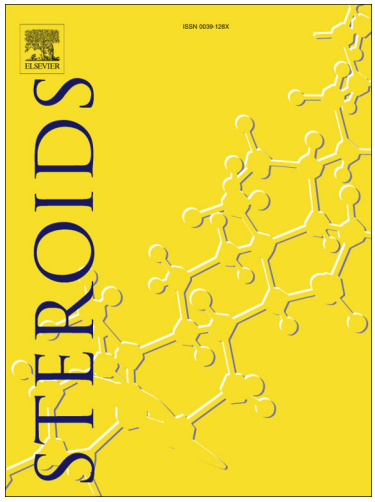

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Synthesis and Antiproliferative Evaluation of Novel Steroid-Benzisoselenazolone Hybrids

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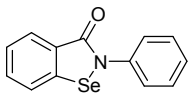
Abstract: The two different types of steroidal benzisoselenazolone hybrids were synthesized by incorporating benzisoselenazolone scaffold into dehydroepiandrosterone and B-norcholesterol. The antiproliferative activity of the synthesized compounds against some carcinoma cell lines were investigated. The results showed that some of these compounds had better inhibitory activity than abiraterone on the proliferation of tumor cells associated with human growth hormone, and had less cytotoxicity on normal human cells. In particular, the IC_{50} values of the compound **8a** and **8f** are 5.4 and 6.5 μmol/L against human ovarian carcinoma (SKOV3) cell line, and possess SI values of 13.9 and 10.5, respectively. The information obtained from the studies may be useful for the design of novel chemotherapeutic drugs.

Keywords: Selenosteroids; Steroid-benzisoselenazolone hybrids; Benzisoselenazolone; dehydroepiandrosterone; B-norcholesterol.

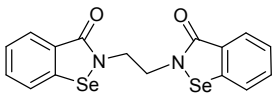
1. Introduction

Selenium is a necessary trace element for the human body and some animals to sustain life. Compared with inorganoselenium compounds, organoselenium compounds have some excellent characteristics, such as higher bioavailability, stronger bioactivity, lower toxicity, smaller environmental pollution, and anti-oxidation, anti-inflammatory and anti-cancer, etc. [1-2]. Karam et al. found that synthetic organoselenium compounds have a stronger antitumor activity and lower toxicity than inorganoselenium compounds [3]. At present, some organoselenium compounds have

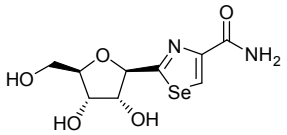
been used in anti-tumor clinical studies and achieved some good results [4], such as ebselen [5-7], ethaselen [8-11] and selenazofurin [12-13], etc.



Ebselen



Ethaselen



Selenazofurin

It can be seen from the structures of ebselen and ethaselen that there is an organoselenium heterocyclic structure of the benzisoselenazolone in both structures, which is the major pharmacophore in the compounds. Some researchers had further introduced the benzisoselenazolone into some different compounds, such as resveratrol [14], sugar [15-16], 1,3,4-thiadiazoles [17-18], 1,2,4-triazole [19], and some of the obtained compounds possessed good anti-tumor activity.

Steroids are an important class of essential physiologically active substances in the human life process, which have high permeability to cells and binding ability to the cell nucleus and cell membrane. Steroidal compounds have been widely used in medicine as anti-inflammatory, anabolic and contraceptive drugs. Changing the steroidal side chain or substitution of the steroidal skeleton, introducing heteroatom or replacing one or more carbon atoms by a heteroatom in steroidal molecule may result in the alteration of its biological activity [20-25].

We have been carrying out the structural modification of steroidal compounds and the research on steroidal compounds as anti-tumor agents. Our research results showed that when different heterocyclic functional groups were introduced into the 17-position of steroid [26-29] or the 6-position of B-norcholesterol [30-31], the compounds obtained had a good inhibitory effect on the proliferation of some tumor cells.

Recently, a few of reports about selenium-containing steroidal compounds have appeared. Some scholars introduced selenocyano [32], selenourea groups [33] or selenide [34] into steroids respectively, and the compounds produced displayed excellent antiproliferative activity on the tested tumor cells. O. Lopez et al. synthesized benzisoselenazolone derivatives of diosgenin, hecogenin and smilagenin, and these compounds behaved as moderate antiproliferative agents, with GI_{50} values ranging from 27 to >100 μM [35]. We recently introduced the selenadiazole into pregnenolone, and the products obtained showed moderate cytotoxicity [36].

However, although some steroidal selenium compounds have been reported recently, the synthesis of organoselenium steroids by introducing Se atoms or selenium-containing groups into steroids and studying their bioactivity, especially anti-tumor activity or antibacterial activity, much work remains to be done in this field [37].

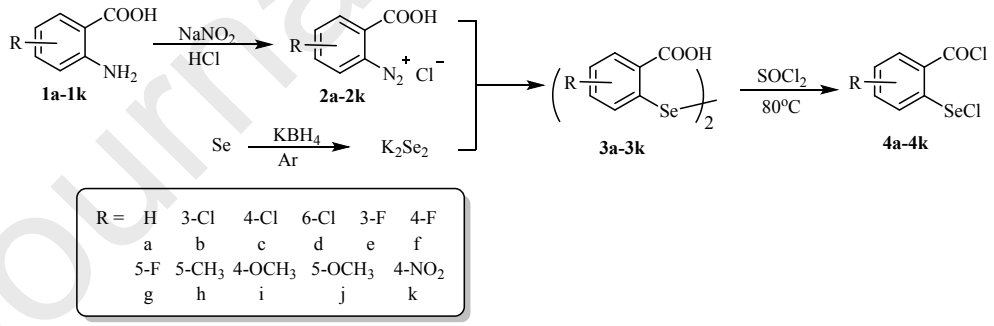
In view of above facts, we report herein the synthesis of some selenium-containing steroidal hybrids by incorporating benzisoselenazolone scaffold into dehydroepiandrosterone or B-norcholesterol, and their antiproliferative activities *in vitro* were evaluated against four types of human hormone-related cancer cell lines, including human ovarian carcinoma (SKOV3), human prostate carcinoma (PC-3), human breast infiltrating duct carcinoma (T47D), human breast adenocarcinoma (MCF-7) and a normal human kidney epithelial cell lines (HEK293T).

2. Result and discussion

2.1 Chemistry

2.1.1 Synthesis of dehydroepiandrosterone-benzisoselenazolone hybrids

First, the key intermediates **4a-4k** with various substituent groups on benzene ring were synthesized according to the method of the reference [38] (Scheme 1). The **4a-4k** were not purified and went straight to the next step reaction.

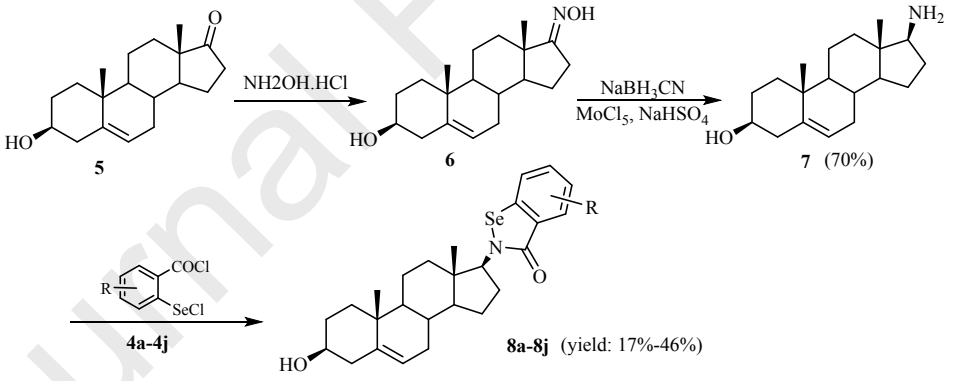


Scheme 1 Synthesis of the intermediates **4a-4k**

Dehydroepiandrosterone is a substance with a wide range of physiological activities [39]. Here, we synthesized the hybrids **8a-8j** of dehydroepiandrosterone by the reaction of compound **7** with **4a-4j**. Scheme 2 outlines the synthetic procedure of compounds **8a-8j**. First, dehydroepiandrosterone (**5**) was transformed to oxime **6** by the reaction of

dehydroepiandrosterone with hydroxylamine hydrochloride. Then, compound **6** was reduced to compound **7** using sodium cyanoborohydride as reductant in the presence of molybdenum pentachloride and sodium hydrogen sulfate [40]. Last, the reaction of compound **7** with the intermediates **4a-4j** afforded corresponding dehydroepiandrosterone-benzisoselenazolone hybrids **8a-8j**.

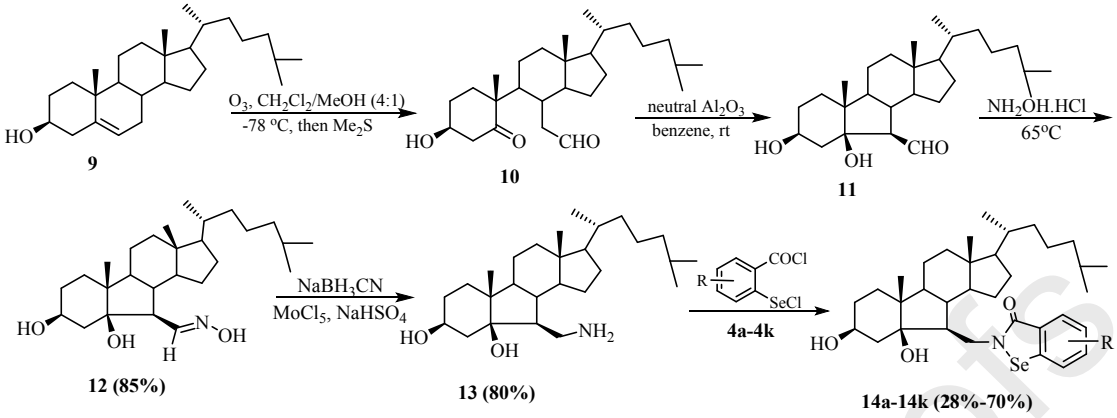
The structure of all synthesized compounds had been confirmed based on analytical and spectral data. In the NMR spectrum of compound **8a**, resonances signals of Ar-H at δ 7.41, 7.73, 8.04ppm and Ar-C at δ 121.07-141.12ppm demonstrate the presence of a benzisoselenazolone group. The triplet of 4.61 ppm (J = 9.4) in the ^{1}H NMR spectrum is the chemical shift of 17-H. It can be seen from the NOESY spectrum of compound **8i** that the 18-methyl group is not correlated with 17-H, so 17-H should be in the α-configuration, that is, the 17-substituent of compounds **8** is in the β-configuration [41]. Moreover, the HREI mass spectrum of compound **8a** exhibits a molecular ion peak ([M+Na]$^+$) at m/z 494.1574 (calcd. for $C_{26}H_{33}NO_2SeNa$ 494.1574). All spectra confirm the structure of compound **8a**. Similarly, the structure of compounds **8b-8j** had been determined by their spectral data.



Scheme 2 Synthesis of dehydroepiandrosterone-benzisoselenazolone hybrids **8a-8j**

2.1.2 Synthesis of B-norcholestane-3β,5β-diol-benzisoselenazolone hybrids

In previous studies, we found that when various functional groups were introduced into the 6-position of B-norcholesterol, the compounds obtained displayed distinct antiproliferative activity. Therefore, we further introduced the benzisoselenazolone pharmacophore into the 6-position of B-norcholesterol and synthesized compounds **14a-14k** (Scheme 3). Meanwhile, the anti-tumor activity of the compounds *in vitro* was investigated.



Scheme 3 Synthesis of B-norcholestane-3β,5β-diol-benzisoselenazolone hybrids **14a-14k**

The configuration of compound **11** at C-6 had been described in reference [42-43] and the synthesis of compound **12** referred to reference [44]. After compound **12** was reduced into **13** using the method in Scheme 2, the **4a-4k** were further incorporated into **13** to give target products **14a-14k**. The structure of the synthesized compounds had been determined by their analysis and spectra data.

2.2 The evaluation of the antiproliferative activity *in vitro*

All synthesized compounds had been evaluated for their antiproliferative activities *in vitro* against SKOV3, PC-3, T47D, MCF-7 and HEK293T cell lines using MTT assay. Abiraterone, a CYP17 inhibitor with a similar structure to compounds **8a-8j**, has been clinically used in the treatment of prostate cancer， using as a positive control. The results were summarized as IC_{50} values in μM in Table 1.

Table 1 *In vitro* antiproliferative activities (IC_{50} in μM) of compounds **8a-8j** and **14a-14k**

Compd.	*SKOV-3*	*PC-3*	*T47D*	*MCF-7*	*HEK-293T*
7	>100	>100	>100	>100	76.58±1.27
8a	5.40±3.61	40.75±0.83	44.37±1.18	40.48±0.66	75.16±1.32
8b	>100	>100	>100	>100	>100
8c	10.41±0.37	16.78±1.04	26.80±1.72	23.39±1.69	70.44±0.16
8d	>100	78.60±6.99	84.42±4.27	15.64±1.54	>100
8e	>100	>100	>100	>100	>100

8f	6.49±0.59	14.26±0.77	35.39±1.35	33.00±2.01	68.19±0.09
8g	>100	>100	>100	>100	>100
8h	14.13±0.29	30.19±0.48	36.46±2.35	23.27±1.53	60.03±0.28
8i	22.95±1.28	29.53±0.19	33.86±0.20	34.24±1.84	51.36±0.60
8j	36.52±0.64	35.16±0.12	36.01±0.31	41.58±2.51	74.10±1.31
14a	15.14±0.56	ND	ND	24.36±1.70	17.75±1.12
14b	>100	>100	>100	>100	>100
14c	21.29±0.39	19.60±1.65	16.68±0.70	24.63±0.06	>100
14d	>100	61.04±4.13	84.60±1.69	75.90±1.10	>100
14e	>100	>100	>100	>100	>100
14f	10.01±0.05	14.45±1.05	16.96±0.59	19.89±0.54	40.72±1.19
14g	17.28±0.92	14.59±0.33	10.25±0.34	22.52±0.24	38.03±0.29
14h	8.72±0.21	7.01±0.14	10.70±0.63	9.59±1.04	53.49±1.09
14i	6.50±0.50	9.07±0.06	11.27±0.66	11.47±0.83	42.03±0.18
14j	>100	>100	61.56±4.14	>100	>100
14k	39.94±10.12	>100	45.45±3.54	27.73±0.06	>100
Abiraterone	51.51±15.98	37.61±0.12	34.66±1.69	44.70±0.67	>100

ND: not determined.

It can be seen from the Table 1 that the synthesized compounds display a different level of inhibitory activity against the proliferation of MCF-7, PC-3, SKOV-3 and T47D cancer cells. For compounds **8a-8j**, there is an important relationship between the antiproliferative activity of compounds and substituent on the benzene ring of benzisoselenazolone. When there is no substituent or there is an electron-withdrawing substituent, such as halogen, on the 6-position of the benzisoselenazolone group, the compounds show an excellent antitumor activity, such as **8a**, **8c** and **8f**, which possess IC_{50} value of less than 10μM to SKOV-3 cell. However, for the same type of substituent, the compound has little cytotoxicity to the tumor cells tested when the substituent is located elsewhere (**8b**, **8d**, **8e**, **8g**). Additionally, when the substituent is the electron-donating group, the compounds show a moderate cytotoxicity (**8h**, **8i**, **8j**). Furthermore, the cytotoxicity of compounds **8a-8j** are higher than that of its precursor compound **7**.

For compounds **14a-14k**, the cytotoxicity of the compounds has nothing to do with the electronic factors of the substituent, and the main determinant factor is the substituting position of functional groups. Similar to compounds **8a-8j**, when the substituent is at the 6-position of the benzisoselenazolone group, the compound has good cytotoxicity (**14f**, **14i**). In addition, compound

14h with 5-methyl substitution also shows good antiproliferative activity to the tested tumor cells. At the same time, it can be seen from Table 1 that the most of these compounds show higher cytotoxicity to these hormone-related tumor cells than the positive control abiraterone.

The Selectivity Index (SI) is defined as the ratio of the cytotoxicity of a compound with respect to normal cells (IC_{50} HEK293T) versus cancer cells and used to determine the criterion of effectiveness of the compounds. It determines the damage degree of a compound to human normal cells. The higher SI value is, the less damage the compound will do to normal cells. The calculated SI values of compound **8a** and **8f** for SKOV-3 cells are 13.9 and 10.5, respectively. So, comparison of the cytotoxicity of the compounds with the SI values suggests that the compound **8a** and **8f** may be potent anticancer agents against human ovarian carcinoma (SKOV-3).

3. Conclusion

In this paper, dehydroepiandrosterone-benzisoselenazolone and B-norcholestane-3β,5β-diol-benzisoselenazolone hybrids were synthesized and their antitumor activity *in vitro* was assayed. The results show that some of these compounds have better inhibitory activity than abiraterone on the proliferation of tumor cells associated with human growth hormone, and have less cytotoxicity on normal human cells. Compounds **8a** and **8f** have a lower IC_{50} value and a higher SI value to human ovarian carcinoma SKOV-3 cells, so they are expected to be candidates for such anti-tumor drugs, which deserve further study.

4. EXPERIMENTAL

4.1 Chemistry

4.1.1 Reagents and Instruments

The cholesterol, dehydroepiandrosterone and various substituted aminobenzoic acid were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. All chemicals and solvents were analytical grade. Melting points were measured on an X_6 apparatus (Beijing Tech Instrument Co. Ltd., Beijing, China), uncorrected. Infrared spectra were determined with a Thermo Scientific Nicolet IS-10 Spectrophotometer (Thermo Scientific, America); The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-600 spectrometer at working frequencies 600 and 150 MHz and a Bruker AV-300 spectrometer at working frequencies 300 and 75 MHz. Chemical shifts are expressed in parts per million (δ) values and coupling constants (J) in Hertz. HREIMS

was measured on an Agilent 6210 TOFMS instrument (Agilent Technologies, America). The cell proliferation assay was undertaken by MTT method using 96-well plates on a MLLTISKAN MK3 analysis spectrometer (Thermo Scientific, Shanghai, China).

4.1.2 Synthesis of compound **2 (2a-2k)**

10mL of HCl solution (concentrated HCl : H_2O = 1 : 1) was added into 4.15 mmol substituted o-aminobenzoic acid, stirred for 30 min in ice bath, then 290 mg (4.2mmol) of sodium nitrite (dissolved in 1.5 mL water) was slowly added. The reaction was continued for 30 min to obtain compound **2**, set aside.

4.1.3 Synthesis of **K_2Se_2**

198 mg (2.5 mmol) of selenium powder was added to a reaction flask, and 3-5 mL of distilled water was added. Another 440 mg (8.02 mmol) of potassium borohydride was added to a constant pressure funnel, and 5 mL of water was added to dissolve it. Under the protection of argon, the potassium borohydride solution was slowly added to the selenium powder in about 10 minutes. After the selenium powder was completely dissolved, 396 mg (5 mmol) of selenium powder was quickly added and stirred at room temperature for 30 min. Then, 4 mL of a 6 M potassium hydroxide solution was added to obtain a K_2Se_2 solution.

4.1.4 Synthesis of compound **3 (3a-3k)**

The K_2Se_2 solution obtained above was transferred to an ice bath, and compound **2** was added slowly under constant agitation. The reaction was continued for 5 min under the ice bath, and then stirred for 2 h at 70 ^oC and 3 h at room temperature. Last, the pH value of the solution was tested.

1) If the pH was less than 7, directly filtered, added the filter cake to 1M sodium carbonate solution, dissolved it until the solution was alkaline, heated until the solution boiled, cooled and filtered, the filtrate was acidified with 1M hydrochloric acid until the pH of solution was less than 1, precipitate appeared. After filtration, solid was dried to give compound **3**.

2) If the pH was greater than 7, directly filtered, the filtrate was acidified with 1M hydrochloric acid until the pH of the solution was less than 1, heated until the solution boiled, cooled and filtered, and the resulting precipitate was added to 1 M sodium carbonate solution to dissolve until the solution was alkaline. The solution was heated to micro-boiling, after cooling, filtered, and the filtrate was acidified with a hydrochloric acid solution until the pH of the solution was less than 1, at which time the precipitate occurred, filtered, and solid was dried to obtain compound **3**.

4.1.5 Synthesis of compounds **4a-4k**

200 mg of compound **3** (**3a-3k**) and 20 mL of $SOCl_2$ were added into the reactive flask and

refluxed for 3 h at 80°C until no starting material was observed. The reaction was stopped and $SOCl_2$ was evaporated under reduced pressure to give an oily compound **4** (**4a**-**4k**). Compound **4** was not further purified and was directly used in the next reaction.

4.1.6 Synthesis of compound **7** and **13**

0.27 mmol of compound **6** was dissolved in 40 mL of anhydrous ethanol, and 1.08 mmol of $NaBH_3CN$, 0.80 mmol $NaHSO_4$, and 0.27 mmol $MoCl_5$ were added. After addition, the solution turned brown and large number of bubbles were produced. The reaction mixture was stirred at room temperature for 2 h until no starting material was observed (the progress of the reaction was monitored by TLC). Then the reaction was stopped and a silica gel short column was used for filtration, and methanol was used for washing until there was no target product. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel with $V_{\text{dichloromethane}}$: V_{methanol} : $V_{\text{ammonia water}}$= 10 : 1 : 1 as mobile phase to afford the corresponding target product **7** as a white solid. Yield: 70%, m.p.154-156°C. IR(KBr) ν/cm^{-1}: 3404, 2930, 1651, 1581, 1434, 1369, 1068, 953, 796; ^{1}H NMR ($CDCl_3$, 300MHz) δ: 0.64 (3H, s, 18-CH_3), 1.00 (3H, s, 19-CH_3), 2.64 (1H, t, J = 9.0, C17-H), 2.26-2.21 (2H, m), 3.54-3.41 (1H, m, C3-H), 5.36 (1H, d, J = 5.1, C6-H); ^{13}C NMR (75MHz, $CDCl_3$): 141.0 (5-C), 121.3 (6-C), 71.4 (3-C), 62.7 (17-C), 53.3 (14-C), 50.3 (9-C), 42.4 (13-C), 42.3 (4-C), 37.3 (1-C), 36.6 (12-C), 32.2 (10-C), 31.6 (7-C), 31.5 (8-C), 31.1 (2-C), 29.7 (16-C), 23.7 (15-C), 20.7 (11-C), 19.4 (19-C), 11.0 (18-C); HREIMS m/z: 290.2505 $[M+H]^+$ (calcd. for $C_{19}H_{32}NO$ 290.2486)。

The synthesis of compound **13** was similar with that of compound **7**:

White solid, Yield: 80%, m.p. 121-123°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.64 (3H, s, 18-CH_3), 0.86 (3H, d, J = 6.6, 26-CH_3 or 27-CH_3), 0.87 (3H, d, J = 6.6, 26-CH_3 or 27-CH_3), 0.91 (3H, s, 19-CH_3), 0.92 (3H, d, J = 6.3, 21-CH_3), 2.78-2.87 (2H, m, C6-H), 3.92 (1H, br s, C3-αH), 3.99 (1H, s, -OH); ^{13}C NMR (75MHz, $CDCl_3$) δ: 12.44 (18-C), 18.72 (19-C), 18.78 (21-C), 21.28 (11-C), 22.50 (26-C or 27-C), 22.76 (26-C or 27-C), 23.76 (23-C), 24.55 (16-C), 27.23 (15-C), 27.91 (25-C), 28.08 (1-C), 28.56 (8-C), 35.57 (2-C), 36.18 (20-C), 39.43 (22-C), 39.81 (6-C), 42.15 (12-C), 44.19 (24-C), 44.53 (13-C), 44.83 (4-C), 46.28 (7-C), 49.92 (10-C), 53.10 (9-C), 55.47 (14-C), 56.52 (17-C), 67.11 (3-C), 82.17 (5-C); IR(KBr) ν/cm^{-1}: 3785, 3695, 3404, 2927, 2868, 2319, 1733, 1594, 1546, 1382, 1075, 716; HREIMS *m/z*: 420.3845 $[M+H]^+$ (calcd. for $C_{27}H_{50}NO_2$, 420.3842).

4.1.7 General procedure for the synthesis of compounds **8a**-**8j** or **14a**-**14k**

The solution of 0.47 mmol compound **7** (or compound **13**) in 20 mL of CH_2Cl_2 was added slowly to the different compound **4** (**4a-4k)** in about 15 minutes, and then 1.5 mL of trimethylamine was added. The reaction mixture was stirred for 3 h at room temperature until no starting material was observed (the progress of the reaction was monitored by TLC). Then the reaction was stopped and 20 mL of CH_2Cl_2 was added. The organic phase was washed with saturated $NaHCO_3$ solution, water and saturated brine, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel with $V_{\text{ethyl acetate}}$: $V_{\text{petroleum ether}}$ = 1 : 2 to afford the corresponding target products **8a-8j** (or **14a-14k**) as a faint yellow solid.

17β-[3'-oxobenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8a)

Faint yellow solid. Yield: 45.84%, m.p. 242-245°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.86 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 3.54 (1H, ddd, J =10.8, 5.6, 5.0, C3-αH), 4.61 (1H, t, J = 9.4, C17-H), 5.37 (1H, d, J = 5.1, C6-H), 7.41 (1H, s, J = 7.4, 3′-ph-H), 7.73-7.52 (2H, m, 3′,4′-ph-H), 8.04 (1H, d, J = 7.8, 6′-ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.01 (18-C), 19.44 (19-C), 20.51 (11-C), 23.78 (15-C), 29.37 (16-C), 31.70 (2-C), 31.43 (8-C), 31.93 (7-C), 32.09 (12-C), 36.56 (1-C), 37.27 (10-C), 42.20 (4-C), 45.17 (13-C), 50.11 (9-C), 52.44 (14-C), 64.21 (17-C), 71.60 (3-C), 121.07 (2′-Ph-C), 123.34 (6-C), 125.98 (6′-Ph-C), 128.22 (5′-Ph-C), 128.65 (3′-Ph-C), 131.70 (4′-Ph-C), 138.35 (1′-Ph-C), 141.12 (5-C), 168.11 (-C=O); IR(KBr) ν/cm^{-1}: 3407.65, 2924.87, 2853.65, 1732.29, 1619.32, 1568.03, 1448.63, 1374.02, 1321.33, 1246.95, 1069.12, 1028.70, 953.91, 737.87, 672.53, 586.69; HREIMS (m/z): 494.1574 $[M+Na]^+$ (calcd. for $C_{26}H_{33}NO_2SeNa$ 494.1574).

17β-[3'-Oxo-7'-chlorobenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8b)

Faint yellow solid. Yield: 32.79%, m.p. 221-222°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.87 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 2.22-2.32 (2H, m, C4-H), 3.51-3.56 (1H, m, C3-αH), 4.61 (1H, t, J = 8.4, C17-H), 5.37 (1H, d, J = 5.4, C6-H), 7.41 (1H, t, J = 7.8, 5′-Ph-H), 7.54 (1H, d, J = 7.8, 4′-Ph-H), 7.94 (1H, d, J = 7.8, 6′-Ph-H)； ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.15 (18-C), 19.56 (19-C), 20.63 (11-C), 23.91 (15-C), 29.82 (16-C), 29.86 (2-C), 31.58 (8-C), 31.73 (7-C), 32.21 (12-C), 36.69 (1-C), 37.34 (10-C), 42.35 (4-C), 45.33 (13-C), 50.19 (9-C), 52.57 (14-C), 64.49 (17-C),

71.80 (3-C), 121.26 (6-C), 126.82 (6′-C), 127.82 (5′-Ph-C), 128.89 (4′-Ph-C), 130.35 (3′-Ph-C), 130.86 (1′-Ph-C), 138.96 (2′-Ph-C), 141.13 (5-C), 168.00 (C=O); IR(KBr) ν/cm^{-1}: 3727.82, 3627.07, 3594.52, 3384.52, 2925.41, 2855.17, 1626.66, 1589.80, 1561.04, 1455.64, 1412.55, 1371.76, 1321.21, 1300.45, 1204.73, 1139.85, 1089.60, 1055.32, 1029.03, 954.12, 908.13, 841.36, 804.01, 742.70, 669.74；HREIMS (m/z): 506.1376 $[M+H]^+$ (calcd. for $C_{26}H_{33}ClNO_2Se$ 506.1365).

17β-[3'-Oxo-6'-chlorobenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8c)

Faint yellow solid. Yield: 17.12%, m.p. 218-221°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.86 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 2.23-2.31 (2H, m, C4-H), 3.50-3.60 (1H, m, C3-αH), 4.62 (1H, t, J = 9.3, C17-H), 5.38 (1H, d, J = 5.1, C6-H), 7.38 (1H, dd, J = 8.4, 1.8, 5′-Ph-H), 7.64 (1H, d, J = 1.8, 3′-Ph-H), 7.95 (1H, d,J = 8.4, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.03 (18-C), 19.43 (19-C), 20.50 (11-C), 23.77 (15-C), 29.60 (16-C), 31.45 (2-C), 31.60 (8-C), 32.08 (7-C), 36.56 (12-C), 37.16 (1-C), 37.24 (10-C), 42.20 (4-C), 45.19 (13-C), 50.07 (9-C), 52.41 (14-C), 64.41 (17-C), 71.63 (3-C), 121.07 (6-C), 123.19 (3′-Ph-C), 126.70 (6′-Ph-C), 126.82 (5′-Ph-C), 129.58 (1′-Ph-C), 138.34 (4′-Ph-C), 139.48 (2′-Ph-C), 141.07 (5-C), 167.23 (-C=O); IR(KBr) ν/cm^{-1}: 3726.72, 3704.38, 3626.24, 3597.83, 3395.08, 2927.72, 2851.64, 1723.12, 1611.58, 1584.71, 1548.04, 1453.43, 1396.54, 1374.04, 1306.68, 1274.16, 1189.51, 1125.07, 1090.86, 1046.65, 953.97, 872.92, 827.26, 760.00, 669.87, 653.72, 617.43, 574.26; HREIMS(m/z): 506.1367 $[M+H]^+$ (calcd. for $C_{26}H_{33}ClNO_2Se$ 506.1365).

17β-[3'-Oxo-4'-chlorobenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8d)

Faint yellow solid. Yield: 35.17%, m.p. 142-144°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 2.19-2.29 (2H, m, C4-H), 2.60 (1H, br s, OH), 3.49-3.59 (1H, m, C3-αH), 4.57 (1H, t, J = 9.3, C17-H), 5.31 (1H, s, C6-H), 7.32-7.41 (2H, m, 3′,4′-Ph-H), 7.52 (1H, dd, J = 7.5, 1.2, 5′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.02 (18-C), 19.40 (19-C), 20.46 (11-C), 23.77 (15-C), 29.68 (16-C), 31.39 (2-C), 31.57 (8-C), 32.02 (7-C), 36.50 (12-C), 37.32 (1-C), 37.39 (10-C), 42.02 (4-C), 45.07 (13-C), 50.14 (9-C), 52.48 (14-C), 64.12 (17-C), 71.42 (3-C), 120.88 (6-C), 122.21 (3′-Ph-C), 123.21 (5′-Ph-C), 128.23 (6′-Ph-C), 131.46 (4′-Ph-C), 136.07 (1′-Ph-C), 141.27 (2′-Ph-C), 141.31 (5-C), 165.95 (-C=O); IR(KBr) ν/cm^{-1}: 3726.56, 3704.80, 3414.50, 2926.82, 2852.16, 2239.85, 1711.13, 1632.72, 1578.35, 1555.32, 1443.36, 1373.68,

1295.18, 1175.02, 1088.87, 1052.14, 1029.67, 953.42, 908.66, 783.32, 731.21, 669.73, 649.03, 586.07, 555.38; HREIMS(m/z): 506.1376 $[M+H]^+$ (calcd. for $C_{26}H_{33}ClNO_2Se$ 506.1365).

17β-[3'-Oxo-7'-fluorobenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8e)

Faint yellow solid. Yield: 41.21%, m.p. 236-238°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.83 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 2.19-2.24 (2H, m, C4-H), 2.56 (1H, br s, OH), 3.48-3.54 (1H, m, C3-αH), 4.54 (1H, t, J = 9.6, C17-H), 5.31 (1H, d, J = 5.4, C6-H), 7.24 (1H, t, J = 8.4, 4′-Ph-H), 7.36-7.39 (1H, m, 5′-Ph-H), 7.80 (1H, d, J = 7.8, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.11 (18-C), 19.55 (19-C), 20.61 (11-C), 23.86 (15-C), 29.81 (16-C), 31.54 (2-C), 31.69 (8-C), 32.17 (7-C), 36.66 (12-C), 37.29 (1-C), 37.41 (10-C), 42.26 (4-C), 45.29 (13-C), 50.23 (9-C), 52.56 (14-C), 64.58 (17-C), 71.61 (3-C), 117.31 ($^2J_{C-F}$=9.75, 4′-Ph-C), 121.05 (6-C), 124.35 (6′-Ph-C), 128.10 (2′-Ph-C), 130.00 (5′-Ph-C), 141.35 (5-C), 156.65 (1′-Ph-C), 158.28 (3′-Ph-C), 167.52 (-C=O); IR(KBr) ν/cm^{-1}: 3425.82, 2933.34, 2849.82, 1641.97, 1574.20, 1471.10, 1365.74, 1325.72, 1300.86, 1247.87, 1200.19, 1087.84, 1059.44, 1030.28, 981.49, 955.10, 799.80, 737.64, 669.59, 648.92; HREIMS (m/z): 490.1654 $[M+H]^+$ (calcd. for $C_{26}H_{33}FNO_2Se$ 490.1661).

17β-[3'-Oxo-6'-fluorobenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8f)

Faint yellow solid. Yield: 46.12%, m.p. 273-275°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 2.24-2.32 (2H, m, C4-H), 3.51-3.59 (1H, m, C3-αH), 4.59 (1H, t, J = 9.3, C17-H), 5.38 (1H, d, J = 5.1, C6-H), 7.10-7.17 (1H, m, 3′-Ph-H), 7.33 (1H, dd, J = 7.8, 2.1, 5′-Ph-H), 8.02 (1H, dd, J = 8.7, 5.1, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.04 (18-C), 19.44 (19-C), 20.48 (11-C), 23.77 (15-C), 29.71 (16-C), 31.45 (2-C), 31.59 (8-C), 32.07 (7-C), 36.56 (12-C), 37.12 (1-C), 37.21 (10-C), 42.21 (4-C), 45.15 (13-C), 50.04 (9-C), 52.35 (14-C), 64.37 (17-C), 71.66 (3-C), 110.03 (5′-Ph-C), 110.73 (3′-Ph-C), 121.14 (6-C), 124.57 (6′-Ph-C), 130.50 (1′-Ph-C), 130.63 (2′-Ph-C), 139.75 (5-C), 141.01 (4′-Ph-C), 167.17 (-C=O); IR(KBr) ν/cm^{-1}: 3727.48, 3448.34, 2924.38, 2852.20, 1605.63, 1470.33, 1409.92, 1375.95, 1309.37, 1245.47, 1215.96, 1174.70, 1089.97, 1061.13, 1027.90, 950.27, 888.46, 859.92, 834.32, 796.51, 762.14, 720.86, 670.73, 611.15, 586.34; HREIMS (m/z): 490.1658 $[M+H]^+$ (calcd. For $C_{26}H_{33}FNO_2Se$ 490.1661).

17β-[3'-Oxo-5'-fluorobenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8g)

Faint yellow solid. Yield: 26.00%, m.p. 236-238°C; ^{1}H NMR (600MHz, $CDCl_3$) δ: 0.84 (3H, s, 18-CH_3), 0.99 (3H, s, 19-CH_3), 2.19-2.25 (2H, m, C4-H), 3.50-3.55 (1H, m, C3-αH), 4.56 (1H, t, J = 9.0, C17-H), 5.35 (1H, d, J = 4.2, C6-H), 7.30-7.33 (1H, m, 4′-ph-H), 7.56 (1H, dd, J = 9.0, 4.8, 3′-ph-H), 7.71 (1H, dd, J = 8.4, 2.4, 6′-ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.10 (18-C), 19.54 (19-C), 20.61 (11-C), 23.88 (15-C), 29.81 (16-C), 31.56 (2-C), 31.56 (8-C), 32.20 (7-C), 36.67 (12-C), 37.30 (1-C), 37.36 (10-C), 42.32 (4-C), 45.34 (13-C), 50.19 (9-C), 52.55 (14-C), 64.63 (17-C), 71.73 (3-C), 114.90 ($^2J_{C\text{-}F}$=11.25, 6′-Ph-C), 120.26 ($^2J_{C\text{-}F}$=12.00, 4′-Ph-C), 121.19 (6-Ph-C), 124.92 ($^3J_{C\text{-}F}$=3.75, 3′-Ph-C), 130.02 ($^3J_{C\text{-}F}$=3.75, 1′-Ph-C), 132.95 (2′-Ph-C), 141.22 (5-C), 161.86 ($^1J_{C\text{-}F}$=122.25, 5′-Ph-C), 167.31 (-C=O); IR(KBr) ν/cm^{-1}: 3377.38, 2925.88, 2852.73, 1714.80, 1613.80, 1576.88, 1461.13, 1331.20, 1272.55, 1219.15, 1143.89, 1107.39, 1090.76, 1057.26, 1029.83, 953.25, 930.84, 879.75, 812.26, 764.18, 730.38, 669.83, 646.36, 600.67, 536.00; HREIMS(m/z): 490.1662 $[M+H]^+$ (calcd. for $C_{26}H_{33}FNO_2Se$ 490.1661).

17β-[3'-Oxo-5'-methylbenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8h)

Faint yellow solid. Yield: 23.65%, m.p. 263-265°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.82 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 2.42 (3H, s, ph-CH_3), 3.49-3.53 (1H, m, C3-αH), 4.56 (1H, t, J = 9.0, C17-H), 5.32 (1H, s, C6-H), 7.36 (1H, t, J = 6.6, 4′-ph-H), 7.47 (1H, t, J = 7.2, 5′-ph-H), 7.81 (1H, d, J = 3.6, 6′-ph-H); ^{13}C NMR (75MHz, $CDCl_3$): 13.09 (18-C), 19.54 (19-C), 20.61 (11-C), 21.12 (5′-Ph-CH3), 23.85 (15-C), 29.65 (16-C), 31.55 (2-C), 31.70 (8-C), 32.16 (7-C), 36.65 (12-C), 37.31 (1-C), 37.40 (10-C), 42.29 (4-C), 45.26 (13-C), 50.24 (9-C), 52.55 (14-C), 64.29 (17-C), 71.63 (3-C), 121.10 (6-C), 123.19 (6′-Ph-C), 128.32 (3′-Ph-C), 128.73 (1′-Ph-C), 133.19 (4′-Ph-C), 135.11 (5′-Ph-C), 136.16 (2′-Ph-C), 141.30 (5-C), 168.23 (-C=O); IR (KBr) ν/cm^{-1}: 3726.79, 3381.71, 2927.72, 2853.08, 1618.63, 1537.51, 1465.03, 1335.11, 1290.91, 1251.42, 1220.53, 1146.81, 1060.50, 953.41, 906.84, 808.14, 767.67, 732.16, 669.59, 649.92, 602.27, 513.20; HREIMS (m/z): 486.1907 $[M+H]^+$ (calcd. for $C_{27}H_{36}NO_2Se$ 486.1911)

17β-[3'-Oxo-6'-methoxylbenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8i)

Faint yellow solid. Yield: 11.58%, m.p. 243-246 °C； ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 2.20-2.31 (2H, m, C4-H), 3.49-3.57 (1H, m, C3-αH), 3.89 (3H, s, ph-OCH_3), 4.57 (1H, t, J = 9.3, C17-H), 5.37 (1H, d, J = 4.8, C6-H), 6.96 (1H, dd, J = 8.7,

2.4, 5′-ph-H), 7.08 (1H, d, *J* = 2.4, 3′-ph-H), 7.92 (1H, d, *J* = 8.7, 6′-ph-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 13.03 (18-C), 19.45 (19-C), 20.50 (11-C), 23.75 (15-C), 29.70 (16-C), 31.46 (2-C), 31.60 (8-C), 32.07 (7-C), 36.56 (12-C), 37.14 (1-C), 37.25 (10-C), 42.19 (4-C), 45.06 (13-C), 50.09 (9-C), 52.35 (14-C), 55.73 (4-Ph-OCH3), 64.10 (17-C), 71.62 (3-C), 106.78 (3′-Ph-C), 114.21 (5′-Ph-C), 121.13 (6-C), 121.32 (1′-Ph-C), 129.68 (6′-Ph-C), 140.16 (5-C), 141.11 (2′-Ph-C), 162.74 (4′-Ph-C), 167.91(-C=O); IR(KBr) *ν*/cm^{-1}: 3380.80, 2929.85, 2852.82, 1596.24, 1480.34, 1437.72, 1416.98, 1364.04, 1317.13, 1256.64, 1187.70, 1043.30, 954.36, 907.38, 880.14, 846.75, 763.98, 731.36, 680.28; HREIMS (m/z): 502.1866 $[M+H]^+$ (calcd. for $C_{27}H_{36}NO_3Se$ 502.1860).

17β-[3'-Oxo-5'-methoxylbenzisoselenazol-2'-(3H)-yl]androst-5-en-3β-ol (8j)

Faint yellow solid. Yield: 8.15%, m.p. 238-241°C； ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.87 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.21-2.32 (2H, m, C4-H), 3.50-3.60 (1H, m, C3-αH), 3.89 (3H, s, ph-OCH_3), 4.61 (1H, t, *J* = 9.3, C17-H), 5.38 (1H, d, *J* = 5.1, C6-H), 7.22 (1H, dd, *J* = 8.7, 2.7, 4′-ph-H), 7.49 (1H, d, *J* = 8.7, 3′-ph-H), 7.55 (1H, d, *J* = 2.7, 6′-ph-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 12.99 (18-C), 19.44 (19-C), 20.52 (11-C), 23.77 (15-C), 29.70 (16-C), 31.47 (2-C), 31.61 (8-C), 32.09 (7-C), 36.57 (12-C), 37.18 (1-C), 37.23 (10-C), 42.22 (4-C), 45.23 (13-C), 50.06 (9-C), 52.41 (14-C), 55.68 (5-Ph-OCH3), 64.34 (17-C), 71.65 (3-C), 110.31 (6′-Ph-C), 121.18 (4′-Ph-C), 121.95 (6-C), 124.09 (3′-Ph-C), 129.21 (1′-Ph-C), 139.58 (2′-Ph-C), 141.03 (5-C), 158.75 (5′-Ph-C), 168.00 (-C=O); IR(KBr) *ν*/cm^{-1}: 3369.77, 2926.41, 2852.94, 1609.03, 1568.82, 1469.44, 1436.58, 1341.20, 1289.76, 1274.50, 1230.54, 1188.71, 1153.22, 1123.39, 1049.84, 1025.64, 955.11, 809.48, 764.25, 729.98, 669.52, 647.90, 605.43; HREIMS (m/z): 502.1864 $[M+H]^+$ (calcd. for $C_{27}H_{36}NO_3Se$ 502.1860).

6β-[3'-Oxobenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14a)

Faint yellow solid. Yield: 43.75%, m.p. 222-223°C; ^{1}H NMR (300MHz, $CDCl_3$) *δ*: 0.71 (3H, s, 18-CH_3), 0.87 (3H, s, 19-CH_3), 0.89 (6H, d, *J* = 6.5, 26- and 27-CH_3), 0.93 (3H, d, *J* = 6.5, 21-CH_3), 3.93-3.86 (2H, m, C6-H), 4.02 (1H, br s, C3-αH), 4.78(1H, s, -OH), 7.47-7.31 (1H, m, 3′-Ph-H), 7.78-7.47 (2H, m, 4′,5′-Ph-H), 8.02 (1H, d, *J* = 7.7, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$)*δ*: 12.60 (18-C), 18.72 (19-C), 18.83 (21-C), 21.41 (11-C), 22.56 (26- or 27-C), 22.83 (26-

or 27-C), 23.88 (23-C), 24.89 (16-C), 27.39 (15-C), 28.02 (25-C), 28.10 (1-C), 28.83 (8-C), 35.67 (2-C), 36.25 (20-C), 39.48 (22-C), 39.87 (6-C), 43.35 (12-C), 44.66 (24-C), 44.81 (13-C), 45.01 (4-C), 46.33 (7-C), 49.96 (10-C), 55.29 (9-C), 55.56 (14-C), 56.68 (17-C), 67.38 (3-C), 80.60 (5-C), 123.66 (2′-ph-C), 125.97 (6′-ph-C), 127.37 (5′-ph-C), 128.58 (3′-ph-C), 131.85 (4′-ph-C), 138.43 (1′-ph-C), 167.84 (-C=O); IR (KBr) ν/cm^{-1}: 3364.18、2927.15、2862.08、1619.20、1564.57、1445.99、1378.98、1352.75、1171.23、1078.73、1020.57、956.54、738.28、675.55; HREIMS (m/z): 624.2939 $[M+Na]^+$ (calcd. for $C_{34}H_{51}NO_3SeNa$ 624.2932).

6β-[3'-Oxo-7'-chlorobenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14b)

Faint yellow solid. Yield: 31.48%,m.p. 198-201°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.69 (3H, s, 18-CH_3), 0.86 (3H, d, J = 6.6, 26 or 27-CH_3), 0.87 (3H, d, J = 6.6, 26 or 27-CH_3), 0.90 (3H, s, 19-CH_3), 0.91 (3H, d, J = 6.6, 21-CH_3), 1.97 (1H, d, J = 15.0, C4-H), 2.04 (1H, d, J = 12.6, C17-H), 2.87 (1H, s, -OH), 3.77 (1H, dd, J = 13.8, 9.6, C6-H), 4.06 (1H, br s, C3-αH), 4.07 (1H, d, J = 13.8, C6-H), 4.39 (1H, s, -OH), 7.37 (1H, t, J = 7.2, 5′-Ph-H), 7.51 (1H, d, J = 7.8, 4′-Ph-H), 7.91 (1H, d, J = 7.2, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 12.71 (18-C), 18.95 (19-C), 19.01 (21-C), 21.54 (11-C), 22.67 (26 or 27-C), 22.94 (26 or 27-C), 24.00 (23-C), 24.92 (16-C), 27.19 (15-C), 28.14 (25-C), 28.6 (1-C), 29.82 (8-C), 35.80 (2-C), 36.36 (20-C), 39.59 (22-C), 39.99 (6-C), 43.51 (12-C), 44.77 (24-C), 44.93 (13-C), 45.24 (4-C), 46.58 (7-C), 49.86 (10-C), 55.20 (9-C), 55.70 (14-C), 56.81 (17-C), 67.64 (3-C), 80.62 (5-C), 126.68 (6′-ph-C), 127.69 (5′-ph-C), 129.13 (4′-ph-C), 129.68 (3′-ph-C), 130.94 (1′-ph-C), 140.17 (2′-ph-C), 167.64 (-C=O); IR(KBr) ν/cm^{-1}: 3726.67, 3627.70, 3298.78, 2927.35, 2861.18, 1628.56, 1559.99, 1456.37, 1424.71, 1379.21, 1348.46, 1273.59, 1206.07, 1172.55, 1147.43, 1078.69, 1016.46, 969.80, 870.18, 802.61, 762.56, 744.85, 669.72; HREIMS (m/z): 636.2722 $[M+H]^+$ (calcd. for $C_{34}H_{51}ClNO_3Se$ 636.2723).

6β-[3'-Oxo-6'-chlorobenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14c)

Faint yellow solid. Yield: 39.61%, m.p. 201-204 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.67 (3H, s, 18-CH_3), 0.85 (3H, d, J = 6.6, 26 or 27-CH_3), 0.86 (3H, d, J = 6.6, 26 or 27-CH_3), 0.87 (3H, s, 19-CH_3), 0.91 (3H, d, J = 6.6, 21-CH_3), 1.94-1.87 (2H, m, C4-H), 2.03 (1H, d, J = 13.6, C17-H), 3.30 (1H, s, OH), 3.74 (1H, dd, J = 13.8, 9.6, C6-H), 4.01 (1H, d, J = 14.4, C6-H), 4.03 (1H, br s, C3-αH), 4.52 (1H, s, OH), 7.33 (1H, dd, J = 12.6, 2.7, 5′-Ph-H), 7.59 (1H, d, J = 2.7,

3′-Ph-H), 7.89 (1H, d, *J* = 12.6, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 12.70 (18-C), 18.91 (19-C), 18.94 (21-C), 21.53 (11-C), 22.68 (26 or 27-C), 22.94 (26 or 27-C), 24.01 (23-C), 24.96 (16-C), 27.44 (15-C), 28.16 (25-C), 28.94 (1-C), 29.82 (8-C), 35.79 (2-C), 36.36 (20-C), 39.59 (22-C), 39.96 (6-C), 43.38 (12-C), 44.67 (24-C), 44.91 (13-C), 45.21 (4-C), 46.49 (7-C), 50.10 (10-C), 55.21 (9-C), 55.68 (14-C), 56.76 (17-C), 67.57 (3-C), 80.68 (5-C), 123.58 (3′-ph-C), 126.12 (6′-ph-C), 126.79 (5′-ph-C), 129.50 (1′-ph-C), 138.48 (4′-ph-C), 141.12 (2′-ph-C), 167.06 (-C=O); IR(KBr) *ν*/cm^{-1}: 3384.08, 3096.66, 2927.18, 2864.60, 1602.53, 1586.39, 1442.70, 1405.91, 1380.02, 1306.97, 1237.91, 1165.77, 1126.33, 1077.60, 1016.10, 951.07, 869.58, 830.21, 762.41, 669.04, 577.26; HREIMS (m/z): 636.2719 $[M+H]^+$ (calcd. for $C_{34}H_{51}ClNO_3Se$ 636.2723).

6β-[3'-Oxo-4'-chlorobenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14d)

Faint yellow solid. Yield: 62.46%, m.p. 196-198^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 0.66 (3H, s, 18-CH_3), 0.84 (3H, d, *J* = 6.6, 26- or 27-CH_3), 0.85 (3H, d, *J* = 6.6, 26- or 27-CH_3), 0.86 (3H, s, 19-CH_3), 0.89 (3H, d, *J* = 6.6, 21-CH_3), 1.97-1.87 (2H, m, C4-H), 2.03 (1H, d, *J* = 12.6, C17-H), 3.63 (1H, dd, *J* = 14.4, 10.2, C6-H), 4.02 (1H, br s, C3-αH), 4.07 (1H, dd, *J* = 14.4, 1.8, C6-H), 4.32 (1H, s, OH), 7.29 (1H, d, *J* = 7.8, 3′-Ph-H), 7.35 (1H, t, *J* = 8.4, 4′-Ph-H), 7.45 (1H, d, *J* = 7.8, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 12.67 (18-C), 18.82 (19-C), 18.93 (21-C), 21.52 (11-C), 22.67 (26 or 27-C), 22.93 (26 or 27-C), 23.99 (23-C), 25.06 (16-C), 27.71 (15-C), 28.10 (25-C), 28.96 (1-C), 29.79 (8-C), 35.78 (2-C), 36.34 (20-C), 39.57 (22-C), 39.94 (6-C), 43.19 (12-C), 44.51 (24-C), 44.86 (13-C), 45.22 (4-C), 46.46 (7-C), 50.39 (10-C), 54.89 (9-C), 55.66 (14-C), 56.60 (17-C), 67.50 (3-C), 80.77 (5-C), 122.41 (3′-ph-C), 122.69 (5′-ph-C), 128.69 (6′-ph-C), 131.50 (4′-ph-C), 136.04 (1′-ph-C), 142.93 (2′-ph-C), 165.91 (-C=O); IR(KBr) *ν*/cm^{-1}: 3382.70, 2926.88, 2865.45, 1580.98, 1556.46, 1444.29, 1379.16, 1334.54, 1309.42, 1259.76, 1169.26, 1131.05, 1075.13, 1018.44, 955.26, 867.54, 778.81, 668.28, 556.98; HREIMS (m/z): 636.2720 $[M+H]^+$ (calcd. For $C_{34}H_{51}ClNO_3Se$ 636.2723).

6β-[3'-Oxo-7'-fluorobenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14e)

Faint yellow solid. Yield: 41.76%, m.p. 219-221^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 0.69 (3H, s, 18-CH_3), 0.85 (3H, d, *J* = 6.6, 26- or 27-CH_3), 0.86 (3H, d, *J* = 6.6, 26- or 27-CH_3), 0.89 (3H, s, 19-CH_3), 0.91 (3H, d, *J* = 6.6, 21-CH_3), 1.98-2.05 (2H, m, C4-H), 3.03 (1H, s, OH), 3.69 (1H, dd,

J = 14.4, 10.6, C6-H), 4.08 (1H, br s, C3-αH), 4.12 (1H, d, *J* = 14.4, C6-H), 4.39 (1H, s, OH), 7.23 (1H, t, *J* = 8.4, 4′-Ph-H), 7.35-7.39 (1H, m, 5′-Ph-H), 7.80 (1H, d, *J* = 7.8, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 12.73 (18-C), 18.97 (19-C), 19.07 (21-C), 21.57 (11-C), 22.70 (26- or 27-C), 22.97 (26- or 27-C), 24.92 (23-C), 27.20 (16-C), 28.16 (15-C), 28.18 (25-C), 28.98 (1-C), 29.85 (8-C), 35.82 (2-C), 36.38 (20-C), 39.61 (22-C), 40.02 (6-C), 43.49 (12-C), 44.67 (24-C), 44.93 (13-C), 45.28 (4-C), 46.60 (7-C), 49.89 (10-C), 55.14 (9-C), 55.73 (14-C), 56.82 (17-C), 67.66 (3-C), 80.63 (5-C), 117.29 ($^2J_{C\text{-}F}$ =18.9, 4′-ph-C), 124.21 (6′-ph-C), 126.24 ($^2J_{C\text{-}F}$ =24.6, 2′-ph-C), 127.90 (5′-ph-C), 130.55 (1′-ph-C), 157.76 ($^1J_{C\text{-}F}$ =244.5, 3′-ph-C), 167.23 (-C=O); IR(KBr) *v*/cm^{-1}: 3371.13, 2923.92, 2866.17, 1632.23, 1575.66, 1473.46, 1429.71, 1379.06, 1352.68, 1272.64, 1247.00, 1204.10, 1169.75, 1132.87, 1075.70, 996.87, 867.55, 800.58, 737.61, 669.36, 615.40; HREIMS (m/z): 620.3018 $[M+H]^+$ (calcd. for $C_{34}H_{51}FNO_3Se$ 620.3018).

6β-[3'-Oxo-6'-fluorobenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14f)

Faint yellow solid. Yield: 27.68%, m.p. 194-196 °C; ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 0.68 (3H, s, 18-CH_3), 0.85 (3H, d, *J* = 6.6, 26- or 27-CH_3), 0.86 (3H, d, *J* = 6.6, 26- or 27-CH_3), 0.87 (3H, s, 19-CH_3), 0.91 (3H, d, *J* = 6.6, 21-CH_3), 1.93-2.04 (2H, m, C4-H), 3.29 (1H, s, -OH), 3.75 (1H, dd, *J* = 14.4, 9.6, C6-H), 3.99 (1H, d, *J* = 14.4, C6-H), 4.03 (1H, br s, C3-αH), 4.56 (1H, s, -OH), 7.08 (1H, td, *J* = 8.4, 1.8, 5′-Ph-H), 7.30 (1H, dd, *J* = 7.8, 1.9, 3′-Ph-H), 7.96 (1H, dd, *J* = 8.4, 4.8, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$)*δ*: 12.68 (18-C), 18.89 (19-C), 18.92 (21-C), 21.51 (11-C), 22.63 (26 or 27-C), 22.92 (26 or 27-C), 23.99 (23-C), 24.94 (16-C), 27.39 (15-C), 28.17 (25-C), 28.91 (1-C), 29.79 (8-C), 35.77 (2-C), 36.34 (20-C), 39.57 (22-C), 39.95 (6-C), 43.37 (12-C), 44.68 (24-C), 44.89 (13-C), 45.18 (4-C), 46.47 (7-C), 50.03 (10-C), 55.24 (9-C), 55.66 (14-C), 56.76 (17-C), 67.55 (3-C), 80.65 (5-C), 110.57 ($^2J_{C\text{-}F}$=25.4, 5′-ph-C), 114.53 ($^2J_{C\text{-}F}$=23.4, 3′-ph-C), 123.95 (1′-ph-C), 130.46 ($^3J_{C\text{-}F}$=9.0, 6′-ph-C), 141.50 ($^3J_{C\text{-}F}$=9.0, 2′-ph-C), 165.15 ($^1J_{C\text{-}F}$=252.0, 4′-ph-C), 167.03 (-C=O); IR(KBr) *v*/cm^{-1}: 3727.06, 3704.22, 3627.01, 3599.97, 3375.24, 2922.88, 2864.18, 1599.65, 1468.74, 1441.94, 1417.27, 1378.65, 1313.59, 1242.15, 1216.14, 1166.58, 1126.59, 1077.81, 1016.07, 951.65, 877.40, 828.87, 763.55, 670.55, 654.71;HREIMS(m/z): 620.3019 $[M+H]^+$ (calcd. for $C_{34}H_{51}FNO_3Se$ 620.3018).

6β-[3'-Oxo-5'-fluorobenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14g)

Faint yellow solid. Yield: 61.99%, m.p. 212-215 °C; ^{1}H NMR (600MHz, $CDCl_3$) δ: 0.67 (3H, s, 18-CH_3), 0.85 (3H, d, J = 6.6, 26- or 27-CH_3), 0.86 (3H, d, J = 6.6, 26- or 27-CH_3), 0.87 (3H, s, 19-CH_3), 0.90 (3H, d, J = 6.6, 21-CH_3), 1.93-2.08 (2H, m, C4-H), 3.41 (1H, s, OH), 3.77 (1H, dd, J = 14.4, 9.6, C6-H), 3.99 (1H, d, J = 14.4, C6-H), 4.03 (1H, br s, C3-αH), 4.57 (1H, s, OH), 7.29-7.31 (1H, td, J = 8.4, 2.4, 4′-Ph-H), 7.55 (1H, dd, J = 8.4, 4.2, 3′-Ph-H), 7.68 (1H, dd, J = 8.4, 2.4, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$)δ: 12.19 (18-C), 18.41 (19-C), 18.43 (21-C), 21.02 (11-C), 22.17 (26 or 27-C), 22.44 (26 or 27-C), 24.43 (23-C), 26.88 (16-C), 27.62 (15-C), 27.67 (25-C), 28.43 (1-C), 29.31 (8-C), 35.28 (2-C), 35.85 (20-C), 39.08 (22-C), 39.46 (6-C), 42.96 (12-C), 44.16 (24-C), 44.41 (13-C), 44.8 (4-C), 46.15 (7-C), 49.51 (10-C), 54.72 (9-C), 55.17 (14-C), 56.27 (17-C), 67.03 (3-C), 80.15 (5-C), 114.18 ($^2J_{C-F}$=22.5, 6′-ph-C), 119.77 ($^2J_{C-F}$=24.0, 4′-ph-C), 124.68-124.73 (3′-ph-C), 128.71 (1′-ph-C), 133.89 (2′-ph-C), 161.27 ($^1J_{C-F}$=244.5, 5′-ph-C), 166.51 (-C=O); IR(KBr) ν/cm^{-1}: 3726.86, 3704.25, 3291.87, 2924.73, 2865.52, 1873.02, 1623.65, 1577.74, 1464.02, 1438.15, 1379.27, 1269.01, 1170.63, 1138.74, 1113.31, 1076.13, 961.10, 883.18, 865.36, 814.56, 762.94, 669.64, 654.15, 619.63, 539.61; HREIMS (m/z): 620.3024 $[M+H]^+$ (calcd. for $C_{34}H_{51}FNO_3Se$ 620.3018).

6β-[3'-Oxo-5'-methylbenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14h)

Faint yellow solid. Yield: 64.75%, m.p. 165-166 °C; ^{1}H NMR (600MHz, $CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.85 (3H, d, J = 6.6, 26- or 27-CH_3), 0.86 (3H, d, J = 6.6, 26- or 27-CH_3), 0.87 (3H, s, 19-CH_3), 0.91 (3H, d, J = 6.6, 21-CH_3), 2.43 (3H, s, Ph-CH_3), 3.49 (1H, s, OH), 3.80 (1H, d, J = 14.4, C6-H), 3.92 (1H, dd, J = 14.4, 8.4, C6-H), 3.97 (1H, br s, C3-αH), 4.89 (1H, s, OH), 7.37 (1H, d, J = 7.8, 4′-Ph-H), 7.46 (1H, d, J = 7.8, 3′-Ph-H), 7.81 (1H, s, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 12.69 (18-C), 14.29 (5-Ph-CH_3), 18.77 (19-C), 18.92 (21-C), 21.10 (11-C), 21.50 (26- or 27-C), 22.92 (26- or 27-C), 24.99 (23-C), 27.54 (16-C), 28.11 (15-C), 28.25 (25-C), 28.92 (1-C), 29.79 (8-C), 35.76 (2-C), 36.35 (20-C), 39.59 (22-C), 39.97 (6-C), 43.44 (12-C), 44.81 (24-C), 44.92 (13-C), 45.07 (4-C), 46.41 (7-C), 50.08 (10-C), 55.51 (9-C), 55.65 (14-C), 56.80 (17-C), 67.44 (3-C), 80.69 (5-C), 123.47 (6′-ph-C), 127.44 (3′-ph-C), 128.79 (1′-ph-C), 133.40 (4′-ph-C), 135.97 (5′-ph-C), 136.20 (2′-ph-C), 167.96 (-C=O); IR(KBr) ν/cm^{-1}: 3374.12, 2927.54, 2863.30, 1616.62, 1562.35, 1465.74, 1378.87, 1277.09, 1237.90, 1167.51, 1129.58, 1078.50, 958.51, 921.51, 865.63, 824.13, 767.76, 730.90, 669.89, 618.26, 538.0; HREIMS (m/z): 616.3279

$[M+H]^+$ (calcd. for $C_{35}H_{54}NO_3Se$ 616.3269).

6β-[3'-Oxo-6'-methoxylbenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14i)

Faint yellow solid. Yield: 62.86%, m.p. 235-236 °C; ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.6, 26- and 27-CH_3), 0.89 (3H, s, 19-CH_3), 0.94 (3H, d, J = 6.3, 21-CH_3), 3.31 (1H, s, OH), 3.80-3.94 (2H, m, C6-H), 3.89 (3H, s, Ph-OCH_3), 4.01 (1H, br s, C3-αH), 4.88 (1H, s, OH), 6.96 (1H, dd, J = 8.7, 2.1, 5′-Ph-H), 7.06 (1H, d, J = 2.1, 3′-Ph-H), 7.91 (1H, d, J = 8.7, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 12.60 (18-C), 18.65 (19-C), 18.82 (21-C), 21.41 (11-C), 22.56 (26 or 27-C), 22.83 (26 or 27-C), 23.88 (23-C), 27.41 (16-C), 28.03 (15-C), 28.17 (25-C), 28.84 (1-C), 29.69 (8-C), 35.68 (2-C), 36.24 (20-C), 39.48 (22-C), 39.86 (6-C), 43.22 (12-C), 44.81 (24-C), 44.96 (13-C), 45.85 (4-C), 46.27 (7-C), 49.97 (10-C), 55.39 (9-C), 55.72 (14-C), 56.68 (17-C), 60.42 (4-Ph-OCH_3), 67.43 (3-C), 80.63 (5-C), 107.14 (3′-ph-C), 114.23 (5′-ph-C), 120.38 (1′-ph-C), 129.70 (6′-ph-C), 141.07 (2′-ph-C), 162.93 (4′-ph-C), 167.67(-C=O); IR(KBr) ν/cm^{-1}: 3726.39, 3261.70, 2932.91, 2864.96, 1872.93, 1781.03, 1598.13, 1481.35, 1462.22, 1438.30, 1379.77, 1337.55, 1257.55, 1173.43, 1129.43, 1078.23, 1043.84, 957.38, 865.49, 835.10, 764.57, 732.68, 679.61; HREIMS (m/z): 654.3040 $[M+Na]^+$ (calcd. for $C_{35}H_{53}NO_3SeNa$ 654.3037).

6β-[3'-Oxo-5'-methoxylbenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14j)

Faint yellow solid. Yield: 67.97%, m.p. 209-210 °C; ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.69 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.3, 26- and 27-CH_3), 0.87 (3H, s, 19-CH_3), 0.92 (3H, d, J = 6.3, 21-CH_3), 3.66 (1H, s, -OH), 3.81-3.88 (2H, m, C6-H), 3.84 (3H, d, Ph-OCH_3), 4.00 (1H, br s, C3-αH), 4.87 (1H, s, -OH), 7.18 (1H, dd, J = 8.7, 2.7, 4′-Ph-H), 7.47 (1H, d, J = 8.7, 3′-Ph-H), 7.49 (1H, d, J = 2.7, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 12.59 (18-C), 18.74 (19-C), 18.84 (21-C), 21.39 (11-C), 22.57 (26- or 27-C), 22.84 (26- or 27-C), 23.88 (23-C), 24.87 (16-C), 27.39 (15-C), 28.01 (25-C), 28.10 (1-C), 28.84 (8-C), 35.67 (2-C), 36.24 (20-C), 39.48 (22-C), 39.85 (6-C), 43.34 (12-C), 44.67 (24-C), 44.80 (13-C), 44.98 (4-C), 46.44 (7-C), 49.91 (10-C), 55.35 (9-C), 55.53 (14-C), 55.64 (17-C), 56.68 (5-Ph-OCH_3), 67.32 (3-C), 80.55 (5-C), 110.21 (6′-ph-C), 121.93 (4′-ph-C), 124.47 (3′-ph-C), 128.30 (1′-ph-C), 130.41 (2′-ph-C), 158.72 (5′-ph-C), 167.70 (-C=O); IR(KBr) ν/cm^{-1}: 3269.67, 2935.69, 2862.10, 1616.41, 1568.18,

1470.64, 1427.48, 1352.40, 1276.45, 1231.53, 1170.31, 1150.74, 1128.47, 1077.13, 1021.74, 977.83, 959.87, 921.86, 866.09, 813.82, 764.44, 732.28, 634.11, 550.86, 506; HREIMS (m/z): 654.3028 $[M+Na]^+$ (calcd. for $C_{35}H_{53}NO_3SeNa$ 654.3037).

6β-[3'-Oxo-6'-nitrobenzisoselenazol-2'-(3H)-ylmethyl]-B-norcholestane-3β,5β-diol (14k)

Faint yellow solid. Yield: 47.59%, m.p. 217-220 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 0.70 (3H, s, 18-CH_3), 0.865 (3H, d, J = 6.6, 26- or 27-CH_3), 0.870 (3H, d, J = 6.6, 26- or 27-CH_3), 0.88 (3H, s, 19-CH_3), 0.91 (3H, d, J = 6.6, 21-CH_3), 1.98-2.07 (2H, m, C4-H), 2.35 (1H, OH), 3.66-3.72 (1H, m, C3-αH), 4.13 (1H, d, J = 13.8, C6-H), 4.15 (1H, OH), 4.25 (1H, d, J = 13.8, C6-H), 8.14 (1H, d, J = 8.4, 6′-Ph-H), 8.21 (1H, d, J = 8.4, 5′-Ph-H), 8.50 (1H, s, 3′-Ph-H); ^{13}CNMR(75MHz,$CDCl_3$) δ: 12.68 (18-C), 18.92 (19-C), 19.03 (21-C), 21.53 (11-C), 22.64 (26 or 27-C), 22.91 (26 or 27-C), 24.00 (23-C), 27.14 (16-C), 28.12 (15-C), 28.20 (25-C), 28.91 (1-C), 29.79 (8-C), 35.78 (2-C), 36.34 (20-C), 39.57 (22-C), 39.95 (6-C), 43.39 (12-C), 44.54 (24-C), 44.91 (13-C), 45.33 (4-C), 46.89 (7-C), 49.96 (10-C), 54.93 (9-C), 55.71 (14-C), 56.75 (17-C), 67.85 (3-C), 80.58 (5-C), 119.57 (3′-ph-C), 120.88 (5′-ph-C), 129.44 (6′-ph-C), 132.22 (1′-ph-C), 140.91 (2′-ph-C), 150.00 (4′-ph-C), 166.00 (-C=O); IR(KBr) v/cm^{-1}:670.18、729.59、1078.91、1164.35、1346.29、1401.15、1496.31、1527.42、1616.28、1653.77、2953.70、3133.78、3429.25; HREIMS (m/z): 649.2956 $[M+H]^+$ (calcd. for $C_{34}H_{51}N_2O_5Se$ 647.2963).

4.2 Biological Studies

Using 96-well plates on a MLLTISKAN MK3 analysis spectrometer, the cell proliferation was tested with MTT method. SKOV3 (ovarian carcinoma), PC-3 (prostatic carcinoma), T47D (breast carcinoma) and MCF-7 (breast carcinoma) cell were obtained by Guangxi Medical University (China).

Materials

The compounds were dissolved by sterile dimethyl sulfoxide (DMSO) (Sigma) at initial concentration of 10 mg/mL. The stock solution was diluted with complete nutrient medium (RPMI-1640) supplemented with 10% heat inactivated fetal bovine serum and 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate.

Cell culture

All cells were grown in the medium (RPMI-1640) supplemented with 10% heat inactivated fetal bovine serum, 0.1 g/L penicillin G + 0.1 g/L streptomycin sulfate. The cell culture was incubated in a humidified atmosphere of 5% CO_2 at 37 ^oC.

Assay for cell viability

Cells (1~2×10^4 cells/mL) were seeded into 96-wells plates for 24 h. The medium contained test compound with different concentrations was added to the cells. After 72 h incubation, 20 μL of the tetrazolium dye (MTT) (5 mg/mL) solution were added to each well. After additional 4 h incubation, the medium was discarded, and 200 μL of DMSO were added to dissolve the purple formazan. The untreated cells were used as controls. Triplicate wells were prepared for each individual dose. The absorbance values (*A*) at 492 nm were determined using a MLLTISKAN MK3 analysis spectrometer (Thermo Scientific Co.). The IC_{50} values were calculated as the concentration of drug yielding 50% cell survival.

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Conflict of Interest

The authors declare no conflict of interest.

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Highlights

1. Two novel steroid-benzisoselenazolone hybrids were synthesized.
2. Hybrids were obtained by condensing of 2-chloroselanylbenzoyl chloride with amino-steroids.
3. The antiproliferative activity of the hybrids was investigated.
4. Some hybrids had excellent inhibitory activity on SKOV3 cells.

Graphical Abstract

Synthesis and Antiproliferative Evaluation of Novel Steroid-Benzisoselenazolone Hybrids

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