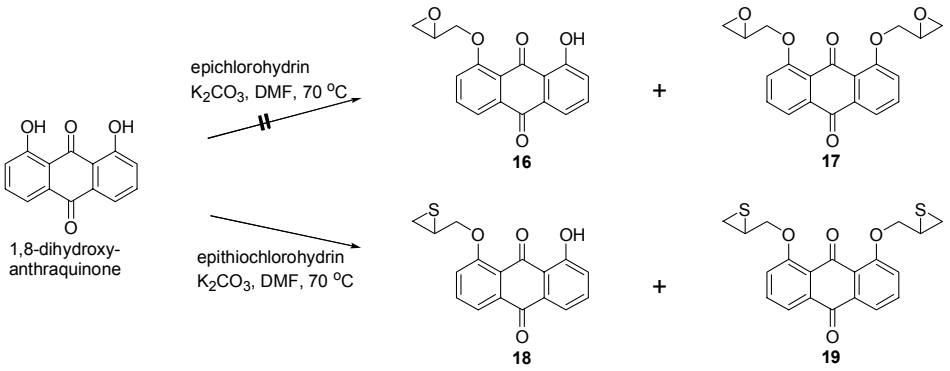
Scheme 1. Synthesis of oxiranyl- and thiiranyl-substituted naphthalenes.



Scheme 2. Synthesis of oxiranyl- and thiiranyl-substituted carbazoles.



Scheme 3. Synthesis of oxiranyl- and thiiranyl-substituted benzophenones.



Scheme 4. Synthesis of oxiranyl- and thiiranyl-substituted anthraquinones.

Table 1. Cytotoxicities of compounds **3**, **6**, **8**, **10**, **15** and **19** against various human cancer cell lines after 24 and 48 h

Cells/compounds	IC_{50}[a] (μM)						
	3	**6**	**8**	**10**	**15**	**19**	Doxorubicin
MDA-MB-231 (breast)	4.8 (9.1)	13.3 (> 20)	3.4 (7.5)	12.9 (> 20)	9.0 (19.8)	3.0 (4.3)	10.2
LNCaP (prostate)	14.8 (18.7)	-	9.3 (11.5)	-	4.1 (5.3)	5.8 (7.5)	1.1 (17.9)
DU145 (prostate)	3.8 (8.4)	8.7 (12.3)	2.9 (4.8)	8.7 (14.5)	6.9 (8.2)	3.8 (4.2)	0.57
PC3 (prostate)	-	-	4.0 (5.7)	-	6.0 (8.2)	2.5 (2.5)	1.2 (3.9)

[a]Each number in parentheses is the value of IC_{50} after 24 h.

poxy substituent was obtained by reacting 2,4-dihydroxybenzophenone with epichlorohydrin and potassium carbonate in acetone and DMF (2:1) at 75 °C, either monoalkylated compound **12** or dialkylated compound **13** was not produced from the reaction (Scheme 3), probably due to the stability of 1-hydroxyl group forming a six-membered intramolecular hydrogen bond. In contrast to case of using epichlorohydrin, treatment with epithiochlorohydrin afforded both monoalkylated product **14** and dialkylated product **15**. This might result from greater reactivity of epithiochlorohydrin than that of epichlorohydrin. Surprisingly, Rf value of the bis-alkylated product **15** was lower than that of the monoalkylated product **14** on a normal silica gel TLC plate and this phenomenon was also found in anthraquinone compounds **18** and **19** which will be discussed later. Probably, the phenomenon might be related to the break of the intramolecular hydrogen bond between the oxygen of the neighboring ketone and the hydrogen of hydroxyl group at 1-position.

As expected, reaction of 1,8-dihydroxyanthraquinone with epichlorohydrin in the similar reaction conditions used for the synthesis of compound **3** did not give any alkylated products **16** and **17**, whereas a reaction with epithiochlorohydrin under the same reaction conditions produced 2,3-thioepoxypropoxy and bis(2,3-thioepoxypropoxy)anthraquinone products **18** and **19**, respectively (Scheme 4).

Bis-alkylated compounds **3**, **6**, **8**, **10**, **15** and **19** were tested for the cytotoxicity against several human cancer cell lines using doxorubicin as a reference. Typical MTT assay procedure was employed for the test. The result is shown in Table 1. All compounds exhibited IC_{50} values of 2.5-14.8 μM after 48 h and bis-thiiranyl-substituted anthraquinone analog **19** showed similar IC_{50} values (2.5-5.8 μM) in all human cancer cell lines tested. Bis-oxiranyl-substituted naphthalene and carbazole analogues **3** and **8** were found to have more potent cytotoxicity in MDA-MB-231 and DU145 cell lines than the other cancer cell lines, and bis-thiiranyl-substituted benzophenone derivative **15** was more cytotoxic in LNCaP cell line than other cell lines. Table 1 indicated that bis-(2,3-epoxypropoxy) compounds, **3** and **8** have more cytotoxic than bis-(2,3-thioepoxypropoxy) compounds, **6** and **10** in cancer cell lines tested. Compounds **3**, **8**, **15** and **19** were more cytotoxic than doxorubicin and especially, compound **19** showed more cytotoxicity against MDA-MB-231 (breast cancer cell) and PC3 (prostate cancer cell) after 24 and/or 48 h. There is a possibility that the synthesized compounds intercalate into a DNA double strand due to their flat structures. Therefore, it was of great interest to survey if the cytotoxicities were derived from the inhibition of topoisomerase II after their intercalation into DNA.

In order to understand the plausible mechanism of cytotoxicity action, topoisomerase II relaxation assay with bis-alkylated compounds **3**, **6**, **8**, **15** and **19** was conducted using human topoisomerase II and etoposide as a positive control. Upon treatment with 100 μM, compounds **3**, **6**, **8** and **15** did not show clear topoisomerase II inhibitory activity, but anthraquinone derivative **19** exhibited a similar efficacy to etoposide, known as a potent topoisomerase II inhibitor (Figure 2).

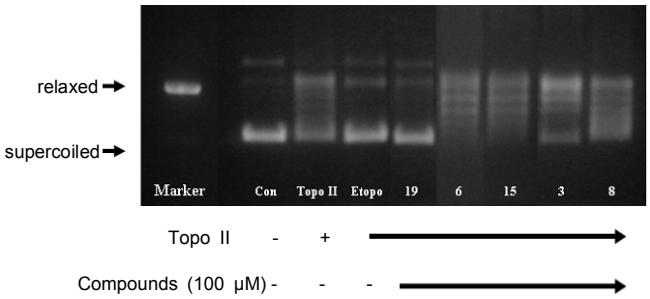


Figure 2. Effects of etoposide and compounds (**3**, **6**, **8**, **15** and **19**) on human topoisomerase II-mediated DNA at 100 μM.

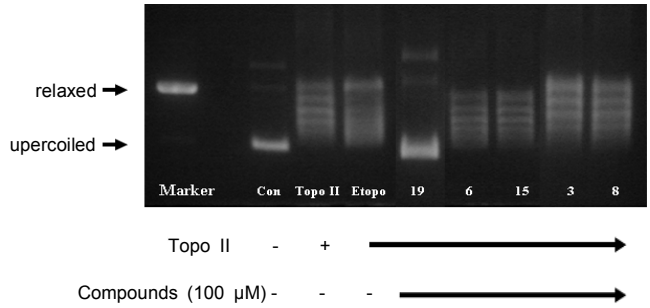


Figure 3. Effects of etoposide and compounds (**3**, **6**, **8**, **15** and **19**) on human topoisomerase II-mediated DNA at 10 μM.

As tested at lower concentration (10 μM), anthraquinone derivative **19** was proved to have more potent topoisomerase II inhibitory activity than the positive control, etoposide (Figure 3). This result indicated that cytotoxicity from the synthesized compounds might be partially related to the inhibition of topoisomerase II. Whether electrophiles such as epoxypropoxy and thioepoxypropoxy react with nucleobase of DNA or not will be published in due course in future.

In conclusion, we have synthesized eleven mono/bis-(2,3-epoxypropoxy)- and mono/bis-(2,3-thioepoxypropoxy)-phenolic compounds. They exhibited potent to moderate cytotoxicity (2.5-14.8 μM) against four cancer cell lines and especially, compound **19** showed more cytotoxicity against MDA-MB-231 (breast cancer cell) and PC3 (prostate cancer cell) than doxorubicin, a positive reference, after 24 and/or 48 h. According to the result of topoisomerase II inhibition experiment, cytotoxicity of the synthesized compounds was partially attributed to their topoisomerase II inhibition. Bis-thiiranyl anthraquinone derivative, **19** was found to have more potent topoisomerase II inhibitory activity than etoposide. These results give that flat phenolic compounds with oxiranyl and thiiranyl substituents can be considered as promising anticancer agents.

Experiments

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity INOVA 400 and Varian Unity AS 500 instruments. Chemical shifts are reported with reference to the respective residual solvent or deuteriated peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 7.27 and δ_C 77.0 for $CDCl_3$). Coupling constants are reported in hertz. The abbreviations used are as follows: s (singlet), d (doublet), m (multiplet), t (triplet), dd (doublet of doublet), br s (broad singlet). All the reactions described below were performed under argon or nitrogen atmosphere and monitored by TLC. All anhydrous solvents were distilled over CaH_2 or Na/benzophenone prior to use.

2,2'-(Naphthalene-1,7-diylbis(oxy))bis(methylene)dioxirane (3). To a stirred solution of 1,7-dihydroxynaphthalene (163.8 mg, 1.02 mmol) in DMF (5 mL) were added potassium carbonate (424.0 mg, 3.07 mmol) and epichlorohydrin (0.4 mL, 1.02 mmol) at room temperature. After being stirred at 70 °C for 5.5 h, aqueous ammonium chloride solution was added to the reaction mixture. The reaction mixture was partitioned between diethyl ether and water and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexane and ethyl acetate (3:1) as the eluent to give the bis-oxiranyl naphthalene **3** (29.5 mg, 11%) as a white solid with a mixture of monooxiranyl compounds, **1** and **2** (7%, the ratio of monooxiranyl compounds = 6:1); **compound 3:** mp 71.0-72.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, 1 H, *J* = 8.8 Hz, 5-H), 7.56 (d, 1 H, *J* = 2.4 Hz, 8-H), 7.38 (d, 1 H, *J* = 7.6 Hz, 4-H), 7.23 (t, 1 H, *J* = 8.0 Hz, 3-H), 7.19 (dd, 1 H, *J* = 2.8, 8.8 Hz, 6-H), 6.79 (d, 1 H, *J* = 8.0 Hz, 2-H), 4.42-4.35 (m, 2 H, 1′-*H*H, 1″-*H*H), 4.10-4.05 (m, 2 H, 1′-H*H*, 1″-H*H*), 3.50-3.46 (m, 1 H, 2′-H), 3.44-3.40 (m, 1 H, 2″-H), 2.97-2.92 (m, 2 H, 3′-*H*H, 3″-*H*H), 2.83-2.80 (m, 2 H, 3′-H*H*, 3″-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.52, 153.59, 130.37, 129.48, 126.57, 123.76, 120.93, 119.45, 105.98, 101.59, 69.50, 69.05, 50.53, 50.36, 45.05, 44.97; LRMS (FAB+) *m/z* 272 $(M+H)^+$; HRMS (FAB+) *m/z* $C_{16}H_{16}O_4$ $(M+H)^+$ calcd 272.1049, obsd 272.1052; **major monooxiranyl product:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, 1 H, *J* = 8.5 Hz, Ar-H), 7.60 (d, 1 H, *J* = 2.5 Hz, Ar-H), 7.39 (d, 1 H, *J* = 8.5 Hz, Ar-H), 7.21 (t, 1 H, *J* = 8.5 Hz, Ar-H), 7.13 (dd, 1 H, *J* = 3.0, 9.0 Hz, Ar-H), 6.78 (d, 1 H, *J* = 7.5 Hz, Ar-H), 5.67 (s, 1 H, OH), 4.40 (dd, 1 H, *J* = 3.0, 11.5 Hz, 1′-*H*H), 4.10 (dd, 1 H, *J* = 6.0, 11.0 Hz, 1′-H*H*), 3.50 (m, 1 H, 2′-H), 3.00 (t, 1 H, *J* = 4.5 Hz, 3′-*H*H), 2.87 (dd, 1 H, *J* = 2.5, 5.0 Hz, 3′-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.72, 153.25, 130.05, 129.72, 126.89, 123.37, 121.07, 118.45, 105.92, 104.42, 69.07, 50.77, 45.07.

2,2'-(Naphthalene-1,7-diylbis(oxy))bis(methylene)dithiirane (6). To a stirred solution of 1,7-dihydroxynaphthalene (59.4 mg, 0.37 mmol) in DMF (2 mL) were added potassium carbonate (102.5 mg, 0.74 mmol) and epithiochlorohydrin (0.13 mL, 1.48 mmol) at room temperature. After being stirred at 70 °C for 3 h, aqueous ammonium chloride solution was added to the reaction mixture. The reaction mixture was partitioned between diethyl ether and water and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexane and ethyl acetate (10:1) as the eluent to give the bis-thiiranyl naphthalene **6** (25.7 mg, 23%) as a white solid with a mixture of monothiiranyl compounds, **4** and **5** (17%, the ratio of monooxiranyl compounds = 4:1); **compound 6:** mp 73.2-75.0

°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, 1 H, *J* = 8.8 Hz, 5-H), 7.56 (d, 1 H, *J* = 2.4 Hz, 8-H), 7.39 (d, 1 H, *J* = 7.6 Hz, 4-H), 7.23 (t, 1 H, *J* = 8.0 Hz, 3-H), 7.18 (dd, 1 H, *J* = 2.8, 9.2 Hz, 6-H), 6.79 (d, 1 H, *J* = 8.0 Hz, 2-H), 4.38-4.32 (m, 2 H, 1′-*H*H, 1″-*H*H), 4.13-4.04 (m, 2 H, 1′-H*H*, 1″-H*H*), 3.40 (quintet, 1 H, *J* = 6.0 Hz, 2′-H), 3.34 (quintet, 1 H, *J* = 6.0 Hz, 2″-H), 2.65 (t, 2 H, *J* = 6.0 Hz, 3′-*H*H, 3″-*H*H), 2.39 (d, 2 H, *J* = 4.8 Hz, 3′-H*H*, 3″-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.46, 153.54, 130.42, 129.55, 126.65, 123.82, 120.92, 119.30, 106.22, 101.94, 73.15, 72.85, 31.79, 31.70, 24.31, 24.25; **major monothiiranyl product:** ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, 1 H, *J* = 9.0 Hz, Ar-H), 7.62 (d, 1 H, *J* = 2.5 Hz, Ar-H), 7.40 (d, 1 H, *J* = 7.5 Hz, Ar-H), 7.21 (t, 1 H, *J* = 8.0 Hz, Ar-H), 7.13 (dd, 1 H, *J* = 3.0, 9.0 Hz, Ar-H), 6.77 (d, 1 H, *J* = 7.5 Hz, Ar-H), 5.32 (s, 1 H, OH), 4.33 (dd, 1 H, *J* = 5.5, 10.0 Hz, 1′-*H*H), 4.12 (dd, 1 H, *J* = 7.0, 10.0 Hz, 1′-H*H*), 3.39 (m, 1 H, 2′-H), 2.65 (d, 1 H, *J* = 6.0 Hz, 3′-*H*H), 2.87 (dd, 1 H, *J* = 1.0, 5.5 Hz, 3′-H*H*).

2-(Oxiran-2-ylmethoxy)-9*H*-carbazole (7). A mixture of 2-hydroxycarbazole (100 mg, 0.54 mmol), K_2CO_3 (188 mg, 1.36 mmol) and epichlorohydrin (0.42 mL, 5.4 mmol) in acetone (3 mL) was stirred at room temperature for 24 h. The mixture was diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue on chromatographic separation (CH_2Cl_2/MeOH = 100:1) gave **7** (64 mg, 49%) as a yellow amorphous solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.12 (s, 1 H, NH), 7.96 (m, 2 H, 4-H, 5-H), 7.40 (d, 1 H, *J* = 8.0 Hz, 8-H), 7.26 (t, 1 H, *J* = 7.2 Hz, 7-H), 7.08 (irregular t, 1 H, *J* = 6.8, 8.0 Hz, 6-H), 6.97 (s, 1 H, 1-H), 6.77 (d, 1 H, *J* = 8.0 Hz, 3-H), 4.38 (dd, 1 H, *J* = 1.2, 11.2 Hz, 1′-*H*H), 3.88 (dd, 1 H, *J* = 6.4, 11.2 Hz, 1′-H*H*), 3.56 (m, 1 H, 2′-H), 2.85 (dd, 1 H, *J* = 4.0, 4.8 Hz, 3′-*H*H), 2.73 (dd, 1 H, *J* = 2.4, 4.8 Hz, 3′-H*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.05, 141.62, 140.44, 124.90, 123.25, 121.63, 119.98, 119.23, 117.15, 111.30, 108.63, 96.02, 69.90, 50.54, 44.48.

2-(Oxiran-2-ylmethoxy)-9-(oxiran-2-ylmethyl)-9*H*-carbazole (8). To a stirred solution of **7** (60 mg, 0.25 mmol) in DMF (2 mL) was added NaH (30 mg of 60% oil dispersion, 0.75 mmol). After stirring for 10 min, epichlorohydrin (0.1 mL, 1.25 mmol) was added. The reaction was stirred at 75 °C for 2 h, diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. Column chromatography (hexane/ethyl acetate = 2:1) gave **8** (30 mg, 40%) as an amorphous yellow solid: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 1 H, *J* = 7.6 Hz, 5-H), 7.94 (d, 1 H, *J* = 8.4 Hz, 4-H), 7.39 (m, 2 H, 7-H, 8-H), 7.22 (m, 1 H, 6-H), 6.97 (br s, 1 H, 1-H), 6.87 (dd, 1 H, *J* = 2.0, 8.4 Hz, 3-H), 4.56 (dd, 1 H, *J* = 3.2, 15.6 Hz, 1′-*H*H), 4.34 (td, 1 H, *J* = 2.8, 11.2 Hz, 1″-*H*H), 4.29 (dd, 1 H, *J* = 4.8, 16.0 Hz, 1′-H*H*), 4.07 (ddd, 1 H, *J* = 2.8, 5.6, 10.8 Hz, 1″-H*H*), 3.42 (m, 1 H, 2′-H), 3.32 (m, 1 H, 2″-H), 2.94 (t, 1 H, *J* = 4.4 Hz, 3′-*H*H), 2.82 ~ 2.80 (m, 2 H, 3′-H*H*, 3″-*H*H), 2.55 (m, 1 H, 3″-H*H*); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 158.21, 142.21, 141.09, 124.98, 123.32, 121.33, 119.81, 119.78, 117.55, 108.78, 108.30, 94.76, 69.55, 50.76, 50.50, 45.49, 45.07, 44.82.

2-(Thiiran-2-ylmethoxy)-9*H*-carbazole and 2-(Thiiran-2-ylmethoxy)-9-(thiiran-2-ylmethyl)-9*H*-carbazole (9 and 10). To a stirred solution of 2-hydroxycarbazole (100 mg, 0.54 mmol) in DMF (3 mL) were added K_2CO_3 (188 mg, 1.36 mmol) and epithiochlorohydrin (0.18 mL, 2.1 mmol). After stirring at 70 °C for 6 h, the reaction mixture was diluted with ethyl acetate and water. The organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue on chromatographic separation (CH_2Cl_2/hexane = 1:2) gave **9** (33 mg, 19%) and **10** (20 mg, 14%) as amorphous yellow solids: **compound 9:** ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1 H, NH), 7.98 (d, 1 H, *J* = 9.0 Hz, 5-H), 7.96 (d, 1 H, *J* = 9.0 Hz, 4-H), 7.40 (d, 1 H, *J* = 8.0 Hz, 8-H), 7.27 (td, 1 H, *J* = 1.0, 7.5 Hz, 7-H), 7.09 (td, 1 H, *J* = 1.0, 8.0 Hz, 6-H), 6.97 (d, 1 H, *J* = 1.5 Hz, 1-H), 6.79 (dd, 1 H, *J* = 1.5, 9.0 Hz, 3-H), 4.23 (dd, 1 H, *J* = 6.0, 10.5 Hz, 1′-*H*H), 4.07 (dd, 1 H, *J* = 6.5, 10.0 Hz, 1′-H*H*), 3.39 (m, 1 H, 2′-H), 2.68 (br d, 1 H, *J* = 6.5 Hz, 1 H, 3′-*H*H), 2.5 (br d, 1 H, *J* = 6.5 Hz, 3′-H*H*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.91, 141.60, 140.44, 124.90, 123.22, 121.63, 119.98, 119.22, 117.18, 111.30, 108.67, 96.14, 73.07, 33.13, 24.49; **compound 10:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, 1 H, *J* = 7.6 Hz, 5-H), 7.95 (d, 1 H, *J* = 8.0 Hz, 4-H), 7.40 (m, 2 H, 7-H, 8-H), 7.22 (m, 1 H, 6-H), 6.94 (br s, 1 H, 1-H), 6.86 (dd, 1 H, *J* = 2.0, 8.0 Hz, 3-H), 4.53 (dd, 1 H, *J* = 6.0, 15.6 Hz, 1′-*H*H), 4.43-4.30 (m, 2 H, 1′-H*H*, 1″-*H*H), 4.02 (td, 1 H, *J* = 6.8, 10.4 Hz, 1″-H*H*), 3.36-3.25 (m, 2 H, 2′-H, 2″-H), 2.64 (d, 1 H, *J* = 6.4 Hz, 3′- *H*H), 2.48 (d, 1 H, *J* = 6.4 Hz, 3′-H*H*), 2.42 (dd, 1 H, *J* = 1.2, 5.2 Hz, 3″-*H*H), 2.38 (d, 1 H, *J* = 5.2 Hz, 3″-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.11, 141.75, 140.68, 124.99, 123.39, 121.49, 119.91, 119.81, 117.68, 108.79, 108.32, 94.84, 73.46, 48.73, 32.04, 31.72, 24.31, 24.30.

(2-Hydroxy-4-(oxiran-2-ylmethoxy)phenyl)(phenyl)methanone (11). To a stirred solution of 2,4-dihydroxybenzophenone (100 mg, 0.47 mmol) in acetone (2 mL) and DMF (1 mL) were added potassium carbonate (129 mg, 0.93 mmol) and epichlorohydrin (0.11 mL, 1.40 mmol) at room temperature. After being stirred at 75 °C for 3 h, the reaction mixture was extracted with ethyl acetate and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using only methylene chloride as the eluent to give the monooxiranyl benzophenone **11** (6.8 mg, 5%) as a white solid: mp 94.6-96.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.64 (s, 1 H, 2-OH), 7.62-7.43 (m, 6 H, Ph, 6-H), 6.50 (d, 1 H, *J* = 3.2 Hz, 3-H), 6.43 (dd, 1 H, *J* = 2.4, 8.8 Hz, 5-H), 4.30 (dd, 1 H, *J* = 2.8, 11.2 Hz, 1′-*H*H), 3.96 (dd, 1 H, *J* = 6.4, 10.8 Hz, 1′-H*H*), 3.75-3.32 (m, 1 H, 2′-H), 2.91 (t, 1 H, *J* = 4.4 Hz, 3′-*H*H), 2.75 (dd, 1 H, *J* = 2.8, 4.4 Hz, 3′-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.31, 166.41, 165.09, 138.37, 135.58, 131.77, 129.07, 128.54, 113.69, 107.82, 101.99, 69.19, 49.92, 44.80.

(2-Hydroxy-4-(thiiran-2-ylmethoxy)phenyl)(phenyl)methanone (14) and (2,4-bis(thiiran-2-ylmethoxy)phenyl)(phenyl) methanone (15). To a stirred solution of 2,4-dihydroxybenzophenone (67.0 mg, 0.31 mmol) in DMF (2 mL) were added potassium carbonate (86.5 mg, 0.63 mmol) and epithiochlorohydrin (0.11 mL, 1.25 mmol) at room temperature. After being stirred at 70 °C for 1 h, aqueous ammonium chloride

solution was added to the reaction mixture. The reaction mixture was partitioned between diethyl ether and water and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexane and ethyl acetate (10:1) as the eluent to give the monothiiranyl benzophenone **14** (26.8 mg, 30%) and bis-thiiranyl benzophenone **15** (35.2 mg, 31%), respectively, as white solids: **compound 14:** mp 83.3-84.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.64 (s, 1 H, 2-OH), 7.62-7.46 (m, 6 H, Ph, 6-H), 6.49 (d, 1 H, *J* = 2.4 Hz, 3-H), 6.41 (dd, 1 H, *J* = 2.4, 8.8 Hz, 5-H), 4.20 (dd, 1 H, *J* = 6.0, 10.0 Hz, 1′-*H*H), 3.99 (dd, 1 H, *J* = 6.8, 10.0 Hz, 1′-H*H*), 3.26 (quintet, 1 H, *J* = 6.0 Hz, 2′-H), 2.61 (d, 1 H, *J* = 6.4 Hz, 3′-*H*H), 2.32 (dd, 1 H, *J* = 1.2, 5.2 Hz, 3′-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.26, 166.44, 165.01, 138.39, 135.62, 131.78, 129.08, 128.55, 113.67, 107.70, 102.01, 72.92, 31.04, 23.97; **compound 15:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.39 (m, 6 H, Ph, 6-H), 6.56 (dd, 1 H, *J* = 2.0, 8.8 Hz, 5-H), 6.45 (d, 1 H, *J* = 2.0 Hz, 3-H), 4.18 (dd, 1 H, *J* = 5.6, 10.0 Hz, 1′-*H*H), 4.11(dd, 1 H, *J* = 5.2, 10.0 Hz, 1″-*H*H), 3.99 (dd, 1 H, *J* = 6.8, 10.4 Hz, 1′-H*H*), 3.70 (dd, 1 H, *J* = 7.2, 10.4 Hz, 1″-H*H*), 3.26 (quintet, 1 H, *J* = 6.0 Hz, 2′-H), 2.79 (quintet, 1 H, *J* = 6.0 Hz, 2″-H), 2.61 (d, 1 H, *J* = 6.0 Hz, 3′-*H*H), 2.32 (dd, 1 H, *J* = 1.2, 5.2 Hz, 3′-H*H*), 2.26 (d, 1 H, *J* = 5.6 Hz, 3″-*H*H), 1.95 (dd, 1 H, *J* = 1.2, 5.6 Hz, 3″-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.97, 162.36, 158.45, 139.48, 132.55, 129.57, 128.31, 122.53, 106.43, 100.57, 73.18, 72.96, 31.31, 30.70, 23.97, 23.81.

1-Hydroxy-8-(2-(thiiran-2-yl)ethoxy)anthracene-9,10-dione (18) and 1,8-bis(2-(thiiran-2-yl)ethoxy)anthracene-9,10-dione (19). To a stirred solution of 1,8-dihydroxyanthraquinone (55.6 mg, 0.23 mmol) in DMF (2 mL) were added potassium carbonate (64.0 mg, 0.46 mmol) and epithiochlorohydrin (0.08 mL, 0.92 mmol) at room temperature. After stirred at 70 °C for 7 h, aqueous ammonium chloride solution was added to the reaction mixture. The reaction mixture was partitioned between diethyl ether and water and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting residue was purified by silica gel column chromatography using hexane and ethyl acetate (4:1) as the eluent to give the monothiiranyl anthraquinone **18** (7.0 mg, 10%) and bis-thiiranyl anthraquinone **19** (11.2 mg, 13%), respectively, as brownish solids: **compound 18:** mp 118.9-120.2 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.98 (s, 1 H, 8-H), 8.00 (dd, 1 H, *J* = 1.0, 8.0 Hz, 5-H), 7.78 (dd, 1 H, *J* = 1.0, 7.5 Hz, 4-H), 7.72 (t, 1 H, *J* = 6.4 Hz, 6-H), 7.63 (t, 1 H, *J* = 6.4 Hz, 3-H), 7.34 (dd, 1 H, *J* = 1.0, 8.5 Hz, 2-H), 7.30 (dd, 1 H, *J* = 1.0, 8.5 Hz, 7-H), 4.47 (dd, 1 H, *J* = 5.0, 10.0 Hz, 1′-*H*H), 4.15 (dd, 1 H, *J* = 7.0, 10.0 Hz, 1′-H*H*), 3.47-3.42 (m, 1 H, 2′-H), 2.08 (dd, 1 H, *J* = 1.0, 5.5 Hz, 3′-*H*H), 2.51 (dd, 1 H, *J* = 1.5, 5.5 Hz, 3′-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.79, 182.85, 162.67, 159.84, 136.12, 135.99, 135.86, 132.86, 124.97, 121.17, 120.73, 119.10, 117.23, 117.09, 74.37, 31.28, 24.20; **compound 19:** mp 136.5-138.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (dd, 2 H, *J* = 1.2, 7.2 Hz, 4-H, 5-H), 7.60 (t, 2 H, *J* = 7.6 Hz, 3-H, 6-H), 7.29 (d, 2 H, *J* = 7.6 Hz, 2-H, 7-H), 4.44-4.39 (m, 2 H, 1′-*H*H, 1″-*H*H), 4.10-4.05 (m, 2 H, 1′-H*H*, 1″-H*H*), 3.44-3.38 (m, 2 H, 2′-H, 2″-H), 2.64 (d, 2 H, *J* = 6.4 Hz, 3′-*H*H, 3″-*H*H), 2.49 (dd, 2 H, *J* = 1.2, 5.2 Hz, 3′-H*H*, 3″-H*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.85, 158.42, 135.06, 134.06, 125.36, 121.66, 120.46, 74.74, 31.54, 24.28; LRMS (FAB+) m/z 385 $(M+H)^+$.

Cell line and culture. LNCaP, MDA-MB-231, PC3 and DU145 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). The cells were grown in DMEM (Gibco® Invitrogen Corporation, Carlsbad, CA) media containing 10% heat-inactivated fetal bovine serum (FBS), 1.25 mM HEPES and 1% penicillin/streptomycin.

Cytotoxicity assays. Cytotoxicity was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described. Briefly, cells were seeded in 96-well microtiter plates at a density of 1×10^3 cells per well. Cells were allowed to attach for 48 h before exposure to compounds. At the end of the treatment period, 15 μL of the MTT (5 mg/mL) reagent was added to each well. After 4 h incubation at 37 °C, the supernatant was aspirated, and formazan crystals were dissolved in 100 μL DMSO at 37 °C for 10 min with gentle agitation. The absorbance per well was measured at 540 nm with a VERS Amax Microplate Reader (Molecular Devices Corp.). Assay was done in triplicate. The IC_{50} values were then determined for each drugs from a plot of log (drug concentration) versus percentage of cell killed.

Topoisomerases II assay. The inhibitory effect of the synthesized compounds on topoisomerases II catalytic activity was performed by using Topoisomerases II Drug Screening Kit (TopoGEN, INC) according to manufacturer's instruction. This assay measures the relaxations of supercoiled pRYG DNA by Topo II enzyme in presence or absence of the synthesized compounds and etoposide as a positive control. For the relaxation assay 250 ng/μL supercoiled pRYG DNA combined with 4 unit Topo II enzyme, double distilled water, assay buffer containing 0.5 M Tris-HCl (pH 8), 1.5 M NaCl, 100 mM $MgCl_2$, 5 mM dithiothreitol, 300 μg BSA/mL, and 20 mM ATP, and the synthesized compounds. Reactions were incubated for 45 min at 37 °C and were terminated by the addition of 10% SDS (1/10 vol.). Topoisomerases II were digested (incubate for 15 min at 37 °C) with 50 μg/mL proteinase K. The reactions were separated with loading buffer (1/10 vol.) on a 1% agarose gel in 1xTAE buffer at 25 V for 2-3 h. The DNA was stained with ethidium bromide (0.5 μg/mL) and visualized under UV light.

Statistical analysis. All the data is represented as the average of the values obtained ± SD. Significance was determined using a paired Student's *t*-test. A p-value < 0.01 was considered statistically significant.

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