Bioorg. Med. Chem. 28 (2020) 115763

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of novel steroidal-chalcone hybrids with potent and selective activity against triple-negative breast cancer

Qiangqiang Hou, Xin Lin, Xiang Lu, Chengfeng Bai, Hanlin Wei, Guoshun Luo *, Hua Xiang *

Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 211198, PR China
Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 211198, PR China

ARTICLE INFO

Keywords:
Steroids
Molecular hybridization
Antiproliferative activity
Apoptosis
Triple-negative
Breast cancer

ABSTRACT

A series of novel steroidal-chalcone derivates were designed and synthesized based on the molecular hybridization strategy and further evaluated for their growth inhibitory activity against three human cancer cell lines. The MTT results indicated that most compounds were apparently more sensitive to human breast cancer cells MDA-MB-231. Compounds **8** and **18** exerted the best cytotoxic activity against triple-negative MDA-MB-231 cells with the IC_{50} values of 0.42 μM and 0.52 μM respectively, which were 23-fold increase or more compared with **5-Fu.** Further mechanism studies demonstrated that compound **8** could induce cells apoptosis through regulating Bcl-2/Bax proteins and activating caspase-3 signaling pathway. Moreover, compound **8** could upregulate the cellular ROS levels which accelerated the apoptosis of MDA-MB-231 cells. In addition, interestingly, cell cycle assay showed that compound **8** could arrest MDA-MB-231 cells at S phase but not commonly anticipated G2/M phase. These evidences fully confirmed that compound **8** could be a potential candidate that deserves further development as an antitumor agent against triple-negative breast cancer.

1. Introduction

Triple-negative breast cancer (TNBC), who is known for lacking in the expression of human epidermal growth factor receptor 2 (HER-2), estrogen receptor (ER) and progesterone receptor, is the most aggressive cancer among all breast cancer subtypes.[1] Currently, the risk factors that led to the progression of TNBC has not been completely elucidated. Consequently, the target therapy such as endocrine therapy is not applicable into TNBC and chemotherapy is the only strategy for TNBC patients.[2,3] Though most TNBC patients respond to chemotherapeutic agents, the defective selectivity caused huge side effects. Therefore, there is urgent need to develop novel drugs with potent and selective activity against TNBC.

Chalcone is one compound that widely exists various plants including fruits, vegetables and tea.[4–8] As is well-known, chalcone exerts wide biological activities containing anti-inflammatory, anti-bacterial,[10] antioxidant[11,12] and anticancer.[7,13,14] However, the pharmacological activity results showed that ordinarily chalcone possessed minor effectiveness on cancer cells, which could be attributed to poor water solubility. To improve its anticancer activity, many chalcone-based derivatives were reported.[15–17] These synthetic chalcones obviously showed more potent activities than natural chalcone. Consequently, chalcone is regarded as a privileged structure in medicinal chemistry for drug discovery.[18]

Steroids, an important family of polycyclic molecules with various structure, have drawn extensive attention due to their diverse bioactivities and highly bioavailable.[19] Naturally occurring steroids possess a wide range of biological activities including anti-microbial,[20,21] anti-inflammatory[9] and anti-cancer.[22–24] Many steroid-based drugs have been applied in clinical treatments and become one of the highest marketed classes of pharmaceuticals.[25] Recent years have seen wide research of steroidal anti-cancer activity by rational modifications of these natural steroidal molecules.[26,27] Different modifications studies of steroid molecules with improved biological activities have been reported and some have entered into clinical use such as Abiraterone and Fulvestrant. Abiraterone is presently used for clinical treatment of advanced prostate cancer, while Fulvestrant have advanced into use for the clinical therapeutics of breast cancer. 3-oxygen-androstene-4-ene is extensively distributed in many steroidal anti-cancer drugs, such as anti-prostate cancer drugs (Oxendolone and Cyproterone) and anti-breast

Abbreviations: DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DMF, N, *N*-dimethylformamide; DIPEA, N, *N*-ethyldiisopropylamine; DMSO, dimethyl sulfoxide; HATu, 2-(7-Azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate; TBDMSCl, *tert*-Butyldimethylsilyl chloride.

* Corresponding authors at: Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 211198, PR China.
E-mail addresses: gsluo@cpu.edu.cn (G. Luo), xianghua@cpu.edu.cn (H. Xiang).

https://doi.org/10.1016/j.bmc.2020.115763
Received 10 July 2020; Received in revised form 27 August 2020; Accepted 6 September 2020
Available online 12 September 2020

cancer drug (Formestane and Exemestane). Theses inspiring evidences proved that 3-oxygen-androstene-4-ene is a promising structure for discovery of novel steroidal drugs.

Hybridization, which usually incorporates two active compounds into one molecule, has emerged as a promising strategy in the discovery of novel anti-cancer drugs with the potential to combat resistance and improve efficacy compared with the parent drugs.[28–31] Various successful hybrid agents including steroids have been reported previously and some have advanced into clinical trials.[32,33] According to the above analysis, we merged chalcone into 3-oxygen-androstene-4-ene to generate a series of structurally novel steroidal hybrids via two hydrophilic amide linkages which could increase water solubility of the molecule. We previously have reported many novel steroidal drugs with excellent growth inhibitory activities against human cancer cells.[34–36] Herein, we designed another kind of new steroidal hybrids using chalcone based on the molecular hybridization approach (Fig. 1). In our continuous efforts to develop new steroidal derivatives with potent anti-tumor activity, this work would provide us a deeper insight into the structural requirements for discovering potent anti-tumor drugs based on steroids.

2. Results and discussion

2.1. Chemistry

The procedure of synthesizing target molecules was outlined in Scheme 1. The starting material was inexpensive pregnenolone acetate which was reduced by H_2 to obtain compound **2**. The intermediate **3** was acquired via a hydrolysis reaction. Subsequently, compound **3** was oxidized to yield the progesterone **4**, whose acetyl group was then oxidized to generate the key intermediate **5**. The compound **5** reacted with N - Boc – ethylenediamine to afford **6**. The compound **6** was first deprotected and then reacted with various carboxylic acid to offer the final productions **7–15** (Table 1). The synthesis of target compounds **17–25** began from the intermediate **6** that was dehydrogenized by DDQ to form the intermediate **16**. Then, the final compounds **17–25** was obtained through deprotection and condensation reaction.

2.2. In vitro antiproliferative activity

In our continuous efforts to develop novel steroidal drugs with potent growth inhibitory activity against cancer cells. Eighteen novel steroidal hybrids **(7–15, 17–25)** were assayed against several cancer cell lines of different origins (MDA-MB-231, A549 and HT-29) using MTT assay. As we can see from Table 1, these steroidal analogues showed varied antiproliferative activities against three cancer cell lines. Apparently, the steroid-chalcone hybrids were more sensitive to triple-negative breast cancer (TNBC) MDA-MB-231 than human colon cancer cells HT-29 and human lung cancer cells A549. For MDA-MB-231 cells, most compounds showed potent growth inhibitory activities. Compounds **8, 12, 14, 17, 18, 20** exerted more excellent activities than positive control **5-Fu**. Among them, compounds **8** and **18** with 2-OMe in the phenyl demonstrated the best cytotoxicity against MDA-MB-231 with the IC_{50} values of 0.42 μM and 0.52 μM respectively, which were 23-fold increase or more compared with **5-Fu**. However, compounds with 3-OMe,4-OMe and 3,4-dimethoxyl (**9**, **10** and **19–21**) exhibited apparently decreased activities (IC_{50} range : 11.45 μM ~ >40 μM). To make matters worse, compounds **11** and **21** with the 3,4,5- trimethoxy were found to be devoid of the activity (IC_{50} > 40 μM). In addition, compared with compounds **9** and **19**, we found that 4-OMe could be substituted by $–NO_2$ and $–CF_3$, which resulted in a subtle effect on growth inhibition of MDA-MB-231. Meanwhile, by analyzing these data, we found that the activities of compounds **7–15** was comparative with compounds **17–25**, which identified that unsaturated bond at the position of C- 1, 2 in the steroidal ring could be tolerated.

For HT-29 and A549 cell lines, we could find compounds **7–15** showed more promising growth inhibitory activities than compounds **17–25** with 1,2-ene in the steroidal skeleton. Among these compounds, compound **8** exhibited comparable activity compared with **5-Fu**, while most compounds showed only moderate or even no activities.

From above discussions, we could conclude that most hybrids showed weak or moderate inhibitory activities against HT-29 and A549 cancer cell lines. However, it was found that the steroidal hybrids were dramatically more sensitive to TNBC cells MDA-MB-231. The most potent compounds **8** and **18** with 2-OMe in the phenyl exhibited extraordinary antiproliferative activities with IC_{50} values of 0.42 μM and

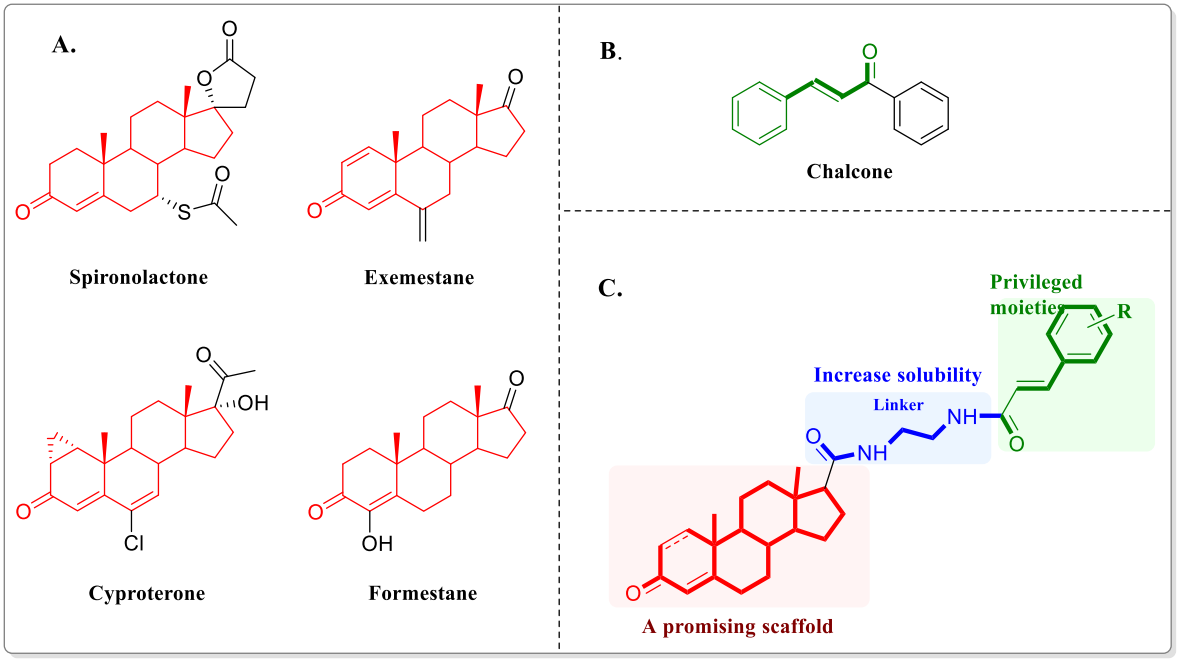
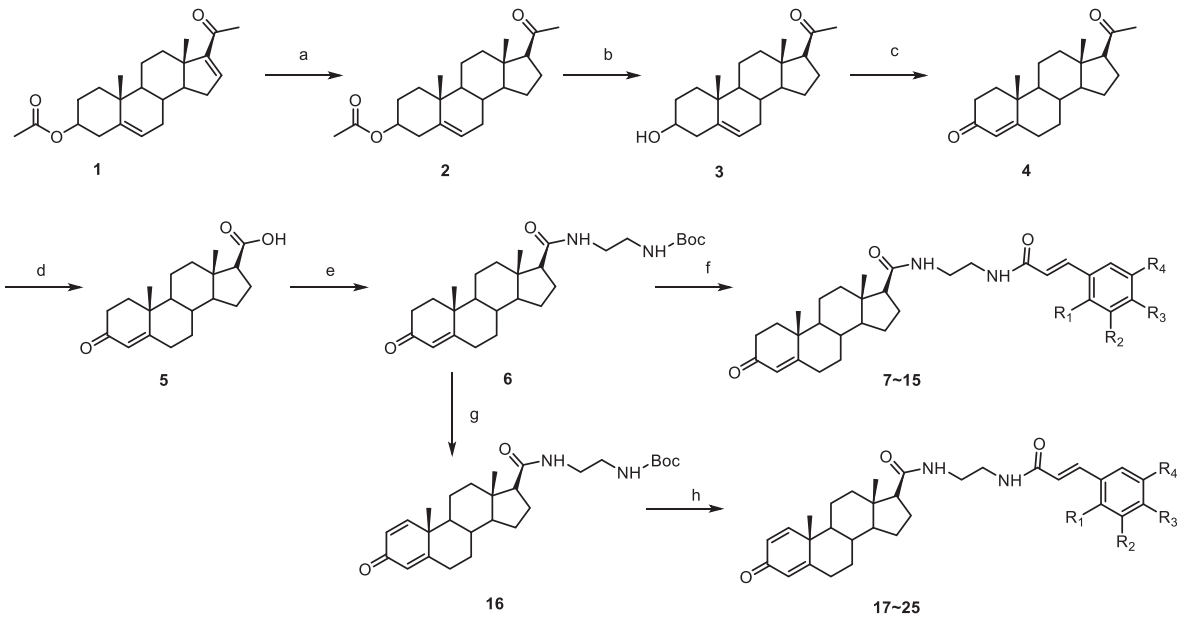


Fig. 1. (A) Several marketed ani-tumor drugs with the structure of 3-oxygen-androstene-4-ene. (B) The structure of chalcone. (C) Designed Steroidal hybrids based on the principle of hybridization.



Scheme 1. The synthetic route of designed target compounds. Regents and conditions: (a) Pd/C, H_2, 30 °C, rt, 6 h; (b) NaOH, CH_3OH, 1,4-dioxane, rt, 30 min; (c) cyclohexanone, aluminium isopropoxide, toluene, reflux, 7 h; (d) NaOH, Br_2, 1,4-dioxane, 0 °C ~ rt, 1.5 h; (e) HATu, DIPEA, DMF, rt, overnight; (f) i, CF_3COOH, CH_2Cl_2, 0 °C, 30 min; ii, HATu, DIPEA, DMF, rt, overnight; (g) DDQ, TBDMSCl, 1,4-dioxane,2h; (h) i, CF_3COOH, CH_2Cl_2, 0 °C, 30 min; ii, HATu, DIPEA, DMF, rt, overnight.

Table 1
In vitro antiproliferative activity of steroidal hybrids.

Comp.	R_1	R_2	R_3	R_4	Antiproliferative (IC_{50}, μM)			SI [a]	SI [b]
					MDA-MB-231	HT-29	A549		
7	H	H	H	H	10.29	9.49	27.32	1.1	2.7
8	OMe	H	H	H	0.42	11.73	30.9	22.5	73.5
9	H	H	OMe	H	22.17	34.96	>40	1.6	1.8
10	H	OMe	OMe	H	15.16	14.73	35.11	1.0	2.3
11	H	OMe	OMe	OMe	>40	>40	>40	–	–
12	H	H	Cl	H	8.19	14.99	35.71	1.8	4.3
13	H	H	CF_3	H	9.21	34.23	>40	3.7	4.3
14	H	H	NO_2	H	12.03	27.82	>40	2.3	3.3
15	H	NO_2	H	H	11.18	25.38	17.7	2.3	1.6
17	H	H	H	H	5.82	12.73	26.94	2.2	4.6
18	OMe	H	H	H	0.52	16.84	29.95	32.3	57.6
19	H	H	OMe	H	11.45	>40	29.61	3.5	2.6
20	H	OMe	OMe	H	5.75	>40	>40	7.0	7.0
21	H	OMe	OMe	OMe	>40	>40	35.91	–	–
22	H	H	Cl	H	>40	>40	38.74	–	–
23	H	H	CF_3	H	12.50	>40	38.07	3.2	3.2
24	H	H	NO_2	H	22.55	>40	>40	1.8	1.8
25	H	NO_2	H	H	13.74	>40	34.25	2.9	2.5
6	–	–	–	–	24.75	>40	>40	–	–
16	–	–	–	–	>40	>40	>40	–	–
Chalcone	–	–	–	–	42.80	45.90	69.19	–	–
5-Fu	–	–	–	–	9.89	12.67	14.53	1.3	1.5

[a] Selective Index (SI): [IC_{50} HT-29]/[IC_{50}[MDA-MB-231].
[b] Selective Index (SI): [IC_{50} A549]/[IC_{50} MDA-MB-231].

0.52 μM respectively. These findings offered us some valuable information in regard to the structural requirements for better potency and would be beneficial to design more potent steroidal drugs selectively targeting TNBC.

2.3. Compounds **8** and **18** inhibited the colony formation of MDA-MB-231 cells

According to the MTT results, we payed further attention to the antitumor effects of compounds **8** and **18** on MDA-MB-231 cells. Colony formation was one of the characteristics of oncogenic proliferation of cancer cells. Consequently, colony formation assay was further carried out to assay whether compounds **8** and **18** could block oncogenic growth of TNBC cells. As showed in Fig. 2, compounds 8 and 18 could significantly repress colony formation of MDA-MB-231 cells.

2.4. The effect of compound **8** on cell cycle distribution

It has been reported that target the cell cycle of cancer cells is a promising anti-cancer strategy.[37] So, we further investigated whether steroidal hybrids designed by us could influence the cell cycle of MDA-MB-231 cells. Compound **8** was chosen to perform the experiment.

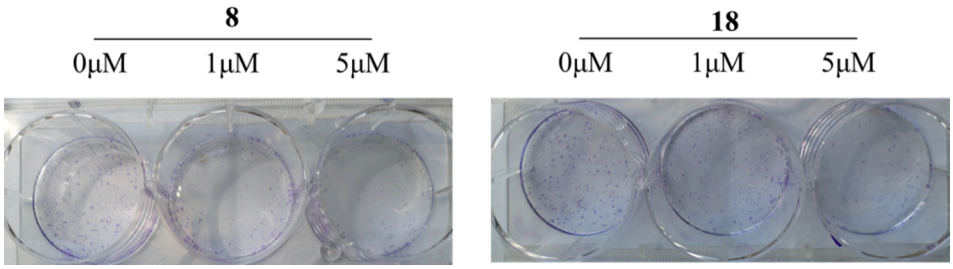


Fig. 2. The effects of compounds **8** and **18** on colony formation of MDA-MB-A231 cells. Cells were treated different concentrations (0, 1, 5 μM).

MDA-MB-231 cells were treated by different concentrations of compound **8** (0, 1, 5, 10, 20 μM) for 48 h and then analyzed by flow cytometry after PI staining. As showed in Fig. 3, compound **8** could arrest the cell cycle of MDA-MB-231 cells at S phase in a concentration-dependent manner.

*2.5. The effect of compound **8** on reactive oxygen species (ROS) accumulation in MDA-MB-231 cells*

ROS has been regarded as a vital mediate factor in various cellular signaling pathways including cell apoptosis.[38] Considering the excellent apoptosis induction of compound **8**, ROS accumulation levels were determined. MDA-MB-231 cells were treated by compound **8** (10 μM) for 0 h, 6 h, 12 h, 24 h, 48 h. As we can see from Fig. 4, the ROS levels varied with the treatment time of compound **8**. When cells were treated by compound **8** for 12 h, the ROS levels were accumulated to maximum that were distinctly higher than 0 h group.

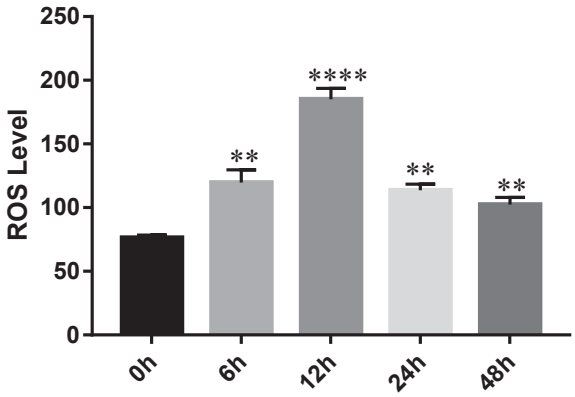


Fig. 4. Compound **8** induced the accumulation of toxic ROS in MDA-MB-231 cells. Experiments were replicated three times. **P < 0.01, ****P < 0.0001 vs the 0 h group.

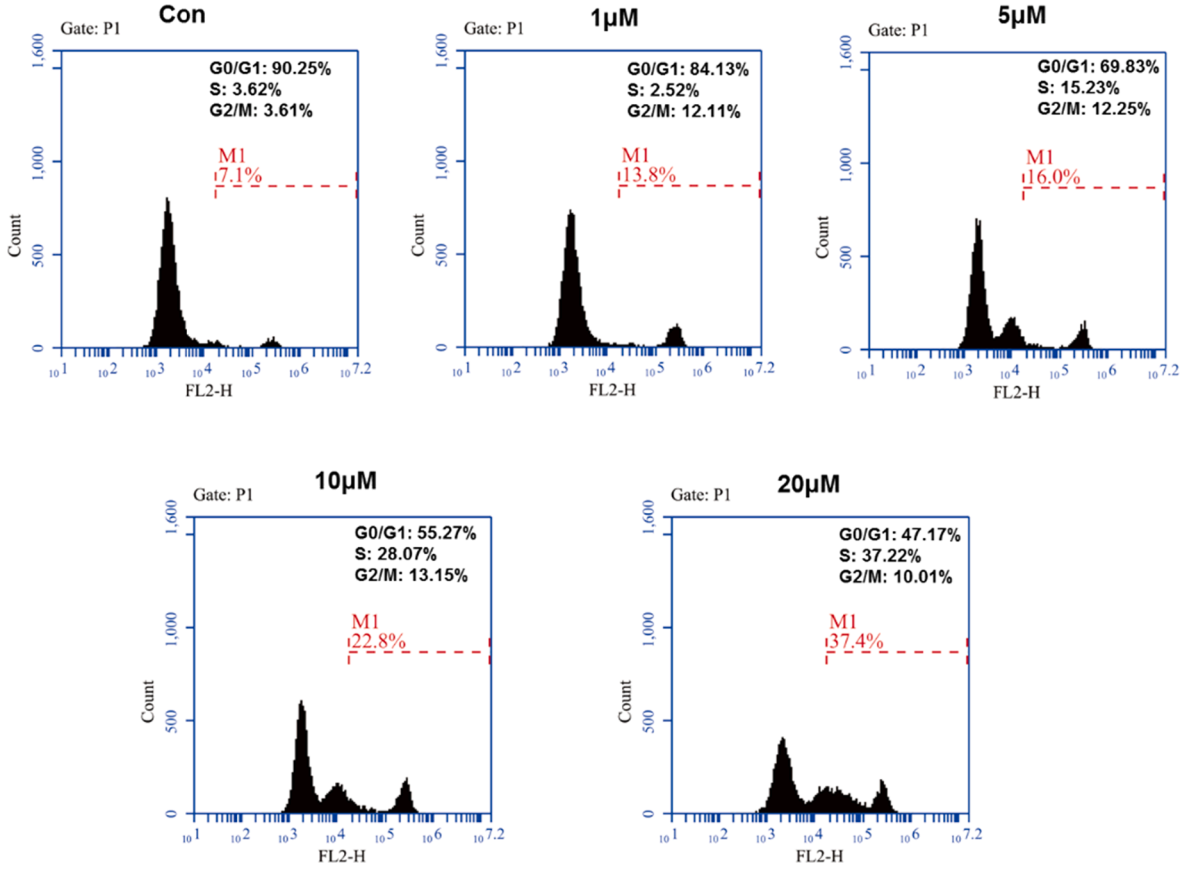


Fig. 3. The effect of compound **8** on cell cycle of MDA-MB-A231 cells. Cells were treated at different concentrations (0, 1, 5, 10, 20 μM) for 48 h.

2.6. Compound **8** induced MDA-MB-231 cells apoptosis

Due to the potent growth inhibitory activity of compound **8** against MDA-MB-231 cells, its apoptotic effect was studied through flow cytometry. Cells were treated by various concentrations of compound **8** (0, 1, 5, 10 μM). Fig. 5**A** showed that compound **8** induced MDA-MB-231 cells apoptosis in a concentration-dependent manner. At the concentration of 10 μM, the percentage of MDA-MB-231 cells was up to 58.50%.

Moreover, Western blot experiments were performed to determine the levels of Bcl-2/Bax. As depicted in Fig. 5**B** revealed that compound **8** significantly decreased the levels of antiapoptotic Bcl-2 but increased the expression pro-apoptotic Bax in MDA-MB-231. Caspase pathway played a key role in cell apoptosis. Consequently, we measured the levels of cleaved caspase-3 and cleaved poly(ADP-ribose)polymerase (PARP) using Western blot. The results confirmed that compound **8** could also dramatically up-regulate the levels of cleaved caspase-3 and PARP in MDA-MB-A231 cells.

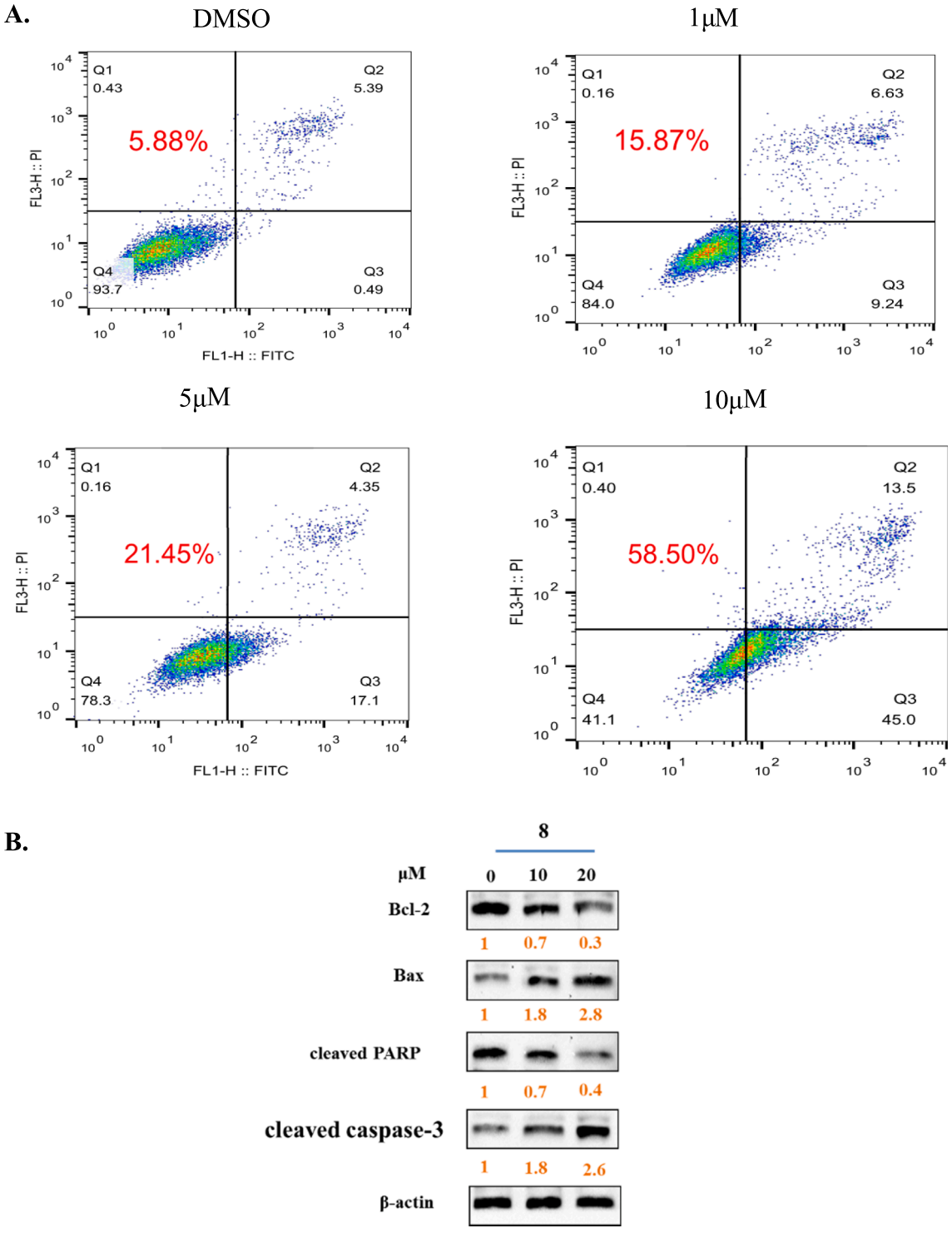


Fig. 5. (**A**) The effect of compound **8** on apoptosis of MDA-MB-A231 cells. Cells were treated at different concentrations (0, 1, 5, 10 μM) for 48 h. (**B**) Western blotting image and gray level analysis of Bcl-2, Bax, cleaved caspase-3 and cleaved PARP. MDA-MB-231 cells were treated with compound **8** for 48 h.

3. Conclusions

Steroids have attracted intense attention because of their wide bioactivities and are promising anti-cancer drugs. According to the principle of molecular hybridization, a series of novel steroidal-chalcone hybrids were designed and synthesized. Meanwhile, preliminary biological activity evaluation was carried out. The antiproliferative results showed that most compounds exerted weak activity against human colon cancer cells HT-29 and lung cancer cells A549. In contrast, most compounds showed excellent growth inhibitory activity against human TNBC cells MDA-MB-231. Among these compounds, compounds **8** and **18** demonstrated the most potent activities with the IC_{50} values of 0.42 μM and 0.52 μM respectively. In order to study the mechanism of steroidal hybrids toward MDA-MB-231 cells, compound **8** was chosen to perform further biological evaluation. Moreover, compound **8** could accumulate the ROS levels in MDA-MB-231 cells and induced cell apoptosis. Western Blot analysis documented that compound **8** could down-regulate antiapoptotic Bcl-2 but up-regulate pro-apoptotic Bax expression. Additionally, compound **8** could also activate the caspase-3 signaling pathway, which accelerated the apoptosis of MDA-MB-231 cells. Further mechanistic studies on the selectivity toward MDA-MB-231 cells and biological activity evaluation are ongoing in our laboratory.

In summary, our studies offered further proof that steroidal derivates occupy an important position in the discovery of anti-tumor compounds. Furthermore, our findings provided some valuable information in terms of the structural optimization for better potency and would be beneficial to design more potent steroidal drugs selectively targeting TNBC.

4. Experimental section

4.1. Chemistry

4.1.1. General

Melting points were determined on XRC-1 a melting point apparatus and are uncorrected. MS spectra were recorded on a Shimadzu GC-MS 2050 (ESI) or an Agilent 1946A-MSD (ESI) Mass Spectrum. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO with a Bruker Avance 300 MHz or 400 MHz spectrometer at 300 K. TMS was used as an internal standard and chemical shifts were reported in parts per million (ppm). All coupling constants (J) are in hertz (Hz), and the signals are designated as follows: s, singlet; d, doublet; t, chemicals and solvents were of analytical grade and, when necessary, were purified and dried by standard methods.

4.1.2. Synthesis of intermediates 3β-hydroxy-pregnane-5-alkene-20-one-3 acetate (2)

To a solution of compound **1** (12 g, 0.0336 mol) dissolved in 180 mL ethyl acetate was added 1.2 g 10% Pd/C. Then, H_2 was blown in and the mixture was stirred at room temperature for 6 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to obtain the crude product with a yield 97.1%. m.p.: 148–151 ℃ (reference, m.p.: 148–150 ℃[39]); ESI-MS *m/z*: 381 $[M+Na]^+$.

4.1.3. General procedure for the synthesis of intermediates 3β-hydroxy-pregnane-5-alkene-20-one (3)

Intermediate **2** (9.6 g, 26.8 mmol) was dissolved in 200 mL methanol and the 20 mL1,4-dioxane. Then, 49 mL 2.2 N water solution of NaOH was added. The mixture was stirred at room temperature for 30 min. Then, 50% acetic acid solution was added to adjust pH 6–7. The mixed solution was concentrated under reduced pressure to remove methanol. The mixture was filtered and washed with water. Finally, the product was dried by an infrared lamb to obtain the white solid **3** with a yield 95%. m.p.: 190–192 ℃ (reference, m.p.: 192–194℃[40]); ESI-MS *m/z*: 339 $[M+Na]^+$.

4.1.4. Synthesis of progesterone (4)

To a solution of compound (7 g, 22 mmol) dissolved in 112 mL dry toluene was added 21 mL dry cyclohexanone and aluminium isopropoxide (1.05 g,5.1 mmol). The mixture was refluxed for 7 h. After cooling, the reaction mixture was poured into 20 mL cool 15% NaOH with stirring for 20 min. The organic layer was separated and the aqueous layer was extracted 3 times with ethyl acetate. All organic fractions were pooled, washed with water, 10% H_2SO_4 and saturated saline, dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to obtain compound **4**. Yellow solid: 6.3 g; yield: 90%. m.p.: 128–130 ℃ (reference, m.p.: 127–129 ℃[41]); ESI-MS *m/z*: 315 $[M+H]^+$.

4.1.5. Synthesis of 3-oxo- androstane-4-ene-17β- carboxylic acid (5)

To a solution of NaOH (5.9 g, 0.14 mol) dissolved in 50 mL water was dropwise added Br_2 (1.9 mL, 0.037 mol) with the temperature below 0 °C to generate fresh solution of NaOBr. Compound **4** (3.5 g, 0.011 mol) was dissolved in 105 mL 1,4-dioxane and then 36 mL waster was added. After the mixture was cooled to 0 °C, NaOBr was dropwise added. The total reaction mixture was stirred for about 1.5 h at room temperature. 19 mL 10% Na $_2SO_3$ was added and the reaction was refluxed for 15 min. After the completion, 7 mL conc.HCl was added to adjust pH 3–4. Cooled to room temperature, the mixture was evaporated under reduced pressure to remove 1,4-dioxane. The precipitate was collected by filtration and dried to obtain compound **5**. m.p.: 240–242 ℃ (reference, m.p.: 240–243 ℃[42]); White solid: 2.6 g; yield: 75%.

4.1.6. Synthesis of 17β-(N-Boc-ethylenediamine formyl) androstane-4-ene-3-one (6)

To a solution of compound **5** (2 g, 0.006 mol) and DIPEA in DMF was added HATu (4.56 g, 0.012 mol) and the mixture was stirred for 5 min. Then, *N*-Boc-ethanediamine (1.15 g, 0.0072 mol) was added and the total reaction mixture was stirred overnight at room temperature. The completion of the reaction was confirmed by TLC. EtOAc and water were added. The aqueous layer was extracted 3 times with ethyl acetate and the combined organic layer was washed several times by saturated NH_4Cl to remove DMF and DIPEA, and then washed with saturated saline, dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to give compound **6**. The crude product was purified by column chromatography. White solid: 1.8 g; yield: 65%. ESI-MS *m/z*: 459 $[M+H]^+$.

4.1.7. Synthesis of N-(2-cinnamamidoethyl)-3-oxo-4-ene-androstane-17-carboxamide (7)

To a solution of **6** (300 mg, 0.65 mmol) in 5 mL CH_2Cl_2 was dropwise added 5 mL CF_3COOH at the ice bath. Maintaining the ice bath for 30 min. After the completion, the solution was evaporated to dry to afford an amine, which was then dissolved in DMF (2 mL).To a solution of cinnamic acid(90 mg, 0.78 mmol) and 993 μL DIPEA in 5 mL DMF were added HATu (456 mg,1.3 mmol). The mixture was stirred for about 5 min. Subsequently, the anmine in DMF was mixed into the solution. The total mixture was stirred overnight at room temperature. The completion of the reaction was confirmed by TLC. EtOAc and H_2O was added. The aqueous layer was extracted 3 times with ethyl acetate and the combined organic layer was washed several times by saturated NH_4Cl to remove DMF and DIPEA, and then washed with saturated saline, dried over anhydrous Na_2SO_4 and evaporated at reduced pressure to give compound **7**. The crude product was purified by column chromatography.

White solid: 110 mg; yield: 35%. m.p.: 130–132 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (t, $J = 5.6$ Hz, 1H), 7.64–7.52 (m, 2H), 7.49 (t, $J = 5.5$ Hz, 1H), 7.47–7.23 (m, 4H), 6.60 (d, $J = 15.8$ Hz, 1H), 5.62 (d, $J = 1.6$ Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 198.55, 172.35, 171.47, 165.76, 139.21, 135.33, 129.94, 129.42, 128.00, 123.61, 122.54. HRMS (ESI) *m/z* calcd for $C_{31}H_{40}N_2O_3[M+H]^+$ 489.6720, found 489.3117.

*4.1.8. Synthesis of N-(2-((E)-3-(2-methoxyphenyl) acrylamido) ethyl)-3-oxo-4-ene-androstane-17-carboxamide (**8**)*

The target compound **8** was synthesized according to the procedure of **7.** Light yellow solid: 205 mg; yield: 61%. m.p.: 136–138 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (d, J = 5.5 Hz, 1H), 7.69 (d, J = 16.1 Hz, 1H), 7.58–7.46 (m, 2H), 7.47–7.24 (m, 1H), 7.10 (d, J = 8.3 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 6.65 (dd, J = 15.9, 2.4 Hz, 1H), 5.64 (s, 1H), 3.88 (d, J = 2.4 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 198.46, 172.29, 171.41, 166.13, 157.98, 134.14, 131.27, 128.19, 123.68, 122.87, 121.15, 112.12, 56.01, 55.35, 53.66, 43.70. HRMS(ESI) *m/z* calcd for $C_{32}H_{42}N_2O_4[M+H]^+$ 519.6980, found 519.3221.

*4.1.9. Synthesis of N-(2-((E)-3-(4-methoxyphenyl) acrylamido) ethyl) −3-oxo-4-ene-androstane-17-carboxamide (**9**)*

The synthesis of target compound 9 was similar to the procedure as described for 7. Light yellow solid: 180 mg; yield: 53%. m.p.: 116–118 □; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.13 (d, J = 5.2 Hz, 1H), 7.55 (d, J = 7.9 Hz, 3H), 7.44 (s, 1H), 7.01 (d, J = 8.3 Hz, 2H), 6.49 (d, J = 15.7 Hz, 1H), 5.65 (s, 1H), 3.82 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 198.58, 172.35, 171.50, 166.12, 160.80, 138.96, 129.60, 127.91, 123.62, 120.03, 114.83, 55.72, 53.68, 43.73. HRMS(ESI) *m/z* calcd for $C_{32}H_{42}N_2O_4[M+H]^+$ 519.6980, found 519.3225.

*4.1.10. Synthesis of N-(2-((E)-3-(3,4-dimethoxyphenyl) acrylamido) ethyl) −3-oxo-4-ene-androstane-17-carboxamide (**10**)*

The synthesis of target compound **10** was similar to the procedure as described for **7.** Light yellow solid: 99 mg; yield: 28%. m.p.: 124–126 □;^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (d, J = 5.4 Hz, 1H), 7.30 (t, J = 5.4 Hz, 1H), 7.16 (d, J = 15.7 Hz, 1H), 7.00–6.89 (m, 2H), 6.78 (d, J = 8.2 Hz, 1H), 6.29 (d, J = 15.7 Hz, 1H), 5.41 (s, 1H), 3.58 (d, J = 3.4 Hz, 6H). ^{13}C NMR (75 MHz, DMSO) δ 198.64, 172.42, 171.58, 166.18, 150.55, 149.32, 139.37, 128.08, 123.58, 121.84, 120.14, 112.07, 110.38, 55.96, 55.83, 55.32, 53.66, 43.72. HRMS(ESI) *m/z* calcd for $C_{33}H_{44}N_2O_5[M+H]^+$ 549.7240, found 549.3335.

*4.1.11. Synthesis of N-(2-((E)-3-(3,4,5-trimethoxy) acrylamido) ethyl)-3-oxo-4-ene-androstane-17-carboxamide (**11**)*

The synthesis of target compound **11** was similar to the procedure as described for **7.** Light brown solid: 207 mg; yield: 55%. m.p.: 126–128 □; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.05 (t, J = 5.4 Hz, 1H), 7.47 (t, J = 5.4 Hz, 1H), 7.37 (d, J = 15.7 Hz, 1H), 6.90 (s, 2H), 6.56 (d, J = 15.7 Hz, 1H), 5.66–5.51 (m, 1H), 3.81 (s, 5H), 3.68 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 198.45, 172.32, 171.41, 165.85, 153.55, 139.35, 139.10, 131.00, 123.59, 121.88, 105.37, 60.55, 56.32, 56.04, 55.37, 53.67, 43.72. HRMS (ESI) *m/z* calcd for $C_{34}H_{46}N_2O_6[M+H]^+$ 579.7550, found 579.3435.

*4.1.12. Synthesis of N-(2-((E)-3-(4-chlorophenyl) acrylamido) ethyl)-3-oxo-4-ene-androstane-17-carboxamide (**12**)*

The synthesis of target compound **12** was similar to the procedure as described for **7.** White solid: 146 mg; yield: 43%. m.p.: 206–208 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.17 (s, 1H), 7.66–7.59 (m, 2H), 7.52–7.37 (m, 4H), 6.63 (d, J = 15.8 Hz, 1H), 5.64 (s, 1H).^{13}C NMR (101 MHz, DMSO) δ 198.47, 172.34, 171.41, 165.54, 137.82, 134.33, 129.69, 123.39, 56.03, 55.36, 53.66, 43.71, 40.38. HRMS(ESI) *m/z* calcd for $C_{31}H_{39}ClN_2O_3[M+H]^+$ 523.1140, found 523.2733.

*4.1.13. Synthesis of N-(2-((E)-3- N-(2-((E)-3-(4-(trifluoromethyl) phenyl) acrylamido) ethyl)-3-oxo-4-ene-androstane-17-carboxamide (**13**)*

The synthesis of target compound **13** was similar to the procedure as described for **7.** Light brown solid: 92 mg; yield: 25%. m.p.: 108–110 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (d, J = 5.5 Hz, 1H), 7.79 (s, 4H), 7.57–7.43 (m, 2H), 6.76 (d, J = 15.8 Hz, 1H), 5.62 (s, 1H). ^{13}C NMR (75 MHz, DMSO) δ 198.43, 172.35, 171.37, 165.27, 139.44, 137.50, 128.62, 126.36, 126.24, 125.38, 123.60, 56.02, 55.35, 53.65, 43.70. HRMS(ESI) *m/z* calcd for $C_{32}H_{39}F_3N_2O_3[M+H]^+$ 557.6702, found 557.2981.

*4.1.14. Synthesis of N-(2-((E)-3- N-(2-((E)-3-(4-(nitrophenyl) phenyl) acrylamido) ethyl)-3-oxo-4-ene-androstane-17-carboxamide (**14**)*

The synthesis of target compound **14** was similar to the procedure as described for **7.** Yellow solid: 87 mg; yield: 25%. m.p.: 128–130 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44–8.22 (m, 3H), 7.98–7.77 (m, 2H), 7.57 (d, J = 16.3 Hz, 2H), 6.83 (d, J = 15.8 Hz, 1H), 5.64 (s, 1H). ^{13}C NMR (75 MHz, DMSO) δ 198.52, 172.37, 171.43, 165.04, 147.94, 141.99, 136.88, 129.08, 126.85, 124.61, 123.61, 56.02, 55.35, 53.65, 43.71, 40.47. HRMS(ESI)*m/z* calcd for $C_{31}H_{39}N_3O_5[M+H]^+$ 534.6690, found 534.2971.

*4.1.15. Synthesis of N-(2-((E)-3- N-(2-((E)-3-(3-(nitrophenyl) phenyl) acrylamido) ethyl)-3-oxo-4-ene-androstane-17-carboxamide (**15**)*

The synthesis of target compound **15** was similar to the procedure as described for **7.** Yellow solid: 105 mg; yield: 30%. m.p.: 128–130 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (t, J = 2.0 Hz, 1H), 8.26–8.16 (m, 2H), 8.03 (dt, J = 7.8, 1.3 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.56 (d, J = 15.8 Hz, 1H), 7.49 (t, J = 5.5 Hz, 1H), 6.81 (d, J = 15.8 Hz, 1H), 5.61 (d, J = 1.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 198.43, 172.35, 171.36, 165.17, 148.75, 137.28, 136.85, 134.29, 131.00, 125.52, 124.18, 123.59, 122.04, 56.03, 55.36, 53.66, 43.71. HRMS(ESI) *m/z* calcd for $C_{31}H_{39}N_3O_5[M+H]^+$ 534.6690, found 534.2966.

*4.1.16. Synthesis of 17β-(N-Boc-ethylenediamine formyl) androstane-1,4-diene-3-one (**16**)*

To a solution of compound **6** (4 g, 8.67 mmol), TBDMSCl (65 mg, 0.43 mmol) in 1,4-dioxane was added DDQ (2.5 g, 11.2 mmol). The mixture was refluxed for about 2 h in a N_2 atmosphere. After the reaction was cooled to room temperature, the solution was filtered and filtrate was evaporated to dry to give the compound **16.** The crude product was purified by column chromatography. White solid: 2.2 g; yield: 56%.^{1}H NMR (300 MHz, DMSO-d_6) δ 7.48 (s, 1H), 7.20 (d, J = 10.1 Hz, 1H), 6.76 (s, 1H), 6.13 (dd, J = 10.3, 1.9 Hz, 1H), 5.99 (s, 1H).

*4.1.17. Synthesis of N-(2-cinnamamidoethyl)-3-oxo-1,4-diene-androstane-17-carboxamide (**17**)*

The detailed procedure of synthesizing target compound **17** was referred to the procedure of **7.** White solid: 180 mg; yield: 57%. m.p.: 120–122 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H), 7.70–7.29 (m, 7H), 7.22–6.97 (m, 1H), 6.73–6.50 (m, 1H), 6.30–5.83 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 185.44, 172.27, 169.89, 165.75, 156.83, 139.17, 135.40, 129.91, 129.41, 127.98, 127.19, 123.51, 122.64, 55.98, 54.93, 52.52, 44.20, 43.94, 43.66. HRMS(ESI) *m/z* calcd for $C_{31}H_{38}N_2O_3[M+H]^+$ 487.2560, found 487.2964.

*4.1.18. Synthesis of N-(2-((E)-3-(2-methoxyphenyl) acrylamido) ethyl)-3-oxo-1,4-diene-androstane-17-carboxamide (**18**)*

The target compound **18** was synthesized according to the procedure of **7.** Beige solid: 205 mg; yield: 61%. m.p.: 208–210 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.18 (d, J = 5.6 Hz, 1H), 7.56 (q, J = 5.9 Hz, 1H), 7.48–7.29 (m, 2H), 7.24–7.12 (m, 2H), 7.09 (dd, J = 10.2, 3.1 Hz, 1H), 6.97 (dd, J = 8.1, 2.5 Hz, 1H), 6.63 (d, J = 15.8 Hz, 1H), 6.11 (dd, J = 10.2, 1.9 Hz, 1H), 5.98 (d, J = 2.1 Hz, 1H), 3.80 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 185.47, 172.26, 170.00, 165.70, 160.05, 156.92, 139.12, 136.80, 130.47, 127.16, 123.50, 122.89, 120.29, 115.62, 113.18, 55.92, 55.55, 54.87, 52.45, 44.19, 43.94, 43.65. HRMS(ESI)*m/z* calcd for $C_{32}H_{40}N_2O_4[M+H]^+$ 517.3065, found 517.3068.

*4.1.19. Synthesis of N-(2-((E)-3-(4-methoxyphenyl) acrylamido) ethyl) −3-oxo-1,4-ene-androstane-17-carboxamide (**19**)*

The synthesis of target compound **19** was similar to the procedure as described for **7.** Beige solid: 183 mg; yield: 54%.m.p.: 128–130 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (s, 1H), 7.62–7.30 (m, 4H), 7.18–6.87 (m, 3H), 6.49 (d, J = 13.9 Hz, 1H), 6.05 (dt, J = 53.7, 9.2 Hz, 3H), 3.80 (dt, J = 10.7, 5.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 185.44, 172.28, 169.93, 166.06, 160.80, 156.88, 138.88, 129.56, 127.96, 127.17,

123.50, 120.16, 114.87, 55.98, 55.74, 54.93, 52.52, 44.20, 43.95, 43.67. HRMS(ESI)*m/z* calcd for $C_{32}H_{40}N_2O_4[M+H]^+$ 517.3065, found 517.3065.

4.1.20. Synthesis of N-(2-((E)-3-(3,4-dimethoxyphenyl) acrylamido) ethyl)-3-oxo-1,4-ene-androstane-17-carboxamide (20)

The detailed procedure of synthesizing target compound **20** was referred to the procedure of **7.** White solid: 146 mg; yield: 41%. m.p.: 98–100 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J = 15.5 Hz, 1H), 7.04 (ddd, J = 20.1, 9.3, 5.9 Hz, 4H), 6.86 (d, J = 8.3 Hz, 1H), 6.63 (dt, J = 21.8, 5.2 Hz, 1H), 6.36 (d, J = 15.6 Hz, 1H), 6.28–6.18 (m, 1H), 6.05 (d, J = 19.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.50, 174.15, 169.50, 167.52, 156.10, 150.72, 149.18, 141.06, 127.69, 123.85, 122.19, 118.35, 111.07, 109.63, 56.74, 55.94, 55.00, 52.36, 44.05, 43.61. HRMS(ESI)*m/z* calcd for $C_{33}H_{42}N_2O_5[M+H]^+$ 547.7080, found 547.3164.

4.1.21. Synthesis of N-(2-((E)-3-(3,4,5-trimethoxy) acrylamido) ethyl)-3-oxo-1,4-diene-androstane-17-carboxamide (21)

The synthesis of target compound **21** was similar to the procedure as described for **7.** Beige solid: 207 mg; yield: 55%. m.p.: 126–128 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17–7.80 (m, 1H), 7.60–7.44 (m, 1H), 7.44–7.32 (m, 1H), 7.09 (dd, J = 10.2, 5.9 Hz, 1H), 6.92 (s, 2H), 6.57 (dd, J = 15.7, 0.9 Hz, 1H), 6.16–6.04 (m, 1H), 5.97 (dt, J = 3.4, 1.5 Hz, 1H), 3.81 (s, 6H), 3.68 (d, J = 1.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 185.43, 172.25, 169.97, 165.81, 156.88, 153.56, 139.33, 139.10, 131.03, 127.15, 123.49, 121.93, 105.38, 60.55, 56.34, 55.96, 54.90, 52.48, 43.95, 43.67. HRMS(ESI)*m/z* calcd for $C_{34}H_{44}N_2O_6[M+H]^+$ 577.3240, found 577.3275.

4.1.22. Synthesis of N-(2-((E)-3-(4–4-chlorophenyl) acrylamido) ethyl)-3-oxo-1,4-diene-androstane-17-carboxamide (22)

The synthesis of target compound **22** was similar to the procedure as described for **7.** White solid: 172 mg; yield: 50%. m.p.: 138–140 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19 (t, J = 5.3 Hz, 1H), 7.63 (d, J = 8.1 Hz, 2H), 7.49 (q, J = 17.3, 14.0 Hz, 3H), 7.10 (d, J = 10.1 Hz, 1H), 6.64 (d, J = 15.7 Hz, 1H), 6.13 (t, J = 9.5 Hz, 1H), 5.99 (s, 1H). HRMS(ESI)*m/z* calcd for $C_{31}H_{37}ClN_2O_4[M+H]^+$ 521.0980, found 521.2557.

4.1.23. Synthesis of N-(2-((E)-3- N-(2-((E)-3-(4-(trifluoromethyl) phenyl) acrylamido) ethyl)-3-oxo-1,4-ene-androstane-17-carboxamide (23)

The synthesis of target compound **23** was similar to the procedure as described for **7.** Light brown solid: 92 mg; yield: 25%. m.p.: 130–132℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38–8.12 (m, 1H), 7.78 (t, J = 6.1 Hz, 4H), 7.63–7.32 (m, 2H), 7.07 (dd, J = 10.1, 4.3 Hz, 1H), 6.75 (d, J = 15.9 Hz, 1H), 6.20–6.00 (m, 1H), 5.97 (q, J = 2.6, 2.1 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 185.40, 172.27, 169.89, 165.21, 156.80, 139.48, 137.46, 128.61, 127.16, 126.30, 125.44, 123.51, 55.95, 54.91, 52.48, 43.94, 43.64. HRMS(ESI)*m/z* calcd for $C_{32}H_{37}F_3N_2O_4[M+H]^+$ 555.6542, found 555.2834.

4.1.24. Synthesis of N-(2-((E)-3- N-(2-((E)-3-(4-(nitrophenyl) phenyl) acrylamido) ethyl)-3-oxo-1,4-ene-androstane-17-carboxamide (24)

The synthesis of target compound **24** was similar to the procedure as described for **7.** Yellow solid: 87 mg; yield: 25%. m.p.: 140–142 ℃; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45–8.15 (m, 3H), 7.98–7.80 (m, 2H), 7.57 (d, J = 15.3 Hz, 2H), 7.14 (dd, J = 10.3, 2.0 Hz, 1H), 6.83 (d, J = 15.8 Hz, 1H), 6.21–6.08 (m, 1H), 6.00 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 185.45, 172.32, 169.97, 165.01, 156.89, 147.96, 142.01, 136.86, 129.05, 127.17, 126.90, 124.59, 123.50, 55.95, 54.91, 52.51, 43.94, 43.66, 38.75, 37.79, 35.33, 33.77, 32.45, 24.83, 23.63, 22.73, 18.83, 13.75. HRMS(ESI)*m/z* calcd for $C_{31}H_{37}F_3N_7O_5[M+H]^+$ 532.6530, found 532.2803.

4.1.25. Synthesis of N-(2-((E)-3- N-(2-((E)-3-(3-(nitrophenyl) phenyl) acrylamido) ethyl)-3-oxo-1,4-diene-androstane-17-carboxamide (25)

The synthesis of target compound **25** was similar to the procedure as described for **7.** Yellow solid: 105 mg; yield: 30%. m.p.: 132–134 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (t, J = 2.0 Hz, 1H), 8.26–8.12 (m, 2H), 8.03 (dt, J = 7.7, 1.3 Hz, 1H), 7.71 (t, J = 8.0 Hz, 1H), 7.62–7.50 (m, 2H), 7.08 (dd, J = 10.2, 3.2 Hz, 1H), 6.81 (d, J = 15.8 Hz, 1H), 6.09 (td, J = 10.4, 2.0 Hz, 1H), 5.95 (dt, J = 4.2, 1.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 185.40, 172.30, 169.85, 165.14, 156.77, 148.73, 137.29, 136.83, 134.30, 130.97, 127.17, 125.55, 124.16, 123.50, 122.00, 55.96, 55.35, 54.91, 52.49, 43.93, 43.63. HRMS(ESI)*m/z* calcd for $C_{31}H_{37}F_3N_7O_5[M+H]^+$ 532.6530, found 532.2807.

4.2. Biological evaluation

4.2.1. MTT assay for anti-proliferative activities

MDA-MB-231, HT-29 and A529 cells were cultured in L15 medium, RPMI1640 and RPMI1640 medium (All mediums containing 10% (v/v) FBS, 100 U/mL Penicillin and 100 mg/mL Streptomycin) respectively in a 5% CO_2-humidified atmosphere at 37 °C. Cells were planted at a density of 5 × 10^4/mL into a 96-well plate (100 μL/well) and incubated at 37 °C, 5% CO_2 atmosphere for 24 h. Then,100 μL/well medium containing test compounds was added and the final concentrations of test compounds was 40 μM/L, 20 μM/L, 10 μM/L, 1 μM/L and 0.1 μM/L. Control wells were added the equivalent volume of medium containing 1% (v/v) DMSO. 20 μL MTT (5 mg/mL) was added and cells were further incubated for 4 h. The culture medium was then removed and 150 μL/well DMSO was added. The cells were shocked for 10 min before reading the absorbance. The absorbance values were read at 490 nm for calculation of IC_{50} values.

4.2.2. Colony formation assay

0.3 × 10^3 MDA-MB-231 cells in a 2 mL of L15 medium (containing 10% (v/v) FBS, 100 U/mL Penicillin and 100 mg/mL Streptomycin) were seeded into a 6-well plate. After incubation for 24 h, compounds were added to provide the indicated concentrations. The cells were continuously incubated in a 5% CO_2-humidified atmosphere at 37 °C for 9 days. Culture medium containing tested compounds was updated twice during incubation. Then, the cells were stained by 0.05% crystal violet.

4.2.3. Cell cycle distribution

3 × 10^5 MDA-MB-231 cells in a 2 mL medium were planted into a 12-well plate. After incubation for 24 h, cells were treated with the tested compound in different concentrations for 48 h. Then, the cells were collected and fixed with 70% ice-cold ethanol following by they were washed with PBS. Subsequently, the cells were stained with PI at 4 °C. The percentage of cells in G1, S and G2/M phase of the cell cycle was determined by using a flow-cytometer (Bekcman, Coulter).

4.2.4. ROS kit to assay ROS levels

Cellular ROS levels were detected by using Reactive Oxygen Species Assay Kit (Beyotime). MDA-MB-231 cells were incubated in a 6-well plate with the density of 3 × 10^5/well for 24 h. Then, cells were treated with compound **8** at a concentration of 10 μM/L for different time (6 h, 12 h, 24 h, 48 h) at 37 °C. DCFH-DA was diluted with serum-free culture medium according to 1:1000 to make the final concentration 10 μM/L. The medium was replaced by diluted DCFH-DA. The cells were incubated for 0.5 h in darkness at 37 °C. Then, the cells were washed three times with serum-free culture medium and collected. Finally, the cells were analyzed by a flow-cytometer (Bekcman, Coulter).

4.2.5. Cell apoptosis assay

MDA-MB-231 cells were planted into a 6-well plate at a density of 3 × 10^5/well. Cells were incubated for 24 h and then the medium was replaced by fresh medium containing different concentrations of tested

compound. The plate was further incubated for 48 h. Cells were harvested and washed twice with cold PBS. Finally, the percentage of cell apoptosis was analyzed using an Annexin V-FITC/PI apoptosis detection kit via a flow-cytometer (Becton, Dickinson).

4.2.6. Western blot analysis

MDA-MB-231 cells were planted into a 6-well plate at a density of 3×10^5/well. Cells were incubated for 24 h and then the medium was replaced by fresh medium containing different concentrations of tested compound. The plate was further incubated for 48 h. Cells were harvested and Western bot experiment was carried out. Briefly, cells were washed with cold PBS and treated with RIPA Lysis buffer (containing 1% PMSF) for 30 min on ice. Then, cells were centrifuged at 12000 rpm/min for 5 min at 4 °C to obtain total protein that was subsequently measured by using a BCA kit. The protein samples were separated by electrophoresis in 10% SDS polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was blocked with 5% skim milk for 2 h at room temperature. Then, the samples were incubated with primary antibodies overnight at 4 °C. Next day, the samples were incubated with secondary antibodies for 2 h. The bands were visualized with super ECL plus by using Bio-RAP (Tanon).

Declaration of Competing Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Acknowledgements

This work was supported by grants from Natural Science Foundation of China (NSFC, 81874286), "Double-First-Class" University Project (CPU 2018PZQ02; CPU 2018GY07) and China Postdoctoral Science Foundation (2019M662007).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2020.115763.

References

1 Abramson VG, Lehmann BD, Ballinger TJ, Pietenpol JA. *Cancer*. 2015;121:8.
2 von Minckwitz G, Martin M. Annals of oncology: official journal of the European Society for. *Med Oncol*. 2012;23 Suppl 6:vi35.
3 Masuda H, Baggerly KA, Wang Y, et al. Clinical cancer research: an official journal of the American Association for. *Can Res*. 2013;19:5533.
4 Zhou B, Xing C. *Med Chem*. 2015;5:388.
5 Batovska DI, Todorova IT. *Curr Clin Pharmacol*. 2010;5:1.
6 Singh P, Anand A, Kumar V. *Eur J Med Chem*. 2014;85:758.
7 Manivannan, E., Karunagaran D., Trivedi P. Recent patents on anti-cancer drug discovery 2015, 10, 97.
8 Nowakowska Z. *Eur J Med Chem*. 2007;42:125.
9 Guo T, Xia R, Liu T, et al. *Chem Biodivers*. 2020;17, e2000025.
10 Xu M, Wu P, Shen F, Ji J, Rakesh KP. *Bioorg Chem*. 2019;91:103133.
11 Wu JZ, Cheng CC, Shen LL, et al. *Int J Mol Sci*. 2014;15:18525.
12 Greeff J, Joubert J, Malan SF, van Dyk S. *Bioorg Med Chem*. 2012;20:809.
13 Rana S, Blowers EC, Tebbe C, et al. *J Med Chem*. 2016;59:5121.
14 Heller L, Schwarz S, Perl V, Köwitsch A, Siewert B, Csuk R. *Eur J Med Chem*. 2015;101:391.
15 Wang Y, Xue S, Li R, Zheng Z, Yi H, Li Z. *Bioorg Med Chem*. 2018;26:8.
16 Abdelbaset MS, Abdel-Aziz M, Ramadan M, et al. *Bioorg Med Chem*. 2019;27:1076.
17 Shankaraiah N, Nekkanti S, Brahma UR, et al. *Bioorg Med Chem*. 2017;25:4805.
18 Zhuang C, Zhang W, Sheng C. *Chem Rev*. 2017;117:7762.
19 Kakati D, Sarma RK, Saikia R, Barua NC, Sarma JC. *Steroids*. 2013;78:321.
20 Cheenpracha S, Karalai C, Ponglimanont C, Subhadhirasakul S, Tewtrakul S. *Bioorg Med Chem*. 2006;14:1710.
21 Khan SA. *Eur J Med Chem*. 2008;43:2040.
22 Banday AH, Mir BP, Lone IH, Suri KA, Kumar HM. *Steroids*. 2010;75:805.
23 El-Kady DS, Abd Rabou AA, Tantawy MA et al. 2019, 188, 635.
24 Salvador JA, Carvalho JF, Neves MA, et al. *Nat Prod Rep*. 2013;30:324.
25 Fernández-Cabezón L, Galán B, García JL. *Front Microbiol*. 2018;9:958.
26 Elmegeed GA, Khalil WKB, Mohareb RM, Ahmed HH, Abd-Elhalim MM, Elsayed GH. *Bioorg Med Chem*. 2011;19:6860.
27 Nikolić AR, Petri ET, Klisurić OR et al. Bioorg Med Chem 2015, 23, 703.
28 Letis AS, Seo E-J, Nikolaropoulos SS, Efferth T, Giannis A, Fousteris MA. *Bioorg Med Chem*. 2017;25:3357.
29 Hampannavar GA, Karpoormath R, Palkar MB, Shaikh MS, Chandrasekaran B. *ACS Med Chem Lett*. 2016;7:686.
30 Koutsourea AI, Fousteris MA, Arsenou ES, Papageorgiou A, Pairas GN, Nikolaropoulos SS. *Bioorg Med Chem*. 2008;16:5207.
31 Singh H, Singh JV, Bhagat K, et al. *Bioorg Med Chem*. 2019;27:3477.
32 Yu B, Qi PP, Shi XJ, et al. *Eur J Med Chem*. 2016;117:241.
33 Sampson PB, Liu Y, Forrest B, et al. *J Med Chem*. 2015;58:147.
34 Lao K, Wang Y, Chen M, Zhang J, You Q, Xiang H. *Eur J Med Chem*. 2017;139:390.
35 Hou Q, He C, Lao K, Luo G, You Q, Xiang H. *Steroids*. 2019;150:108384.
36 Lao K, Sun J, Wang C, et al. *Steroids*. 2017;124:29.
37 Diaz-Moralli S, Tarrado-Castellarnau M, Miranda A, Cascante M. *Pharmacol Ther*. 2013;138:255.
38 Dharmaraja AT. *J Med Chem*. 2017;60:3221.
39 Moorthy JN, Singhal N, Senapati K. *Tetrahedron Lett*. 2008;49:80.
40 Shoppee C, Summers G. *J Chem Soc (resumed)*. 1957.
41 Daglish AF, Green J, Poole VD. *J Chem Soc (Resumed)*. 1954;2627.
42 Woodward RB, Sondheimer F, Taub D. *J Am Chem Soc*. 1951;73:3547.