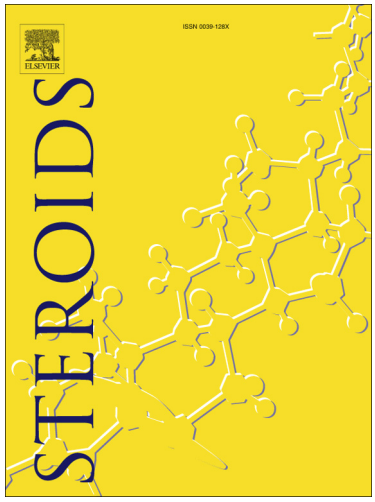

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PII: S0039-128X(18)30191-0
DOI: https://doi.org/10.1016/j.steroids.2018.10.004
Reference: STE 8326

To appear in: *Steroids*

Received Date: 13 September 2018
Revised Date: 25 September 2018
Accepted Date: 1 October 2018

Please cite this article as: Cui, J., Pang, L., Wei, M., Gan, C., Liu, D., Yuan, H., Huang, Y., Synthesis and AntiproliferativeActivity of 17-[1',2',3']-selenadiazolylpregnenolone compounds, *Steroids* (2018), doi: https://doi.org/10.1016/j.steroids.2018.10.004

Synthesis and AntiproliferativeActivity of 17-[1′,2′,3′]-selenadiazolylpregnenolone compounds

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ABSTRACT

Using pregnenolone as a starting material, some 3-substituted 17-[1′,2′,3′]-selenadiazolylpregnenolone derivatives were synthesized, and their structures were characterized by IR, NMR and HRMS. The *in vitro* antitumor activity of the compounds was assayed against PC-3、SKOV3、T47D、MCF-7 and HEK293T cell lines. The results show that some compounds display selective antiproliferative activity against PC-3 and SKOV3 cells lines and are almost inactive to normal kidney epithelial cells (HEK293T). The IC_{50} value are much better than that of abiraterone (positive control).

KEYWORDS

17-[1′,2′,3′]-selenadiazolylpregnenolone; antiproliferative activity; [1′,2′,3′]-selenadiazole; Pregnenolone; Organoselenium compounds.

1. Introduction

Selenium is a key component of severalmajor metabolic pathways in human, including thyroidhormone metabolism, antioxidant defense system, andimmune function[1]. Organoselenium compounds have attracted special attention over the last years mainly because of their diversified biological activity. Compared with inorganoselenium compound, organoselenium compound has some excellent characteristics, such as higher bioavailability, stronger bioactivity, lower toxicity, smaller environmental pollution, and anti-oxidation, anti-inflammatory and anti-cancer, etc. [2-3]. Karam et al. found that synthetic organoselenium compounds had a stronger antitumor activity and lower toxicity than inorganoselenium compounds [4]. At present, some organoselenium compounds have been used in anti-tumor clinical studies and achieved some good results, such as

ebselen [5-6], ethaselen [7-9] and selenazofurin [10-11], etc.

Selenazoles are interesting five-membered heterocycles which are being reported for their potential pharmacological features [12-15]. In addition, some selenadiazole derivatives have triggered intense research interest due to their excellent anticancer biological activities[16-20]. [1,2,3]-selenadiazole compounds are a type of compounds in which a selenium atom is directly connected with two nitrogen atoms. Because of its simple preparation [21-22] and good bioactivity [23-25], it has received great attention. Recently, Tibor Pasinszki synthesized a 4,5-dihydrobenzoferroceno[1,2-d][1,2,3]selenadiazole and its benzo-fused analogue, and found that the antiproliferative effect of both compounds were active against cervical HeLa, ovarian A2780 and breast MDA-MB-231 cell lines[26].

Steroidal compounds are a kind of essential physiological active substances in the human life process, which have high permeability to cells and binding ability to the cell nucleus and cell membrane. Small changes in steroids often lead to widespread changes in their biological activity, and may be used to treat different diseases in humans. In recent years, pharmacologist and chemist have been committed to the structure transformation of steroid compounds through different ways, such as introducing different hetero atoms and functional groups or heterocyclic groups into the steroid, or changing the structure of steroid nucleus or branched chain, and then further explore their biological activity and clinical application [27].However, 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 wasn't carried out in this field [28-31].

Recently, Fernández-Herrera M. A. et al. introduced a selenocyano group into 22-oxo-26-hydroxycholestane and synthesized two novel 22-oxo-26-selenocyanocholestanic steroids [32]. Romero-Hernández L. L. et al. introduced a selenourea into the diosgenin to prepare a selenourea-steroid compound [33]. Fuentes-Aguilar A. et al. introduced abselen into diosgenin, hecogenin and smilagenin, and prepared three steroidal ebselen analogues [34]. The result of bioactivity tests showed that all the steroidal selenium compounds obtained above had a good antiproliferative activity *in vitro*.

In previous studies, we synthesized some novel heterosteroids[35-38], and the results of antiproliferative test showed that some B-norcholesteryl benzimidazoles, thiazoles and

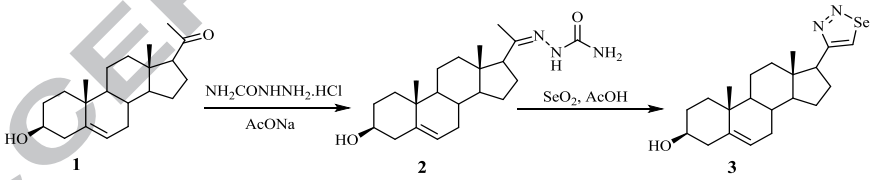
17-hydrazone aromatic steroidal heterocycle derivatives exhibited an excellent antiproliferative activity. In continuation of our work on the synthesis and anticancer activity evaluation of various heterosteroids, herein, we designed and synthesized a series of novel heterosteroids possessing [1,2,3]selenadiazole ring as the side chain by using pregnenolone as a starting material. The antiproliferative activity of compounds had been evaluated against SKOV3, PC-3, T47D and MCF-7 cancer cell lines.

2. Result and discussion

2.1 Chemistry

2.1.1 Synthesis of pregnenolone-17-[1′,2′,3′] selenadiazole

First, pregnenolone was transformed to compound **2** by the reaction with semicarbazide and CH_3CH_2ONa. Then, the reaction of compound **2** with selenium dioxide and acetic acid afforded corresponding pregnenolone-17-[1′,2′,3′]selenadiazole (**3**) (Scheme 1). The structure of compound **3** had been confirmed on analytical and spectral data. In the NMR spectrum of compound **3**, resonances signals of Ar-H at δ 8.86 ppm and Ar-C at δ 137.79, 164.49ppm demonstrate the presence of a selenadiazole ring. The HREI mass spectrum of compound **3** exhibits a molecular ion peak ($[M+H]^+$) at *m*/*z* 407.1604. All spectra confirm the structure of compound **3**.

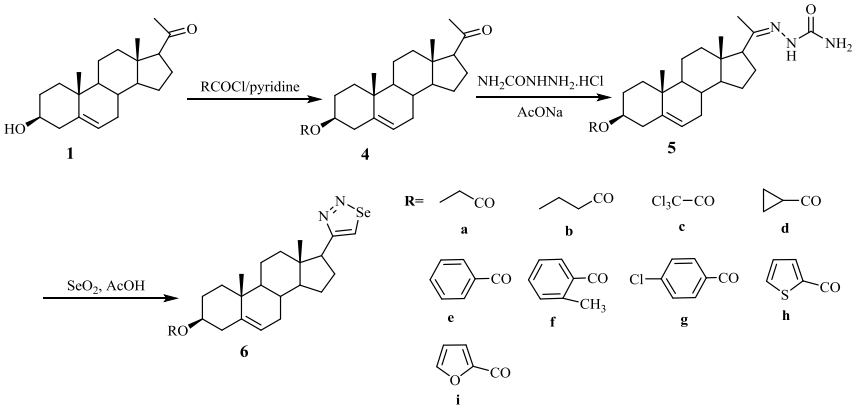


1 2 3

Scheme 1 The synthesis of compound pregnenolone-17-[1′,2′,3′] selenadiazole

2.1.2 Synthesis of pregnenolone-17-[1′,2′,3′]selenadiazole derivatives

To investigate the effect of different 3-substituted group in compound **3** to antiproliferative acitivity, pregnenolone-17-[1′,2′,3′]selenadiazole derivatives **6a**-**6i** were prepared (Scheme 2) and evaluated for their antiproliferative activity.



Scheme 2 The synthesis of pregnenolone-17-[1′,2′,3′]selenadiazole derivatives

Firstly, the reaction of compound **1** with various acyl chlorides afforded corresponding carboxylic pregnenoloneesters **4a-4i**. After that, compounds **5a-5i** were obtained by the reaction of compounds **4a-4i** with semicarbazide. Last, the reaction of compounds **5a-5i** with selenium dioxide gave the target compounds **6a-6i**. The structure of all synthesized compounds had been confirmed on analytical and spectral data.

2.2 The evaluation of the antiproliferative activity*in vitro*

All synthesized compounds were evaluated for their antiproliferative activities *in vitro* against PC-3 (human prostate carcinoma), SKOV3 (human ovarian carcinoma), T47D (human breast infiltrating duct carcinoma), MCF-7(human breast adenocarcinoma) and HEK293T (normal kidney epithelial cells) cell lines using a MTT assay. The results were summarized as IC_{50} values in μmol/L in Table 1.

Tab.1 *In vitro* antiproliferative activities (IC_{50} inμM) of pregnenolone selenadiazole compounds

compd	SKOV3	PC-3	T47D	MCF-7	HEK-293T
3	57.59±0.86	37.35±0.06	71.28±1.31	>100	>100
6a	42.35±0.66	13.24±0.41	40.65±0.10	>100	>100
6b	36.60±1.05	31.74±0.32	41.65±0.24	>100	>100
6c	55.31±11.02	56.31±2.01	>100	>100	>100
6d	35.79±0.47	12.48±0.44	>100	>100	>100
6e	>100	>100	>100	>100	>100
6f	>100	59.85±5.48	>100	>100	>100
6g	>100	>100	>100	>100	>100

6h	33.88±0.13	18.56±0.82	37.39±0.60	>100	>100
6i	18.26±0.49	21.40±0.37	46.67±0.19	>100	>100
abiraterone	51.51±15.98	37.61±0.12	34.66±1.69	44.70±0.67	>100

As showed in Table 1, all pregnenolone selenadiazole compounds do not exhibit obvious inhibitory activity against MCF-7 and T47D breast cancer cell lines, but show selective antiproliferative activity against PC-3 and SKOV3 cell lines. From the perspective of SKOV3 cells, compounds (**6a**, **6b** and **6c**) display obvious inhibitory activity when the substituted group R is the alkyl groups. When the substituent R is phenyl or substituted phenyls (**6e**, **6f**, **6g**), compounds show no obvious anticancer activity to SKOV3 cells. However, when R is the thiophenyl or furanyl of 5-member heterocycle, the compounds manifest good antiproliferative activity. For example, IC_{50} of compound **7i** is 18.26 μmol/L against SKOV3 cells which is vastly superior to the 51.51μmol/L of the positive control abiraterone.

When the substituent R is propyl, cyclopropyl or thiophenyl and furanyl, the compounds (**6a**, **6d** and **7h**, **7i**) display very good inhibitive effect on the proliferation of PC-3 cells with IC_{50} value of 13.24, 12.48, 18.56 and 21.40 μmol/L, respectively. The result is much better than the 37.61μmol/L of abiraterone.

Furthermore, all tested compounds are almost inactive to normal kidney epithelial cells (HEK293T).

3. Conclusion

Using pregnenolone as a starting material, some 3-substituted 17-[1′,2′,3′]-selenadiazolylpregnenolone compounds were synthesized, and the structures of all investigated compounds were characterized by IR, NMR and HRMS. The *in vitro* antitumor activity of the compounds was assayed against PC-3、SKOV3、T47D、MCF-7 and HEK293T cell lines. The results show that some compounds display selective inhibitory activity against PC-3 and SKOV3 cells when the substituted group R is the alkyl groups, thiophenyl or furanyl, and the IC_{50} value are much better than that of abiraterone (positive control). Moreover, all compounds are almost inactive to normal kidney epithelial cells (HEK293T). The information obtained from the studies is valuable for the design of novel steroidal chemotherapeutic drugs.

4. EXPERIMENT

4.1 Chemistry

4.1.1 Reagent and Instrument

The sterols were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai, China. All chemicals and solvents are analytical grade. Melting points were determined on an X_4 apparatus (Beijing Tech Instrument Co. Ltd., Beijing, China) and were uncorrected. Infrared spectra were measured with a Thermo Scientific Nicolet IS-10 Spectrophotometer (Thermo Scientific, America). The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ 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, respectively. 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 a MTT method using 96-well plates on a MLLTISKAN MK3 analysis spectrometer (Thermo Scientific, Shanghai, China).

4.1.2 General procedure for the synthesis of compounds **4a**-**4i**.

600μL of acyl chloride was injected slowly to a solution of pregnenolone (500mg,1.6mol) in 15 mL of pyridine. The mixture was stirred at 45°C until no starting material was observed (the progress of the reaction was monitored by TLC). 2 mL of ice water was added to terminate the reaction. Then 30 mL of 1M hydrochloric acid and 50 mL of ethyl acetate were added, organic phase was separated and water phase was extracted with ethyl acetate. The combined organic phase was washed twice with 1 M hydrochloric acid and one time with 10mL solution of saturated copper sulfate to ensure residual pyridine removed. Then the organic phase was washed in turn with saturated $NaHCO_3$, water and saturated brine, dried with anhydrous sodium sulfate. After solvent was removed under reduced pressure, the crude product was purified by the column chromatography on silica gel using CH_3COOEt/petroleum ether (1:20) as the eluent to give the target product.

3β-Propionyloxypregnenolone (**4a**)

White solid, Yield: 57.52%, m.p. 121-122°C; ^1H NMR (300 MHz, $CDCl_3$) δ: 0.58 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3)， 1.08 (3H, t, J = 7.5, 3′-CH_3), 2.07 (3H, s, 21-CH_3), 2.26 (2H, q, J = 7.5, 2′-CH_2), 2.49 (1H, t, J = 8.7, C4-βH), 4.50-4.61 (1H, m, C3-αH), 5.31 (1H, d, J = 4.8, C6-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 9.14 (3′-C), 13.16 (18-C), 19.26 (19-C), 20.98 (11-C), 22.75 (15-C), 24.43 (16-C), 27.69 (2′-C), 27.82 (2-C), 31.49 (21-C), 31.71 (8-C), 31.76 (7-C), 36.54

(10-C), 36.95 (1-C), 38.04 (4-C), 38.72 (12-C), 43.90 (13-C), 49.81 (9-C), 56.76 (14-C), 63.58 (17-C), 73.51 (3-C), 122.19 (6-C), 139.64 (5-C), 173.80 (-C=O), 209.34 (20-C); IR(KBr) ν/cm^{-1}: 3728.81, 3628.14, 3068.31, 2963.20, 2939.87, 2899.06, 2874.33, 2825.44, 1728.49, 1469.90, 1383.11, 1364.35, 1310.29, 1260.18, 1188.78, 1136.16, 1088.55, 1044.45, 1015.68, 1000.50, 957.92, 900.90, 803.86, 669.88, 603.80; HREIMS (m/z): 395.2564 $[M+Na]^+$ (calcd. For $C_{24}H_{36}NaO_3$ 395.2562).

3β-Butanoyloxypregnenolone **(4b)**

White solid, Yield: 67.81%, m.p. 88-91 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.60 (3H, s, 18-CH_3), 0.91 (3H, t, J = 7.5, 4′-CH_3), 0.99 (3H, s, 19-CH_3), 1.61 (2H, q, J = 7.5, 3′-CH_3), 2.09 (2H, s, 21-CH_3), 2.22 (2H, t, J = 7.5, 2′-CH_2), 2.51 (1H, t, J = 9.0, C4-βH), 4.53-4.64 (1H, m, C3-αH), 5.34 (1H, d, J = 4.5, C6-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.18 (18-C), 13.61 (4′-C), 18.42 (3′-C), 19.28 (19-C), 20.99 (11-C), 22.77 (15-C), 24.44 (16-C), 27.73 (2-C), 31.51 (21-C), 31.72 (8-C), 31.77 (7-C), 36.30 (2′-C), 36.50 (10-C), 36.56 (1-C), 38.07 (4-C), 38.74 (12-C), 43.92 (13-C), 49.83 (9-C), 56.78 (14-C), 63.60 (17-C), 73.46 (3-C), 122.21 (6-C), 139.65 (5-C), 173.03 (1′-C), 209.40 (20-C); IR(KBr) ν/cm^{-1}: 2943.08, 2872.36, 2849.21, 1734.72, 1708.76, 1467.82, 1386.16, 1356.89, 1300.75, 1226.88, 1189.11, 1135.60, 1111.55, 1013.52, 985.43, 955.69, 922.28, 797.96, 592.42; HREIMS (m/z): 409.2716 $[M+Na]^+$ (calcd. For $C_{25}H_{38}NaO_3$ 409.2719).

3-Trichloroacetoxypregnenolone **(4c)**

White solid, Yield: 86.05%, m.p. 177-180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.61 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.10 (3H, s, 21-CH_3), 2.52 (1H, t, J = 9.0, C4-βH), 4.68-4.77 (1H, m, C3-αH), 5.42 (1H, d, J = 4.8, C6-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.20 (18-C), 18.42 (19-C), 19.28 (11-C), 21.02 (15-C), 22.79 (16-C), 24.45 (2-C), 27.04 (21-C), 31.54 (8-C), 31.73 (7-C), 36.53 (10-C), 36.73 (1-C), 37.23 (4-C), 38.69 (12-C), 43.91 (13-C), 49.75 (9-C), 56.73 (14-C), 63.57 (17-C), 79.57 (3-C), 90.18 (CCl_3), 123.32 (6-C), 138.60 (5-C), 161.25 (-C=O), 209.37 (20-C); IR(KBr) ν/cm^{-1}: 3123.78, 2967.14, 2933.09, 2860.77, 2829.75, 1758.20, 1705.66, 1450.45, 1379.93, 1358.97, 1319.66, 1256.33, 1175.34, 1155.51, 1137.14, 1089.24, 1007.24, 980.66, 943.15, 927.38, 858.25, 827.67, 754.80, 680.41, 639.39, 597.26, 577.54; HREIMS (m/z): 483.1239 $[M+Na]^+$ (calcd. For $C_{23}H_{31}Cl_3NaO_3$ 483.1236).

3-Cyclopropylformyloxypregnenolone **(4d)**

White solid, Yield: 49.75%, m.p. 217-219 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.54 (3H, s,

18-CH_3), 0.73-0.76 (2H, m, 3′-CH_2), 0.84-0.89 (2H, m, 4′-CH_2), 0.94 (3H, s, 19-CH_3), 2.03 (3H, s, 21-CH_3), 2.45 (1H, t, *J* = 8.7, C4-βH), 4.44-4.55 (1H, m, C3-αH), 5.28 (1H, d, *J* = 3.3, C6-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 8.25 (3′,4′-C), 13.01 (18-C), 13.13 (2′-C), 19.24 (19-C), 20.93 (11-C), 22.72 (15-C), 24.41 (16-C), 27.69 (2-C), 31.44 (21-C), 31.69 (8-C), 31.72 (7-C), 36.50 (10-C), 36.94 (1-C), 38.04 (4-C), 38.68 (12-C), 43.84 (13-C), 49.78 (9-C), 56.71 (14-C), 63.51 (17-C), 73.59 (3-C), 122.14 (6-C), 139.61 (5-C), 174.05 (1′-C=O), 209.14 (20-C); IR(KBr) *ν*/cm^{-1}: 3448.45, 2939.46, 2852.15, 1699.24, 1469.21, 1450.99, 1384.04, 1356.75, 1291.05, 1229.42, 1196.29, 1133.57, 979.01, 927.14, 882.35, 854.98, 839.47, 784.52, 764.10, 722.21, 592.69; HREIMS (m/z): 407.2566 $[M+Na]^+$ (calcd. For $C_{25}H_{36}NaO_3$ 407.2562).

3-Benzoyloxypregnenolone **(4e)**

White solid, Yield: 78.41%, m.p. 203-204 °C; ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.63 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 2.13 (3H, s, 21-CH_3), 2.54 (1H, t, *J* = 9.0, C4-βH), 4.81-4.91 (1H, m, C3-αH), 5.42 (1H, d, *J* = 4.2, C6-H), 7.42 (2H, t, *J* = 7.5, 3′, 5′-Ph-H), 7.54 (1H, t, *J* = 6.0, 4′-Ph-H), 8.04 (2H, d, *J* = 6.9, 2′,6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 13.22 (18-C), 19.37 (19-C), 21.05 (11-C), 22.81 (15-C), 24.48 (16-C), 27.84 (2-C), 31.56 (21-C), 31.78 (8-C), 31.81 (7-C), 36.64 (10-C), 37.02 (1-C), 38.16 (4-C), 38.76 (12-C), 44.00 (13-C), 49.86 (9-C), 56.80 (14-C), 63.65 (17-C), 74.45 (3-C), 122.46 (6-C), 128.27 (3′- or 5′-Ph-C), 128.41 (3′- or 5′-Ph-C), 129.52 (2′- or 6′-Ph-C), 130.09 (2′- or 6′-Ph-C), 130.71 (1′-Ph-C), 132.77 (4′-Ph-C), 139.61 (5-C), 166.01 (-C=O), 209.75 (20-C); IR(KBr) *ν*/cm^{-1}: 3387.33, 2945.99, 2899.52, 2882.82, 2834.51, 1714.35, 1701.92, 1450.62, 1382.87, 1350.71, 1312.01, 1271.81, 1253.84, 1225.41, 1178.60, 1163.08, 1111.36, 1066.60, 1026.07, 994.46, 980.21, 946.75, 837.23, 711.89, 683.58, 592.83; HREIMS (m/z): 443.2561 $[M+Na]^+$ (calcd. For $C_{28}H_{36}NaO_3$ 443.2562).

3-(2′-Methylbenzoyloxy)pregnenolone **(4f)**

White solid, Yield: 77.48%, m.p. 187-189 °C; ^{1}H NMR (300MHz, $CDCl_3$) *δ*: 0.64 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 2.13 (3H, s, 21-CH_3), 2.54 (1H, t, *J* = 9.0, C4-βH), 2.60 (3H, s, Ph-CH_3), 4.80-4.91 (1H, m, C3-αH), 5.43 (1H, d, *J* = 4.2, C6-H), 7.26-7.21 (2H, m, 3′,5′-Ph-H), 7.38 (1H, t, *J* = 6.3, 4′-Ph-H), 7.91 (1H, d, *J* = 8.4, 6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 13.23 (18-C), 19.37 (19-C), 21.05 (11-C), 21.78 (Ph-CH_3), 22.82 (15-C), 24.49 (16-C), 27.91 (2-C), 31.56 (21-C), 31.78 (8-C), 31.82 (7-C), 36.66 (10-C), 37.07 (1-C), 38.23 (4-C), 38.77 (12-C), 43.98 (13-C), 49.88 (9-C), 56.80 (14-C), 63.65 (17-C), 74.30 (3-C), 122.44 (6-C), 125.66

(5′-Ph-C), 130.23 (6′-Ph-C), 130.42 (1′-Ph-C), 131.61 (3′-Ph-C), 131.75 (4′-Ph-C), 139.64 (2′-Ph-C), 139.82 (5-C), 167.12 (-C=O), 209.60 (20-C); IR(KBr) ν/cm^{-1}: 3060.45, 2967.81, 2935.60, 2852.84, 1700.45, 1601.26, 1438.21, 1383.57, 1352.77, 1292.48, 1258.04, 1192.13, 1166.55, 1131.30, 1087.40, 1068.81, 1051.83, 1025.85, 1011.38, 978.45, 947.51, 838.15, 801.19, 742.23, 592.72; HREIMS (m/z): 457.2718 $[M+Na]^+$ (calcd. For $C_{29}H_{38}NaO_3$ 457.2719).

3-(4′-Chlorobenzoyloxy)pregnenolone **(4g)**

White solid, Yield: 73.94%, m.p. 185-187 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.61 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.10 (3H, s, 21-CH_3), 2.45 (2H, d, J = 7.8, C7-H), 2.51 (1H, t, J = 9.0, C4-βH), 4.77-4.88 (1H, m, C3-αH), 5.39 (1H, d, J = 4.5, C6-H), 7.38 (2H, d, J = 8.4, 3′,5′-Ph-H), 7.95 (2H, d, J = 8.4, 2′,6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.20 (18-C), 19.33 (19-C), 21.03 (11-C), 22.80 (15-C), 24.46 (16-C), 27.79 (2-C), 31.53 (21-C), 31.77 (8-C), 31.78 (7-C), 36.61 (10-C), 36.98 (1-C), 38.10 (4-C), 38.14 (12-C), 43.9 (13-C), 49.84 (9-C), 56.77 (14-C), 63.61 (17-C), 74.72 (3-C), 122.55 (6-C), 128.57 (1′-Ph-C), 129.17 (3′,5′-Ph-C), 130.92 (2′,6′-Ph-C), 139.12 (4′-Ph-C), 139.45 (5-C), 165.00 (-C=O), 209.40 (20-C); IR(KBr) ν/cm^{-1}: 2942.67, 2883.00, 2834.21, 1787.23, 1716.31, 1701.31, 1592.60, 1486.42, 1448.21, 1399.50, 1382.55, 1354.13, 1321.34, 1274.44, 1190.53, 1167.36, 1114.81, 1089.94, 1067.79, 1013.97, 993.63, 979.10, 946.03, 850.83, 760.35, 682.79, 593.66; HREIMS (m/z): 477.2169 $[M+Na]^+$ (calcd. For $C_{28}H_{35}ClNaO_3$ 477.2172).

3-(2′-Thiophenoyloxy)pregnenolone **(4h)**

White solid, Yield: 87.16%, m.p. 225-226 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.64 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 2.14 (3H, s, 21-CH_3), 2.46 (2H, d, J = 7.8, C7-H), 2.55 (1H, t, J = 9.0, C4-βH), 4.77-4.88 (1H, m, C3-αH), 5.42 (1H, d, J = 3.9, C6-H), 7.09 (1H, dd, J = 5.1, 3.9, 4′-thiophene-H), 7.54 (1H, dd, J = 5.1, 1.5, 5′-thiophene-H), 7.79 (1H, dd, J = 3.9, 1.5, 3′-thiophene-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.23 (18-C), 19.36 (19-C), 21.05 (11-C), 22.82 (15-C), 24.48 (16-C), 27.81 (2-C), 31.56 (21-C), 31.77 (8-C), 31.81 (7-C), 36.62 (10-C), 36.99 (1-C), 38.12 (4-C), 38.76 (12-C), 44.03 (13-C), 49.85 (9-C), 56.81 (14-C), 63.68 (17-C), 74.79 (3-C), 122.54 (6-C), 128.03 (4′-thiophene-C), 132.19 (5′-thiophene-C), 133.23 (3′-thiophene-C), 134.77 (2′-thiophene-C), 139.54 (5-C), 161.76 (-C=O), 209.95(20-C); IR(KBr) ν/cm^{-1}: 3377.77, 3097.14, 2967.31, 2943.69, 2895.70, 2853.16, 1697.32, 1526.21, 1434.16, 1416.09, 1382.74, 1355.99, 1281.41, 1258.55, 1235.69, 1191.14, 1168.75, 1131.47, 1099.14, 1080.54, 1036.82,

1013.02, 976.72, 943.93, 914.14, 862.53, 820.88, 796.39, 753.71, 738.98, 652.33, 591.80; HREIMS (m/z): 449.2127 $[M+Na]^+$ (calcd. For $C_{26}H_{34}SNaO_3$ 449.2126).

3-(2′-Furoyloxy)pregnenolone **(4i)**

White solid, Yield: 41.97%, m.p. 209-211 ºC; ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.60 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 2.11 (3H, s, 21-CH_3), 2.43 (2H, d, J = 7.8, C7-H), 2.55 (1H, t, C17-H), 4.78-4.85 (1H, m, C3-αH), 5.38 (1H, d, C6-H), 6.47-6.79 (1H, m, 3′-furan-H), 7.40 (1H, d, 5′-furan-H), 7.69 (1H, d, 4′-furan-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.22 (18-C), 19.33 (19-C), 21.04 (11-C), 22.82 (15-C), 24.48 (16-C), 27.79 (2-C), 31.56 (21-C), 31.78 (8-C), 31.82 (7-C), 36.63 (10-C), 37.00 (1-C), 38.10 (4-C), 38.78 (12-C), 43.98 (13-C), 49.89 (9-C), 56.83 (14-C), 63.68 (17-C), 74.56 (3-C), 111.76 (3′-Ph-C), 117.69 (4′-Ph-C), 122.61 (6-C), 139.48 (5-C), 145.07 (2′-C), 146.09 (2′-furan-C), 158.21 (-C=O), 209.54 (20-C); IR(KBr) ν/cm^{-1}: 3136.84, 3107.95, 2945.57, 2894.70, 2871.60, 2852.11, 2835.41, 1715.29, 1699.61, 1569.01, 1470.03, 1451.96, 1437.14, 1393.25, 1380.34, 1355.51, 1321.00, 1294.77, 1230.99, 1172.66, 1122.00, 1079.55, 1015.47, 983.44, 950.14, 884.00, 772.54, 597.97; HREIMS (m/z): 433.2353 $[M+Na]^+$ (calcd. For $C_{26}H_{34}NaO_4$ 433.2355).

4.1.3 General procedure for the synthesis of congpound **2** and compounds **5a**-**5i**.

5.5 mmol of semicarbazide hydrochloride and 6.8 mmol of sodium acetate trihydrate were added into 30 mL of acetonitrile and stirred for 2 h at 80 ºC. During the process of the reaction, some white precipitation appeared in the solution. After the solid was filtrated, 1.0 mmol of compound **1** or compounds **4a**-**4i** was added into the filter liquor. After the solution was heated to 80 ºC, the mixture was refluxed and stirred at the temperature for 8 h (the progress of the reaction was monitored by TLC). Then, the reaction was terminated and solvent was removed under reduced pressure. 400mL of distilled water and 10mL of ethanol was added to the solid, and the product was sonicated for 10 min under ultrasonication, filtered and dried to obtain the target product.

Pregnenolon-20-semicarbazone **(2)**

White solid, Yield: 91.32%, m.p. 206-209℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.61(3H, s, 18-CH_3), 1.03 (3H, s, 18-CH_3), 1.82 (3H, s, 21-CH_3), 3.55 (1H, br s, C3-αH), 5.37 (1H, s, C6-H), 7.44 (1H, s, -CONH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.25 (18-C), 16.79 (21-C), 19.44 (19-C),

21.09 (11-C), 23.24 (15-C), 24.15 (16-C), 31.61 (8-C), 31.74 (2-C), 32.06 (7-C), 36.53 (10-C), 37.25 (1-C), 38.92 (12-C), 42.25 (4-C), 43.97 (13-C), 50.09 (9-C), 56.4(17-C), 58.80 (14-C), 71.97 (3-C), 121.45 (6-C), 140.77 (5-C), 150.06 (20-C), 157.55 (-$CONH_2$); IR(KBr) ν/cm^{-1}: 3468.21, 3263.71, 3201.53, 2931.29, 2846.33, 1688.58, 1569.41, 1435.61, 1374.25, 1241.27, 1155.73, 1108.67, 1080.69, 1060.09, 955.60, 834.54, 800.59, 767.16, 742.52, 671.47, 616.98, 574.60; HREIMS (m/z): 396.2627 $[M+Na]^+$ (calcd. For $C_{22}H_{35}N_3NaO_2$ 396.2627).

3β-Propionyloxypregnenolon-20-semicarbazone **(5a)**

White solid, Yield: 76.15%, m.p. 238-240℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.59 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 1.14 (3H, t, J = 7.5, 3′-CH_3), 1.84 (3H, s, 21-CH_3), 2.31 (2H, t, J = 7.5, 2′-CH_2), 4.57-4.67 (1H, m, C3-αH), 5.38 (1H, d, J = 4.5, C6-H), 8.13 (1H, s, -CONH); ^{13}CNMR (75MHz, $CDCl_3$) δ: 9.18 (3′-C), 13.20 (18-C), 17.16 (19-C), 19.34 (11-C), 21.03 (21-C), 23.29 (15-C), 24.14 (2-C), 27.75 (2′-C), 27.91 (17-C), 31.73 (8-C), 32.01 (7-C), 36.61 (16-C), 37.00 (1-C), 38.10 (10-C), 38.84 (4-C), 43.91 (12-C), 50.00 (13-C), 56.34 (9-C), 58.79 (14-C), 73.62 (3-C), 122.30 (6-C), 139.73 (5-C), 150.33 (20-C), 158.08 (-$CONH_2$), 173.96 (-COO); IR(KBr) ν/cm^{-1}: 3467.35, 3190.50, 2967.64, 2941.32, 2848.50, 1733.60, 1695.75, 1579.42, 1439.71, 1376.55, 1237.86, 1195.66, 1110.65, 1082.62, 1026.40, 769.26, 670.06, 577.19; HREIMS (m/z): 452.2890 $[M+Na]^+$ (calcd. For $C_{25}H_{39}NaN_3O_3$ 452.2889).

3β-Butanoyloxypregnenolon-20-semicarbazone **(5b)**

White solid, Yield: 82.32%, m.p. 224-226 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.60 (3H, S, 18-CH_3), 0.93 (3H, t, J = 7.5, 4′-CH_3), 1.03 (3H, s, 19-CH_3), 1.66 (2H, q, J = 7.5, 3′-CH_3), 1.84 (3H, s, 21-CH_3), 2.27 (2H, t, J = 7.2, 2′-CH_2), 2.33 (1H, d, J = 6.9, C4-H), 4.58-4.68 (1H, m, C3-αH), 5.39 (1H, d, J = 4.8, C6-H), 7.95 (1H, s, -CONH); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.21 (18-C), 13.64 (4′-C), 17.06 (19-C), 18.54 (3′-C), 19.35 (11-C), 21.03 (21-C), 23.28 (15-C), 24.14 (2-C), 27.77 (17-C), 31.77 (8-C), 32.02 (7-C), 36.57 (2′-C), 36.61 (16-C), 37.00 (1-C), 38.12 (10-C), 38.85 (4-C), 43.92 (12-C), 50.00 (13-C), 56.34 (9-C), 58.79 (14-C), 73.55 (3-C), 122.30 (6-C), 139.73 (5-C), 150.28 (20-C), 157.88 (-$CONH_2$), 173.17 (-COO); IR(KBr) ν/cm^{-1}: 3745.89, 3471.00, 3194.10, 2964.65, 2940.57, 1732.40, 1696.79, 1668.28, 1569.73, 1447.94, 1374.75, 1256.52, 1188.64, 1106.14, 999.82, 957.04, 768.19; HREIMS (m/z): 466.3044 $[M+Na]^+$ (calcd. For $C_{26}H_{41}NaN_3O_3$ 466.3046).

3-Trichloroacetoxypregnenolon-20-semicarbazone **(5c)**

White solid, Yield: 74.14%, m.p. 240-242 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.61 (3H, S, 18-CH_3), 1.07 (3H, s, 19-CH_3), 1.84 (3H, s, 21-CH_3), 2.48 (1H, d, J = 9.0, C4-βH), 4.73-4.84 (1H, m, C3-αH), 5.46 (1H, d, J = 4.8, C6-H), 7.85 (1H, s, -CONH); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.22 (18-C), 17.02 (18-C), 19.33 (11-C), 21.04 (21-C), 23.27 (15-C), 24.14 (2-C), 27.07 (17-C), 31.73 (8-C), 31.97 (7-C), 36.57 (16-C), 36.76 (1-C), 37.27 (10-C), 38.80 (4-C), 43.92 (12-C), 49.92 (13-C), 56.29 (9-C), 58.77 (14-C), 79.65 (3-C), 90.19 (2′-C), 123.40 (6-C), 138.66 (5-C), 150.16 (20-C), 157.72 (-$CONH_2$), 161.37 (-COO); IR(KBr) v/cm^{-1}: 3465.29, 3204.36, 2939.84, 2845.71, 1761.22, 1693.94, 1570.32, 1439.72, 1370.45, 1243.20, 1107.43, 1084.43, 984.41, 925.83, 860.43, 828.33, 768.26, 681.19; HREIMS (m/z): 540.1569 $[M+Na]^+$ (calcd. For $C_{24}H_{34}Cl_3NaN_3O_3$ 540.1563).

3-Cyclopropylformyloxypregnenolon-20-semicarbazone **(5d)**

White solid, Yield: 74.31%, m.p.231-233℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.60 (3H, s, 18-CH_3), 0.86 (2H, d, J = 7.8, 3′-CH_2), 0.98 (2H, d, J = 7.8, 4′-CH_2), 1.04 (3H, s, 19-CH_3), 1.83 (3H, s, 21-CH_3), 2.35 (2H, d, J = 9.0, C4-H), 4.57-4.68 (1H, m, C3-αH), 5.39 (1H, d, J = 4.5, C6-H), 7.78 (1H, s, -CONH); ^{13}C NMR (75MHz, $CDCl_3$) δ: 8.36 (3′,4′-C), 13.15 (18-C), 13.22 (2′-C), 16.97 (19-C), 19.35 (11-C), 21.03 (21-C), 23.27 (15-C), 24.14 (2-C), 27.78 (17-C), 31.73 (8-C), 32.02 (7-C), 36.61 (16-C), 37.00 (1-C), 38.13 (10-C), 38.86 (4-C), 43.94 (12-C), 50.00 (13-C), 56.34 (9-C), 58.79 (14-C), 73.76 (3-C), 122.29 (6-C), 139.76 (5-C), 150.21 (20-C), 157.65 (-$CONH_2$), 174.34 (-COO); IR(KBr) v/cm^{-1}: 3466.51, 3238.33, 2935.45, 2849.37, 1717.99, 1694.07, 1579.59, 1474.95, 1439.46, 1399.47, 1372.66, 1300.98, 1233.12, 1186.43, 1118.99, 1084.19, 1014.28, 951.86, 756.55, 669.92, 611.09; HREIMS (m/z): 464.2887 $[M+Na]^+$ (calcd. For $C_{26}H_{39}NaN_3O_3$ 464.2889).

3-Benzoyloxypregnenolon-20-semicarbazone **(5e)**

White solid, Yield: 80.42%, m.p.221-223 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.62 (3H, s, 18-CH_3), 1.09 (3H, s, 19-CH_3), 1.84 (3H, s, 21-CH_3)， 2.50 (1H, d, J = 7.5, C4-H), 4.82-4.92 (1H, m, C3-αH), 5.45 (1H, d, J = 5.4, C6-H), 6.09 (1H, br s, -NH), 7.45 (2H, t, J = 7.5, 3′,5′-Ph-H), 7.56 (1H, s, 4′-Ph-H), 8.09 (2H, d, J = 7.2, 2′,6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.25 (18-C), 16.87 (19-C), 19.42 (11-C), 21.06 (21-C), 23.25 (15-C), 24.15 (2-C), 27.85 (17-C), 31.76 (8-C), 32.04 (7-C), 36.68 (16-C), 37.04 (1-C), 38.18 (10-C), 38.87 (4-C), 43.97 (12-C), 50.02 (3-C), 56.34 (9-C), 58.79 (14-C), 74.45 (3-C), 122.51 (6-C), 128.28 (3′,5′-Ph-C), 129.53

(2′,6′-Ph-C), 130.74 (1′-Ph-C), 132.77 (4′-Ph-C), 139.68 (5-C), 150.14 (20-C), 157.41 (-$CONH_2$), 166.03 (-COO); IR(KBr) ν/cm^{-1}: 3466.75, 3237.49, 2934.47, 2849.05, 1712.17, 1582.67, 1450.81, 1373.42, 1317.23, 1277.37, 1180.07, 1116.03, 1085.67, 1069.70, 1028.84, 952.40, 769.88; HREIMS (m/z): 478.3073 $[M+H]^+$ (calcd. For $C_{29}H_{40}N_3O_3$ 478.3070).

3-(2′-Methylbenzoyloxy)pregnenolon-20-semicarbazone **(5f)**

White solid, Yield: 67.47%, m.p.226-228 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.62 (3H, s, 18-CH_3), 1.08 (3H, s, 19-CH_3), 1.84 (3H, s, 21-CH_3), 2.49 (2H, d, J = 6.9, C17-H), 2.61 (3H, s, Ph-CH_3), 4.81-4.92 (1H, m, C3-αH), 5.45 (1H, d, J = 5.2, C6-H), 7.25 (1H, d, J = 7.2, 3′-Ph-H), 7.40 (1H, t, J = 7.5, 5′-Ph-H), 7.79 (1H, s, 4′-Ph-H), 7.91 (1H, d, J = 8.4, 6′-Ph-H); ^{13}C NMR (75MHz ,$CDCl_3$) δ: 13.24 (18-C), 16.99 (19-C), 19.41 (11-C), 21.06 (21-C), 21.75 (2′-Ph-CH_3), 23.28 (15-C), 24.16 (2-C), 27.79 (17-C), 31.76 (8-C), 32.04 (7-C), 36.03 (16-C), 36.69 (1-C), 37.08 (10-C), 38.24 (4-C), 43.96 (12-C), 50.03 (13-C), 56.35 (9-C), 58.80 (14-C), 74.33 (3-C), 122.48 (6-C), 125.66 (5′-Ph-C), 130.28 (6′-Ph-C), 130.40 (1′-Ph-C), 131.61 (3′-Ph-C), 131.74 (4′-Ph-C), 139.70 (2′-Ph-C), 139.81 (5-C), 150.22 (20-C), 157.66 (-$CONH_2$), 167.19 (-COO); IR(KBr) ν/cm^{-1}: 3474.03, 3196.31, 2940.47, 2851.65, 1696.34, 1571.98, 1437.22, 1372.65, 1293.22, 1257.71, 1132.87, 1079.89, 1050.66, 995.80, 767.82, 739.26; HREIMS (m/z): 514.3046 $[M+Na]^+$ (calcd. For $C_{30}H_{41}NaN_3O_3$ 514.3046).

3-(4′-Chlorobenzoyloxy)pregnenolon-20-semicarbazone **(5g)**

White solid, Yield: 80.42%, m.p. 241-243℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.62 (3H, s, 18-CH_3), 1.09 (3H, s, 19-CH_3), 1.86 (3H, s, 21-CH_3)， 2.48 (2H, d, J = 7.8, C4-H), 4.81-4.88 (1H, m, C3-αH), 5.45 (1H, d, J = 4.5, C6-H), 6.12 (1H, br s, -NH), 7.42 (2H, d, J = 8.4, 3′,5′-Ph-H), 7.99 (2H, d, J = 8.4, 2′,6′-Ph-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.23 (18-C), 17.03 (19-C), 19.40 (11-C), 21.05 (21-C), 23.29 (15-C), 24.15 (2-C), 27.81 (17-C), 31.75 (8-C), 32.04 (7-C), 36.67 (16-C), 37.01 (1-C), 38.13 (10-C), 38.86 (4-C), 43.97 (12-C), 50.01 (13-C), 56.35 (9-C), 58.80 (14-C), 74.79 (3-C), 122.63 (6-C), 128.63 (3′,5′-Ph-C), 130.94 (1′-Ph-C), 131.35 (2′,6′-Ph-C), 139.19 (4′-Ph-C), 139.54 (5-C), 150.72 (20-C), 158.00 (-$CONH_2$), 165.17 (-COO); IR(KBr) ν/cm^{-1}: 3456.96, 3126.77, 2936.34, 2847.62, 1720.42, 1691.47, 1575.77, 1440.20, 1399.81, 1374.13, 1274.88, 1237.22, 1168.53, 1114.21, 1086.07, 1014.32, 949.13, 843.57, 758.93; HREIMS (m/z): 512.2680 $[M+H]^+$ (calcd. For $C_{29}H_{39}ClN_3O_3$ 512.2680).

3-(2′-Thiophenoyloxy)pregnenolon-20-semicarbazone **(5h)**

White solid, Yield: 88.50%, m.p.227-228 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.61 (3H, s, 18-CH_3), 1.08 (3H, s, 19-CH_3), 1.84 (3H, s, 21-CH_3), 2.48 (1H, d, J = 7.5, C4-H), 4.78-4.89 (1H, m, C3-αH), 5.44 (1H, d, J = 2.1, C6-H), 6.11 (1H, br s, -NH), 7.11 (1H, dd, J = 4.8, 3.9, 4′-thiophene-H), 7.56 (1H, dd, J = 4.8, 0.9, 5′-thiophene-H), 7.81 (1H, s, 3′-thiophene-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.23 (18-C), 16.98 (19-C), 19.40 (11-C), 21.05 (21-C), 23.28 (15-C), 24.15 (2-C), 27.82 (17-C), 31.76 (8-C), 32.04 (7-C), 36.65 (16-C), 37.01 (1-C), 38.15 (10-C), 38.87 (4-C), 43.95 (12-C), 50.02 (13-C), 56.35 (9-C), 58.50 (14-C), 74.78 (3-C), 122.56 (6-C), 127.65 (4′-thiophene-C), 132.11 (5′-thiophene-C), 133.17 (3′-thiophene-C), 134.47 (2′-thiophene-C), 139.60 (5-C), 150.20 (20-C), 157.67 (-$CONH_2$), 161.72 (-COO); IR(KBr) v/cm^{-1}: 3459.67, 3252.06, 3188.23, 2937.12, 2880.93, 2847.83, 1690.35, 1569.84, 1527.64, 1420.08, 1360.08, 1259.80, 1237.29, 1224.00, 1100.09, 1075.82, 1035.18, 768.75, 750.34, 707.04; HREIMS (m/z): 506.2456 $[M+Na]^+$ (calcd. For $C_{27}H_{37}NaSN_3O_3$ 506.2453).

3-(2′-Furoyloxy)pregnenolon-20-semicarbazone **(5i)**

White solid, Yield: 65.71%, m.p.244-246 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.61 (3H, s, 18-CH_3), 1.07 (3H, s, 19-CH_3), 1.84 (3H, s, 21-CH_3), 2.47 (1H, d, J = 7.5, C4-H), 4.82-4.93 (1H, m, C3-αH), 5.44 (1H, d, J = 4.5, C6-H), 6.52 (1H, d, J = 3.3, 4′-furan-H), 7.19 (1H, d, J = 3.3, 3′-furan-H), 7.59 (1H, s, NH), 7.67 (1H, s, 5′-furan-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.23 (18-C), 16.90 (19-C), 19.37 (11-C), 21.04 (21-C), 23.27 (15-C), 24.14 (2-C), 27.80 (17-C), 31.74 (8-C), 32.03 (7-C), 36.64 (16-C), 37.00 (1-C), 38.12 (10-C), 38.86 (4-C), 43.95 (12-C), 50.01 (13-C), 56.33 (9-C), 58.79 (14-C), 74.57 (3-C), 111.78 (4′-furan-C), 117.70 (3′-furan-C), 122.65 (6-C), 139.50 (5-C), 145.06 (2′-furan-C), 146.10 (5′-furan-C), 150.16 (20-C), 157.53 (-$CONH_2$), 158.23 (-COO); IR(KBr) v/cm^{-1}: 3465.68, 2933.02, 2848.60, 1717.76, 1693.73, 1580.81, 1476.97, 1440.35, 1400.30, 1371.72, 1305.18, 1232.61, 1189.29, 1118.85, 1085.62, 1015.43, 755.60; HREIMS (m/z): 490.2680 $[M+Na]^+$ (calcd. For $C_{27}H_{37}NaN_3O_4$ 490.2682).

4.1.4 General procedure for the synthesis of congpound **3** and compounds **6a**-**6i**.

0.33mmol of compound **2** or compounds **5a**-**5i** was dissolved in 30mL of glacial acetic acid with vigorous stirring and gentle heating to 45-50 ºC. Selenium dioxide powder (3.0mmol) was added into the solution and the mixture was kept under vigorous stirring. After about 0.5hr, the color of the mixture became red color gradually. The reaction mixture was stirred until no starting

material was observed (the progress of the reaction was monitored by TLC, eluent: $V_{\text{ethyl acetate}}$:$V_{\text{petroleum ether}}$=1:4). Then the reaction was stopped and the roduct was extracted with 30 mL of ethyl acetate. The solution was washed with water (30 mL×2), saturated $NaHCO_3$ (20 mL×4) and saturated brine, dried with anhydrous sodium sulfate, and evaporated under reduced pressure. The crude product was separated by the column chromatography using ethyl acetate/petroleum ether (eluent: $V_{\text{ethyl acetate}}$:$V_{\text{petroleum ether}}$=1:20) to give the target product.

17-[1′,2′,3′-Selenadiazol-4′-yl]pregnenolon (**3**)

Faint red solid, Yield: 57.83%; m.p.158-159℃。^{1}H NMR (300MHz, $CDCl_3$) δ: 0.55 (3H, s, 18-CH_3), 1.20 (3H, s, 19-CH_3), 3.33 (1H, t, J = 9.6, C4-βH), 3.56-3.65 (1H, m, C3-αH), 4.18 (1H, s, -OH), 5.73 (1H, d, J = 3.3, C6-H), 8.86 (1H, s, 21-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.00 (18-C), 20.24 (19-C), 21.05 (11-C), 24.67 (16-C), 25.34 (15-C), 27.42 (8-C), 32.06 (2-C), 32.26 (7-C), 36.12 (10-C), 36.96 (1-C), 37.76 (12-C), 44.62 (4-C), 50.33 (13-C), 51.38 (9-C), 56.21 (14-C), 63.64 (17-C), 72.45 (3-C), 128.43 (6-C), 137.79 (21-CSe), 142.88 (5-C), 164.49 (20-C); IR(KBr) ν/cm^{-1}: 3475.47, 3071.65, 2964.16, 2934.15, 2896.62, 2854.90, 2822.57, 1663.90, 1472.13, 1450.35, 1424.11, 1364.90, 1337.61, 1312.27, 1256.84, 1200.62, 1177.42, 1116.22, 1069.53, 1050.80, 1029.66, 985.50, 967.67, 902.92, 866.29, 823.17, 801.68, 758.34, 644.60; HREIMS (m/z): 407.1604 $[M+H]^+$ (calcd. For $C_{21}H_{31}N_2OSe$ 407.1601).

3β-Propionyloxy-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon (**6a**)

Faint red solid, Yield: 34.98%, m.p. 148-150℃; ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.54 (3H, S, 18-CH_3), 1.04 (3H, s, 19-CH_3), 1.14 (3H, t, J = 7.5, 3′-CH_3), 2.32 (2H, q, J = 7.5, 2′-CH_2), 3.32 (1H, t, J = 7.5, C17-H), 4.59-4.70 (1H, m, C3-αH), 5.43 (1H, d, J = 4.8, C6-H), 8.86 (1H, s, C21-H); ^{13}C NMR (75MHz, $CDCl_3$) δ: 9.20 (3′-C), 13.01 (18-C), 19.36 (19-C), 20.71 (11-C), 24.71 (16-C), 27.43 (15-C), 27.76 (2′-C), 27.92 (2-C), 31.88 (8-C), 32.29 (7-C), 36.72 (1-C), 37.03 (10-C), 37.78 (4-C), 38.14 (12-C), 44.62 (13-C), 50.15 (9-C), 51.43 (14-C), 55.98 (17-C), 73.66 (3-C), 122.29 (6-C), 137.82 (21-CSe), 139.88 (5-C), 164.54 (20-C), 173.96 (-COO); IR(KBr) ν/cm^{-1}: 2967.09, 2941.72, 2904.77, 2888.75, 2854.57, 2826.25, 1732.73, 1457.86, 1382.56, 1362.60, 1330.74, 1310.73, 1196.02, 1135.89, 1082.29, 1023.95, 957.92, 797.53; HREIMS (m/z): 463.1860 $[M+H]^+$ (calcd. For $C_{24}H_{35}N_2O_2Se$ 463.1864).

3β-Butanoyloxy-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon (**6b**)

Brownish red solid, Yield: 37.01%，m.p. 154-157℃; ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.54 (3H,

s, 18-CH_3), 0.96 (3H, t, *J* = 7.5, 4′-CH_3), 1.04 (3H, s, 19-CH_3), 1.66 (2H, q, *J* = 7.5, 3′-CH_2), 2.27 (2H, t, *J* = 7.5, 2′-CH_2), 3.32 (1H, t, *J* = 9.6, C17-H), 4.59-4.70 (1H, m, C3-αH), 5.43 (1H, d, *J* = 5.1, C6-H), 8.86 (1H, s, 21-C); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 13.01 (18-C), 13.67 (4′-C), 18.55 (3′-C), 19.37 (19-C), 20.71 (11-C), 24.71 (16-C), 27.43 (15-C), 27.78 (2-C), 31.88 (8-C), 32.28 (7-C), 36.58 (2′-C), 36.72 (1-C), 37.03 (10-C), 37.77 (4-C), 38.15 (12-C), 44.62 (13-C), 50.13 (9-C), 51.43 (14-C), 55.97 (17-C), 73.58 (3-C), 122.29 (6-C), 137.83 (21-CSe), 139.87 (5-C), 164.55 (20-C), 173.17 (-C=O); IR(KBr) *v*/cm^{-1}: 3728.81, 3628.14, 3068.31, 2963.20, 2939.87, 2899.06, 2874.33, 2825.44, 1728.49, 1469.90, 1383.11, 1364.35, 1310.29, 1260.18, 1188.78, 1136.16, 1088.55, 1044.45, 1015.68, 1000.50, 957.92, 900.90, 803.86, 669.88, 603.80; HREIMS (m/z): 477.2018 $[M+H]^+$ (calcd. For $C_{25}H_{37}N_2O_2Se$ 407.2020).

3-Trichloroacetoxy-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon **(6c)**

Faint yellow solid, Yield: 35.03%, m.p. 162-163℃; ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 0.55 (3H, S, 18-CH_3), 1.08 (3H, s, 19-CH_3), 2.49 (2H, d, *J* = 6.9, C4-H), 3.33 (1H, t, *J* = 9.6, C17-H), 4.74-4.83 (1H, m, C3-αH), 5.48 (1H, d, *J* = 5.1, C6-H), 8.86 (1H, s, C21-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 13.00 (18-C), 19.33 (19-C), 20.74 (11-C), 24.69 (16-C), 27.09 (15-C), 27.42 (2-C), 31.89 (8-C), 32.25 (7-C), 36.68 (1-C), 36.80 (10-C), 37.30 (4-C), 37.76 (12-C), 44.61 (13-C), 50.09 (9-C), 51.42 (14-C), 55.95 (17-C), 79.69 (3-C), 90.22 (2′-C), 123.36 (6-C), 137.80 (-CSe), 138.82 (5-C), 161.35 (20-C), 164.47 (1′-C=O); IR(KBr) *v*/cm^{-1}: 3097.89, 2938.54, 2892.05, 1762.51, 1472.02, 1447.25, 1368.02, 1311.57, 1248.91, 1199.86, 1140.69, 982.42, 926.69, 899.03, 859.32, 836.62, 821.70, 753.84, 675.50, 618.45; HREIMS (m/z): 551.0524 $[M+H]^+$ (calcd. For $C_{23}H_{30}Cl_3N_2O_2Se$ 551.0538).

3-Cyclopropylformyloxy-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon **(6d)**

Faint yellow solid, Yield: 37.85%, m.p.153-154℃; ^{1}H NMR (300MHz, $CDCl_3$) *δ*: 0.54 (3H, S, 18-CH_3), 0.85 (2H, d, *J* = 7.8, 3′-CH_2), 0.99 (2H, d, *J* = 7.8, 4′-CH_2), 1.04 (3H, s, 19-CH_3), 2.36 (2H, d, *J* = 7.2, C4-H), 3.32 (1H, t, *J* = 9.6, C17-H), 4.58-4.65 (1H, m, C3-αH), 5.42 (1H, d, *J* = 5.1, C6-H), 8.85 (1H, s, C21-H); ^{13}C NMR (75MHz, $CDCl_3$) *δ*: 8.34 (3′,4′-C), 13.00 (18-C), 13.15 (2′-C), 19.36 (19-C), 20.72 (11-C), 24.70 (16-C), 27.43 (15-C), 27.79 (2-C), 31.88 (8-C), 32.29 (7-C), 36.71 (1-C), 37.04 (10-C), 37.79 (4-C), 38.17 (12-C), 44.62 (13-C), 50.15 (9-C), 51.44 (14-C), 55.98 (17-C), 73.79 (3-C), 122.27 (6-C), 137.79 (21-CSe), 139.90 (5-C), 164.53 (20-C), 174.31 (-C=O); IR(KBr) *v*/cm^{-1}: 3077.48, 2931.14, 2896.45, 2862.32, 1727.71, 1469.46, 1445.11,

1390.90, 1308.05, 1196.37, 1170.78, 1141.49, 1095.09, 1063.46, 998.67, 959.31, 921.17, 894.80, 834.91, 805.51, 745.96, 668.71, 618.83; HREIMS (m/z): 475.1866 $[M+H]^+$ (calcd. For $C_{25}H_{35}N_2O_2Se$ 475.1864).

3-Benzoyloxy-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon **(6e)**

White solid, Yield: 56.58%, m.p.168-169℃; ^{1}H NMR (300MHz, $CDCl_3$) δ: 0.56 (3H, s, 18-CH_3), 1.10 (3H, s, 19-CH_3), 2.51 (2H, d, J = 7.2, C4-H), 3.34 (1H, t, J = 9.9, C17-H), 4.85-4.95 (1H, m, C3-αH), 5.48 (1H, d, J = 4.8, C6-H), 7.45 (2H, t, J = 7.5, 3′,5′-Ph-H), 7.56 (1H, t, J = 7.5, 4′-Ph-H), 8.07 (2H, d, J = 7.5, 2′,6′-Ph-H), 8.87 (1H, s, 21-CH); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.02 (18-C), 19.43 (19-C), 20.75 (11-C), 24.72 (16-C), 27.45 (15-C), 27.86 (2-C), 31.92 (8-C), 32.32 (7-C), 36.79 (1-C), 37.08 (10-C), 37.81 (4-C), 38.22 (12-C), 44.64 (13-C), 50.19 (9-C), 51.45 (14-C), 56.00 (17-C), 74.49 (3-C), 122.49 (6-C), 128.27 (21-CSe), 129.54 (3′,5′-Ph-C), 130.79 (2′,6′-Ph-C), 132.74 (1′-Ph-C), 137.81 (4′-Ph-C), 139.83 (5-C), 164.54 (-C=O), 166.00 (20-C); IR(KBr) v/cm^{-1}: 3394.60, 3184.26, 3076.10, 2922.24, 2851.39, 1714.90, 1646.54, 1469.14, 1450.42, 1379.91, 1310.99, 1272.33, 1203.33, 1175.38, 1111.23, 1066.34, 1025.28, 974.69, 953.80, 900.73, 903.46, 737.82, 711.47, 684.00; HREIMS (m/z): 533.1683 $[M+Na]^+$ (calcd. For $C_{25}H_{36}N_2O_2SeNa$ 533.1683).

3-(2′-MethylBenzoyloxy)-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon **(6f)**

Faint red solid, Yield: 33.17%, m.p. 162-164℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.56 (3H, s, 18-CH_3), 1.09 (3H, s, 19-CH_3), 2.51 (2H, d, J = 5.1, C4-H), 2.62 (3H, s, ph-CH_3), 3.33 (1H, t, J = 9.6, C17-H), 4.83-4.94 (1H, m, C3-αH), 5.48 (1H, d, J = 4.8, C6-H), 7.25 (1H, m, 3′,5′-Ph-H), 7.40 (1H, t, J = 7.2, 4′-Ph-H), 7.91 (1H, d, J = 7.2, 6′-Ph-H), 8.86 (1H, s, 21-C); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.02 (18-C), 19.43 (19-C), 20.75 (11-C), 21.76 (2′-Ph-CH_3), 24.72 (16-C), 27.45 (15-C), 27.93 (2-C), 31.92 (8-C), 32.32 (7-C), 36.80 (1-C), 37.12 (10-C), 37.80 (4-C), 38.28 (12-C), 44.64 (13-C), 50.18 (9-C), 51.45 (14-C), 55.99 (17-C), 74.37 (3-C), 122.47 (6-C), 125.65 (5′-Ph-C), 130.31 (21-CSe), 130.41 (6′-Ph-C), 131.60 (1′-Ph-C), 131.72 (3′-Ph-C), 137.82 (4′-Ph-C), 139.82 (2′-Ph-C), 139.86 (5-C), 164.55 (-C=O), 167.19 (20-C); IR(KBr) v/cm^{-1}: 3068.39, 2940.90, 2898.57, 2854.51, 2825.16, 1715.04, 1489.84, 1469.67, 1453.32, 1367.56, 1310.96, 1255.70, 1200.59, 1142.24, 1082.85, 1014.02, 957.32, 900.13, 802.70, 731.74, 669.41; HREIMS (m/z): 563.1577 $[M+K]^+$ (calcd. For $C_{29}H_{36}N_2O_2SeK$ 563.1579).

3-(4′-Chlorobenzoyloxy)-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon **(6g)**

Faint yellow solid, Yield: 40.58%, m.p.163.165°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.56 (3H, s, 18-CH_3), 1.09 (3H, s, 19-CH_3), 2.50 (2H, d, J = 7.5, C4-H), 3.34 (1H, t, J = 9.6, C17-H), 4.83-4.94 (1H, m, C3-αH), 5.47 (1H, d, J = 4.5, C6-H), 7.43 (2H, d, J = 8.7, 3′,5′-Ph-H), 8.00 (2H, d, J = 8.7, 2′,6′-Ph-H), 8.86 (1H, s, 21-C); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.01 (18-C), 19.41 (19-C), 20.74 (11-C), 24.71 (16-C), 27.44 (15-C), 27.83 (2-C), 31.91 (8-C), 32.31 (7-C), 36.77 (1-C), 37.05 (10-C), 37.80 (4-C), 38.17 (12-C), 44.64 (13-C), 50.17 (9-C), 51.45 (14-C), 55.99 (17-C), 74.82 (3-C), 122.61 (6-C), 128.61 (1′-Ph-C), 129.23 (21-CSe), 130.94 (3′,5′-Ph-C), 137.80 (2′,6′-Ph-C), 139.16 (4′-Ph-C), 139.69 (5-C), 164.54 (-COO), 165.14 (20-C); IR(KBr) ν/cm^{-1}: 3418.26, 3088.98, 2939.09, 2848.20, 1716.17, 1593.42, 1485.33, 1447.58, 1399.30, 1367.96, 1312.52, 1275.25, 1199.78, 1169.91, 1120.41, 1088.93, 1065.64, 1014.08, 977.18, 850.29, 798.76, 758.97, 732.34, 683.97; HREIMS (m/z): 545.1476 $[M+H]^+$ (calcd. for $C_{28}H_{34}ClN_2O_2Se$ 545.1474).

3-(2′-Thiophenoyloxy)-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon **(6h)**

White solid, Yield: 13.39%, m.p. 217-220°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.56 (3H, s, 18-CH_3), 1.09 (3H, s, 19-CH_3), 2.49 (2H, d, J = 7.5, C4-H), 3.33 (1H, t, J = 9.6, C17-H), 4.80-4.91 (1H, m, C3-αH), 5.46 (1H, d, J = 4.8, C6-H), 7.11 (1H, t, J = 4.5, 4′-thiophene-H), 7.56 (1H, d, J = 4.8, 5′-thiophene-H), 7.82 (1H, d, J = 3.9, 3′-thiophene-H), 8.86 (1H, s, 21-C); ^{13}C NMR (75MHz, $CDCl_3$) δ: 13.02 (18-C), 19.42 (19-C), 20.75 (11-C), 24.72 (16-C), 27.44 (15-C), 27.83 (2-C), 31.92 (8-C), 32.31 (7-C), 36.76 (1-C), 37.05 (10-C), 37.80 (4-C), 38.10 (12-C), 44.64 (13-C), 50.17 (9-C), 51.45 (14-C), 55.99 (17-C), 74.83 (3-C), 122.55 (6-C), 127.65 (4′-thiophene-C), 132.10 (21-CSe), 133.16 (5′-thiophene-C), 134.51 (3′-thiophene-C), 137.80 (2′-thiophene-C), 139.74 (5-C), 161.71 (-COO), 164.54 (20-C); IR(KBr) ν/cm^{-1}: 3077.07, 2938.21, 2853.12, 1705.98, 1523.95, 1448.70, 1417.80, 1360.71, 1312.36, 1277.37, 1256.20, 1224.58, 1202.45, 1131.62, 1097.72, 1077.92, 1064.37, 1036.43, 1014.49, 989.92, 972.86, 952.82, 899.65, 860.46, 800.13, 751.55, 737.11, 620.29; HREIMS(m/z): 539.1243 $[M+Na]^+$ (calcd. for $C_{26}H_{33}N_2O_2SeNaS$ 539.1247).

3-(2′-Furoyloxy)-17-[1′,2′,3′-selenadiazol-4′-yl]pregnenolon **(6i)**

Faint yellow solid, Yield: 24.60%, m.p.154-156°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.55 (3H, s, 18-CH_3), 1.07 (3H, s, 19-CH_3), 2.48 (2H, d, J = 7.2, C4-H)， 3.32 (1H, t, J = 9.6, C17-H), 4.83-4.91 (1H, m, C3-αH), 5.46 (1H, d, J = 4.5, C6-H), 6.51 (1H, dd, J = 3.6, 1.8, 4′-furan-H),

7.19 (1H, d, *J* = 4.2, 3′-furan-H), 7.58 (1H, d, *J* = 1.5, 5′-furan-H), 8.86 (1H, s, 21-C); ^{13}C NMR (75MHz, $CDCl_3$): 13.01 (18-C), 19.38 (19-C), 20.73 (11-C), 24.71 (16-C), 27.43 (15-C), 27.80 (2-C), 31.90 (8-C), 32.29 (7-C), 36.74 (1-C), 37.03 (10-C), 37.78 (4-C), 38.14 (12-C), 44.63 (13-C), 50.15 (9-C), 51.43 (14-C), 55.97 (17-C), 74.61 (3-C), 111.78 (4′-furan-C), 117.70 (3′-furan-C), 122.64 (6-C), 137.83 (21-CSe), 139.63 (5-C), 145.07 (2′-furan-C), 146.09 (5′-furan-C), 158.22 (-COO), 164.53 (20-C); IR(KBr) ν/cm^{-1}: 2942.40, 2907.12, 2852.97, 2829.04, 1717.34, 1475.95, 1399.41, 1303.91, 1234.21, 1191.46, 1119.98, 1013.77, 952.87, 797.77, 753.80; HREIMS (m/z): 501.1662 $[M+H]^+$ (calcd. for $C_{26}H_{33}N_2O_3Se$ 501.1656).

4.2 Biological studies

Using 96-well plates on a MLLTISKAN MK3 analysis spectrometer, the cell proliferation was tested with MTT method. PC-3 (human prostate carcinoma), SKOV3 (human ovarian carcinoma), T47D (human breast infiltrating duct carcinoma), MCF-7 (human breast adenocarcinoma) and HEK293T (normal kidney epithelial cells) cell lines were obtained by Guangxi Medical University (China).

4.2.1 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.

4.2.2 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 ^{o}C.

4.2.3 Assay for cell viability

Cells ($1\sim2\times10^4$ cells/mL) were seeded into 96-wells plates for 24 hr. The medium contained test compound with different concentrations was added to the cells. After 72 hr incubation, 20 μL of the tetrazolium dye (MTT) (5 mg/mL) solution were added to each well. After additional 4 hr 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.

Acknowledgments

The authors acknowledge the financial support of 'The National Natural Science Foundation of China', China (No.: 21462009; No: 21562007), the Nanning technology development project. of China（20171125-5）and Universities Key Laboratory Funds of Synthetic and Natural Functional Molecular Chemistry.

Conflict of Interest

The authors declare no conflict of interest.

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Current knowledge in species-related bioavailability of selenium

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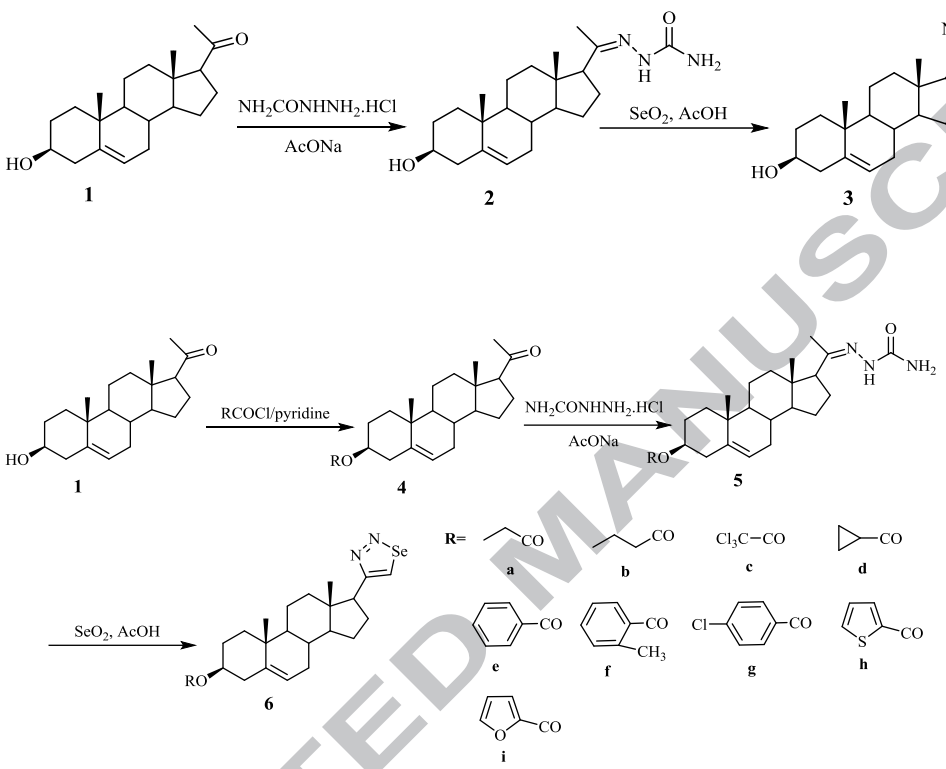
Graphical Abstract:



Research Highlights

1. 3-substituted 17-[1′,2′,3′]-selenadiazolylpregnenolone derivatives were synthesized.

2. The structures of all synthesized compounds had been were characterized.

3. The antiproliferative activity of synthesized compounds was assayed.

4. Some compounds show a distinct antiproliferative activity.