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Research Article

Synthesis and Antimetastatic Effects of *E*-Salignone Amide Derivatives

Hong-Ling Wang,[1†] Nan Qin,[2†] Jia Liu,[3] Mei-Na Jin,[3] Xiang Zhang,[1] Mei-Hua Jin,[3] Dexin Kong,[2,3] Shen-De Jiang[1], and Hong-Quan Duan[2,3]*

[1]*School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, China*
[2]*Research Center of Basic Medical Sciences, Tianjin Medical University, Tianjin 300070, China*
[3]*Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics (Theranostics), School of Pharmacy, Tianjin Medical University, Tianjin 300070, China*

Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The preparation of novel *E*-salignone derivatives and their biological evaluation as potential antimetastatic agents is described. The *E*-salignone amide derivatives were prepared from epiandrosterone and androsterone, and characterized by analytical ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The derivatives were evaluated for antimetastatic activity in MDA-MB-231 cells by using a transwell assay. Comparing with the positive control, LY294002, compounds **19b**, **19d**, and **19e** exhibited significant inhibitory effects on the EGF-induced invasion of MB-MDA-231 cells. Moreover, compound **19b** also had antimigration effects in wound-healing assay. Compound **19b** may represent a novel antimetastatic agent for treating breast cancer. Drug Dev Res 75 : 76–87, 2014.

Key words: *E*-salignone; amide derivatives; antimetastasis; breast cancer cells

INTRODUCTION

Metastasis, the dissemination and growth of neoplastic cells in an organ distinct from that in which they originated, is a major cause of mortality in millions of cancer patients, especially in breast cancer, the leading cause of cancer-related death in women worldwide [Jemal et al., 2011]. Preventing or inhibiting breast cancer metastasis is key to prolonging and/or enhancing the quality of life of patients. Treatment options for metastatic breast cancer often focus on relieving symptoms together with systemic therapies to control primary tumor growth, e.g., chemotherapy and hormonal therapy. Unfortunately, only limited progress has been made in developing antimetastatic agents [Chen et al., 2010].

Cell migration plays a central role in metastasis and involves multiple steps, including: loss of cell–cell adhesion capacity; the degradation and invasion of basement membrane and extracellular matrix constituents; the ability to enter the bloodstream or lymphatic system, and then survive in the circulation; and the consequent establishment of new tumors [Mego et al., 2010; Yachida et al., 2010].

Natural products (NPs) are a rich source of new drugs. *E*-Salignone, an NP isolated from *Pachysandra terminalis*, has inhibitory effects on the migration of human MDA-MB-231 breast cancer cells induced

†These authors contributed equally to this work.

*Correspondence to: Hong-Quan Duan, Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics (Theranostics), School of Pharmacy, Tianjin Medical University, Tianjin 300070, China.
E-mail: duanhq@tijmu.edu.cn

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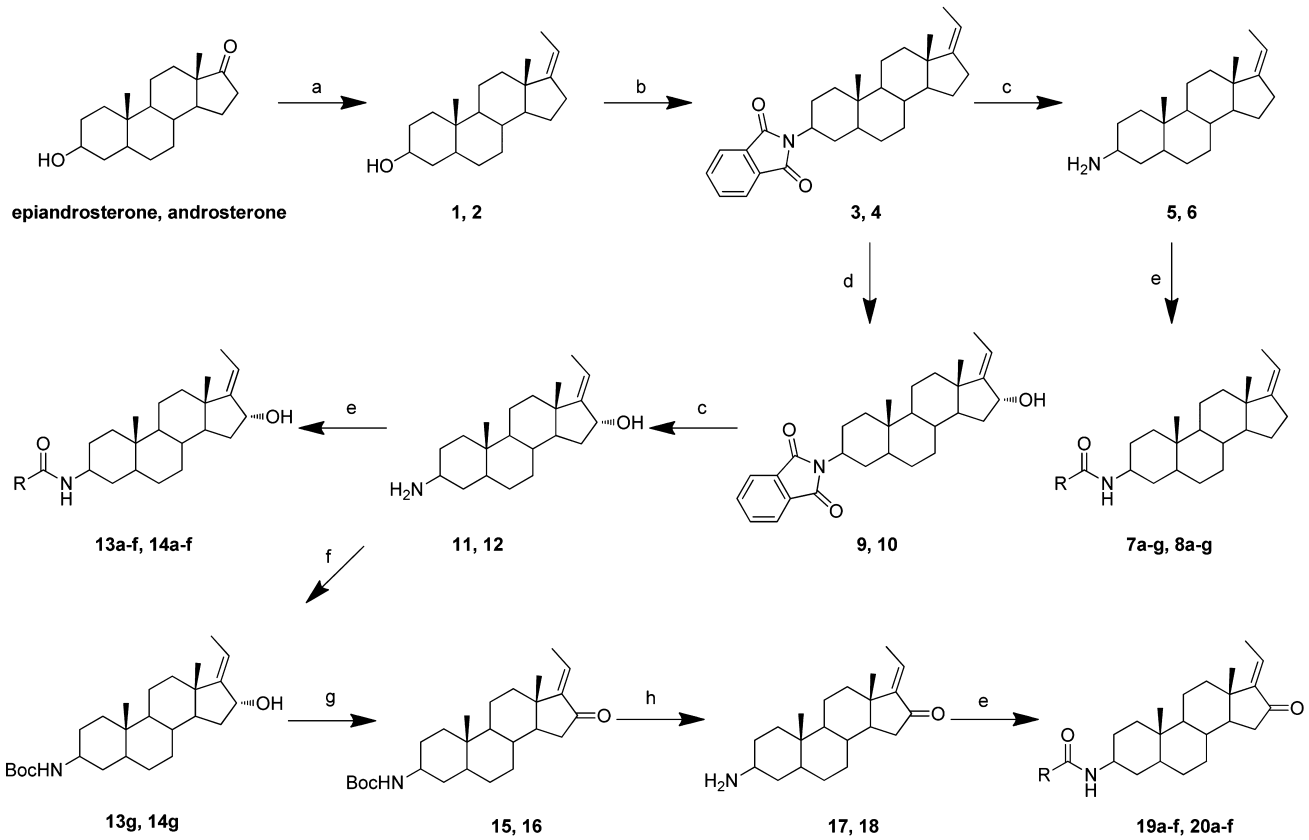

Fig. 1. The synthesis of *E*-salignone and its amide derivatives.

by the chemokine epidermal growth factor (EGF-IC_{50} = 0.87 μM) [Zhai et al., 2012]. As a result, the synthesis of a series of novel *E*-salignone amide derivatives was undertaken that were tested for their antitumor metastatic activity.

Materials and Methods

All solvents and reagents were obtained from commercial sources and used without purification. Reactions were monitored by thin-layer chromatography (TLC) using precoated silica gel aluminum plates containing a fluorescence indicator. The column chromatography was carried out on silica gel (300–400 mesh) (Qingdao Haiyang Chemical Co. Ltd., Qingdao, Shandong province, China). ^{1}H, ^{13}C NMR spectra were taken using a Brüker AVANCE III 400 instrument (Bruker Biospin AG, Fallanden, Switzerland). ^{1}H NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using tetramethylsilane (TMS) as internal standard. Mass spectra were taken in ESI mode on an Agilent 1200 LC-MS (Agilent, Palo Alto, CA, USA).

Synthetic Procedures

Wittig olefination of epiandrosterone and androsterone as starting material gave intermediates **1** and **2** stereoselectively [Deng et al., 1999] with the 3-OH of **1** and **2** being converted to phthalimide group by Mitsunobu reaction (Fig. 1). This method of inversion of the stereochemistry from the 3α-ol of **1** represented a substantial advantage over other literature methods as the 3β-amine was isolated as a single diastereoisomer in good yield without the need for chromatographic purification of the intermediates [Hamilton et al., 2012]. The intermediates **3** and **4** were oxidized by Riley reaction to provide 16α-ol of **9** and **10** [Bruttomesso et al., 1999]. After the protection of the 3-NH_2 group by di-tert-butyl dicarbonate, the 16α-ol of **13g** and **14g** were oxidized by activity MnO_2 to obtain the derivatives **15** and **16**. After the deprotection by TFA, the C-3 amino intermediates **17** and **18** were obtained. Finally, all the amide derivatives were prepared by condensation of the acids with 1,1'-Carbonyldiimid-azole (CDI) or the acid chlorides with 4-Dimethylaminopyridine (DMAP). The chemical structures of all target compounds (Fig. 2) were

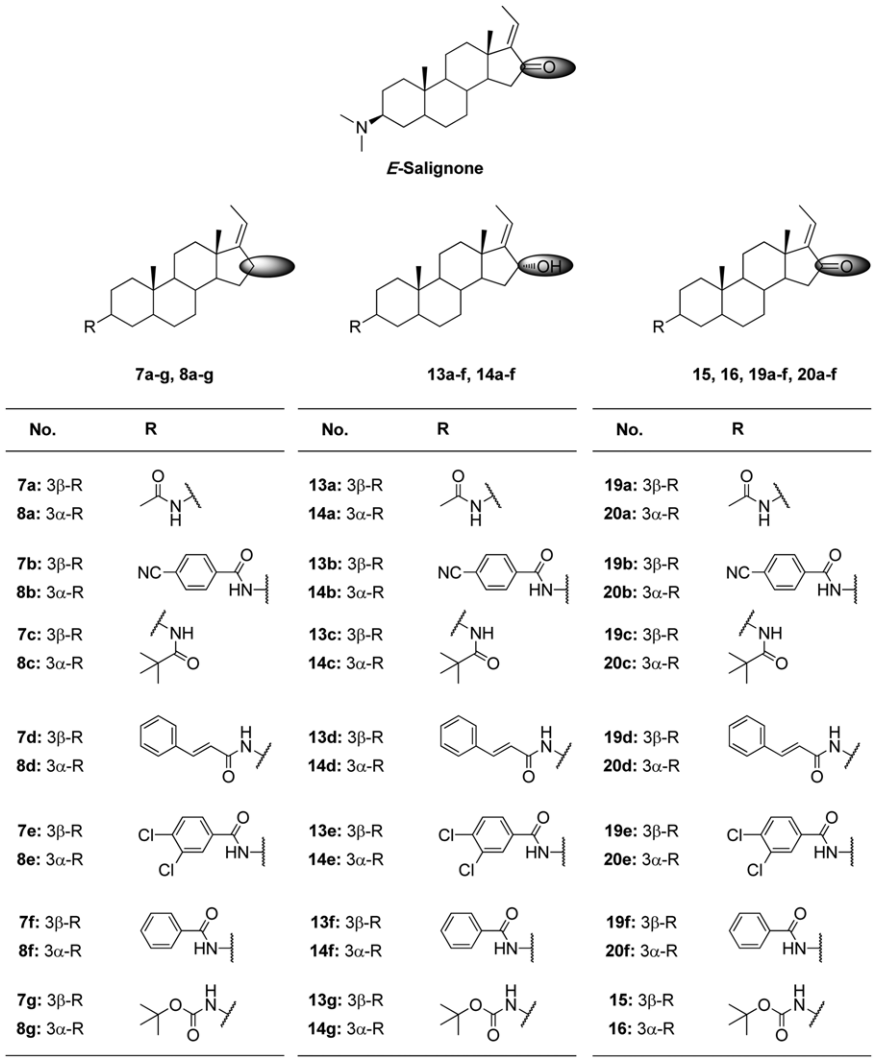

Fig. 2. The structures of *E*-salignone and its amide derivatives.

characterized by ^{1}H NMR, ^{13}C NMR, and ESI-MS spectral data analyses.

General procedure of compounds 1 and 2

A mixture of *t*-BuOK (4.0 eq) and Ph_3PEtBr (4.0 eq) was dissolved in anhydrous tetrahydrofuran (THF), stirred at room temperature for 1 h, then was added the starting material androsterone or epiandrosterone (1.0 eq). The mixture was refluxed for 4 h, and saturated NH_4Cl aqueous solution was added to quench the reaction. The reaction mixture was extracted with dichloromethane, washed with water, and dried over $MgSO_4$ and concentrated under reduced pressure to obtain the crude product, which was purified by recrystallization with methanol to give white needles.

(17*Z*)-Pregn-17(20)-en-3*β*-ol (1)

Yield: 93.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.11 (q, J = 6.9 Hz, 1H), 3.59 (m, 1H), 1.65 (d, J = 6.9 Hz, 3H), 0.87 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.5, 113.2, 71.3, 56.3, 54.4, 44.8, 44.4, 38.2, 37.2, 37.0, 35.5, 31.9, 31.4, 28.7, 24.4, 21.4, 16.9. ESI-MS *m/z*: 301 $[M-H]^-$.

(17*Z*)-Pregn-17(20)-en-3*α*-ol (2)

Yield: 91.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.12 (qt, J_1 = 2.0, J_2 = 7.1 Hz, 1H), 4.06 (s, 1H), 1.67 (dt, J_1 = 2.0, J_2 = 7.1 Hz, 3H), 0.88(s,3H), 0.81(s,3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.5, 113.2, 66.6, 56.3, 54.3, 44.4, 39.1, 37.2, 36.2, 35.9, 35.0, 32.2, 31.9, 31.4, 29.0, 28.5, 24.3, 21.0, 16.9, 13.1, 11.2. ESI-MS *m/z*: 301 $[M-H]^-$.

General procedure of compounds 3 and 4

Ph_3P (1.1 eq), Phathalimide (1.1 eq), and Compound **1** or **2** (1.0 eq) were added to anhydrous THF. The mixture was stirred in ice bath for 1 h, then Diisopropyl azodicarboxylate (DIAD) (1.1 eq) was added, stirred continuously hermetically for 18 h, and distilled water was added to quench the reaction. THF was evaporated to dryness. The reaction mixture was extracted with dichloromethane, washed with water, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to obtain the crude product, which was purified by recrystallization with methanol and dichloromethane to give white solid.

(17*Z*)-3β-(1,3-Dioxoisoindolin-2-yl)-pregn-17(20)-en (3)

Yield: 66.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82–7.79 (m, 2H), 7.70–7.68 (m, 2H), 5.13 (qt, J_1 = 1.9, J_2 = 7.2 Hz, 1H), 4.18 (m, 1H), 1.66 (dt, J_1 = 1.9, J_2 = 7.2 Hz, 3H), 0.97 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5, 150.5, 133.8, 132.1, 123.0, 113.2, 56.2, 54.1, 50.6, 46.1, 44.4, 37.8, 37.2, 35.6, 35.1, 31.8, 31.7, 31.4, 28.6, 25.1, 24.4, 21.3, 16.9, 13.1, 12.2. ESI-MS *m/z*: 430 $[M-H]^-$.

(17*Z*)-3α-(1,3-Dioxoisoindolin-2-yl)-pregn-17(20)-en (4)

Yield: 61.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81–7.82 (m, 2H), 7.71–7.69 (m, 2H), 5.12 (q, J = 6.8 Hz, 1H), 4.51 (br s, 1H), 1.66 (d, J = 6.8 Hz, 3H), 0.89 (s, 3H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 155.6, 133.8, 132.1, 122.9, 119.3, 74.4, 54.5, 52.4, 47.5, 44.5, 40.4, 37.4, 35.1, 35.0, 34.4, 32.7, 31.6, 31.6, 28.5, 24.6, 20.9, 17.6, 13.2, 12.4. ESI-MS *m/z*: 430 $[M-H]^-$.

General procedure for compounds 5, 6, 11, and 12

Compounds **3**, **4**, **9**, or **10** were dissolved in methanol, 85% hydrazine hydrate was added, then refluxed for 1 h (Fig. 1). The 4 mol/L NaOH of aqueous solution was added and stirred for 0.5 h, extracted with dichloromethane, washed with water and dried. The solvent was evaporated under reduced pressure to obtain the crude product which was purified by gel permeation chromatography HW-40C (dichloromethane/methanol = 2/1) to give a white solid.

(17*Z*)-Pregn-17(20)-en-3β-amine (5)

Yield: 88.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.10 (qt, J_1 = 2.0, J_2 = 6.8 Hz, 1H), 2.67 (br s, 1H), 1.64 (dt, J_1 = 2.0, J_2 = 6.8 Hz, 3H), 0.86 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.5, 113.2, 56.3, 54.5, 51.1, 45.5, 44.4, 37.6, 37.2, 35.6, 35.1, 31.9, 31.4, 31.3, 28.7, 28.6, 24.4, 21.3, 16.9, 13.1, 12.3. ESI-MS *m/z*: 300 $[M-H]^-$.

(17*Z*)-Pregn-17(20)-en-3α-amine (6)

Yield: 89.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.10 (q, J = 6.8 Hz, 1H), 3.16 (br s, 1H), 1.64 (d, J = 6.8 Hz, 3H), 0.86 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.2, 113.1, 56.2, 54.4, 49.4, 45.6, 44.3, 39.1, 37.1, 36.3, 34.9, 32.0, 31.8, 31.3, 28.9, 28.6, 24.3, 20.9, 16.8, 13.0, 11.2. ESI-MS *m/z*: 300 $[M-H]^-$.

(17*E*)-3β-Aminopregn-17(20)-en-16α-ol (11)

Yield: 88.1% ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57 (q, J = 6.8 Hz, 1H), 4.42 (d, J = 4.4 Hz, 1H), 2.66 (br s, 1H), 1.73 (d, J = 6.8 Hz, 3H), 0.86 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 119.3, 74.4 54.5, 52.5, 51.1, 45.5, 44.5, 39.4, 37.6, 37.4, 35.6, 35.1, 34.4, 32.6, 31.9, 28.7, 21.2, 17.6, 13.2, 12.3. ESI-MS m/z: 316 $[M-H]^-$

(17*E*)-3α-Aminopregn-17(20)-en-16α-ol (12)

Yield: 89.4%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.58 (q, J = 7.2 Hz, 1H), 4.43 (d, J = 4.9 Hz, 1H), 3.21 (br s, 1H), 1.74 (dt, J = 7.2 Hz, 3H), 0.87 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.5, 119.3, 74.3, 54.4, 52.5, 45.8, 44.6, 39.1, 37.4, 36.4, 36.0, 35.1, 32.0, 31.8, 28.9, 28.6, 20.8, 17.6, 13.2, 11.3. ESI-MS *m/z*: 316 $[M-H]^-$.

General procedure for compounds 9 and 10

SeO_2 (1.0 eq) and *t*-BuOOH (1.6 eq) were dissolved in CH_2Cl_2 solution, stirred in ice bath for 1 h (Fig. 1). Compound **3** or **4** (1.0 eq) was dissolved in CH_2Cl_2 solution, then was added into the mixture, the reaction mixture was stirred continuously in ice bath for 3.5 h, 10% $NaHSO_3$ was added then stirred for 15 min to quench the reaction. The reaction mixture was extracted with dichloromethane, washed with water, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to obtain the crude product, which was purified by column chromatography over silica gel (PE/EA = 4/1 ~ 1/1) to give white solid.

(17*E*)-3β-(1,3-Dioxoisoindolin-2-yl)-pregn-17(20)-en-16α-ol (9)

Yield: 76.4%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82–7.80 (m, 2H), 7.70–7.68 (m, 2H), 5.58 (q,

J = 7.2 Hz, 1H), 4.43 (d, J = 5.2 Hz, 1H), 4.18 (m, 1H), 1.74 (d, J = 7.2 Hz, 3H), 0.99 (s, 3H), 0.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5, 155.6, 133.8, 132.1, 123.0, 119.4, 74.4, 54.1, 52.5, 50.5, 46.1, 44.5, 37.7, 37.4, 35.6, 35.1,34.4, 31.8, 31.7, 28.5, 25.1, 21.2, 17.6, 13.2, 12.2. ESI-MS *m/z*: 446 $[M-H]^-$.

(17*E*)-3α-(1,3-Dioxoisoindolin-2-yl)-pregn-17(20)-en-16α-ol (10)

Yield: 71.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82–2.80 (m, 2H), 7.71–7.69 (m, 2H), 5.56 (q, J = 7.2 Hz, 1H), 4.49 (br s, 1H), 4.41 (d, J = 5.4 Hz, 1H), 1.72 (d, J = 7.2 Hz, 3H), 0.87 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 155.6, 133.8, 132.1, 122.9, 119.4, 74.4, 54.5, 52.4, 47.5, 44.5, 40.4, 37.4, 35.1, 34.9, 34.5, 32.7, 31.6, 28.5, 24.6, 20.9, 17.6, 13.2, 12.4. ESI-MS *m/z*: 446 $[M-H]^-$.

General Procedure for Compounds 15 and 16

Compound **13g** or **14g** (1 eq) was dissolved in dichloromethane, and activity MnO_2 powder (20 eq) was added. The mixture was refluxed for 2 h, filtered with Celite (Fig. 1). The solvent was evaporated under reduced pressure to obtain the crude product, which was purified by column chromatography over silica gel (PE/EA = 4/1) to give yellow oil.

(17*E*)-3β-(*tert*-Butoxycarbonylamino)-pregn-17(20)-en-16-one (15)

Yield: 74.0%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.49 (q, J = 7.2 Hz, 1H), 4.40 (br s, 1H), 3.42 (br s, 1H), 1.84 (d, J = 7.2 Hz, 3H), 1.44 (s, 9H), 1.01 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.6, 148.0, 129.0, 54.0, 50.0, 45.4, 43.4, 37.9, 37.1, 36.3, 35.7, 35.6, 34.2, 31.9, 29.1, 28.4, 28.3, 20.8, 17.6, 13.2, 12.2. ESI-MS *m/z*: 414 $[M-H]^-$.

(17*E*)-3α-(*tert*-Butoxycarbonylamino)-pregn-17(20)-en-16-one (16)

Yield: 75.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.49 (q, J = 7.2 Hz, 1H), 4.83 (br s, 1H), 3.85 (br s, 1H), 1.84 (d, J = 7.2 Hz, 3H), 1.45 (s, 9H), 1.01 (s, 3H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.5, 155.2, 147.9, 129.0, 79.0, 54.2, 50.1, 45.7, 43.4, 40.6, 37.9, 36.3, 36.2, 34.1, 33.2, 32.7, 31.9, 28.5, 28.2, 26.3, 20.5, 17.6, 13.2, 11.4. ESI-MS *m/z*: 414 $[M-H]^-$.

General procedure for compounds 17 and 18

Compound **15** or **16** was dissolved in dichloromethane, CF_3COOH was added, and then the mixture was stirred in ice bath for 1 h, evaporated just to dryness with a vacuum evaporator. $NaHCO_3$ solution was added until the mixture reached neutral (Fig. 1). The reaction mixture was extracted with dichloromethane, washed with $NaHCO_3$ solution, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to obtain the colorless oil.

(17*E*)-3β-Aminopregn-17(20)-en-16-one (17)

Yield: 92.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.48 (q, J = 7.6 Hz, 1H), 2.67 (br s, 1H), 1.84 (d, J = 7.6 Hz, 3H), 1.01 (s, 3H), 0.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.6, 148.0, 129.0, 54.2, 51.1, 50.1, 45.5, 43.4, 39.3, 38.0, 37.3, 36.4, 35.7, 34.2, 32.5, 32.0, 28.5, 20.9, 17.7, 13.2, 12.3. ESI-MS *m/z*: 314 $[M-H]^-$.

(17*E*)-3α-Aminopregn-17(20)-en-16-one (18)

Yield: 96.1%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.48 (q, J = 7.2 Hz, 1H), 3.24 (br s, 1H), 1.84 (d, J = 7.2 Hz, 3H), 1.01 (s, 3H), 0.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.6, 148.0, 128.9, 54.1, 50.1, 45.8, 43.4, 39.0, 37.9, 36.5, 36.3, 35.8, 34.2, 31.9, 31.8, 28.7, 28.4, 20.5, 17.7, 13.2, 12.3. ESI-MS *m/z*: 314 $[M-H]^-$.

General procedure for compounds 7g, 8g, 13g, and 14g

Compound **5**, **6**, **11**, or **12** (1.0 eq) was dissolved in THF, then TEA (2.0 eq) was added, when the mixture was stirred in ice bath, and THF solution of Boc_2O (1.2 eq) was added (Fig. 1). The mixture was then stirred at room temperature for 17 h, evaporated just to dryness with a vacuum evaporator. The reaction mixture was extracted with ethyl acetate, washed with water, then washed with saturated brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to obtain the crude product, which was purified by column chromatography over silica gel to give white solid.

(17*Z*)-3β-(*tert*-Butoxycarbonylamino)-pregn-17(20)-en (7g)

Yield: 87.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.11 (qt, J_1 = 1.9, J_2 = 7.1 Hz, 1H), 4.37 (s, 1H), 3.41 (br s, 1H), 1.64 (dt, J_1 = 1.9, J_2 = 7.1 Hz, 3H), 1.44 (s, 9H), 0.86 (s, 3H), 0.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.3, 150.4, 113.2, 79.0, 56.2, 54.3, 50.1, 45.4, 44.3, 37.4, 37.2, 35.8, 35.5, 35.0, 31.8, 31.4, 29.2, 28.5, 28.4, 24.4, 21.3, 16.9, 13.1, 12.2. ESI-MS *m/z*: 400 $[M-H]^-$.

(17*Z*)-3α-(*tert*-Butoxycarbonylamino)-pregn-17(20)-en (8g)

Yield: 82.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.11 (qt, J_1 = 2.0 Hz, J_2 = 7.1 Hz, 1H), 3.84 (s, 1H), 1.65

(dt, J_1 = 2.0 Hz, J_2 = 7.1 Hz, 3H), 1.45 (s, 9H), 0.86 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.4, 113.2, 56.4, 54.5, 44.4, 37.2, 36.1, 35.0, 33.3, 31.8, 31.4, 28.5, 26.4, 24.3, 21.0, 16.9, 13.1, 11.4. ESI-MS *m/z*: 400 $[M-H]^-$.

(17*E*)-3β-(*tert*-Butoxycarbonylamino)-pregn-17(20)-en-16α-ol (13g)

Yield: 80.1%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.57 (q, J = 6.8 Hz, 1H), 4.41 (d, J = 4.8 Hz, 1H), 3.41 (br s, 1H), 1.73 (d, J = 6.8 Hz, 3H), 1.73 (d, J = 6.8 Hz, 3H), 1.44 (s, 9H), 0.85 (s, 3H), 0.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.5, 119.4, 74.4, 54.3, 52.5, 45.4, 44.5, 37.4, 35.8, 35.5, 35.1, 34.4, 31.7, 29.2, 28.5, 21.2, 17.6, 13.2, 12.2. ESI-MS *m/z*: 416 $[M-H]^-$.

(17*E*)-3α-(*tert*-Butoxycarbonylamino)-pregn-17(20)-en-16α-ol (14g)

Yield: 86.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.58 (q, J = 7.1 Hz, 1H), 4.42 (d, J = 4.9 Hz, 1H), 3.84 (br s, 1H), 1.45 (s, 9H), 0.85 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.5, 155.3, 119.4, 74.3, 54.5, 52.5, 44.5, 40.7, 37.4, 36.1, 35.1, 34.4, 33.3, 33.0, 31.8, 28.5, 28.4, 26.4, 20.8, 17.6, 13.2, 11.4. ESI-MS *m/z*: 416 $[M-H]^-$.

General Procedure for Compounds 7a, 8a, 7c, 8c, 13c, 14c, 19a, 20a, 19c, and 20c

The compound **5**, **6**, **11**, **12**, **17**, or **18** (1 eq) was dissolved in CH_2Cl_2, then DMAP (1.2 eq) was added at room temperature. The acid chloride (1.2 eq) was added into the mixture. The mixture was stirred at room temperature for 24 h, and then evaporated just to dryness with a vacuum evaporator. The reaction mixture was extracted with CH_2Cl_2, washed with water, then washed with saturated brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to obtain the crude product which was purified by column chromatography over silica gel to give white solid.

(17*Z*)-3β-Acetamidopregn-17(20)-en (7a)

Yield: 87.4%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.67 (d, J = 8.0 Hz, 1H), 5.10 (qt, J_1 = 2.0, J_2 = 7.2 Hz, 1H), 3.74 (m, 1H), 1.95 (s, 3H), 1.64 (dt, J_1 = 2.0, J_2 = 7.2 Hz, 3H), 0.86 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3, 150.3, 113.2, 56.2, 54.3, 49.0, 45.3, 44.3, 37.3, 37.2, 35.5, 35.4, 35.0, 31.8, 31.4, 28.8, 28.5, 24.4, 23.5, 21.3, 16.9, 13.1, 12.2. ESI-MS *m/z*: 342 $[M-H]^-$.

(17*Z*)-3α-Acetamidopregn-17(20)-en (8a)

Yield: 90.0%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.84 (br s, 1H), 5.10 (qt, J_1 = 1.9, J_2 = 7.2 Hz, 1H), 4.10 (d, J = 7.0 Hz, 1H), 1.98 (s, 3H), 1.64 (dt, J_1 = 1.9, J_2 = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 150.4, 113.3, 56.4, 54.6, 44.8, 44.4, 41.0, 37.2, 36.1, 35.0, 33.2, 32.8, 31.8, 31.4, 28.5, 28.4, 26.0, 24.3, 23.7, 21.0, 16.9, 13.1, 11.4. ESI-MS *m/z*: 342 $[M-H]^-$.

(17*Z*)-3β-Pivalamidopregn-17(20)-en (7c)

Yield: 85.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41 (d, J = 7.6 Hz, 1H), 5.11 (q, J = 7.1 Hz, 1H), 3.74 (m, 1H), 1.65 (d, J = 7.1 Hz, 3H), 1.18 (s, 9H), 0.87 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.5, 150.4, 113.2, 56.2, 54.3, 48.7, 45.3, 44.3, 38.5, 37.4, 37.2, 35.5, 35.4, 35.1, 31.8, 31.4, 28.8, 28.5, 27.6, 24.4, 21.3, 16.9, 13.1, 12.2. ESI-MS *m/z*: 384 $[M-H]^-$.

(17*Z*)-3α-Pivalamidopregn-17(20)-en (8c)

Yield: 82.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (d, J = 5.8 Hz, 1H), 5.12 (qt, J_1 = 1.8, J_2 = 7.2 Hz, 1H), 4.09 (br s, 1H), 1.65(dt, J_1 = 1.8, J_2 = 7.2 Hz, 3H), 1.21 (s, 9H), 0.87 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 150.3, 113.3, 56.3, 54.7, 44.3, 41.4, 38.7, 37.2, 36.1, 35.0, 33.4, 32.8, 31.8, 31.4, 28.5, 27.7, 25.9, 24.3, 21.0, 16.9, 13.1, 11.4. ESI-MS *m/z*: 384 $[M-H]^-$.

(17*E*)-3β-Acetamidopregn-17(20)-en-16-one (19a)

Yield: 92.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.49 (q, J = 7.5 Hz, 1H), 5.30 (d, J = 8.1 Hz, 1H), 3.76 (m, 1H), 1.96 (s, 3H), 1.85 (d, J = 7.5 Hz, 3H), 1.01 (s, 3H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.5, 169.2, 148.0, 129.0, 54.0, 49.9, 48.9, 45.3, 43.4, 37.9, 37.0, 36.3, 35.6, 35.3, 34.2, 31.8, 28.8, 28.3, 23.6, 20.8, 17.7, 13.1, 12.2. ESI-MS *m/z*: 356 $[M-H]^-$.

(17*E*)-3α-Acetamidopregn-17(20)-en-16-one (20a)

Yield: 86.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (q, J = 7.6 Hz, 1H), 5.75 (br s, 1H), 4.14 (br s, 1H), 2.00 (s, 3H), 1.85 (d, J = 7.6 Hz, 3H), 1.02 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.3, 169.2, 147.9, 129.0, 65.5, 54.3, 50.2, 44.7, 43.4, 41.0, 37.9, 36.4, 36.2, 34.1, 32.9, 32.7, 31.9, 28.2, 25.9, 23.7, 20.5, 17.6, 13.1, 11.4. ESI-MS *m/z*: 357 $[M-H]^-$

(17*E*)-3β-Pivalamidopregn-17(20)-en-16-one (19c)

Yield: 86.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.49 (q, J = 7.5 Hz, 1H), 5.42 (d, J = 7.9 Hz, 1H), 3.74

(m, 1H), 1.84 (d, J = 7.5 Hz, 3H), 1.18 (s, 9H), 1.01 (s, 3H), 0.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.6, 177.6, 148.0, 129.0, 53.9, 50.0, 48.6, 45.3, 43.4, 38.5, 37.9, 37.1, 36.3, 35.6, 35.3, 34.2, 31.9, 28.7, 28.3, 27.6, 20.8, 17.7, 13.2, 12.3. ESI-MS *m/z*: 398 $[M-H]^-$.

(17*E*)-3α-Pivalamidopregn-17(20)-en-16-one (20c)

Yield: 88.6%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.50 (q, J = 7.6 Hz, 1H), 5.89 (d, J = 5.2 Hz, 1H), 4.10 (br s, 1H), 1.85 (d, J = 7.6 Hz, 3H), 1.21 (s, 9H), 1.02 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.4, 177.4, 147.9, 129.0, 54.4, 50.2, 44.2, 43.4, 41.4, 38.8, 37.9, 36.3, 36.2, 34.2, 33.2, 32.7, 31.9, 28.3, 27.7, 25.8, 20.6, 17.7, 13.2, 11.4. ESI-MS *m/z*: 398 $[M-H]^-$.

General procedures for compounds 7b, 7d–f, 8b, 8d–f, 13a–b, 13d–f, 14a–b, 14d–f, 19b, 19d–f, 20b, and 20d–f

Acid (1.0 eq) was dissolved in CH_2Cl_2, then CDI (1.2 eq) was added, when the mixture was stirred in ice bath for 2 h. The amine (1.2 eq), such as compound **5**, **6**, **11**, **12**, **17** or **18**, was added into the mixture. The mixture was stirred at room temperature for 24 h, and then evaporated just to dryness with a vacuum evaporator. The reaction mixture was extracted with CH_2Cl_2, washed with water, then washed with saturated brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to obtain the crude product which was purified by column chromatography over silica gel to give white solid.

(17*Z*)-3β-Paracyanobenzamidopregn-17(20)-en (7b)

Yield: 82.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (d, J = 8.1 Hz, 2H), 7.73 (d, J = 8.1 Hz, 2H), 6.07 (d, J = 7.8 Hz, 1H), 5.14 (q, J = 7.2 Hz, 1H), 3.98 (br s, 1H), 1.67 (d, J = 7.1 Hz, 3H), 0.89 (s, 3H), 0.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 150.3, 139.0, 132.4, 127.6, 118.0, 114.9, 113.3, 56.2, 54.3, 49.9, 45.3, 44.3, 37.3, 37.2, 35.5, 35.3, 35.1, 31.8, 31.4, 29.7, 28.5, 24.4, 21.3, 16.9, 13.1, 12.2. ESI-MS *m/z*: 429 $[M-H]^-$.

(17*Z*)-3α-Paracyanobenzamidopregn-17(20)-en (8b)

Yield: 76.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 8.4 Hz, 2H), 6.44 (d, J = 7.1 Hz, 1H), 5.11 (qt, J_1 = 1.8, J_2 = 7.1 Hz, 1H), 4.32 (br s, 1H), 1.64 (dt, J_1 = 1.8, J_2 = 7.1 Hz, 3H), 0.87 (s, 3H), 0.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.0, 150.2, 139.1, 132.4, 127.7, 118.1, 114.8, 113.3, 56.3, 54.6, 45.7, 44.3, 41.3, 37.2, 36.2, 34.9, 33.4, 32.7, 31.8, 31.4, 28.4, 25.9, 24.3, 21.0, 16.9, 13.1, 11.4. ESI-MS *m/z*: 429 $[M-H]^-$.

(17*Z*)-3β-Cinnamamidopregn-17(20)-en (7d)

Yield: 67.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, J = 15.6 Hz, 1H), 7.49–7.47 (m, 2H), 7.36–7.33 (m, 3H), 6.36 (d, J = 15.6 Hz, 1H), 5.54 (d, J = 8.0 Hz, 1H), 5.11 (d, J_1 = 1.8, J_2 = 7.6 Hz, 1H), 3.91 (m, 1H), 1.65 (d, J_1 = 1.8, J_2 = 7.6 Hz, 1H), 0.87 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.0, 150.4, 140.7, 135.0, 129.5, 128.8, 127.7, 121.2, 113.2, 56.2, 54.3, 49.2, 45.3, 44.4, 37.4, 37.2, 35.5, 35.1, 31.8, 31.4, 29.7, 28.9, 28.6, 24.4, 21.3, 16.9, 13.1, 12.2. ESI-MS *m/z*: 430 $[M-H]^-$.

(17*Z*)-3α-Cinnamamidopregn-17(20)-en (8d)

Yield: 53.9%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, J = 15.6 Hz, 1H), 7.52–7.50 (m, 2H), 7.37–7.35 (m, 3H), 6.44 (d, J = 15.6 Hz, 1H), 5.90 (d, J = 7.0 Hz, 1H), 5.12 (d, J = 7.1 Hz, 1H), 4.29 (br s, 1H), 1.65 (d, J = 7.1 Hz, 1H), 0.87 (s, 3H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.0, 150.4, 140.8, 135.0, 130.0, 128.8, 127.8, 121.2, 113.3, 56.4, 54.6, 45.0, 44.4, 41.1, 37.2, 36.2, 35.0, 33.3, 32.8, 31.8, 31.4, 28.4, 26.0, 24.3, 21.0, 16.9, 13.1, 11.5. ESI-MS *m/z*: 430 $[M-H]^-$.

(17*Z*)-3β-(3, 4-Diclorobenzamido)-pregn-17(20)-en (7e)

Yield: 88.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 6.20 (d, J = 6.9 Hz, 1H), 5.11 (q, J = 7.1 Hz, 1H), 3.92 (br s, 1H), 1.65 (d, J = 7.1 Hz, 3H), 0.86 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.5, 149.3, 134.6, 133.8, 131.9, 129.5, 128.1, 125.1, 112.3, 55.2, 53.2, 48.8, 44.3, 43.3, 36.3, 36.2, 34.5, 34.0, 30.7, 30.4, 27.7, 27.5, 23.4, 21.7, 20.3, 15.9, 13.1, 11.2. ESI-MS *m/z*: 473 $[M-H]^-$.

(17*Z*)-3α-(3,4-Diclorobenzamido)-pregn-17(20)-en (8e)

Yield: 85.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, J = 1.9 Hz, 1H), 7.59 (dd, J_1 = 1.9 Hz, J_1 = 8.3 Hz 1H), 7.52 (d, J = 8.3 Hz, 1H), 6.28 (d, J = 6.0 Hz, 1H), 5.12 (q, J = 7.2 Hz, 1H), 4.31 (br s, 1H), 1.65 (d, J = 7.2 Hz, 3H), 0.88 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.5, 150.3, 135.7, 135.0, 133.1, 130.6, 129.1, 126.1, 113.3, 56.3, 54.6, 45.6, 44.4, 41.3, 37.2, 36.2, 35.0, 33.4, 32.7, 31.7, 31.4, 28.4, 26.0, 24.3, 21.0, 16.9, 13.1, 11.4. ESI-MS *m/z*: 473 $[M-H]^-$.

(17*Z*)-3β-Benzamidopregn-17(20)-en (7f)

Yield: 82.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76–7.74 (m, 2H), 7.48–7.40 (m, 3H), 5.96 (d, J = 7.8 Hz, 1H), 5.12 (qt, J_1 = 1.9, J_2 = 7.1 Hz, 1H), 3.98 (m, 1H), 1.65 (dt, J_1 = 1.9, J_2 = 7.1 Hz, 3H), 0.87 (s, 3H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 150.4, 135.1, 133.5, 131.3, 130.1, 128.5, 128.4, 126.8, 113.2, 56.2, 54.3, 49.4, 45.4, 44.4, 37.4, 37.2, 35.6, 35.5, 35.1, 31.8, 31.4, 28.9, 28.6, 24.4, 21.3, 16.9, 13.1, 12.3. ESI-MS *m/z*: 420 $[M-H]^-$.

(17*Z*)-3α-Benzamidopregn-17(20)-en (8f)

Yield: 79.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79–7.76 (m, 2H), 7.52–7.42 (m, 3H), 6.41 (d, J = 6.6 Hz, 1H), 5.12 (qt, J_1 = 1.6, J_2 = 7.2 Hz, 1H), 4.34 (m, 1H), 1.65 (dt, J_1 = 1.6, J_2 = 7.2 Hz, 3H), 0.88 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 150.4, 135.2, 131.3, 130.1, 128.6, 128.4, 126.9, 113.3, 56.3, 54.6, 45.3, 44.4, 41.3, 37.2, 36.2, 35.0, 33.4, 32.8, 31.8, 31.4, 28.5, 26.1, 24.3, 21.0, 16.9, 13.1, 11.5. ESI-MS *m/z*: 420 $[M-H]^-$.

(17*E*)-3β-Acetamidopregn-17(20)-en-16α-ol (13a)

Yield: 62.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.58 (dd, J_1 = 1.1, J_2 = 7.2 Hz, 1H), 5.35 (d, J = 7.5 Hz, 1H), 4.43 (d, J = 5.2 Hz, 1H), 3.77 (m, 1H), 1.96 (s, 3H), 1.74 (dd, J_1 = 1.1, J_2 = 7.2 Hz, 3H), 0.87 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 155.5, 119.4, 74.4, 54.3, 52.4, 48.9, 45.3, 44.5, 37.4, 37.3, 35.5, 35.4, 35.0, 34.4, 31.7, 28.9, 28.4, 23.6, 21.2, 17.6, 13.2, 12.2. ESI-MS *m/z*: 358 $[M-H]^-$.

(17*E*)-3α-Acetamidopregn-17(20)-en-16α-ol (14a)

Yield: 57.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73 (br s, 1H), 5.35 (qd, J_1 = 1.6, J_2 = 7.2 Hz, 1H), 4.79 (d, J = 5.2 Hz, 1H), 4.13 (s, 1H), 1.99 (s, 3H), 1.78 (d, J = 7.2 Hz, 3H), 0.82 (s, 3H), 0.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 156.2, 117.5, 71.1, 55.0, 51.7, 44.7, 44.1, 41.1, 36.7, 36.2, 35.9, 34.6, 33.2, 32.8, 31.9, 28.4, 26.0, 23.7, 20.8, 20.6, 14.6, 11.4. ESI-MS *m/z*: 358 $[M-H]^-$.

(17*E*)-3β-Paracyanobenzamidopregn-17(20)-en-16α-ol (13b)

Yield: 61.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 8.0 Hz, 2H), 7.72 (d, J = 8.0 Hz, 2H), 6.09 (d, J = 7.9 Hz, 1H), 5.33 (q, J = 7.0 Hz, 1H), 4.79 (d, J = 5.5 Hz, 1H), 3.98 (m, 1H), 1.79 (d, J = 7.0 Hz, 3H), 0.85 (s, 3H), 0.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.9, 156.2, 139.0, 132.4, 127.6, 118.0, 117.6, 114.9, 71.2, 54.7, 51.6, 49.8, 45.5, 44.1, 37.3, 36.7, 35.9, 35.7, 35.3, 34.7, 31.8, 28.8, 28.5, 21.1, 20.6, 14.6, 12.3. ESI-MS *m/z*: 445 $[M-H]^-$.

(17*E*)-3α-Paracyanobenzamidopregn-17(20)-en-16α-ol (14b)

Yield: 64.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, J = 8.3 Hz, 2H), 7.72 (d, J = 8.3 Hz, 2H), 6.42 (d, J = 7.1 Hz, 1H), 5.56 (q, J = 7.1 Hz, 1H), 4.40 (d, J = 5.0 Hz, 1H), 4.31 (br s, 1H),1.71 (d, J = 7.1 Hz, 3H), 0.84 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.0, 155.3, 139.1, 132.4, 127.7, 119.6, 118.1, 114.9, 113.3, 74.3, 54.6, 52.5, 45.7, 44.5, 41.3, 37.3, 36.2, 35.0, 34.3, 33.3, 32.7, 31.7, 28.3, 25.9, 20.9, 17.6, 13.2, 11.4. ESI-MS *m/z*: 445 $[M-H]^-$.

(17*E*)-3β-Pivalamidopregn-17(20)-en-16α-ol (13c)

Yield: 50.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 (d, J = 8.0 Hz, 1H), 5.30 (q, J = 6.8 Hz, 1H), 4.77 (d, J = 5.6 Hz, 1H), 3.73 (m, 1H), 1.78 (d, J = 6.8 Hz, 3H), 1.17 (s, 9H), 0.82 (s, 3H), 0.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.6, 156.2, 117.5, 71.2, 54.6, 51.6, 48.7, 45.5, 44.1, 38.5, 37.4, 36.7, 36.0, 35.7, 35.4, 34.7, 31.9, 28.8, 28.5, 27.6, 21.1, 20.6, 14.6, 12.3. ESI-MS *m/z*: 400 $[M-H]^-$.

(17*E*)-3α-Pivalamidopregn-17(20)-en-16α-ol (14c)

Yield: 57.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (d, J = 6.1 Hz, 1H), 5.59 (q, J = 7.2 Hz, 1H), 4.43 (d, J = 5.0 Hz, 1H), 4.10 (br s, 1H), 1.74 (d, J = 7.2 Hz, 3H), 1.22 (s, 9H), 0.87 (s, 3H), 0.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.4, 155.4, 119.5, 74.4, 54.7, 52.6, 44.5, 44.3, 41.4, 37.4, 36.2, 35.0, 34.3, 33.4, 32.7, 31.7, 28.4, 27.7, 25.9, 20.9, 17.6, 13.2, 11.4. ESI-MS *m/z*: 400 $[M-H]^-$.

(17*E*)-3β-Cinnamamidopregn-17(20)-en-16α-ol (13d)

Yield: 47.0%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, J = 16.0 Hz, 1H), 7.50 (m, 5H), 6.46 (d, J = 16.0 Hz, 1H), 6.34 (d, J = 15.6 Hz, 1H), 5.58 (q, J = 7.2 Hz, 1H), 5.42 (d, J = 8.0 Hz, 1H), 4.42 (d, J = 5.2 Hz, 1H), 3.91 (m, 1H), 1.73 (d, J = 7.2 Hz, 1H), 0.86 (s, 3H), 0.83 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 165.0, 155.4, 140.8, 135.0, 129.5, 128.8, 127.8, 121.2, 119.4, 74.4, 54.6, 52.6, 44.9, 44.5, 41.4, 37.4, 36.2, 35.0, 34.4, 33.2, 32.9, 31.8, 28.4, 26.0, 20.9, 17.6, 13.2, 11.4. ESI-MS *m/z*: 446 $[M-H]^-$.

(17*E*)-3α-Cinnamamidopregn-17(20)-en-16α-ol (14d)

Yield: 46.8%. ^{1}H NMR (400 MHz, DMSO): δ 7.63 (d, J = 15.6 Hz, 1H), 7.53–7.51 (m, 2H), 7.39–7.34

(m, 3H), 6.44 (d, J = 15.6 Hz, 1H), 5.92 (d, J = 7.3 Hz, 1H), 5.58 (q, J = 6.8 Hz, 1H), 4.43 (d, J = 5.2 Hz, 1H), 4.31 (m, 1H), 1.74 (d, J = 6.8 Hz, 3H), 0.87 (s, 3H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, DMSO): δ 165.0, 155.4, 140.8, 135.0, 129.5, 128.8, 127.8, 121.2, 119.4, 74.4, 54.6, 52.6, 44.9, 44.5, 41.4, 37.4, 36.2, 35.0, 34.4, 33.2, 32.9, 31.8, 28.4, 26.0, 20.9, 17.6, 13.2, 11.4. ESI-MS *m/z*: 446 [M-H]$^-$.

(17*E*)-3β-(3,4-Diclorobenzamido)-pregn-17(20)-en-16α-ol (13e)

Yield: 44.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (s, 1H), 7.57 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 5.96 (d, J = 7.6 Hz, 1H), 5.33 (q, J = 6.8 Hz, 1H), 4.78 (d, J = 5.6 Hz, 1H), 3.93 (br s, 1H), 1.78 (d, J = 6.8 Hz, 3H), 0.83 (s, 3H), 0.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.5, 156.2, 135.7, 134.8, 133.0, 130.6, 129.1, 126.1, 117.6, 71.2, 54.6, 51.6, 49.7, 45.5, 44.2, 37.3, 36.7, 35.9, 35.7, 35.3, 34.7, 31.9, 28.8, 28.5, 21.1, 20.6, 14.6, 12.3. ESI-MS *m/z*: 489 [M-H]$^-$.

(17*E*)-3α-(3,4-Diclorobenzamido)-pregn-17(20)-en-16α-ol (14e)

Yield: 45.1%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, J = 1.9 Hz, 1H), 7.59 (dd, J_1 = 1.9 Hz, J_1 = 8.2 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 6.29 (d, J = 6.7 Hz, 1H), 5.32 (q, J = 6.9 Hz, 1H), 4.79 (d, J = 5.6 Hz, 1H), 4.31 (br s, 1H), 1.78 (d, J = 6.9 Hz, 3H), 0.86 (s, 3H), 0.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.6, 156.1, 135.7, 135.0, 133.1, 130.6, 129.1, 126.1, 117.6, 71.2, 54.9, 51.7, 45.6, 44.2, 41.4, 36.7, 36.3, 35.8, 34.6, 33.4, 32.7, 31.8, 28.4, 25.9, 20.8, 20.6, 14.6, 11.5. ESI-MS *m/z*: 489 [M-H]$^-$.

(17*E*)-3β-Benzamidopregn-17(20)-en-16α-ol (13f)

Yield: 52.9%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75–7.73 (m, 2H), 7.50–7.39 (m, 3H), 6.02 (d, J = 8.0 Hz, 1H), 5.59 (qd, J_1 = 1.1, J_2 = 7.2 Hz, 1H), 4.42 (d, J = 5.3 Hz, 1H), 3.97 (m, 1H), 1.72 (dd, J_1 = 1.1, J_2 = 7.2 Hz, 3H), 0.85 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 155.4, 135.0, 131.3, 128.5, 126.8, 119.4, 74.4, 54.3, 52.4, 49.4, 45.4, 44.5, 37.4, 37.3, 35.6, 35.4, 35.1, 34.4, 31.7, 28.9 28.5, 21.2, 17.6, 13.2, 12.2. ESI-MS *m/z*: 420 [M-H]$^-$.

(17*E*)-3α-Benzamidopregn-17(20)-en-16α-ol (14f)

Yield: 50.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78–7.76 (m, 2H), 7.52–7.43 (m, 3H), 6.38 (d, J = 6.8 Hz, 1H), 5.58 (q, J = 7.1 Hz, 1H), 4.42 (d, J = 5.2 Hz, 1H), 4.34 (t, J = 3.2 Hz, 1H), 1.74 (d, J = 7.1 Hz, 3H), 0.87 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 155.4, 135.3, 131.3, 128.6, 126.9, 119.5, 54.6, 52.5, 45.2, 44.5, 41.3, 37.4, 36.2, 35.0, 34.3, 33.4, 32.8, 31.7, 28.4, 26.0, 20.9, 17.6, 13.2, 11.4. ESI-MS *m/z*: 420 [M-H]$^-$.

(17*E*)-3β-Paracyanobenzamidopregn-17(20)-en-16-one (19b)

Yield: 79.5%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 6.49 (q, J = 7.5 Hz, 1H), 6.00 (d, J = 7.9 Hz, 1H), 4.00 (m, 1H), 1.85 (d, J = 7.5 Hz, 3H), 1.02 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.4, 164.9, 147.9, 138.9, 132.4, 129.0, 127.6, 53.9, 50.0, 49.8 45.3, 43.4, 37.9, 37.0, 36.3, 35.7, 35.2, 34.2, 31.8, 28.7, 28.3, 20.9, 17.7, 13.2, 12.3. ESI-MS *m/z*: 443 [M-H]$^-$.

(17*E*)-3α-Paracyanobenzamidopregn-17(20)-en-16-one (20b)

Yield: 74.2%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 8.0 Hz, 2H), 6.50 (q, J = 7.4 Hz, 1H), 4.36 (m, 1H), 1.84 (d, J = 7.4 Hz, 3H), 1.03 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.3, 165.0, 147.8, 139.1, 132.4, 129.2, 127.7, 118.1, 114.9, 54.3, 50.2, 45.7, 43.3, 41.2, 37.9, 36.2, 34.1, 33.0, 32.7, 31.9, 29.7, 28.2, 25.9, 20.6, 17.7, 13.2, 11.5. ESI-MS *m/z*: 443 [M-H]$^-$.

(17*E*)-3β-Cinnamamidopregn-17(20)-en-16-one (19d)

Yield: 72.9%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (d, J = 15.6 Hz, 1H), 7.51–7.49 (m, 2H), 7.36–7.35 (m, 3H), 6.49 (q, J = 7.5 Hz, 1H), 6.35 (d, J = 15.6 Hz, 1H), 5.48 (d, J = 7.6 Hz, 1H), 3.94 (m, 1H), 1.85 (d, J = 7.5 Hz, 3H), 1.01 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.6, 165.1, 148.0, 140.9, 134.9, 130.9, 129.6, 129.0, 128.8, 127.8, 120.9, 54.0, 50.0, 49.1, 45.2, 43.4, 37.9, 37.0, 36.3, 35.6, 35.3, 34.2, 31.8, 28.8, 28.3, 20.8, 17.7, 13.2, 12.2. ESI-MS *m/z*: 444 [M-H]$^-$.

(17*E*)-3α-Cinnamamidopregn-17(20)-en-16-one (20d)

Yield: 42.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, J = 15.6 Hz, 1H), 7.51–7.49 (m, 2H), 7.37–7.34 (m, 3H), 6.50 (q, J = 7.5 Hz, 1H), 6.48 (d, J = 15.6 Hz, 1H), 6.08 (br s, 1H), 4.31 (t, J = 6.7 Hz, 1H), 1.85 (d, J = 7.5 Hz, 3H), 1.02 (s, 3H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.5, 165.1, 148.0, 140.7, 135.0, 130.9, 129.5, 129.2, 128.8, 127.7, 121.2, 54.3, 50.2, 44.9,

43.4, 41.0, 37.9, 36.3, 36.2, 34.1, 33.0, 32.8, 31.9, 28.2, 26.0, 20.5, 17.7, 13.2, 11.5. ESI-MS *m/z*: 444 $[M-H]^-$.

(17*E*)-3*β*-(3,4-Diclorobenzamido)-pregn-17(20)-en-16-one (19e)

Yield: 43.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (d, *J* = 2.0 Hz, 1H), 7.56 (dd, J_1 = 2.0, J_2 = 8.4 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 6.50 (q, *J* = 7.6 Hz, 1H), 5.85 (d, *J* = 8.0 Hz, 1H), 3.96 (m, 1H), 1.85 (d, *J* = 7.6 Hz, 3H), 1.02 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.4, 164.5, 147.9, 135.7, 134.8, 133.0, 130.6, 129.0, 126.1, 54.0, 50.0, 49.7, 45.3, 43.4, 37.9, 37.0, 36.3, 35.7, 35.3, 34.2, 31.8, 28.8, 28.3, 20.9, 17.7, 13.2, 12.3. ESI-MS *m/z*: 487 $[M-H]^-$.

(17*E*)-3*á*-(3,4-Diclorobenzamido)-pregn-17(20)-en-16-one (20e)

Yield: 48.1%.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, *J* = 2.0 Hz, 1H), 7.64 (dd, J_1 = 2.0, J_2 = 8.4 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 6.64 (d, *J* = 7.2 Hz, 1H), 6.49 (q, *J* = 7.6 Hz, 1H), 4.35 (br s, 1H), 1.85 (d, *J* = 7.6 Hz, 3H), 1.03 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.4, 164.6, 147.9, 135.6, 134.9, 132.8, 130.5, 129.3, 129.1, 126.4, 54.2, 50.3, 45.5, 43.4, 41.0, 37.9, 36.3, 36.2, 34.1, 32.9, 32.7, 31.9, 29.7, 28.1, 25.9, 20.5, 17.7, 13.2, 11.4. ESI-MS *m/z*: 487 $[M-H]^-$.

(17*E*)-3*β*-Benzamidopregn-17(20)-en-16-one (19f)

Yield: 55.8%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75–7.74 (m, 2H), 7.49–7.41 (m, 3H), 6.49 (q, *J* = 7.6 Hz, 1H), 5.95 (d, *J* = 7.6 Hz, 1H), 3.99 (m, 1H), 1.85 (d, *J* = 7.6 Hz, 3H), 1.02 (s, 3H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.6, 166.7, 148.0, 135.0, 131.3, 129.0, 128.5, 126.8, 54.0, 50.0, 49.3, 45.3, 43.4, 37.9, 37.1, 36.3, 35.7, 35.4, 34.2, 31.9, 28.8, 28.3, 20.9, 17.7, 13.2, 12.3. ESI-MS *m/z*: 418 $[M-H]^-$.

(17*E*)-3*α*-Benzamidopregn-17(20)-en-16-one (20f)

Yield: 59.7%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80–7.78 (m, 2H), 7.52–7.44 (m, 3H), 6.49 (q, *J* = 7.5 Hz, 1H), 6.34 (d, *J* = 6.4 Hz, 1H), 4.35 (br s, 1H), 1.85 (d, *J* = 7.5 Hz, 3H), 1.03 (s, 3H), 0.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 206.4, 166.7, 147.9, 135.2, 131.3, 129.1, 128.6, 126.9, 54.3, 50.1, 45.2, 43.4, 41.2, 37.9, 36.3, 34.1, 33.1, 32.8, 31.8, 28.2, 26.0, 20.6, 17.7, 13.2, 11.5. ESI-MS *m/z*: 418 $[M-H]^-$.

Cell Viability Assay

Cell viability assay was performed as described previously [Wu et al., 2010].

Invasion assay

The chemotaxis invasion assay was performed as reported as previously [Zhai et al., 2012]. Briefly, after pretreatment with the different concentrations of compounds for 24 h, MDA-MB-231 cells suspended in binding medium (RPMI 1640, 0.1% bovine serum albumin) were loaded into the upper chambers. Chemoattractant (EGF, 1 ng/mL) was loaded into the lower chemotaxis chamber. The 8 μm filter membranes, which had previously been pretreated with 0.001% fibronectin, were inserted between the upper and lower chambers. After further incubation for 3.5 h, the filter membrane was rinsed, fixed, and stained. The number of migrating cells was counted under a microscope (Olympus Corp., CKX41, Tokyo, Japan). The inhibitory ratio (IR) was calculated as follows: IR% = (1 − number of migrated cells in sample/number of migrated cells in control) × 100%, and the median inhibitory concentration (IC_{50}) was obtained. Data were analyzed by using SPSS 16.0 software package. LY294002 (Camarillo, CA, USA), and a phosphoinositide 3-kinase (PI3K) inhibitor [Kong and Yamori, 2009] reported to inhibit metastasis [Sun et al., 2005] was used as a positive control.

Wound healing assay

The assay was carried out as previously reported [Kong and Yamori, 2010]. MDA-MB-231 cells were seeded in 6-well plates and grown to full confluence. The cell monolayer was wounded with a sterile micropipette tip to generate a clean wound area across the center of the well. Subsequently, cellular debris was rinsed with PBS, and then incubated in medium with or without compound for 24 h. Cell migration into the wound was monitored and photographed under microscope.

RESULTS AND DISCUSSION

The synthetic steps are outlined in Figure 1. Firstly, The C-C double bond was formed by Wittig reaction with the Z-configuration [Deng et al., 1999]. Then, the C-N single bond at C-3 position was introduced by a Mitsunobu reaction. To induce the C-3 amino group, a trial-and-error approach is required, e.g., introduction of C-3 nitro group, C-3 azides group [Czako et al., 2009], or C-3 phthalimide group [Hamilton et al., 2012]. Only the C-3 phthalimide steroid was obtained successfully from C-3 hydroxyl steroid by a Mitsunobu reaction in a 66.3% yield. After hydrazinolysis, the C-3 amino steroids were obtained in above 90% yield. Meanwhile, the allylic position of C-17 (20) double bond could be oxidized to obtain the C-16 hydroxyl or carbonyl group. Due to the limitation

TABLE 1. Inhibition of Cancer Cell Invasion by *E*-Salignone Amide Derivatives

No.	IC_{50}[a] (μM)	No.	IC_{50}[a] (μM)	No.	IC_{50}[a] (μM)
7a	27.54 ± 0.35	**13a**	39.48 ± 0.04	**19a**	16.12 ± 0.44
7b	10.63 ± 0.43	**13b**	36.11 ± 0.39	**19b**	0.24 ± 0.04
7c	39.17 ± 0.08	**13c**	>50	**19c**	25.24 ± 0.27
7d	1.63 ± 0.16	**13d**	6.95 ± 0.11	**19d**	0.91 ± 0.12
7e	2.48 ± 0.31	**13e**	25.61 ± 0.33	**19e**	0.62 ± 0.09
7f	27.31 ± 0.05	**13f**	37.85 ± 1.20	**19f**	15.13 ± 0.13
7g	24.38 ± 0.56	**13g**	46.54 ± 0.05	**15**	4.01 ± 0.05
8a	31.22 ± 1.13	**14a**	>50	**20a**	24.24 ± 0.47
8b	21.12 ± 0.06	**14b**	41.63 ± 0.65	**20b**	3.46 ± 0.07
8c	44.34 ± 0.37	**14c**	>50	**20c**	33.02 ± 0.06
8d	19.15 ± 0.51	**14d**	42.73 ± 0.08	**20d**	9.39 ± 0.14
8e	23.76 ± 0.47	**14e**	36.57 ± 0.36	**20e**	6.543 ± 1.12
8f	31.91 ± 0.14	**14f**	48.26 ± 0.23	**20f**	23.07 ± 0.53
8g	22.44 ± 0.61	**14g**	>50	**16**	9.61 ± 0.51
LY294002	0.38 ± 0.06				

[a]IC_{50} values are the mean of three independent experiments; standard deviation is given.

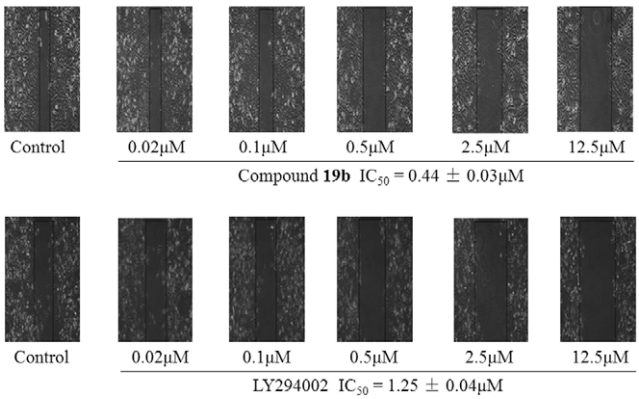

Fig. 3. The effects of compound **19b** and LY294002 (positive control) on the migration of MDA-MB -231 cells. Quantitative assessment of the migrated cells to the wound area was expressed as IC_{50} values with the mean ± SD of three independent experiments.

of hydrazinolysis with ketone, the allylic position had to be oxidized to a carbonyl group indirectly, while protecting the amino group to avoid oxidation. Finally, the synthesis of amide derivatives was accomplished by the reaction of various acids or acid chlorides with corresponding C-3 amino steroids, including compounds **5**, **6**, **11**, **12**, **17**, and **18**.

Synthetic small molecules and NPs are potent antimetastatic agents, acting via complex pathways that involve inhibition of cell proliferation and blockade of cell migration [Molinski et al., 2009; Scott et al., 2009; Oskarsson et al., 2010]. Accordingly, in order to achieve an understanding of the mechanism responsible for antimetastatic actions, cytotoxic effects of small molecules must be deemphasized while maintaining their ability to inhibit cancer cell migration. The in vitro cytotoxic activities of the novel *E*-salignone amide derivatives against breast adenocarcinoma MDA-MB-231 were evaluated using MTT assays. The results (data not shown) show that the majority of the compounds do not display positive effects on the inhibition of cancer cell proliferation below a concentration of 70 μM.

An important characteristic of tumor metastasis is the invasive and migratory ability of tumor cells. The synthesized amide derivatives were evaluated in the transwell invasion assay, where they inhibited MDA-MB-231 cell invasion with IC_{50} values in the range 0.24–50 μM (Table 1). Compound **19b** exhibited the most potent inhibitory effect with an IC_{50} value of 0.24 μM compared with the positive control, LY294002 (IC_{50} = 0.38 μM). The structure–activity relationships (SAR) were also studied. It is notable that the 3β-substituted steroid derivatives exhibited a better anti-invasion activities than the 3α-substituted derivatives. Thus, compound **19b** is 15-fold more potent than that of **20b**, while compound **19e** is 10-fold more potent than **20e**. On the other hand, the C-16 carbonyl derivatives had better activities than other two types of derivatives, including compounds **7a–g**, **8a–g**, **13a–g**, and **14a–g**. However, an accurate SAR could not be established due to the insufficient number of compounds. Compound **19b** was also evaluated in wound healing assay (Fig. 3) where it was more potent (IC_{50} = 0.44 μM) than the positive control, LY294002 (IC_{50} = 1.25 μM).

Other natural steroids and synthetic steroid derivatives also inhibit invasion or migration in tumor cells. For example, α-Chaconine isolated from *Solanum tuberosum* inhibited metastatic A549 cell invasion/migration in wound healing and Boyden chamber assays and had antiproliferative and proapoptotic effects on the growth of cancer cells originating from human skin, liver, prostate, breast, and colon [Shih et al., 2007]. Another steroid derivative, PG545, was evaluated in a phase I clinical trial in cancer patients with more potent antimetastatic activities in both HUVECs (human umbilical vein endothelial cells) and *in vivo* models [Ferro et al., 2012]. Dehydroepiandrosterone (DHEA), the most abundant adrenal steroid in humans resulted in an inhibition of around 30% in cell migration in MDA-MB-231 cells at a concentration of 100 μM at 24 h [Lopez-Marure et al., 2011]. At the same concentration, it decreased the number of cells migrating into the wound area in MDA-MB-231 cells.

In the present study, compound **19b**, a steroid derived from *E*-salignone, was effective at lower concentrations than DHEA, *E*-salignone, and LY294002 in transwell and wound-healing assays in MDA-MB-231 cells. These results suggest that *E*-salignone amide derivatives, like compound **19b**, may be promising lead

candidates for the development of new antimetastasis agents for the treatment of human breast cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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