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New 11β-aryl-substituted steroids exhibit both progestational and antiprogestational activity

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The syntheses of three 11β-aryl-19-norpregna-4,9-dien-3-one derivatives with 17-spirolactone and 17β-hydroxy-17α-cyanoethyl substitutions are described. The progesterone agonist/antagonist activities of the new compounds are investigated using a recently developed tissue culture system that relies on the progesterone agonist up-regulation of the prostate-specific antigen (PSA) gene in female breast tumor cell lines. Two of the newly synthesized compounds exhibit mixed agonistic/antagonistic progestational activity. (*Steroids* **63**:523–530, 1998) © *1998 by Elsevier Science Inc.*

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Introduction

The discovery of the first competitive progesterone antagonist mifepristone (Figure 1, RU-486, 1) has initiated an intense search for more potent and more selective antiprogestins.[1,2] Various attempts to dissociate the antiprogestin from the antiglucocorticoid activity of mifepristone and other analogs have not been very successful, and the design and synthesis of new compounds having antiprogestational activity devoid of antiglucocorticoid are highly desirable, both in terms of clinical applications and basic endocrine research.[3,4]

Steroid receptors are closely related structurally and in their mechanism of action. Slight modifications of the steroidal structure have been found to induce important affinity and specificity variations for the corresponding receptors. The most prominent structural feature of mifepristone is the 4-(dimethylamino)phenyl group in the 11β-position of a 19-nor steroid. Without this substituent, the molecule would be expected to act as a progestin. Replacement of the 4-(dimethylamino)phenyl group with a 4-acetylphenyl (ZK112993, 2)[5] leads to equal or more potent antiprogestins, and minor changes at the C-17 position dramatically produces antiprogestins with reduced antiglucocorticoid activity.[4,6] For example, CDB-2914 (compound **3**) with a 17α-acetoxy-17β-acetyl group exhibits activity similar to mifepristone.[7] In view of the significance of C-17 in reducing antiglucocorticoid activity, introduction of other substituents at C-17 position of 11β-aryl substituted 19-norsteroids may be useful in producing an antiprogestin with reduced antiglucocorticoid activity. Modifications incorporating hydrophobic 17α-substituents such as 17α-ethyl (compound **4**), and 17α-(1′-pentynyl, compound **5**) have given rise to in vivo antiprogestational activity superior to that of mifepristone.[2] Philibert et al.[8] have demonstrated that a spirotetrahydrofuran at C-17 (compounds **6** and **7**) causes marked increase in the ratio of antiprogestational/antiglucocorticoid activity. The corresponding 17-spirolactone with a 6β-methyl substituent was synthesized by van den Heuvel and Groen[9] and exhibited an improved dissociation between progesterone receptor binding and glucocorticoid receptor binding relative to mifepristone. However, due to a decreased progesterone receptor binding relative to mifepristone, this compound was not tested for antiprogestational activity. We felt it was desirable to synthesize 17-spirolactone derivatives without any substituents at the C-6 position and to evaluate their antiprogestational activity. Conveniently, the synthetic scheme chosen for these compounds (Figure 2) allowed a facile conversion of intermediate **14b** to the 17β-hydroxy-17α-cyanoethyl derivative **16**. This compound is structurally similar to a 17β-hydroxy-17α-cyanomethyl antiprogestational derivative synthesized by Cook et al.[7] In the present communication, we describe the synthesis and biologic data of these derivatives.

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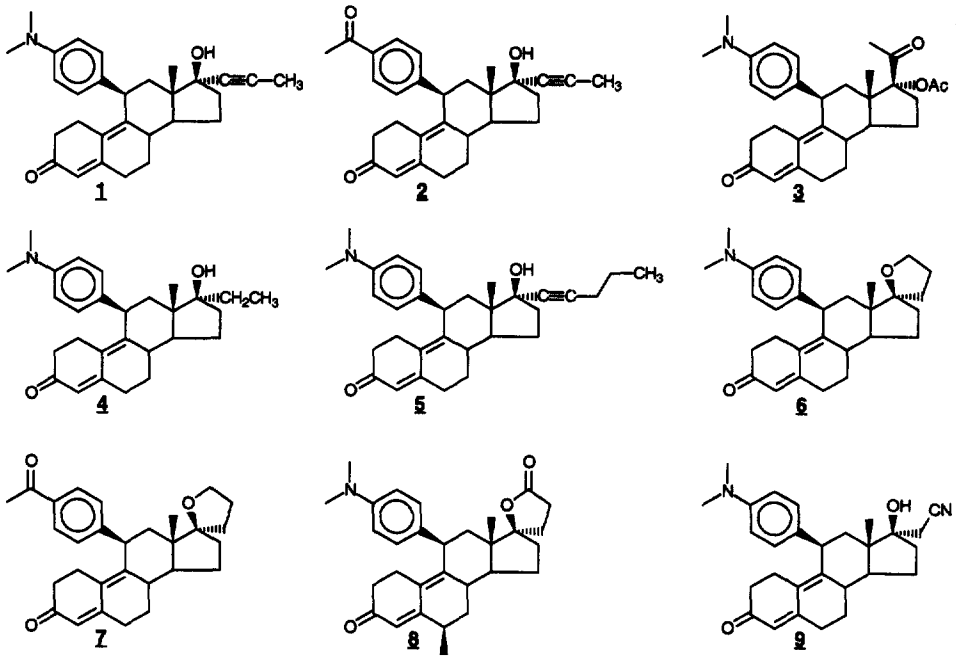


Figure 1. Antiprogestins.

Experimental

Chemistry

Melting points were determined on a Thomas–Hoover apparatus and are uncorrected. ^{1}H NMR were determined in deuterochloroform either at 300 MHz using a General Electric QE-300 spectrometer or at 90 MHz using a Varian EM-390 spectrometer. Infrared spectra were recorded on a Perkin–Elmer model 1600 FTIR instrument equipped with a diffuse reflectance accessory using a KBr matrix. Mass

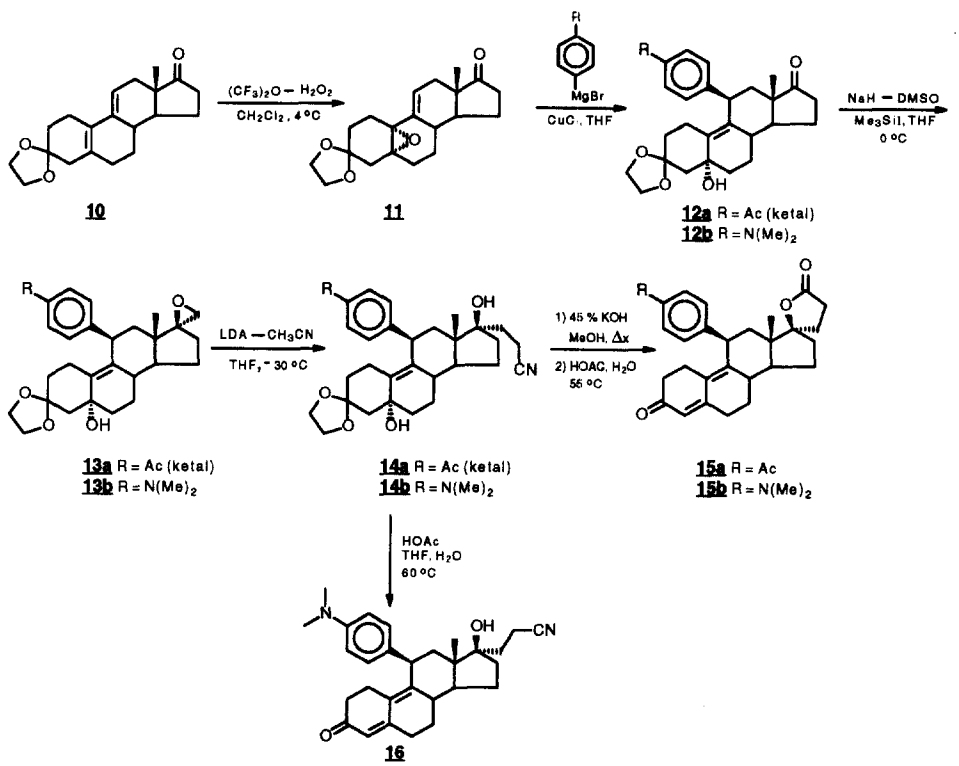


Figure 2. Synthesis of 11β-Aryl steroids.

spectral analyses (EI) were conducted by Dr. Susan Weintraub of the University of Texas Health Science Center at San Antonio using a Finnigan-MAT model 4615 mass spectrometer. Combustion analyses were performed by Midwest MicroLabs Ltd., Indianapolis Indiana. "Flash column" chromatography was performed on 32–64 μM silica gel obtained from Scientific Absorbents Inc., Atlanta Georgia. TLC Analyses were carried out on silica gel GF (Analtech) glass plates (2.5 × 10 cm with 250 μM layer and prescored).

Most chemicals and solvents were analytical grade and used without further purification. 4-Bromoacetophenone ethylene ketal was prepared according to the procedure of Detty et al.[10]

3,3-Ethylenedioxy-5α,10α-epoxyestr-9,11-en-17-one (**11**)

Hydrogen peroxide (30%, 21.3 mL, 208 mmol) was added to a mixture of hexafluoroacetone trihydrate (17 mL, 121.93 mmol) in CH_2Cl_2 (320 mL) cooled to 0°C in an ice bath. Solid Na_2HPO_4 (17.2 g, 121 mmol) was added and the mixture stirred mechanically at 0°C for 20 min. A solution of the 3-ketal **10** (31.84 g, 101.3 mmol) in methylene chloride (320 mL) was then added dropwise over a period of 15 min. The reaction mixture was then stirred at 0–4°C for 18 h. The reaction was extracted with CH_2Cl_2 (3 times), and the organic fractions were washed successively with 10% Na_2SO_3 solution (2 times), saturated sodium bicarbonate solution (2 times), and brine (1 time). The organic layers were combined, dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo. Crystallization of the residue from diisopropyl ether (2 times) afforded the pure 5α,10α-epoxide **11** (20 g, 59.76%). m.p. = 153–155°C (158°C[11]). ^{1}H NMR (300 MHz) δ 0.810 (s, 18-CH_3), 3.8–3.9 (m, -OCH_2CH_2O-), 5.972–6.005 (m, 11α CH). IR (cm^{-1}) 2938, 2876, 2842, and 1736.

3,3-Ethylenedioxy-5α-hydroxy-11β-[4-(2-methyl-1,3-dioxolan-2-yl)phenyl]-estr-9-en-17-one (**12a**)[12]

Under anhydrous conditions, magnesium turnings (3.2 g, 132.4 mmol) were weighed into a 1-L round bottom four-neck flask equipped with a stirring shaft, thermometer, addition funnel, and a reflux condenser. The system was flushed with dry argon, and a few crystals of iodine and 2 drops of 1,2-dibromoethane were added. The contents of the flask were then agitated for a few minutes to activate the magnesium surface. Dry THF (100 mL) was added followed by a slow (30 min.) dropwise addition of a solution of 4-bromoacetophenone ethylene ketal (32.2 g, 132.4 mmol)[10] in dry THF (100 mL). Most of the magnesium had reacted after stirring 1.5 h at room temperature. Solid copper (I) chloride (1.3 g, 13.2 mmol) was added, and the reaction mixture was stirred an additional 30 min at room temperature. The reaction was then cooled to −30°C, and a THF (100 mL) solution of the α-epoxide (**11**, 7.0 g, 21.18 mmol) was added dropwise. The reaction was then stirred at −30 ~ −15°C for 3 h. Ammonium chloride solution (33 g in 150 mL water) was added dropwise to quench the reaction. To oxidize Cu (I) to Cu (II), air was drawn through the reaction mixture for 30 min via a 6-inch needle inserted through a rubber septum and a slight vacuum applied to the top of the condenser. The reaction was extracted with ethyl acetate (3 times). The organic fractions were washed with 10% NH_4Cl solution (2 times) and brine (1 time), combined, dried over Na_2SO_4, filtered, and concentrated in vacuo. Flash chromatography (hexanes/ethyl acetate 2:1) of the residue followed by trituration with ether/hexanes gave the pure adduct (**12a**, 8.17 g, 85.37%). m.p. = 190–191°C. ^{1}H NMR (300 MHz) δ 0.466 (s, 18-CH_3), 1.637 (s, CH_3 of 11β-substituent), 3.742–4.061 (m, -OCH_2CH_2O-), 4.310 (d, J = 4.8 Hz, 11αCH), 7.189 (d, J = 8.4 Hz, aromatic 2′-H and 6′-H), 7.350 (d, J = 8.4 Hz, aromatic 3′-H and 5′-H) ppm. IR (cm^{-1}) 3520, 2943, 1739, 1609, 1506. MS (m/z) M^+ = 494. Analysis calculated for $C_{30}H_{38}O_6$: C, 72.85; H, 7.74. Found: C, 73.03; H, 7.87.

3,3-Ethylenedioxy-5α-hydroxy-11β-[4-(N,N-dimethylamino)phenyl]-estr-9-en-17-one (**12b**)

Following the same procedure used in the preparation of **12a**, a solution of 4-bromo-*N,N*-dimethylaniline (26.5 g, 132.4 mmol) in dry THF (100 mL) was added to activated magnesium (3.2 g, 132.4 mmol) in dry THF (100 mL). After the reaction had initiated, the mixture was stirred at ambient temperature for 1.5 h. Solid copper (I) chloride (1.3 g, 13.2 mmol) was added and the mixture stirred at room temperature for an additional 30 min. The reaction was then cooled to −30°C and a THF (100 mL) solution of the α-epoxide (**11**, 7.0 g, 21.18 mmol) was added dropwise. The reaction was then stirred at −30 ~ −20°C for 3 h. The reaction was quenched and worked up in an identical manner as carried out for the preparation of compound **12a**. Flash chromatography (hexanes/ethyl acetate 1:1) followed by trituration with ether gave the pure adduct **12b** (8.17 g, 85.37%) as a light green solid. A small portion of this material was recrystallized from ethyl acetate/petroleum ether for characterization. m.p. = 176–177°C (176°C). [13] ^{1}H NMR (300 MHz) δ 0.518 (s, 18-CH_3), 2.909 (s, NMe_2), 3.905–4.025 (m, -OCH_2CH_2O-), 4.245 (d, J = 6.9 Hz, 11α CH), 6.642 (d, J = 8.7 Hz, aromatic 3′-H and 5′-H), 7.065 (d, J = 8.7 Hz, aromatic 2′-H and 6′-H) ppm. IR (cm^{-1}) 3510, 2946, 1737, 1612, 1519. MS (m/z) M^+ = 451. Analysis calculated for $C_{28}H_{37}NO_4$: C, 74.47; H, 8.26; N, 3.10. Found: C, 74.67; H, 8.24; N, 3.23.

3,3-Ethylenedioxy-11β-[4-(2-methyl-1,3-dioxolan-2-yl)phenyl]spiro[estr-9-en-17β,2′-oxirane] **13a**

Under nitrogen, 60% sodium hydride (0.45 g, 11.3 mmol) in mineral oil was weighed into a 100 mL three neck round bottom flask equipped with a magnetic stir bar, a reflux condenser, glass stopper, and a rubber septum. The sodium hydride suspension was washed free of mineral oil using petroleum ether (3 times). Dry DMSO (21 mL, 296 mmol) was introduced via syringe and the mixture was stirred and heated at 70°C for 45 min. The reaction was cooled to room temperature, diluted with dry THF (35 mL), and further cooled to −5°C. A solution of trimethylsulfonium iodide (2.1 g, 10.35 mmol) in dry DMSO (15 mL) was added and the reaction stirred at −5 ~ 0°C for 35 min. A solution of

the Grignard adduct **12a** (1.2 g, 2.43 mmol) in dry THF (12 mL) was introduced and the reaction mixture stirred at 0°C for 3 h. The reaction was poured into cold water and extracted with CH_2Cl_2 (3 times). The organic fractions were washed with brine (2 times), combined, dried over Na_2SO_4, filtered and concentrated in vacuo. Crystallization of the residue from ether gave the pure 17-oxirane **13a** (1.0 g, 81.3%) as a white solid. m.p. = 126–128°C. ^{1}H NMR (90 MHz) δ 0.47 (s, 18- CH_3), 1.62 (s, CH_3 of 11β-substituent), 2.59 and 2.94 (both d, J = 6.3 Hz, 17α-CH_2), 3.73–4.13 (m, -OCH_2CH_2O-), 4.2–4.37 (m, 11α H plus OH), 7.15 (d, J = 9 Hz, aromatic 2′-H and 6′-H), 7.35 (d, J = 9 Hz, aromatic 3′-H and 5′-H) ppm. IR (cm^{-1}) 3533, 2928, 1679, 1602, 1505. MS (m/z) M^+ = 508. Analysis calculated for $C_{31}H_{40}O_6$: C, 73.20; H, 7.93. Found: C, 73.38; H, 7.88.

*3,3-Ethylenedioxy-11β-[4-(*N,N-*dimethylamino)phenyl]spiro[estr-9-en-17β,2′-oxirane]* **13b**

Following the same procedure used in the preparation of **13a**, Grignard adduct **12b** (4.0 g, 8.86 mmol) in THF (50 mL) was reacted with a mixture prepared from 60% sodium hydride suspension (1.64 g, 41.02 mmol) in DMSO (50 mL) and THF (120 mL) and trimethylsulfonium iodide (7.65 g, 37.74 mmol) in DMSO (50 mL). The reaction mixture was stirred at 0°C for 3 h and worked up in an identical fashion to provide after crystallization from ether the pure 17-oxirane **13b** (3.4 g, 82.4%). m.p. = 145–150°C (d). ^{1}H NMR (300 MHz) δ 0.522 (s, 18- CH_3), 2.598 and 2.939 (both d, J = 5.0 Hz, 17α-CH_2), 2.900 (s, NMe_2), 3.904–4.043 (m, -OCH_2CH_2O-), 4.192 (d, J = 6 Hz, 11α H), 6.626 (d, J = 9 Hz, aromatic 3′-H and 5′-H), 7.017 (d, J = 9 Hz, aromatic 2′-H and 6′-H) ppm. IR (cm^{-1}) 3511, 2940, 1612, 1518. MS (m/z) M^+ = 465.

Analysis calculated for $C_{29}H_{39}NO_4$: C, 74.80; H, 8.44; N, 3.01. Found: C, 74.52; H, 8.54; N, 3.01.

3,3-Ethylenedioxy-5α,17β-dihydroxy-11β-[4-(2-methyl-1,3-dioxolan-2-yl)phenyl]-19-nor- 17α-pregn-9-ene-21-carbonitrile **14a**

Under nitrogen, a 1.5-M cyclohexane solution of lithium diisopropylamide mono(tetrahydrofuran) (10 mL, 15 mmol) was added to dry THF (40 mL), and the mixture was cooled to −30°C. Dry acetonitrile (1.0 mL, 16 mmol) was added, and the mixture was stirred at −30°C for 30 min. A solution of the 17-oxirane **4a** (1.36 g, 2.67 mmol) in dry THF (15 mL) was added dropwise over a period of 10 min and the reaction was then allowed to warm to ambient temperature and stirred for 3 h. The reaction mixture was then poured into saturated ammonium chloride solution and extracted with ethyl acetate (3 times). The organic fractions were washed with brine (once), combined, dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified via flash chromatography (hexanes/ethyl acetate 1:1) followed by crystallization from ethyl acetate/hexanes to give the 21-carbonitrile **14a** (0.8 g, 54.4%) as a white solid. m.p. = 191–193°C. ^{1}H NMR (300 MHz) δ 0.451 (s, 18- CH_3), 1.641 (s, CH_3 of 11β-substituent), 3.7–4.1 (m, -OCH_2CH_2O-), 4.328 (d, J = 7.5 Hz, 11α H), 7.170 (d, J = 8.1 Hz, aromatic 2′-H and 6′-H), 7.350 (d, J = 8.1 Hz, aromatic 3′-H and 5′-H) ppm. IR (cm^{-1}) 3470, 2940, 2242, 1609, 1505. MS (m/z) M^+ = 549. Analysis calculated for $C_{33}H_{43}NO_6 \cdot 4/5\ H_2O$: C, 70.26; H, 7.97; N, 2.48. Found: C, 70.08; H, 7.67; N, 2.43.

*3,3-Ethylenedioxy-5α,17β-dihydroxy-11β-[4-(*N,N-*dimethylamino)phenyl]-19-nor-17α-pregn-9-ene-21-carbonitrile* **14b**

Following the same procedure used in the preparation of **14a**, a mixture of 1.5 M cyclohexane solution of lithium diisopropylamide mono(tetrahydrofuran) (20 mL, 30 mmol), THF (40 mL), and acetonitrile (2 mL, 30 mmol) was reacted with a solution of the 17-oxirane **13b** (2.0 g, 4.3 mmol) in THF (15 mL) at ambient temperature for 6 h. The reaction was quenched and worked up in an identical manner as carried out for the preparation of compound **14a**. Purification via flash chromatography (ethyl acetate/hexanes 1:1) gave the 21-carbonitrile compound **14b** (1.25 g, 57%) as a foam. ^{1}H NMR (300 MHz) δ 0.506 (s, 18- CH_3), 2.915 (s, NMe_2), 3.9–4.1 (m, -OCH_2CH_2O-), 4.255 (d, J = 6.9 Hz, 11α H), 6.655 (d, J = 9 Hz, aromatic 3′-H and 5′-H), 7.047 (d, J = 9 Hz, aromatic 2′-H and 6′-H) ppm. IR (cm^{-1}) 3506, 2944, 2245, 1612, 1517. MS (m/z) M^+ = 506. Analysis calculated for $C_{31}H_{42}N_2O_4 \cdot 1/4$ EtOAc: C, 72.69; H, 8.39; N, 5.30. Found: C, 72.93; H, 7.47; N, 5.37.

17β-hydroxy-11β-(4-acetylphenyl)-19-nor-17α-pregna-4,9-diene-21-carboxylic acid, γ-lactone **15a**

Under nitrogen, a mixture of the 21-carbonitrile **14a** (0.8 g, 1.46 mmol) 45% potassium hydroxide (2 mL, 16 mmol), and methanol (10 mL) was heated to reflux for 5 hours. The reaction was cooled to room temperature, and water (7 mL) and acetic acid (22 mL) were added. The reaction was then heated to 50°C for 5 h. The solvents were removed in vacuo and the residue extracted with CH_2Cl_2 (3 times). The organic fractions were washed with water (once) and brine (once), combined, dried over sodium sulfate, filtered, and concentrated in vacuo. Crystallization of the residue from ethyl acetate gave the 21,17-carbolactone **15a** (0.54 g, 83.5%) as a yellow solid. m.p. = 251–252°C. ^{1}H NMR (300 MHz) δ 0.585 (s, 18-CH_3), 2.587 (s, 4′-acetyl), 4.488 (d, J = 7.2 Hz, 11α H), 5.810 (s, 4-CH), 7.268 (d, J = 8.1 Hz, aromatic 2′-H and 6′-H), 7.894 (d, J = 8.1 Hz, aromatic 3′-H and 5′-H) ppm. IR (cm^{-1}) 2945, 1771, 1671, 1654, 1601. MS (m/z) M^+ = 444. Analysis calculated for $C_{29}H_{32}O_4 \cdot 1/4$ EtOAc: C, 77.23; H, 7.34. Found: C, 77.19; H, 7.32. Analysis by HPLC (CH_3CN/H_2O, 1:1; Waters Associates NovaPak C_{18}; 1 mL/min; λ = 260 nm) indicated the product to be > 99% pure with a retention time of 3.74 min.

*17β-Hydroxy-11β-[4-(*N,N-*dimethylamino)phenyl]-19-nor-17α-pregna-4,9-diene-21-carboxylic acid, γ-lactone* **15b**

Following the same procedure used in the preparation of **15a**, the 21-carbonitrile **14b** (0.6 g, 1.18 mmol) was reacted with 45% potassium hydroxide (2 mL, 16 mmol) in meth-

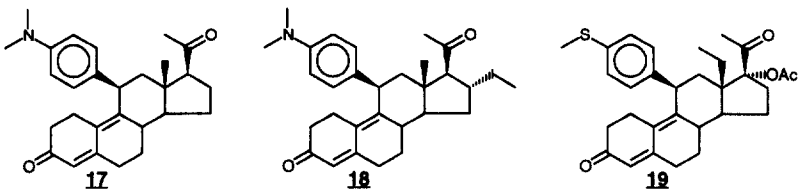


Figure 3. Previously synthesized 11β-aryl-substituted steroids with agonist or mixed agonist/antagonist progestational activity.

anol (10 mL) at reflux for 4.5 h followed by the addition of acetic acid (22 mL) and water (7 mL) and reaction at 60°C for 5 h. The reaction was worked up in an identical manner as carried out for the preparation of compound **15a**. Purification via Flash chromatography (ethyl acetate/hexanes 3:2) gave the 21,17-carbonitrile compound **15b** (0.37 g, 70.2%) as a yellow foam.

^{1}H NMR (300 MHz) δ 0.645 (s, 18- CH_3), 2.921 (s, NMe_2), 4.367 (d, J = 5.7 Hz, 11α H), 5.773 (s, 4-CH), 6.659 (d, J = 9 Hz, aromatic 3′-H and 5′-H), 6.982 (d, J = 9 Hz, aromatic 2′-H and 6′-H) ppm. IR (cm^{-1}) 2945, 1775, 1660, 1612, 1518. MS (m/z) M^+ = 445. Analysis calculated for $C_{29}H_{35}NO_3$: C, 78.17; H, 7.92; N, 3.14. Found: C, 78.07; H, 7.81; N, 3.14. Analysis by HPLC (CH_3CN/H_2O, 1:1; Waters Associates NovaPak C_{18}; 2 mL/min; λ = 260 nm) indicated the product to be > 99% pure with a retention time of 7.1 min.

17β-Hydroxy-11β-[4-(N,N-dimethylamino)phenyl]-19-nor-17α-pregna-4,9-diene-21-carbonitrile **16**

Under nitrogen, a solution of the 21-carbonitrile **14b** (0.3 g, 0.59 mmol) in acetic acid (14 mL), THF (6 mL), and water (6 mL) was heated to 60°C for 5 h. The solvents were removed in vacuo and the residue extracted with CH_2Cl_2 (3 times). The organic fractions were washed with saturated sodium bicarbonate solution (2 times) and brine (two times), combined, dried over sodium sulfate, filtered, and concentrated in vacuo. Crystallization of the residue from ether gave product **16** (0.178 g, 67.6%) as a yellow solid. m.p. = 241–242°C. ^{1}H NMR (300 MHz) δ 0.580 (s, 18-CH_3), 2.916 (s, NMe_2), 4.365 (d, J = 6.6 Hz, 11 α H), 5.760 (s, 4-CH), 6.659 (d, J = 8.7 Hz, aromatic 3′-H and 5′-H), 7.002 (d, J = 8.7 Hz, aromatic 2′-H and 6′-H) ppm. IR (cm^{-1}) 3362, 2950, 2242, 1641, 1600, 1518. MS (m/z) M^+ = 444. Analysis calculated for $C_{29}H_{36}N_2O_2$: C, 78.34; H, 8.16; N, 6.30. Found: C, 78.32; H, 8.24; N, 6.31. Analysis by HPLC (CH_3CN/H_2O, 1:1; Waters Associates NovaPak C_{18}; 2 mL/min; λ = 260 nm) indicated the product to be 97.6% pure with a retention time of 2.75 min.

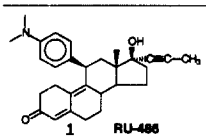
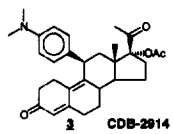
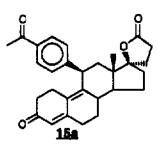
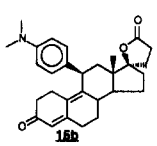
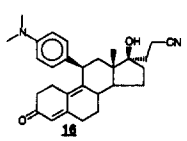
Assessment of biological activity

Growth and maintenance of cells

The breast cancer cell lines T47-D and BT-474 were obtained from the American Type Culture Collection (ATCC), Rockville, MD 20852. Both cell lines were cultured according to the instructions of the ATCC.

T47-D and BT-474 cells were initially grown in flasks at 37°C and 5% CO_2 in RPMI 1640 medium (Gibco BRL, Gaithersburg MD), supplemented with 10% fetal calf serum (FCS), 0.2 I.U. bovine insulin per mL, and 29 g/L glutamine. Cells were grown until confluency in the flask, then detached by trypsin-EDTA treatment to be split or subcultured in 48-well microtiter plates for experimentation. The subculture medium was the same as above except charcoal stripped FCS was used instead of the regular FCS, as it is devoid of any steroid hormones. Cell clumps were minimized by passing cells and media through an 18-gauge syringe. Cells were grown as monolayers until confluency,

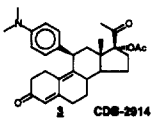
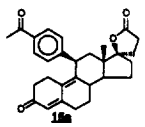
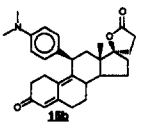
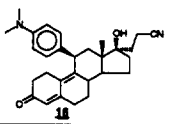
Table 1. Progestational/Androgenic Activity

Compound	BT-474 cells PSA, ng/L[a]	BT-474 cells % Agonist Activity[b]	T-47D cells PSA, ng/L[a]	T-47D cells % Agonist Activity[b]
 1 RU-486	7	0.8	45	8
 3 CDB-2914	5	0.6	58	9
 15a	<1	<0.1	0	0
 15b	336	40	373	54
 16	118	14	190	27

[a]The amount of PSA present in the tissue culture is proportional to the progestational/androgenic activity of these compounds. All experiments were done in duplicate and results were reproducible within ±10%.

[b]Comparison to norgestrel, a progestin, whose activity was arbitrarily set at 100%.

Table 2. Antiprogestational/Antiandorgenic Activity of Compounds, as Tested in a Tissue Culture System

Compound	Concentration M[a]	% Blocking of Norgestrel Activity[b,c]	% Blocking of Norgestimate Activity[b,c]	% Blocking of Dihydrotestosterone Activity[b,d]
	$10^{-8}/10^{-7}$	96	100	86
	$10^{-10}/10^{-9}$	100	–	80
	$10^{-8}/10^{-7}$	40	0	0
	$10^{-10}/10^{-9}$	27	–	0
	$10^{-8}/10^{-7}$	100	100	91
	$10^{-10}/10^{-9}$	93	–	64
	$10^{-8}/10^{-7}$	84	100	87
	$10^{-10}/10^{-9}$	50	–	54

[a]Denotes concentration of stimulant/blocker under the conditions of the experiments.
[b]All experiments were repeated twice and results were reproducible within ±10%.
[c]Indicates antiprogestational activity. For details, see "Methods."
[d]Indicates antiandrogen activity. For details, see "Methods."

with approximately 2×10^5 cells per well and each well contained 1 mL of medium.

Agonistic activity

BT-474 and T47-D breast cancer cells were grown to confluency in 48-well and 24-well plates, respectively, under the conditions described, and stimulated with compounds CDB-2914, **15a**, **15b**, or **16** at final concentration of 10^{-7} and 10^{-9} M. All compounds were diluted with pure anhydrous ethyl alcohol. Positive controls were norgestrel (a progestin), testosterone (an androgen) and norgestimate (a progestin devoid of androgenic activity) (Sigma Chemical Co., St. Louis, MO 63178). Alcohol was the negative control. Mifepristone (Roussel-UCLAF, Romainville, France) was used as a known antiprogestin with weak progestational activity[13] Tissue culture supernatants were collected after 7 days (see below).

Antagonistic activity

T47-D cells were grown to confluency under standard conditions in 48-well microtiter plates. The cells were incubated in 1 mL media with the candidate antagonists CDB-2914, **15a**, **15b**, or **16** for 1 h at final concentrations of 10^{-7} or 10^{-9} M. Norgestrel or dihydrotestosterone were then added at final concentrations of 10^{-8} or 10^{-10} M, or norgestimate at 10^{-8} M. Controls did not contain the antagonist (only stimulating steroid) or the stimulating steroid (only antagonist). Alcohol was the negative control. Incubation time was 7 days.

Harvesting of supernatant

Supernatant was separated from the cells through aspiration, using plastic pipettes. Supernatant was immediately transferred into labeled Eppendorf tubes, and either immediately used for PSA assay or stored at −20°C until analysis.

PSA assay

PSA protein was measured with a highly sensitive immunofluorometric procedure that can measure PSA at levels of 1 ng/L or higher (up to 10,000 ng/L) with a precision of <10%. This assay uses a mouse anti-PSA capture antibody coated to polystyrene microtiter wells, a biotinylated mouse monoclonal detection antibody, and alkaline phosphatase-labeled streptavidin. The details and evaluation of this assay are described elsewhere.[14]

Calculations

The agonistic activity of the various steroids tested was expressed both as ng/L of PSA in the tissue culture supernatant at 7 days stimulation as well as a percentage of norgestrel's agonistic activity. The antagonistic activity was expressed as a percentage of blocking of either norgestrel (antiprogestational/antiandrogenic), norgestimate (antiprogestational) or dihydrotestosterone (antiandrogenic) activity. For this, we evaluated the PSA concentration at 7 days in the tissue culture supernatant without any blocker (0% blocking) or in the presence of the blocker (blocking from 0–100%).

Results and discussion

Chemistry

The syntheses of the key 17-oxirane intermediates **4a-b** (Figure 2) was carried out following the general strategy of Cook et al..[7] The Regioselective 5,10-epoxidation of 3,3-ethylenedioxyestra-5(10),9(11)-dien-17-one[16] **1** was achieved using hexafluoroacetone hydroperoxide generated in situ from hexafluoroacetone trihydrate and hydrogen peroxide following the general procedure of Teutsch et al.[17] From the resulting epoxide mixture, the pure 5α,10α-isomer **11** could be isolated in 60% yield. The copper (I) catalyzed addition of the Grignard reagent prepared from the appropriate aryl bromide (4-bromodimethylaniline and 4-bromoacetophenone ketal[10]) gave the corresponding Grignard adducts **12a-b** in 85% yield. Reaction of **12a-b** with dimethylsulphonium methylide gave the 17-oxirane intermediates **13a-b** in 81–82% yield. Condensation of the 17-oxirane intermediates **13a-b** with in situ generated acetonitrile anion according to Faraj et al.[18] afforded the 17α-cyanoethyl-17β-hydroxy intermediates **14a-b** in 54–57% yields. A one step alkaline hydrolysis-acid treatment of compounds **14a-b** then gave the desired 17,21-carbolactone products **15a-b** in 70–84% yields. Acid hydrolysis of intermediate **14b** gave the 17α-cyanoethyl derivative **16**.

Biology

We have previously found that the prostate specific antigen (PSA) gene can be regulated by steroid hormones in the breast carcinoma cell lines T47-D and BT-474.[13] This effect is mediated by the steroid hormone receptor system. When a steroid is added to the tissue culture supernatant, it binds to its cognate receptor and then it regulates a number of genes, one of which is PSA. PSA is a secreted protein and can be conveniently quantified in the tissue culture supernatant after 7 days of stimulation. We have previously found that progestins and androgens, and to a lesser extent glucocorticoids and mineralocorticoids, upregulate this gene. For assessing the antagonistic activity of steroids, we use the same system but the candidate antagonist is added first, followed by the agonist. The % blocking (inhibition) can then be calculated. With this system, we have evaluated previously a number of agonists and antagonists and have shown that the system works well.

In Table 1 we present the structures of compounds RU-486 (control), CDB-2914, **15a**, **15b**, and **16** along with their agonistic activity with T47-D and BT-474 cells. At 10^{-7} M concentration, the most potent agonistic activity (progestin and/or androgen and/or glucocorticoid and/or mineralocorticoid) is associated with compound **15b**, followed by compound **16**. Significantly less agonistic activity is associated with compound CDB-2914, while compound **15a** has very little or no agonistic activity. The ability of these compounds to block the activity of norgestrel, norgestimate and dihydrotestosterone is also shown in Table 1. Compound CDB-2914 blocks effectively the activity of all three agonists, indicating that it is a potent antiprogestational/antiandrogenic agent. The same applies to compounds **15b** and **16**. Compound **15a** has weak or no antiprogestational or antiandrogenic activity.

The evidence presented here indicates the importance of D-ring substituents in determining the balance of agonist/antagonist activity. This is consistent with the findings of Cook et al.[19] that a derivative of CDB-2914 lacking the 17α-acetoxy group was found to have mixed agonist/antagonist progestational activity and its 16α-ethyl-derivative was found to be a pure agonist. A similar reversal of antiprogestational activity has been observed upon 18-methylation of an 11β-[4-(methylthio)phenyl]-derivative of CDB-2914.[20] At the present time, the data are insufficient for a definitive structure activity relationship with regard to agonist/antagonist activity, but it is evident that modifications of or near C-17 substituents play a key role in determining agonist/antagonist progestational activity of these compounds.

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