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Oxiranylmethyloxy or thiiranylmethyloxy-azaxanthones and -acridone analogues as potential topoisomerase I inhibitors

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ABSTRACT

A total of seven new oxyranylmethyloxy or thiiranylmethyloxy group substituted 5-azaxanthones and -acridones analogues were synthesized and tested for their biological activities for cancer cell lines and topoisomerases. Among the compounds, compound **5**, 3-thiiranylmethyloxy-1-hydroxy-5-azaxanthone, showed effective topoisomerase I inhibitory activity, 50% and 27% inhibition ratio at 100 and 20 μM, respectively. This result is the first finding of the function of 5-azaxanthone compounds for topoisomerase I inhibition and can provide a novel skeleton for the anticancer drug development process.

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Topoisomerases, generally classified as types I and II, are critical cellular enzymes necessary for cell proliferation by solving topological hurdles in the process of DNA replication.[1] Topoisomerase I mediates the breaking and rejoining of single strand of DNA duplex to relax the supercoiled condition of chromosomes. On the other hand, topoisomerase II produces the relaxation of DNA double helices by scissoring and religating two strands. Due to the critical role of these enzymes for the cell proliferative process, topoisomerases have been one of the major targets in the anticancer drug development area. To date, numerous compounds have been reported or commercialized for cancer therapy via topoisomerases inhibitory activity, such as doxorubicin and camptothecin. Among these compounds, xanthone derivatives have been known to belong to a group of representative topoisomerase II inhibitors.

Some acridone compounds, such as 7-chloro-1,3-dihydroxyacridone (**1**) and 1,3,7-trihydroxyacridone (**2**), are also known to be effective topoisomerase II inhibitory agents. Compound **1** was also considered selective inhibitors of herpes simplex virus (HSV) replication in the cell culture system.[2] Other acridone derivatives bearing carboxamides were suggested to exert anti-herpes activity through topoisomerase II inhibitory activity.[3] Azapyranoxanthone analogues (**3**) have also been prepared and reported for their antitumor activities against HT-29 cancer cells.[4] The results showed that replacement with aza group from parent compounds changed biological activities.

Previously, we reported the biological experimental results of some epoxy group tethered xanthone analogues.[5] These compounds showed significant topoisomerase II inhibitory activities and bis-oxiran substituted compound (**4**) was the most efficient among the compounds tested. As a continuous work, we designed and synthesized isosteric analogues of xanthone cores bearing oxiran or thiiran groups as substituents to test their biological activity alteration compared to parent compounds. For this purpose, we prepared a series of thiiranylmethyloxy and oxyranylmethyloxy-5-azaxanthone or -acridone analogues as target compounds and tested their topoisomerases I and II inhibitory activities.

Synthetic methods for target compounds are described in Scheme 1. First, 5-aza-1,3-dihydroxyxanthone was prepared with the modified method previously reported.[4] Coupling of this compound with epichlorohydrin or epithiochlorohydrin as electrophiles under Cs_2CO_3 basic condition in acetone provided the desired compounds. To accomplish the reaction, the ratio of Cs_2CO_3 (2–3 equiv) and electrophiles (2–12 equiv) were controlled. In ^{1}H NMR spectrum, compounds **5** and **7** showed a singlet peak around δ 12.0 corresponding to C1–O*H*, which confirms mono substitution of electrophiles at C3-oxygen. On the contrary, in compound **6**, we observed disappearance of proton peak of C1–O*H* and two sets of proton peaks corresponding to thiiranylmethyloxy- (δ 2.37–4.44) or oxiranylmethyloxy- (δ 2.81–4.43) protons in the aliphatic region. All other spectral data supported the supposed structures.

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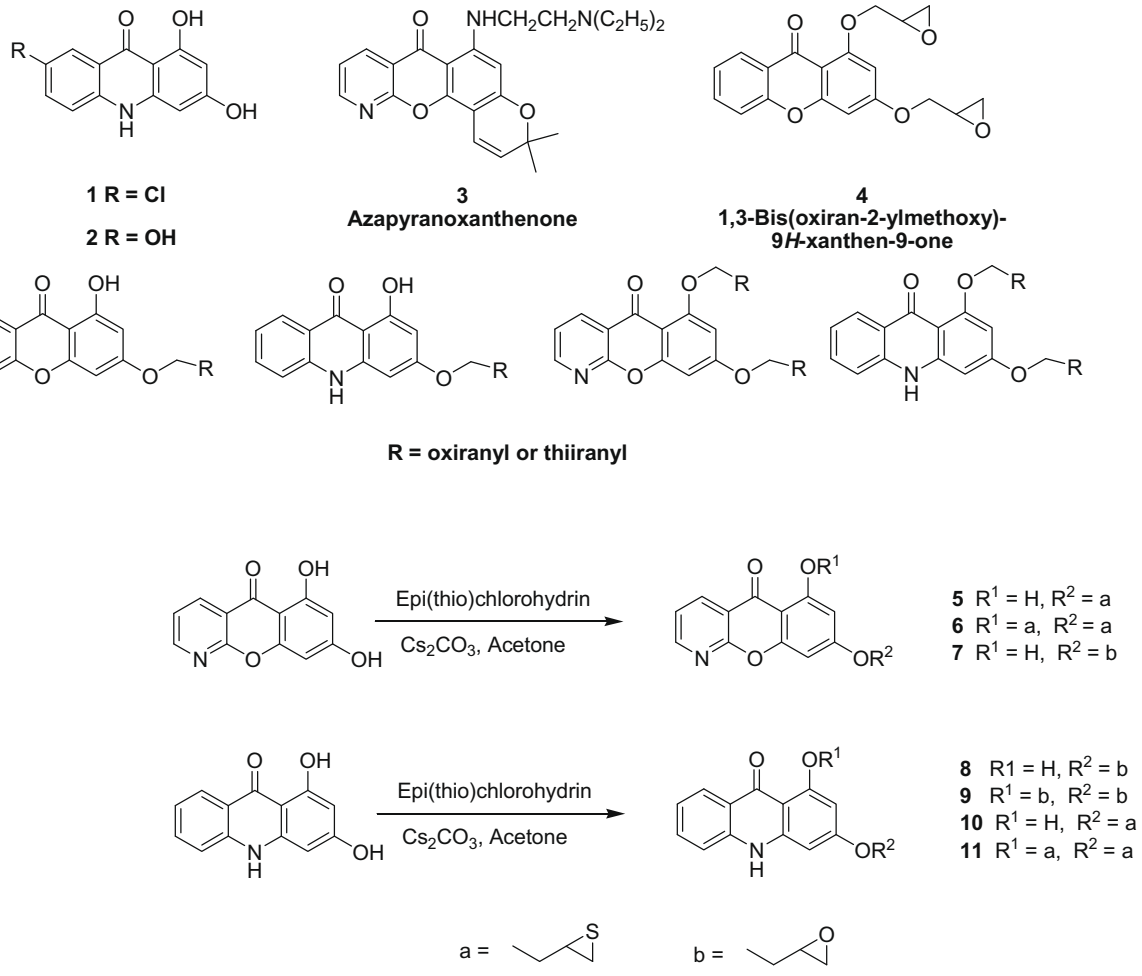

Scheme 1. Synthetic method for compounds **5**–**11**.

Table 1
Cytotoxicities of compounds **5**–**11** against various cancer cells

Compd/cells	IC_{50}[a] (μM)				
	HeLa	HT-29	DU 145	MDA-MB231	HL-60
Adriamycin	1.08 ± 0.01	3.90 ± 0.08	2.54 ± 0.60	1.67 ± 0.04	0.88 ± 0.02
Camptothecin	0.58 ± 0.04	1.07 ± 0.02	1.00 ± 0.03	0.68 ± 0.01	0.067 ± 0.00
Etoposide	2.15 ± 0.37	6.32 ± 0.10	5.16 ± 0.05	3.50 ± 0.11	0.93 ± 0.03
5	6.90 ± 0.14	>50	15.81 ± 1.31	18.17 ± 0.37	20.05 ± 0.42
6	9.96 ± 0.18	>50	21.00 ± 1.17	13.02 ± 0.36	10.45 ± 0.48
7	22.05 ± 0.12	26.32 ± 0.54	8.11 ± 1.78	24.34 ± 0.47	24.76 ± 0.90
8	>50	>50	>50	18.59 ± 0.29	12.53 ± 0.57
9	>50	29.26 ± 0.72	30.94 ± 1.61	28.86 ± 0.49	22.02 ± 0.25
10	>50	33.01 ± 2.84	23.00 ± 0.85	21.35 ± 0.52	22.97 ± 0.56
11	11.12 ± 0.11	23.19 ± 1.03	23.21 ± 1.39	8.02 ± 0.20	7.73 ± 0.40

[a] Each data point represents mean ± SD from three different experiments performed in triplicate. Cell lines used are HeLa, human cervix tumor cell line; HT-29, human colorectal adenocarcinoma cell line; DU 145, human prostate tumor cell line; MDA-MB231, human breast tumor cell line; HL-60, human myeloid leukemic tumor cell line.

1,3-Dihydroxyacridone was prepared according to the literature method.[6] This compound was coupled with epichlorohydrin or epithiochlorohydrin by employing previous methods as well. All the spectral information obtained corresponded to the suggested structures. Analytical data for prepared compounds are indicated in the reference.[7]

Biological tests were conducted with cytotoxicity and topoisomerases I and II inhibitory activities. First, cytotoxicity of compounds **5**–**11** were tested against human cancer cell lines with adriamycin as a positive reference. Method applied for the test is typical MTT assay procedure according to the literature.[5] The -cytotoxic activities of the compounds were not very effective (Table 1). Compound **11** showed the most effective cytotoxic activity among the series compounds in the MDA-MB231 and HL-60 cell lines, but it was lower than the reference. In this test we could not pull out the structure correlation of cytotoxic activities between azaxanthone (compounds **5**–**7**) and acridone compounds (compounds **8**–**11**).

Topoisomerase relaxation assay was conducted using human topoisomerases I and II (Topogen) with camptothecin and etoposide as positive controls. The data were analyzed and calculated with LABWORK 4.5 Software for the inhibition ratio. Most compounds were inactive to topoisomerase II relaxation activity. But, interestingly, compound **5** effectively inhibited the topoisomerase I function with 50% and 27% inhibition ratios at 100 and 20 μM, respectively (Fig. 1 and Table 2). Because xanthone analogues are well-known conventional topoisomerase II inhibitors, this result is quite surprising. Although inhibitory activity of compound **5** is lower than that of camptothecin, this result implicates that azaxanthone cores can be possible topoisomerase I inhibitor resources with elaborate calibration of substituents. Without further mechanism study, we suspect that the dramatic conversion from topo II to topo I inhibition of compound **5** compared to 1,3-bis(oxyranylmethyloxy)-9*H*-xanthen-9-one (**4**) we previously reported and other traditional xanthone analogues might be caused by a combination effect of two factors. One is electronic property change by replacement of carbon in xanthone with nitrogen isostere and the other is binding ability difference between thiiran and oxiran ring, which change the electronic interaction between azaxanthone analogues and topo enzymes or DNA during ternary complex formation.

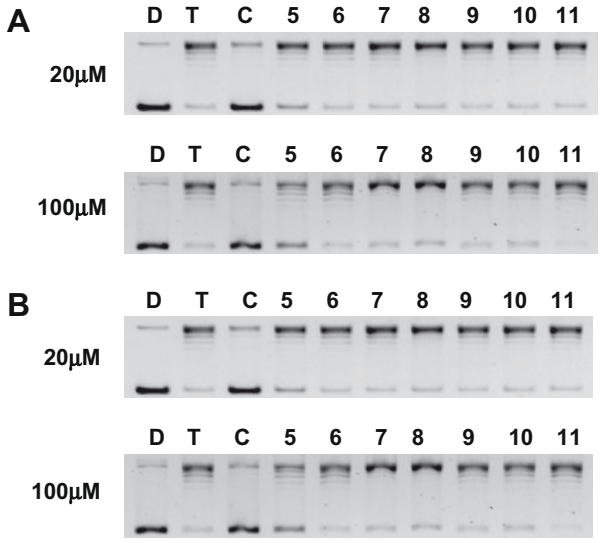

Figure 1. Topoisomerase I (A) and II (B) inhibitory activities of compounds. Compounds were examined in a final concentration of 20 and 100 μM, respectively. (A) Lane D: pBR322 only, Lane T: pBR322 + Topo I, Lane C: pBR322 + Topo I + camptothecin, Lanes 1–7: pBR322 + Topo I + compounds in designated concentrations. (B) Lane D: pBR322 only, Lane T: pBR322 + Topo II, Lane E: pBR322 + Topo II + etoposide, Lanes 1–7: pBR322 + Topo II + compounds in designated concentrations.

Table 2
Topoisomerases I and II inhibitory activities of compounds **5**–**11**

Compounds	Topo I (% inhibition)		Topo II (% inhibition)	
	20 μM	100 μM	20 μM	100 μM
Camptothecin	67.1	84.5	—	—
Etoposide	—	—	13.4	55.4
5	26.7	50.3	9.2	19.5
6	5.2	0.0	8.6	6.7
7	1.8	0.0	8.3	30.3
8	10.8	9.1	12.9	18.1
9	2.5	0.0	8.7	13.2
10	0.0	2.3	8.1	8.7
11	0.0	0.0	8.5	6.9

The values of % inhibition are the means from at least three independent experiments. The mark '—' indicates that the experiment was not performed.

Acridone analogues we tested have not shown any inhibitory activities against topoisomerase II, which suggests that substituents on 1 or 3-hydroxyl oxygen in acridone cores might control the topoisomerase II inhibitory activities. This result also implied the importance of xanthone core to retain topo II inhibitory activity. Compound **11** which has same structure with compound **4** except nitrogen isostere of oxygen in xanthone core completely lost topo II inhibitory activity. This observation also might implicate the importance of electronic interaction of oxygen in xanthone in the topoisomerases involved DNA relaxation process.

In conclusion, we prepared seven new acridone and azaxanthone analogues tethered with thiranylmethyloxy or oxiranylmethyloxy at C1 and C3-oxygen. Among this series, compound **5** exhibited significant topoisomerase I inhibitory activity. This finding can provide novel skeletons which are effective for topoisomerase I function in the anticancer drug development process.

Acknowledgments

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7. *Compound* **5**: Yield: 17.7%; mp 150–152 °C; R_f 0.74 (EtOAc:*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (d, J = 6.0 Hz, 1H), 2.65 (d, J = 6.0 Hz, 1H), 3.27–3.33 (m, 1H), 4.05 (dd, J = 10.0, 6.8 Hz, 1H), 4.27 (dd, J = 10.0, 4.4 Hz, 1H), 6.40 (s, 1H), 6.58 (s, 1H), 7.45 (dd, J = 7.8, 4.4 Hz, 1H), 8.63 (dd, J = 7.8, 2.0 Hz, 1H), 8.72 (dd, J = 4.4, 2.0 Hz, 1H), 12.49 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 23.9, 30.8, 73.3, 94.5, 98.2, 104.1, 116.0, 121.4, 136.7, 154.5, 157.4, 160.5, 163.8, 166.1, 181.1 ppm; EI-MS (*m*/*z*) 301 $[M]^+$.*Compound* **6**: Yield: 5.1%; mp 160–161 °C; R_f 0.47 (EtOAc:*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (dd, J = 4.8, 1.6 Hz, 1H), 2.53 (d, J = 4.8 Hz, 1H), 2.66 (d, J = 6.4 Hz, 1H), 2.71 (d, J = 6.4 Hz, 1H), 3.28–3.31 (m, 1H), 3.44–3.47 (m, 1H), 4.00 (dd, J = 10.0, 7.2 Hz, 1H), 4.10 (dd, J = 10.0, 6.8 Hz, 1H), 4.24 (dd, J = 10.0, 6.0 Hz, 1H), 4.44 (dd, J = 10.0, 4.8 Hz, 1H), 6.40 (d, J = 2.0 Hz, 1H), 6.62 (d, J = 2.0 Hz, 1H), 7.40 (dd, J = 7.2, 5.2 Hz, 1H), 8.64 (d, J = 5.2 Hz, 1H), 8.65 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 23.8, 24.5, 30.9, 31.1, 73.2, 73.8, 95.0, 97.7, 107.7, 118.0, 121.3, 137.5, 153.3, 159.5, 159.8, 161.0, 164.2, 175.6 ppm; EI-MS (*m*/*z*) 373 $[M]^+$.*Compound* **7**: Yield: 14.0%; mp 147–148 °C; R_f 0.61 (EtOAc:*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (dd, J = 4.0, 2.8 Hz, 1H), 2.96 (dd, J = 4.4, 4.0 Hz, 1H), 3.39–3.43 (m, 1H), 4.03 (dd, J = 11.2, 6.4 Hz, 1H), 4.36 (dd, J = 11.2, 2.8 Hz, 1H), 6.41 (d, J = 1.6 Hz, 1H), 6.59 (d, J = 1.6 Hz, 1H), 7.44 (dd, J = 8.0, 5.2 Hz, 1H), 8.64 (d, J = 8.0 Hz, 1H), 8.72 (d, J = 5.2 Hz, 1H), 12.50 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 44.8, 49.8, 69.6, 94.6, 98.3, 104.2, 116.0, 121.4, 136.7, 154.5, 157.3, 160.5, 163.8, 166.2, 181.2 ppm; EI-MS (*m*/*z*) 285 $[M]^+$.*Compound* **8**: Yield: 13.1%; mp 192–193 °C; R_f 0.52 (EtOAc:*n*-hexane = 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.74–2.76 (m, 1H), 2.87 (dd, J = 4.4, 4.4 Hz, 1H), 3.37 (dd, J = 4.0, 4.0 Hz, 1H), 3.90 (dd, J = 11.2, 6.4 Hz, 1H), 4.45 (dd, J = 11.2, 2.0 Hz, 1H), 6.19 (d, J = 1.6 Hz, 1H), 6.39 (d, J = 1.6 Hz, 1H), 7.28 (dd, J = 8.0, 7.2 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.74 (dd, J = 8.0, 7.2 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 40.5, 50.2, 69.9, 90.6, 95.3, 104.7, 117.7, 119.6, 122.3, 125.7, 134.7, 141.5, 143.8, 164.2, 164.8, 181.0 ppm; EI-MS (*m*/*z*) 283 $[M]^+$.*Compound* **9**: Yield: 24.1%; mp 201–203 °C; R_f: 0.23 (EtOAc:*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.70–2.72 (m, 1H), 2.80 (dd, J = 7.2, 4.8 Hz, 1H), 2.94–2.98 (m, 2H), 3.3–3.43 (m, 1H), 3.46–3.49 (m, 1H), 4.00–4.04 (m, 1H), 4.29 (dd, J = 16.8, 5.2 Hz, 1H), 4.40 (ddd, J = 11.2, 3.2, 2.8 Hz, 1H), 4.78 (d, J = 16.8 Hz, 1H), 6.31 (d, J = 2.0 Hz, 1H), 6.48 (d, J = 2.0 Hz, 1H), 7.31 (dd, J = 7.2, 6.8 Hz, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.73 (dd, J = 7.2, 6.8 Hz, 1H), 8.46 (dd, J = 8.0, 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 44.9, 45.3, 48.5, 50.1, 50.2, 69.4, 115.3, 122.2, 127.0, 134.6, 142.7, 144.9, 165.1, 166.3, 181.2 ppm, other peaks are not detected; EI-MS (*m*/*z*) 339 $[M]^+$.*Compound* **10**: Yield: 28.6%; mp 137–138 °C; R_f: 0.58 (EtOAc:*n*-hexane = 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.47–2.49 (m, 1H), 2.65 (d, J = 6.4 Hz, 1H), 3.34–3.37 (m, 1H), 4.04 (dd, J = 10.4, 6.8 Hz, 1H), 4.26 (dd, J = 10.4, 5.6 Hz, 1H), 6.15 (d, J = 2.0 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H), 7.26 (dd, J = 8.0, 7.2 Hz, 1H), 7.46 (d,

J = 8.0 Hz, 1H), 7.72 (dd, J = 8.0, 7.2 Hz, 1H), 8.13 (d, J = 8.0 Hz, 1H), 11.98 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 24.4, 32.6, 72.9, 90.6, 95.3, 104.7, 117.7, 119.5, 122.3, 125.7, 134.8, 141.4, 143.8, 164.2, 164.7, 181.0 ppm; EI-MS (m/z) 299 [M]$^+$.*Compound* **11**: Yield: 12.8%; mp 148–149 °C; R_f: 0.19 (EtOAc:*n*-hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.38 (dd, J = 5.2, 1.6 Hz, 1H), 2.55 (dd, J = 5.2, 1.6 Hz, 1H), 2.63–2.67 (m, 2H), 3.28–3.37 (m, 2H), 4.06–4.18 (m, 1H), 4.26 (dd, J = 10.4, 6.0 Hz,, 1H), 4.54 (dd, J = 16.4, 6.0 Hz, 1H), 4.64 (dd, J = 16.4, 5.2 Hz, 1H), 6.31 (d, J = 2.0 Hz, 1H), 6.50 (d, J = 2.0 Hz, 1H), 7.33 (dd, J = 7.2, 7.2 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.73 (dd, J = 8.0, 8.0 Hz, 1H), 8.49 (dd, J = 8.0, 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 23.9, 29.9, 30.9, 31.2, 50.8, 73.0, 91.1, 94.9, 105.6, 115.0, 121.4, 122.2, 127.3, 134.5, 142.1, 144.4, 165.1, 166.6, 181.1 ppm; EI-MS (m/z) 371 [M]$^+$.