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Synthesis and Antiproliferative Activity of Epoxy and Bromo Compounds Derived from Estrone

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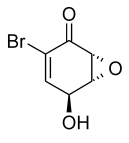
Abstract—Based on biological properties of epoxyquinols from natural sources, bromo and epoxyquinols derived from estrone were synthesized and screened against Fem-X, HeLa and K_{562} cell lines. Evidence was found that the bromine atom and the epoxy moiety significantly increase the antiproliferative activity within the series.

During our efforts aimed at incorporating bioactive substructures into the steroidal skeleton,[1] we found that oxidation of estrone, using a non-metal oxidizing system [consisting of MCPBA/$(BzO)_2$/*hν*], could afford the epoxyquinol **2** in satisfactory yield.[2]

Since structures with an epoxyquinol moiety represent an important class of biologically active compounds,[3] we proposed that transformations of epoxyquinol **2** could be a reasonable way for further research on structure–activity relationships.

Our first objective was to establish the influence of the C(3)-substituent and epoxy ring on the antitumor activity. The second one was to synthesize the steroidal analogue of bromoxone (**1**), that is, to incorporate the bromine atom into the epoxyquinol substructure. Bromoxone (**1**) and its acetate were isolated from marine acorn worms *Phylum hemichordata* (order Enteropneusta).[4] The acetylated derivative displayed strong antitumor activity toward P388 cells (IC_{50} = 49 nM).



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Here, we present a short synthesis of vinyllic bromo compounds **4** and **6** starting with epoxyquinol **2**, and the results of in vitro antitumor activity screening of all compounds given in Scheme 1.

Chemistry

Reduction of compound **2** with $NaBH_4$ in methanol at room temperature afforded triol **3** in a good yield (Scheme 1).[5] Diepoxy compound **5** was obtained after H_2O_2 epoxidation of epoxyquinol **2** under mild reaction conditions. It is important to note that the formation of the second oxirane is stereoselective, only the 1β,2β-epoxide was formed. Introduction of the bromine atom (**2**→**4**; **5**→**6**) was achieved by nucleophilic epoxide ring opening with bromide ion, and subsequent, in situ dehydration using acidic resin Amberlyst-15.[6] The reaction **5**→**6** is regioselective, and the attempt to prepare the corresponding 2,4-dibromoquinol failed.

Herewith, the preparation of compounds **4** and **5** as a typical example is given. 10β-Hydroxy-4β,5β-epoxy-2-bromoestr-1-en-3,17-dione (**6**)[7] was prepared from **5** in analogy with reaction **2**→**4**.

10β-Hydroxy-4-bromoestra-1,4-diene-3,17-dione (4). To a solution of epoxyquinol **2** (200 mg, 0.66 mmol) in acetone (5 mL), LiBr (180 mg, 2.07 mmol) and Amberlyst-15 (450 mg) were added and the suspension was stirred

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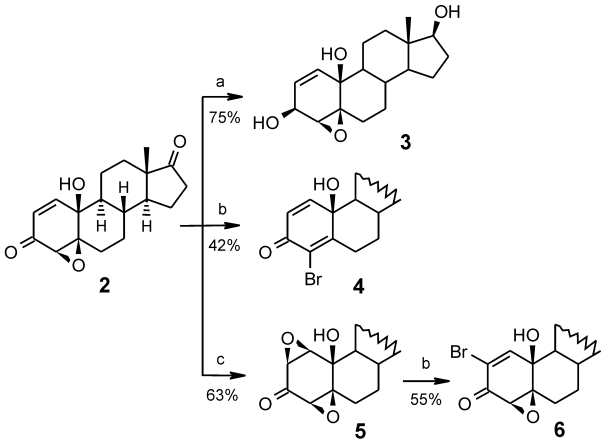
at room temperature for 6 h. After filtration and evaporation to dryness, the reaction mixture was separated using SiO_2 column chromatography. Elution with toluene/EtOAc (7:3) and crystallization from a toluene/CH_2Cl_2 mixture gave 101.7 mg (42%) of 10β-hydroxy-4-bromoestra-1,4-diene-3,17-dione (**4**) as colorless needles.[8] IR absorption bands at 3433, 1654 and 1631 cm^{-1} correspond to quinol substructure, molecular ions at 365 and 367 *m/e* in DEI mass spectrum (1:1 ratio), and peaks at 7.12 and 6.31 ppm (both doublets, $J = 10.2$ Hz) in the ^1H NMR spectrum indicate an insertion of bromine at C(4) position.

10β-Hydroxy-1β,2β,4β,5β-diepoxyestrane-3,17-dione (5). To a warm ethanolic solution of epoxyquinol **2** (1.00 g, mmol in 10 mL of EtOH) 3.6 mL of a freshly prepared solution of hydrogen peroxide in aqueous sodium carbonate (1 mL 30% H_2O_2, 0.20 g Na_2CO_3, 5 mL H_2O) was added. The stirred reaction mixture was heated to 60 °C for 15 min, diluted with water (50 mL), neutralized with dil. HCl, and extracted with $CHCl_3$ (3×20 mL). The combined organic extracts were washed with $NaHCO_3$ (20 mL) and H_2O (20 mL), dried over anhyd Na_2SO_4, filtered and evaporated. Crystallization from benzene gave 660 mg (63%) of 10β-hydroxy-1β,2β,4β,5β-diepoxyestrane-3,17-dione (**5**) as colorless needles.[9] Molecular ion at 319 *m/e* in DCI mass spectrum corresponds to diepoxide (MH^+). In the ^1H NMR spectrum appear signals at 3.84 (d, $J_{1,2} = 4.0$ Hz), 3.50 (dd, $J_{1,2} = 4.0$ Hz; $J_{2,4} = 2.4$ Hz) and 3.29 ppm (d, $J_{2,4} = 2.4$ Hz). Doublets at 3.84 and 3.29 ppm belong to H-C(1) and H-C(4) [both coupled to H-C(2)], while a doublet of doublets at 3.50 ppm belongs to H-C(2). W-coupling through carbonyl function between H-C(2) and H-C(4) is possible only if the epoxy rings are *cis*.

Antiproliferative Activity

The antiproliferative activity (IC_{50}) of the synthesized compounds was determined after 48 h exposure to various cell lines (Table 1). Cytotoxicity of some compounds with high antiproliferative action was also determined after 72 h (Table 2). The compounds were screened against malignant Fem-X and K562 cells, and against normal human peripheral blood mononuclear cells, PBMC [resting, or stimulated for proliferation with phytohaemaglutinin (PHA)].



Scheme 1. Synthesis of estrone-derived epoxy and bromo compounds. Reagents and conditions: (a) $NaBH_4$/MeOH, rt, 1 h; (b) LiBr, Amberlyst-15, acetone, rt, 6 h; (c) H_2O_2, Na_2CO_3, H_2O, EtOH, 60 °C, 15 min.

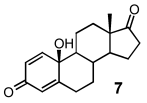
Cell culture

Human cervix carcinoma, HeLa cells, human melanoma Fem-x cells, were maintained as a monolayer culture, while human myelogenous leukemia, K562 cells were grown as a suspension culture in the same nutrient medium [RPMI 1640 medium supplemented with L-glutamine (3 mmol/L), streptomycin (100 μg/mL), and penicillin (100 IU/mL), 10% heat inactivated fetal bovine serum, FBS and 25 mM Hepes, adjusted to pH 7.2 by bicarbonate solution]. The cells were grown at 37 °C in a humidified air atmosphere with 5% CO_2.

Treatment of adherent HeLa and Fem-X cells

Target cells were seeded in 0.1 mL of nutrient medium into one group of 96-well microtiter plates, 2000 cells per well. Twenty hours later, to one series of wells, various dilutions of investigated compounds were added. All samples were done in triplicate. Nutrient medium

Table 1. Antiproliferative activity of estrone-derived steroids after 48 h of continuous action

Compound	IC_{50} (μM)[a]		
	Fem-X[b]	HeLa[c]	K_{562}[d]
 7	>100	>100	>100
2	7.10	5.73	6.22
3	>270	>270	>270
4	14.5	6.17	13.67
5	1.55	1.35	1.50
6	6.00	2.50	1.20

[a]The concentration of a drug required for inhibiting cell survival by 50%.
[b]Human melanoma.
[c]Human cervix carcinoma.
[d]Human myelogenous leukemia.

Table 2. Antiproliferative activity and cytotoxicity of estrone-derived steroids after 72 h of continuous action

Compound	IC_{50} (μM)[a]			
	Fem-X[b]	K_{562}[c]	PBMC[d]	
			−PHA[e]	+PHA[e]
4	4.6	5.90	12.3	5.20
5	0.65	1.50	0.65	0.47
6	1.00	0.70	2.90	1.30

[a]The concentration of a drug required for inhibiting cell survival by 50%.
[b]Human melanoma.
[c]Human leukemia.
[d]Peripheral mononuclear blood cells.
[e]Phytohemaglutinine, 5 μg/mL.

with corresponding agent concentrations, but without target cells was used as blank, also in triplicate.

Treatment of K562 cells

K562 cells were seeded, 3000 cells per well, in 0.1 mL of nutrient medium. Four hours later, cells were treated with various concentrations of investigated agents in the same way as described for the treatment of adherent cells.

Preparation and treatment of peripheral blood mononuclear cells

PBMC were separated from whole heparinized blood of healthy volunteers (age range 20–50 years) by Lymphoprep™ gradient centrifugation. Interface cells, washed three times with Haemaccel® aqueous solution supplemented with 145 mM Na^+, 5.1 mM K^+, 6.2 mM Ca^{2+}, 145 mM Cl^- and 35 g/L gelatine polymers, pH = 7.4, were counted and resuspended in nutrient medium. Target cells were seeded (100,000 cells per well) in nutrient medium [enriched or not enriched, with 5 μg/mL phytohemaglutinin, (PHA)] in 96-well microtiter plates. Two hours later, five different concentrations of compounds with the most pronounced cytotoxic action on malignant cells, were added to the PBMC in triplicate (to the same final concentrations used for treatment of malignant cells) except to the control wells where a nutrient medium was added to the cells. Nutrient medium with corresponding concentrations of compounds, but void of cells was used as blank.

Determination of target cell survival

Cell survival was determined by MTT test according to the method of Mosmann,[10] modified by Ohno and Abe,[11] 48 or 72 h after the drug addition. Briefly, 20 μL of MTT solution (5 mg/mL PBS) were added to each well. Samples were incubated for a further 4 h at 37 °C in a humidified atmosphere with 5% CO_2. Then, 100 μL of 10% SDS in 0.01 M HCl were added to the wells. The optical density (OD) at 570 nm was read the next day. To get cell survival (%), the optical density at 570 nm of a sample with cells grown in the presence of various concentrations of agent (OD), was divided with control optical density ODc (the OD of cells grown only in nutrient medium)×100. Concentration IC_{50} was defined as the concentration of a drug required for inhibiting cell survival by 50%, compared with vehicle-treated control.

Results and Discussion

Results presented in Table 1 show that the absence of a carbonyl function at C(3) is responsible for a decrease of antiproliferative activity (**3** vs **2**), while introduction of another epoxy ring results in significantly enhanced activity (**5** vs **2**). In both cases, the presence of bromine increases antiproliferative activity (**4** vs **7**[1,12] and **6** vs **2**), especially in the epi-bromoxone type substructure (**6**).

We have shown that steroidal bromo- and epoxy-compounds can be synthesized stereoselectively in good yields, using mild reaction conditions. As we envisaged, introduction of another oxirane ring and a bromine atom into the A-ring of estrone derived compounds, leads to a significant increase in antiproliferative activity, while the substitution of a carbonyl at C(3) with a hydroxyl group has the opposite effect. On the other hand, in cytotoxicity tests all of synthesized compounds have shown low selectivity between neoplastic and normal (healthy) cells.

Acknowledgements

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5. Data for **3**: mp 203–206 °C (colorless plates, methanol). IR (KBr) cm^{-1}: 3380, 3034, 2984, 1657, 1268, 834. ^1H NMR (250 MHz, DMSO-d_6): 5.36 (dd, J = 10.6, 2.2 Hz, H-C(1)), 5.15 (br s, exchangeable with D_2O, HO-C(3)), 5.12 (dt, J = 10.6, 1.6 Hz, H-C(2)), 4.78 (s, exchangeable with D_2O, HO-C(10)), 4.40 (d, J = 5.0 Hz, exchangeable with D_2O, HO-C(17)), 4.27 (dd, J = 6.1, 1.7 Hz, after addition D_2O appears as d, J = 1.8 Hz, H-C(3)), 3.37 (t, J = 8.4 Hz, H_α-C(17)), 3.06 (t, J = 2.0 Hz, H_α-C(4)), 2.27–2.05 (m, 1H), 0.62 (s, H_3C-C(13)). ^{13}C NMR (60 MHz, DMSO-d_6): 132.17, 125.74 (C(1) and C(2)), 80.19, 71.65, 64.62, 63.20, 61.58 (C(10), C(3), C(4), C(5), and C(17)), 52.95, 49.59, 42.78, 36.28, 34.44, 29.92, 29.67, 29.45, 23.37, 11.30 (C(18)). MS (DE-IMS, 70 eV, m/z (%)): 306 (M^+, 17), 288 (M^+-H_2O, 12), 270 (M^+-2H_2O, 30), 260 (50), 211 (56), 147 (58), 133 (90), 91 (90), 73 (93), 55 (100), 41 (93). $[\alpha]_D^{25}$ +53 (c 1.0, MeOH). Calcd for $C_{18}H_{26}O_4\times4$/3 MeOH (349.13): C 66.51, H 9.05, found: C 66.54, H 8.93.
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7. Molecular ions at 381 and 383 m/e (1:1 ratio) correspond to the proposed bromoepoxyquinol, while a singlet at 7.08 ppm [H-C(1)] in the ^1H NMR spectrum indicates that a bromine is in the C(2) position.

Data for **6**: mp 281–283 °C (colorless rhombus, n-heptane/CH_2Cl_2). IR (KBr) cm^{-1}: 3296, 2953, 1725, 1704, 1610, 1402. ^1H NMR (200 MHz, $CDCl_3$): 7.08 (s, H-C(1)), 3.50 (s, H-C(3)), 0.95 (s, H_3C-C(13)). ^{13}C NMR (50 MHz, $CDCl_3$): 220.38 (C(17)), 187.92 (C(3)), 150.31 (C(1)), 117.55 (C(2)), 74.78 (C(10)), 64.87 (C(5)), 60.44 (C(4)), 53.58 (C(9)), 49.84 (C(14)), 47.45 (C(13)), 35.45 (C(16)), 34.02 (C(8)), 30.65 (C(15)), 28.15 (C(6)), 28.01 (C(12)), 21.72 (C(7)), 20.89 (C(11)), 13.57 (C(18)). MS (DEI–MS, 70 eV, m/z (%)): 383 (M^+ + 2, 100), 381 (M^+, 99), 365 (M^+-O, 17), 301 (M^+-Br, 6). $[\lambda]_{max}^{MeOH}$: 208 (7000), 261 nm (6000). calcd for $C_{18}H_{21}BrO_4$ (381.29): C 56.70, H 5.56, found: C 56.41, H 5.44.
8. Data for **4**: mp 231–233 °C (colorless needles, toluene/

CH_2Cl_2). IR (KBr) cm^{-1}: 3433, 2937, 1737, 1654, 1631. ^1H NMR (200 MHz, $CDCl_3$): 7.12 (d, $J=10.2$ Hz, H-C(1)), 6.31 (d, $J=10.2$ Hz, H-C(2)), 3.20 (dt, $J=12.2$, 3.1 Hz, 1H), 2.78 (td, $J=13.2$, 4.5 Hz, 1H), 2.48 (dd, $J=18.5$, 8.7 Hz, 1H), 2.44 (s, 1H), 0.97 (s, H_3C-C(13)). ^{13}C NMR (50 MHz, $CDCl_3$): 220.21 (C(17)), 177.90 (C(3)), 163.49 (C(5)), 150.50 (C(1)), 126.62 (C(2)), 121.52 (C(4)), 73.04 (C(10)), 55.12 (C(9)), 49.96 (C(14)), 47.73 (C(13)), 35.58 (C(16)), 34.59 (C(8)), 32.16 (C(7)), 31.15 (C(6)), 30.98 (C(11)), 22.41 (C(12)), 21.95 (C(15)), 13.70 (C(18)). MS (DEI–MS, 70 eV, *m/z* (%)): 367 ($M^+ + 2$, 48), 365 (M^+, 44), 348 ($M^+ - OH$, 22), 347 ($M^+ - H_2O$, 17), 303 (100), 285 ($M^+ - Br$, 81). $[\lambda]_{max}^{MeOH}$: 208.5 (10,000), 240 nm (12,000). Calcd for $C_{18}H_{21}BrO_3 \times 1/2PhMe$ (411.36): C 62.77, H 6.14, found: C 62.97, H 6.14.

9. Data for **5**: mp 244–250 °C (colorless needles, PhH). IR (KBr) cm^{-1}: 3542, 3500–3300, 2950, 1737, 1709, 1260, 915, 900. ^1H NMR (200 MHz, $CDCl_3$): 3.84 (d, $J=4.0$ Hz, H-C(1)), 3.50 (dd, $J_{1,2}=4.0$, $J_{2,4}=2.4$ Hz, H-C(2)), 3.29 (d, $J_{2,4}=2.4$ Hz, H-C(4)), 2.60–2.35 (m, 2H), 0.97 (s, H_3C-C(13)). ^{13}C NMR (DEPT, 50 MHz, $CDCl_3$): 220.18 (C(17)), 199.46 (C(3)), 71.41 (C(10)), 70.10 (C(5)), 63.45 (C(4)), 62.47 (C(2)), 57.16 (C(1)), 50.04 (C(9)), 48.83, 47.32 (C(13)), 35.54, 33.81 (C(8)), 30.81, 30.36 (C(6)), 28.10, 21.82, 20.36, 13.60 (C(18)). MS (DCI-MS, isobutane, 150 eV, *m/z* (%)): 319 (MH^+, 100), 301 ($MH^+ - H_2O$, 70). $[\alpha]_D^{25} = +96.8$ (*c* 0.8, chl).

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