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Synthesis and antiprogestational properties of novel 17-fluorinated steroids

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ABSTRACT

Progesterone receptor (PR) plays a key role in reproductive functions, and compounds that inhibit progesterone action (antiprogestins) have potential use in the treatment of estrogen- and progesterone-dependent diseases, including uterine leiomyomas and breast cancer. In the present study, we chemically synthesized novel 17-fluorinated steroids and evaluated the cytotoxicity profiles of these compounds in T47D breast cancer cells compared to the activity of known antiprogestins, including ZK230 211, RU-486, CDB2914, CDB4124 and ORG33628. We analyzed *in vitro* receptor-binding assays and PR-transactivation assays to establish the antiprogestational activity of these molecules. The representative antiprogestin EC304 was found to inhibit *in vitro* tumorigenicity in a dose-dependent fashion in T47D cells by a colony formation assay at 1 and 10 nM concentrations. The potent *in vivo* antiprogestational activity of EC304 was also demonstrated in an antinidation assay for the interruption of early pregnancy in rats. The strong antiprogestational activity and absence of antiglucocorticoid activity in EC compounds may demonstrate their utility in the treatment of leiomyoma, endometriosis and breast cancer.

1. Introduction

Progesterone receptor (PR) is a ligand-dependent transcription factor that plays a crucial role in the maintenance and development of the female reproductive system [1,2]. The biological activity of progesterone is mediated by a cascade of events induced upon binding of progesterone with its receptor in the classical pathway of PR activation. Thus, antiprogestins have considerable potential as therapeutics for the treatment of several gynecological disorders and breast cancer [1]. Independent of inhibiting endogenous progesterone levels, antiprogestins have been shown to inhibit estrogen-dependent proliferation in the uterus and in the mammary gland [3,4]. The mouse mammary gland has served as an important *in vivo* surrogate in modeling normal human breast development and breast cancer. Mouse models have also underscored the critical role of PR in mammary tumorigenesis, with PR knockout mice exhibiting reduced susceptibility to chemically induced mammary tumors [5]. The progestin medroxyprogesterone acetate (MPA) can induce ER/PR-positive mammary adenocarcinomas in female BALB/c mice [6], and spontaneous mammary tumors that develop in p53-null mammary gland transplants are highly dependent on P_4 [7].

The majority of breast tumors are of the luminal epithelial phenotype, and over 75% of tumors express ER/PR, suggesting that luminal mammary epithelial cells expressing ER/PR are major targets for breast tumorigenesis [8,9]. An increased PR-A/PR-B ratio was found to be associated with high-grade breast tumors, poor clinical outcome and resistance to endocrine therapy [10]. Transgenic mice that over-express PR-A exhibit excessive ductal side branching and disruption of the normal interactions between the epithelial cells and basement membrane [11]. These data collectively indicate that PR is potentially a valuable therapeutic target in breast cancer. Preliminary clinical trials have been performed with first-generation PR antagonists (mifepristone and onapristone), with promising responses obtained with either these molecules alone or in association with other agents [12–14]. However, the anti-glucocorticoid (anti-GR) activity of mifepristone and the *in vivo* hepatic toxicity of onapristone limit their long-term use [15].

A new finding that a subgroup of breast cancer patients might especially benefit from a antiprogestin treatment has increased the interest in antiprogestins for breast cancer again.

Women with mutations in the breast cancer susceptibility gene BRCA1 are predisposed to breast and ovarian cancers. Treatment of

Abbreviations: PR, progesterone receptor; GR, glucocorticoid receptor; MEM, minimal essential medium.

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Brca1/p53-deficient mice with the progesterone antagonist mifepristone (RU-486) prevented mammary tumorigenesis. These findings reveal a tissue-specific function for the BRCA1 protein and raise the possibility that antiprogesterone treatment may be useful for breast cancer prevention in individuals with BRCA1 mutations [16].

The field of progesterone receptor antagonists (antiprogestins) has progressed in two directions since the first compound of this class, mifepristone (RU-486), was discovered by Teutsch et al [17]. Full receptor antagonists have been investigated for applications in post-coital contraception, abortion and breast cancer, whereas mixed agonist/antagonists have been considered for gynecological indications such as endometriosis and uterine fibroids [18].

Over the years, knowledge about the structure/activity relationship of antiprogestins has grown considerably [19,20]. Mifepristone's use is limited by its strong antiglucocorticoid activity.

Replacement of the 17-propynyl group in mifepristone with 3-hydroxypropyl [21], 17-acetoxy [22] or 17,17spiro ether [23] gave rise to potent antiprogestins with significantly reduced antiglucocorticoid activity, as shown in Scheme 1.

Recently, 17-perfluoralkyl derivatives have been reported that exhibit very strong antiprogestational activity and minimal antiglucocorticoid activity. The compound ZK230 211 was selected from this class of compounds based on its extraordinary potency and its highest receptor selectivity observed to date [24].

All these compounds exhibit strong antiprogestational activity with only marginal agonistic activity. Asoprisnil, however, is the first representative of a new class of antiprogestins with a dual antagonistic/agonistic profile [25].

In an earlier study [26] the authors have reported the synthesis of steroidal antiprogestins with partial agonistic activity for potential application in the treatment of fibroids and endometriosis.

In this study the synthesis of pure antiprogestins is described. The synthesized compounds are evaluated for anti-progestational activity *in vitro* and *in vivo* andcytotoxic activity inhuman breast cancer T47D cells.

2. Experimental

2.1. General

Nuclear magnetic resonance spectra were recorded on a Bruker ARX (300 MHz) spectrometer as solutions in deuterated chloroform ($CDCl_3$) using tetramethylsilane (TMS) as an internal standard ($\delta = 0$), unless noted otherwise. 'Flash column' chromatography was performed on 32-64 μM silica gel obtained from EM Science, Gibbstown, New Jersey. Thin-layer chromatography (TLC) analyses were carried out on prescored silica gel GF (Analtech) glass plates (2.5 cm × 10 cm with 250 μM silica layer). Chemicals and solvents were analytical grade and used without further purification, unless otherwise noted. Most chemicals and solvents were analytical grade and used without further purification. Commercial reagents were purchased from Aldrich Chemical Company (Milwaukee, WI).

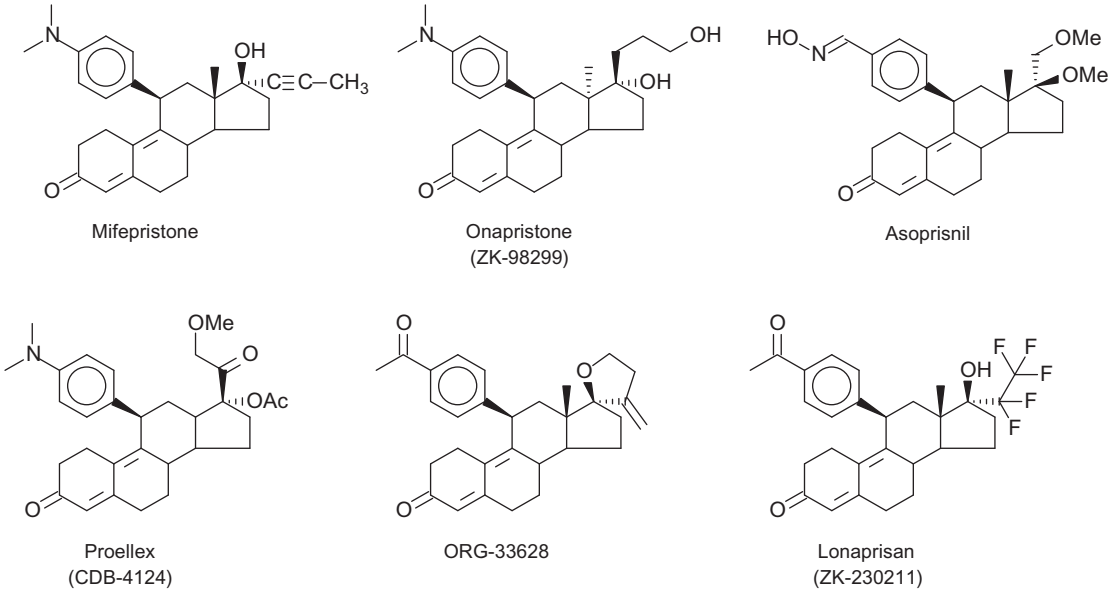
2.2. Chemical synthesis

2.2.1. 3,3-Ethylenedioxy-5α,17β-dihydroxy-17-(3,3,3-trifluoro-1-propynyl)-11′-{4′-[1′,1′-(ethylenedioxy)-ethyl]phenyl}-estr-9-ene (**3**)

n-Butyllithium (55 mL, 2.5 N, 137.5 mmol) was added to a solution of diisopropylamine (21.6 mL, 154 mmol) in THF (40 mL) at −78 °C under argon over 10 min, and the resulting mixture was stirred for 30 min. This LDA solution was added slowly over 20 min to a solution of 2-bromo-3,3,3-trifluoropropene (12 g, 68.5 mmol) in THF (80 mL), precooled to −78 °C. The mixture was stirred for 15 min, after which a solution of 3,3-ethylenedioxy-5α-hydroxy-11β-{4′-[1′,1′-(ethylenedioxy)-ethyl]-phenyl}-estra-9-ene-17-one (**2**) (6 g, 12.1 mmol) in THF (80 mL) was introduced over 15 min, stirred at −78 °C for an additional 1 h, and slowly warmed to room temperature over 15 h The reaction mixture was quenched with aqueous ammonium chloride (50 mL) and extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with water and brine, dried over sodium sulfate and evaporated in vacuo to afford the crude product, which was purified on a silica gel using 25% ethyl acetate in hexane to afford **3** (5.0 g, 70%).

^{1}H NMR (δ, 300 MHz) 0.45 (s, 3H), 1.63 (s, 3H), 1.1–2.5 (m, 19H), 3.7–4.1 (m, 8H), 4.34 (d, J = 6.3 Hz, 1H), 4.44 (s, 1H), 7.17 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.2 Hz).

^{13}C NMR (75 MHz) 13.5, 23.4, 23.9, 24.1, 27.5, 35.1, 38.3, 38.7, 39.21, 39.25, 47.37, 47.49, 50.1, 59.54, 64.15, 64.53, 64.6, 64.76, 70.1, 74.1 (q, J = 52 Hz) 80.0 (d, J = 1.1 Hz), 90.5 (q, J = 6.5 Hz), 108.7, 108.9, 114 (q, J = 255 Hz), 125.2, 127.0, 133.2, 135.1, 140.6, 146.2



Scheme 1.

*2.2.2. 11β-(4′-Acetyl-phenyl)-17β-hydroxy-17-(3,3,3-trifluoro-1-propynyl)-estra-4,9-diene-3-one (**1a,** EC301)*

Fifty percent sulfuric acid (2.2 mL) was added to a solution of 3,3-ethylenedioxy-5α,17 β -dihydroxy-17-(3,3,3-trifluoro-1-propynyl)-11β-{4′-[1′,1′-(ethylenedioxy)-ethyl]phenyl}-estr-9-ene (**3**) (3.5 g, 6 mmol) in methanol (35 mL) at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was carefully quenched with a solution of sodium bicarbonate (15 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic layer was washed with water and brine, dried over sodium sulfate and evaporated in vacuo to afford the crude product, which was purified on a silica gel using 25% ethyl acetate in hexane to afford **1a** (2.5 g, 87%).

^{1}H NMR (δ, 300 MHz) 0.52 (s, 3H), 1.3–2.9 (m, 17H), 2.58 (s, 3H), 4.0 (bs, 1H), 4.46 (d, *J* = 7.1 Hz, 1H), 5.81 (s, 1H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.89 (d, *J* = 8.4 Hz, 2H).

^{13}C NMR (75 MHz) 13.6, 23.4, 25.8, 26.4, 27.3, 31.0, 36.5, 38.3, 39.071, 39.169, 40.6, 47.5, 50.1, 73.5 (q, *J* = 52 Hz), 79.3 (d, *J* = 1 Hz), 90.8 (q, *J* = 6.3 Hz), 114.3 (q, *J* = 256 Hz), 123.3, 126.8, 127.2, 128.8, 130.3, 134.9, 144.0, 150.4, 156.5, 197.9, 199.6.

*2.2.3. 3,3-Ethylenedioxy-5α,17β-dihydroxy-17-(3,3,3-trifluoroprop-1(E)-enyl)-11′-{4′-[1′,1′-(ethylenedioxy)-ethyl]phenyl}-estr-9-ene (**4**)*

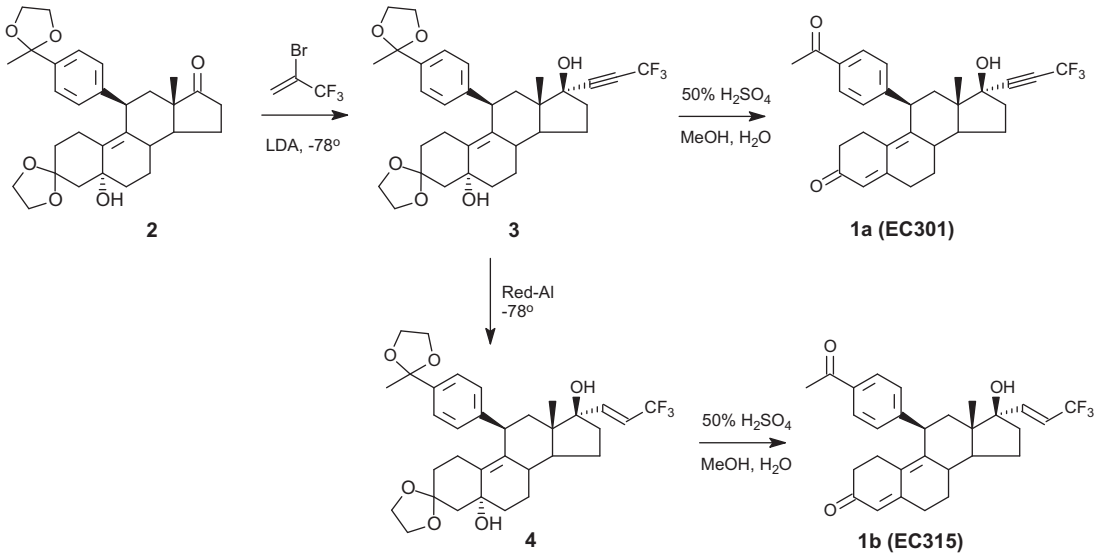
A ⩾ 65% wt. solution of sodium bis (2-methoxyethoxy) aluminum hydride in toluene (2.1 mL, 7.1 mmol) was added to a slurry of 3,3-ethylenedioxy-5α,17β-dihydroxy-17-(3,3,3-trifluoro-1-propynyl)-11β-{4′-[1′,1′-(ethylenedioxy)-ethyl]phenyl}-estr-9-ene (**3**) (1.2 g, 2 mmol) in ether (10 mL) and toluene (10 mL) at −78 °C. The resulting reaction mixture was stirred for 3 h at −78 °C, then warmed to room temperature over 1 h. The reaction mixture was quenched with a solution of saturated ammonium chloride (20 mL) and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined and washed with water and brine, dried over sodium sulfate and evaporated under vacuum to afford **7** (1.2 g crude product).

^{1}H NMR (δ, 300 MHz) 0.51 (s, 3H), 1.64 (s, 3H), 1.1–2.5 (m, 21H), 3.7–4.1 (m, 8H), 4.30 (d, *J* = 6 Hz, 1H), 4.45 (s, 1H), 5.80-6.00 (m, 1H), 6.54 (d, *J* = 15.5 Hz, 1H), 7.19 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.3 Hz, 2H).

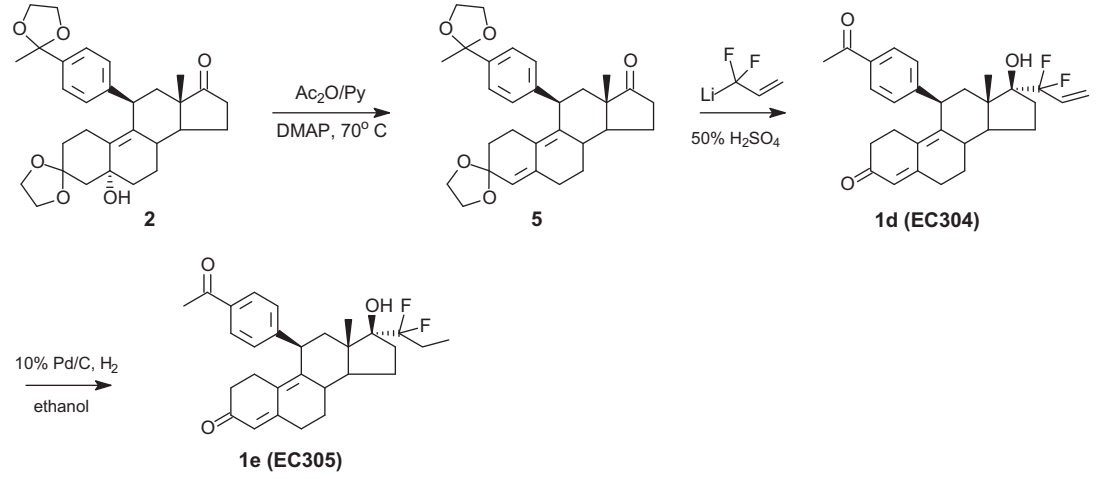
*2.2.4. 11β-(4′-Acetyl-phenyl)-17β-hydroxy-17-(3,3,3-trifluoroprop-1(E)-enyl)-estra-4,9-diene-3-one (**1b,** EC315)*

Following the procedure outlined for the synthesis of compound **1a**, compound **4** (1.2 g) was hydrolyzed in 50% sulfuric acid to give 700 mg of compound **1b** after workup and purification.

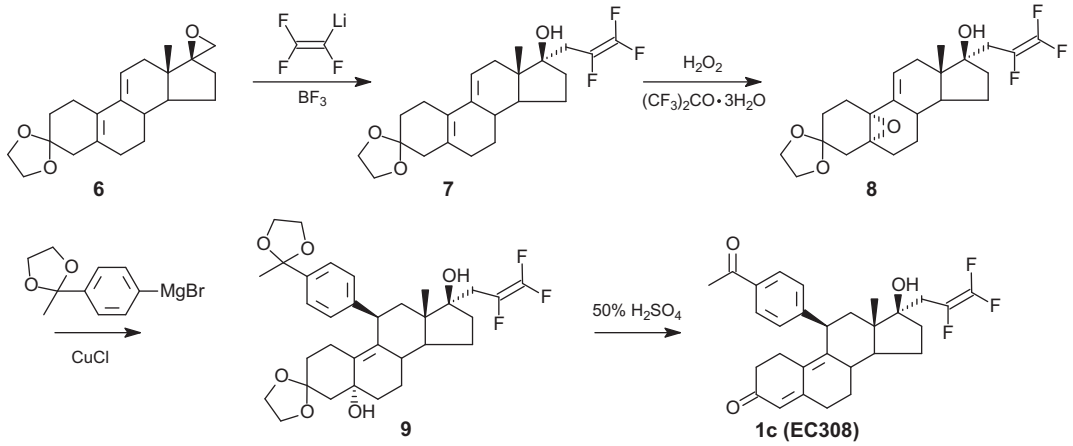
^{1}H NMR (δ, 300 MHz) 0.59 (s, 3H), 2.57 (s, 3H), 1.3-2.8 (m, 19H), 4.42 (d, *J* = 7.0 Hz, 1H), 5.80 (s, 1H), 5.80-6.00 (m, 1H), 6.57 (d, *J* = 15.5 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 8.2 Hz, 2H).



Scheme 2.



Scheme 3.



Scheme 4.

Table 1
EC compounds inhibit cell proliferation of T47D cells: Cytotoxicity screening of EC compounds in 47D cells demonstrates their potent antiproliferative activity compared with known antiprogestins in the MTT assay.

Compounds	IC50 (EC300)
RU-486	>100 nM
CDB 2914	>100 nM
CDB 4124	>100 nM
ORG33628	100 pM
ZK230211	5.1 nM
EC301	10 nM
EC304	0.5 nM
EC305	<1 pM
EC308	5.9 nM
EC315	1 pM

Table 2
MMTV-Luc regulation.

Compound	EC_{50} [nM]	IC_{50} [nM]
Progesterone	0.43	---
RU-486	---	0.124
EC300	ND	0.034
EC304	ND	0.047
EC315	ND	0.259

ND = none detected.

Table 3
PR and GR-antagonist screen.

CMPD	Invitrogen receptor profiling	
	Progesterone	Glucocorticoid
	Antagonist (Rel to RU-486)	Antagonist (Rel to RU-486)
EC301	<6%	ND
EC304	119%	21%
EC305	191%	10%
EC308	21%	5%
EC315	33%	22%
ZK230 211	81%	3
CDB 4124	186%	ND

ND = none detected.

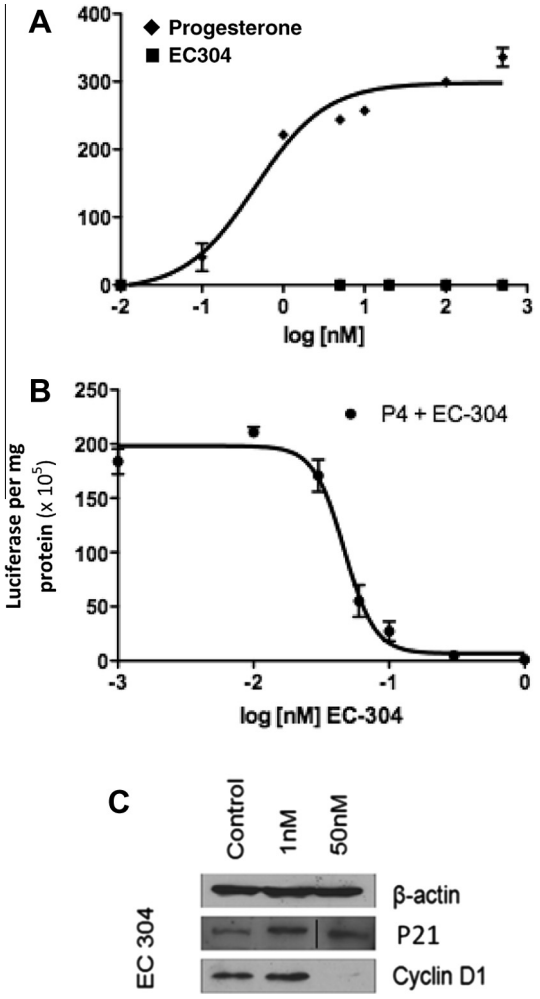


Fig. 1. (A) Estrogen stimulated 3-dimensional growth of T47D cells: The 3-dimensional structures of T47D cells in Matrigel were completely destroyed within 48 h of EC304 treatment. The treated cells were determined to be cytostatic and did not grow further. (B) EC compounds induce cell-cycle arrest at G1 phase: One of the leading candidates, EC304, induced apoptosis and G1-phase cell-cycle arrest when co-incubated with E2 for 24 h. The percentage of cells gated for G1 phase in a control reaction compared to those treated with 1 or 10 nM EC304 were 40.45, 50.21 and 54.22, respectively. (C) EC304 inhibits cell proliferation at protein level: Antiprogestin EC304 inhibits cell proliferation by inhibiting cyclin D1 and stabilizing the cyclin-dependent kinase inhibitor p21.

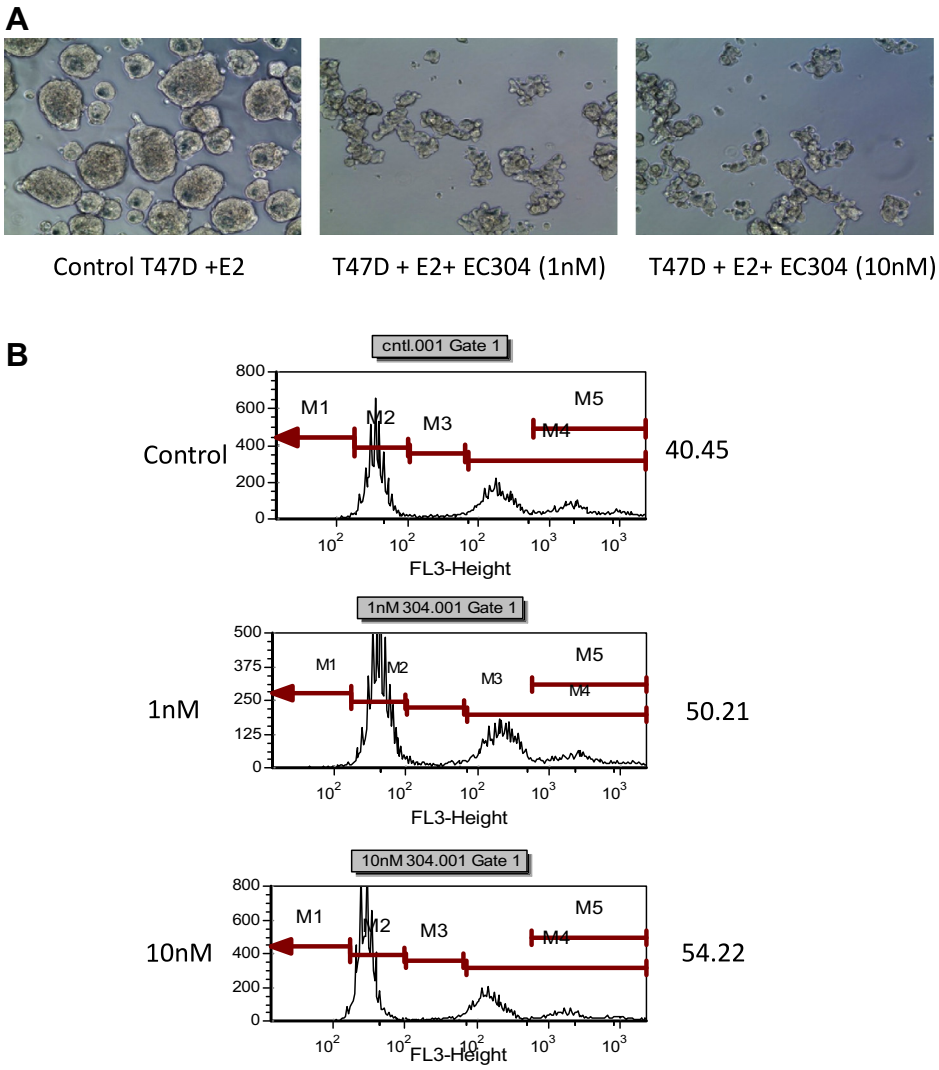


Fig. 2. MMTV-Luciferase reporter gene assay in breast cancer cells. (A) Agonist mode. T47D cells infected with adenovirus MMTV-Luc (MOI = 3) were treated with increasing amounts of progesterone (0.1, 1, 5, 10, 100 or 500 nM) or EC compounds (5, 20, 100 or 500 nM) for 24 h. Luciferase activity was measured in triplicate as described in the Experimental section, with data normalized to protein concentration and values indicating the average Luc units per mg protein ± SEM. The data were graphed as Luc units/mg protein vs. log of the concentration (nM) of compounds and were fit by non-linear regression using GraphPad Prism 5.0. (B) Antagonist mode. T47D cells infected with adenovirus MMTV-Luc (MOI = 3) were treated for 24 h with 5 nM P4 alone or 5 nM P4 supplemented with increasing amounts of EC304 (0.01, 0.03, 0.06, 0.1 or 0.3 nM) or RU-486 (0.03, 0.1, 0.3, 1 or 3 nM). Luciferase activity was measured and the data were quantified and graphed as in panel A.

*2.2.5. 3,3-Ethylenedioxy-11β-{4′-[1′,1′-(ethylenedioxy)-ethyl]phenyl}-estra-4,9-diene-17-one (**5**)*

DMAP (150 mg) and acetic anhydride (3 mL) were added to a solution of 3,3-ethylenedioxy-5α-hydroxy-11β-{4′-[1′,1′-(ethylenedioxy)-ethyl]phenyl}-estra-9-ene-17-one (**2**) (3 g, 6 mmol) in pyridine (30 mL), and the resulting reaction mixture was heated at 70 °C for 30 h. The reaction mixture was concentrated under vacuum and directly purified on a silica gel using 10% ethyl acetate in hexane containing 1% TEA to afford **5** (2.3 g, 80%).

^{1}H NMR (δ, 300 MHz) 0.48 (s, 3H), 1.2–2.8 (m, 17H), 1.63 (s, 3H), 3.7–4.1 (m, 8H), 4.31 (d, J = 7.1 Hz, 1H), 5.4 (s, 1H), 7.18 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.3 Hz, 2H).

^{13}C NMR (75 MHz) 14.5, 22.0, 24.3, 27.4, 27.5, 30.5, 33.2, 35.5, 37.5, 37.7, 39.6, 47.8, 51.0, 64.5, 64.6, 64.7, 106.2, 108.9, 121.9, 125.4, 127.0, 130.4, 137.5, 139.3, 140.7, 144.6, 219.6.

*2.2.6. 11β-(4′-Acetyl-phenyl)-17β-hydroxy-17-(1,1-difluoroprop-2-enyl)-estra-4,9-diene-3-one (**1d**, EC304)*

n-BuLi (1.3 mL, 2.5 M, 3.2 mmol) was added drop wise to a solution of 3,3-ethylenedioxy-11β-{4′-[1′,1′-(ethylenedioxy)-ethyl]-phenyl}-estra-4,9-diene-17-one (**5**) (400 mg, 0.84 mmol) and 3-bromo-3,3-difluoropropene (530 mg, 3.4 mmol) in a (4:1:1) THF:ether:pentane mixture (6 mL) at −100 °C. The reaction mixture was stirred for 90 min at −95 °C and warmed to room temperature over 3 h, then quenched with ammonium chloride solution (20 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were concentrated to dryness, dissolved in methanol (5 mL) and treated with 50% sulfuric acid (0.25 mL) at 0 °C. The reaction was stirred at room temperature for 2 h and carefully quenched with a solution of sodium bicarbonate (15 mL). The solution was extracted with dichloromethane (3 × 10 mL) and the combined dichloromethane layers were dried over sodium sulfate, concentrated under vacuum, and purified on a silica gel column using 25% ethyl acetate in hexane to afford **1d** (160 mg, 40%).

^{1}H NMR (δ, 300 MHz) 0.52 (s, 3H), 1.2-2.8 (m, 17H), 2.52 (s, 3H), 4.42 (bs, 1H), 5.50 (d, J = 11.1 Hz, 1H), 5.68 (d, J = 17.3 Hz, 1H), 5.74 (s, 1H), 6.0-6.3 (m, 1H), 7.26 (d, J = 8.1 Hz, 2H), 7.83 (d, J = 8.3 Hz, 2H).

^{13}C NMR (75 MHz) 16.9, 24.5, 25.8, 26.5, 27.7, 31.1, 33.6, 36.7, 38.8, 39.5, 41.0, 48.2, 51.0, 85.1 (t, J = 26 Hz), 120.7 (t, J = 9.5 Hz),

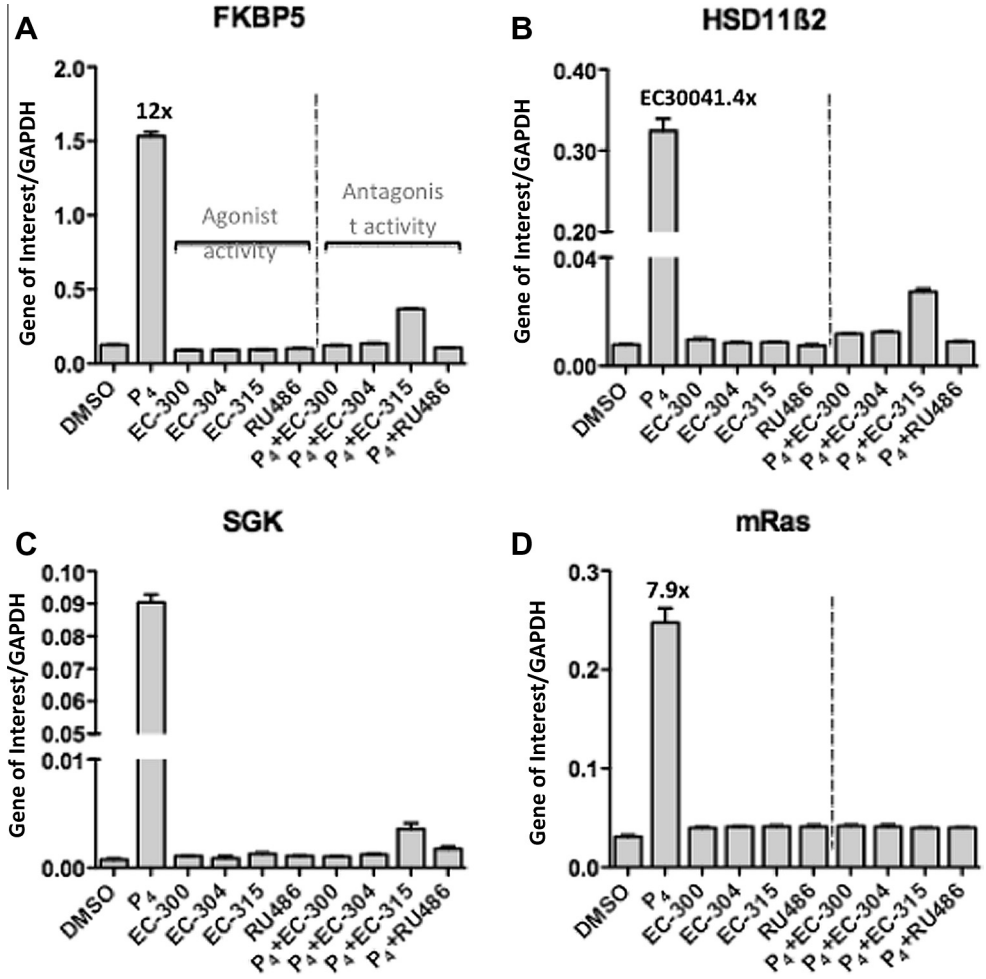


Fig. 3. Regulation of endogenous PR-target genes: T47D cells were treated for 24 h with 10 nM P4, or 20 nM of either an EC compound or RU-486 alone, or, 5 nM P4 plus antagonist (1 nM of EC300 or EC304 or 3 nM of EC315, EC317 or RU-486). Total RNA was isolated and used in a PanomicsQuantiGenePlex 2.0 assay to quantify expression of RNA from twenty-seven different endogenous target genes. The RNA expression of four representative genes, normalized to GAPDH expression, are shown as panels A–D: (A) FKBP5, (B) HSD11ß2, (C) SGK, and (D) mRas. The factor by which P4 is induced is indicated for each gene.

123.0 (t, J = 247 Hz), 123.2, 127.1, 128.7, 129.9, 131.2 (t, J = 25.2 Hz), 134.9, 144.3, 150.7, 156.3, 197.7, 199.3.

*2.2.7. 11β-(4′-Acetyl-phenyl)-17β-hydroxy-17-(1,1-difluoropropyl)-estra-4,9-diene-3-one (**1e,** EC305)*

A solution of 11β-(4′-acetyl-phenyl)-17β-hydroxy-17-(1,1-difluoroprop-2-enyl)-estra-4,9-diene-3-one (**1d**) (160 mg, 0.34 mmol) in ethanol (5 mL) containing 10% Pd/C (20 mg) was stirred under positive hydrogen pressure for 2 h. The catalyst was removed by filtration through a cotton plug, the solvent was removed under vacuum, and the crude product was purified on silica gel to yield **1e** (120 mg, 75%).

^{1}H NMR (δ, 300 MHz) 0.53 (s, 3H), 1.03 (t, J = 7.4 Hz, 3H), 1.1–2.8 (m, 19H), 2.55 (s, 3H), 4.44 (bs, 1H), 5.76 (s, 1H), 7.28 (d, J = 8.4 Hz), 7.86 (d, J = 8.4 Hz).

^{13}C NMR (75 MHz) 1.0, 5.5 (t, J = 6.1 Hz), 17.1, 24.7, 25.9, 26.1, 26.6, 27.7, 31.1, 34.1, 36.8, 39.1, 39.4, 41.2, 48.6, 51.4, 85.7 (t, J = 26 Hz), 123.3, 127.2, 127.8 (t, J = 249 Hz), 128.8, 130.0, 135.0, 144.3, 150.9, 156.3, 197.7, 199.3.

*2.2.8. 3,3-Ethylenedioxy-17β-hydroxy-17-(2,3,3-trifluoroprop-2-enyl)-5(10),9(11)-estradiene (**7**)*

n-BuLi (2.5 M, 2.4 mL, 6.1 mmol) was added to a solution of 1,1,1,2-tetrafluoroethane (820 mg, 8 mmol) in ether (10 mL) at −78 °C over 10 min, and the mixture was stirred for 1 h at −78 °C. A solution of spiro-2′-(1′-oxacyc1opropane)-17(S)-[3,3-(ethylenedioxy)-5(10),9(11)-estradiene] (**6**) (1 g, 3.04 mmol) in ether (7 mL) was added to the mixture, followed by the drop wise addition of boron trifluorideetherate (0.38 mL, 3.04 mmol). The reaction mixture was stirred at −78 °C for 1 h and warmed to room temperature over 1 h, then quenched with a solution of sodium bicarbonate (20 mL) and extracted with ethyl acetate (3 X 15 mL). The organic layers were combined and washed with water and brine, dried over sodium sulfate and evaporated in vacuo to afford the crude product, which was purified on silica using 20% ethyl acetate in hexane to afford **7** (430 mg, 35%).

^{1}H NMR (δ, 300 MHz) 0.90 (s, 3H), 1.0–2.7 (m, 20H), 3.99 (s, 4H), 5.50–5.60 (bs, 1H).

^{13}C NMR (75 MHz) 14.4, 23.6, 24.6, 27.6, 31.2, 31.3, 32.8, 33.7 (dd, J = 2.8, 18.6 Hz), 34.7, 39.4, 41.3, 45.3, 46.2, 46.8, 64.36, 64.49, 82 (m), 108.2, 117.7, 126.1, 125–130 (m), 130.2, 136.5, 154.8 (ddd, J = 285, 271, 47.2 Hz).

*2.2.9. 3,3-Ethylenedioxy-5α,10α-epoxy-17β-hydroxy-17-(2,3,3-trifluoroprop-2-enyl)-estr-9(11)-ene (**8**)*

Hydrogen peroxide (0.18 mL, 30%, 1.6 mmol) was added to an ice-cold solution of hexafluoroacetonetrihydrate (350 mg, 1.6 mmol) in dichloromethane (3 mL). Solid Na_2HPO_4 (180 mg, 1.3 mmol) was introduced, and the reaction mixture was stirred for 1 h at 0 °C. An ice-cold solution of 3,3-ethylenedioxy-17β-hydroxy-17-(2,3,3-trifluoroprop-2-enyl)-5(10),9(11)-estradiene (**7**) (410 mg, 1 mmol) in dichloromethane (3 mL) was added, and the

Table 4
Endogenous gene regulation in T47D breast cancer cells.

Gene	Accession	Agonist mode: fold induction		Antagonist mode: % inhibition	
		P4	EC304	RU-486	EC304
S100P	NM_005980.2	125.2	0.9	98.8	98.2
SGK1	NM_005627.3	117.9	2.0	98.1	98.6
ZBTB16	NM_006006.4	660.0	6.6	97.8	98.3
HSD11B2	NM_000196.3	41.4	1.1	97.2	96.2
NDRG1	NM_006096.3	30.0	1.0	96.8	95.7
F3	NM_001993.4	16.2	0.4	95.9	96.1
FKBP5	NM_004117.3	12.0	0.7	93.0	91.1
CDKN1C	NM_000076.2	22.9	1.3	90.9	94.3
KLF15	NM_014079.3	12.2	1.6	89.9	84.9
MT2A	NM_005953.3	11.4	1.1	86.6	86.9
MRAS	NM_012219.4	7.9	1.3	83.8	83.3
CALD1	NM_033138.3	3.9	0.7	83.6	76.1
FGF18	NM_003862.2	12.3	1.5	80.7	84.3
ARHGAP26	NM_015071.4	4.6	1.3	77.3	76.4
FOXO1	NM_002015.3	4.2	0.9	72.4	73.5
EGFR	NM_005228.3	3.0	1.1	69.4	60.2
SOX4	NM_003107.2	4.0	1.9	68.0	68.8
GREB1	NM_014668.3	2.0	0.8	61.5	47.1
NFIC	NM_205843.2	2.0	1.0	58.6	57.3
RGS2	NM_002923.3	1.6	0.6	51.1	56.6
IGF1	NM_000618.3	3.7	2.9	50.9	25.9
MAP3K6	NM_004672.3	2.2	1.4	44.3	32.3
TRAF5	NM_004619.3	1.1	1.0	NA	NA
CCND1	NM_053056.2	1.3	0.9	NA	NA
E2F1	NM_005225.2	1.4	1.1	NA	NA
MYC	NM_002467.4	1.1	0.5	NA	NA
NDRG2	NM_201535.1	0.5	0.3	NA	NA

NA – not applicable (not induced by P4).

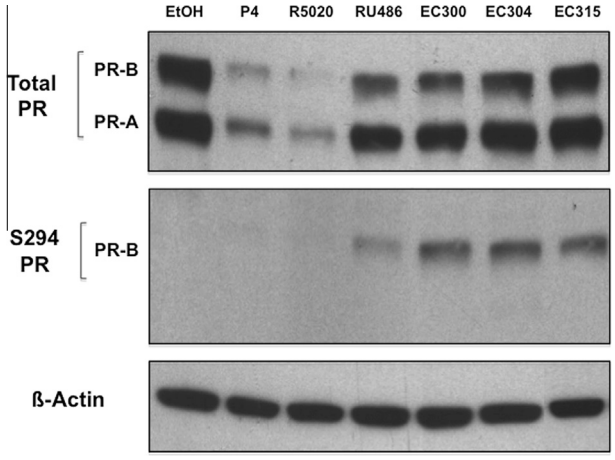


Fig. 4. Down-regulation and phosphorylation of PR: T47D cells grown in medium supplemented with 2% dextran-coated charcoal-treated FBS were treated for 24 h with 100 nM of P4, R5020, RU-486, or an EC compound. Cell lysates were analyzed by immunoblot assay for total PR protein (PR-A and PR-B) content with the monoclonal antibody (MAb) 1294 (top panel) or with a MAb that recognizes phosphorylation of serine 294 of PR-B (middle panel). An immunoblot to β-actin is shown as a loading control (bottom panel).

mixture was stirred at 0 °C for 3 h, then at 5 °C for 15 h. The reaction mixture was diluted with dichloromethane (15 mL), washed with a 10% sodium sulfite solution (15 mL) and water, dried over sodium sulfate and concentrated under vacuum to obtain a mixture of crude epoxides. Separation of the epoxide isomers was carried out on a silica gel column using 20% ethyl acetate in hexane to afford the pure α-isomer **8** (240 mg, 56%).

^{1}H NMR (δ, 300 MHz) 0.9 (s, 3H), 1.0–2.8 (m, 20H), 3.8–4.0 (m, 4H), 5.90–6.10 (m, 1H).

*2.2.10. 3,3-Ethylenedioxy-5α,17β-dihydroxy-17-(2,3,3-trifluoroprop-2-enyl)-11β-{4′-[1′,1′-(ethylenedioxy)-ethyl]phenyl}-estr-9-ene (**9**)*

A slurry of magnesium (220 mg, 9 mmol) in THF (10 mL) containing a crystal of iodine was heated to reflux for 10 min until colorless. A solution of 2-(4-bromophenyl)-2-methyl-1,3-dioxolane (2.1 g, 8.5 mmol) in THF (5 mL) was added to the slurry over 5 min and the resulting mixture refluxed for 1 h. The mixture was cooled on ice prior to the addition of solid CuCl (150 mg, 1.5 mmol). The reaction was stirred at 0 °C for 30 min. Finally, a solution of 3,3-ethylenedioxy-5α,10α-epoxy-17β-hydroxy-17-(2,3,3-trifluoroprop-2-enyl)-estr-9(11)-ene (**8**) (730 mg, 1.7 mmol) in THF (5 mL) was added to the cuprate solution and stirred for 2 h at 0 °C, after which the reaction was quenched with aqueous ammonium chloride solution (30 mL) and extracted with ethyl acetate (3× 25 mL). The organic layers were combined and washed with water and brine, dried over sodium sulfate and evaporated in vacuo to afford the crude product, which was purified on silica gel using 25% ethyl acetate in hexane to afford **9** (810 mg, 80%).

^{1}H NMR (δ, 300 MHz) 0.48 (s, 3H), 0.8–2.7 (m, 24H), 3.6-4.6 (m, 10H), 6.79 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 8.2 Hz, 2H), 7.30–7.40 (m, 3H).

*2.2.11. 11α-(4′-Acetyl-phenyl)-17β-hydroxy-17-(2,3,3-trifluoroprop-2-enyl)-estra-4,9-diene-3-one (**1c**, EC308)*

Following the procedure outlined for the synthesis of compound **1a**, compound **9** (1.5 g) was hydrolyzed in 50% sulfuric acid to give 1.1 g of compound **1c** after workup and purification.

^{1}H NMR (δ, 300 MHz) 0.56 (s, 3H), 1.0–2.8 (m, 22H), 4.48 (d, J = 6.7 Hz, 1H), 5.80 (s, 1H), 7.29 (d, J = 8.4 Hz, 2H), 7.88 (d, J = 8.4 Hz, 2H).

^{13}C NMR (75 MHz) 15.2, 22.7, 23.5, 25.8, 26.4, 27.4, 30.9, 33.5 (d, J = 18.7 Hz), 34.3, 36.6, 37.1, 39.3, 40.5, 46.7, 50.0, 82.8 (d, J = 2.7 Hz), 123.4, 125–130 (m), 127.0, 128.6, 130.2, 134.9, 143.9, 150.0, 154.7 (ddd, J = 47, 272, 286 Hz), 155.9, 197.4, 198.9.

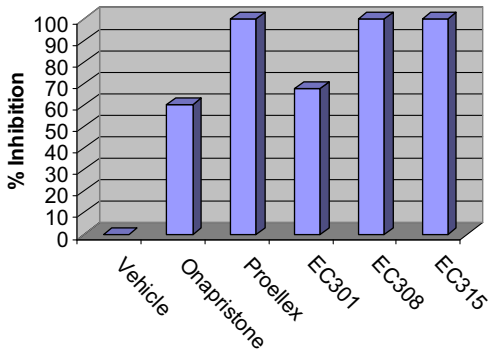


Fig. 5. EC compounds exhibit potent anti-nidation activity. The antinidation activities EC compounds were screened in Sprague-Dawley rats. Three control rats (vehicle treated, s.c.), three rats treated (s.c.) with a known progestin antagonist and three rats treated (s.c.) with the test compound (3 mg/day) were used. Female rats were placed with male rats for three to four days, and their vaginas were examined for sperm plugs every morning. The presence of a sperm plug indicated day one of pregnancy. Pregnant rats were treated daily with 3 mg of an EC compound beginning on day five of pregnancy. On day 9, the rats were euthanized, and their nidation sites were counted. Since the sample size of this experiment is limited to calculate statistical significance, the observations from this experiment may be considered as a pilot data.

2.3. Biological assays

Antiprogestational and antiglucocorticoid activity of EC304 was determined as previously described using *select screen* assay system (Invitrogen-Life Technologies) [26,27]. Briefly, PR-UAS-bla HEK 293T and GR-UAS-bla HEK 293T cells were used for the PR antogonistic screen and GR antagonistic screen respectively. Cells were activated by R5020 (PR agonist) and dexamethasone (GR agonist) for anti-PR and anti-GR screening. 32 μL of cell suspension was added to the wells and pre-incubated at 37 °C/5% CO2 in a humidified incubator with compounds and control antagonist titration for 30 min. 4 μL of 10× control agonist R5020 at the pre-determined EC80 concentration was added to wells containing the control antagonist or compounds. The plate was incubated for 16–24 h at 37 °C/5% CO2 in a humidified incubator. 8 μL of 1 μM Substrate Loading Solution was added to each well and the plate was incubated for 2 h at room temperature. The plate was read on a fluorescence plate reader.

2.4. Cell culture

T47D breast cancer cells were maintained in α-MEM media containing 5% fetal bovine serum (FBS) as previously described [2,28]. For MMTV-Luc assays, cells were plated in 12-well dishes at a density of 150,000 cells/well and, after 24 h, an adenovirus vector expressing MMTV-Luciferase (Luc) was added at an MOI of 3 particles/cell, as previously described [2]. Twenty-four hours after transduction, cells were switched to MEM supplemented with 2% dextran-charcoal-treated FBS and were subjected to the new compounds for 24 h at the concentrations indicated in the figure legends. Identical experiments were conducted in three wells. For RNA expression and protein immunoblot assays, cells were plated at 350,000–400,000 cells/well in 6-well dishes and treated with the new compounds in triplicate wells, as described above.

2.5. Luciferase assay

Cell monolayers were washed with PBS before scraping the cells into 200 μL of 1× reporter lysis buffer (Promega). After centrifugation, cell lysates (30 μL were injected with 50 μL of luciferase assay reagent (Promega), and light output was measured for 2 s using a LUMIstar Omega luminometer. Luciferase light units were normalized to protein concentrations in cell lysates as determined by Bradford assay [2,28].

2.6. Quantitative RNA assays

The total RNA from T47D cells was isolated using RNeasy columns (Qiagen) according to the manufacturer's instructions. QuantigenePlex 2.0 assays [3] were performed using 100 ng of RNA from each treatment group according to the manufacturers recommendation. RNA concentration and quality were determined by NanoDrop assay. Assays were performed with the help of the Genomic and RNA Expression Profiling Core at Baylor College of Medicine [29]. Relative RNA levels were normalized to GAPDH, and values obtained in triplicate were reported as the average ± SEM.

2.7. Western blot analysis

Cell monolayers were washed with PBS and lysed directly with 200 μL of a buffer containing 50 mM Tris pH 7.4, 50 mM KCl, 4 mM EDTA, 2 mM EGTA, 1% NP-40 and a protease inhibitor tablet (Roche). The lysates were centrifuged at 14,000 rpm for 20 min at 4 °C, and protein concentrations were measured by Bradford assay. Equal amounts of protein (30 μg) were subjected to electrophoresis on 7.5% SDS-polyacrylamide gels and immunoblotted with a mouse monoclonal antibody to total PR-A and PR-B (clone 1294) or to phosphoserine 294 of PR (clone B608) and detected by chemiluminescence, as previously described [2,30,31]. PR-A and B specific antibodies were indigenouslyprepared in Dr. Edwards's lab, p21 actin and cyclin D1 were purchased from Santacruz laboratories, Santacruz, CA.

2.8. Cytotoxicity screening

The *in vitro* cytotoxicity of the newly synthesized antiprogestins was analyzed by the MTT (ATCC, Manassas, VA) assay. Briefly, 5000 human breast cancer T47D cells were seeded per well in a 96-well plate (Corning, Lowell, MA) containing phenol red-free RPMI-1640 medium (Invitrogen, Austin, TX) supplemented with 0.1 nM estradiol (Sigma-Aldrich, St. Louis, MO), 10% charcoal-stripped serum (Invitrogen) and various concentrations of the test compounds. T47D cells were selected for use in this experiment based on their logarithmic growth phase at 70% confluence, and cultivated for 48 h in phenol red-free charcoal-stripped RPMI medium supplemented with 1% penicillin-streptomycin (Invitrogen). Once seeded, the cells were grown for 3 days before supplementing the medium with test compounds and E2. The inhibition of cell proliferation was measured on the 7th day by the addition of 20 μL of MTT solution to each well. After 4 h, the formation of the tetrazolium salt was measured with a Thermo Multiskan Spectrum spectrophotometer (Thermo Labsystems, Waltham, MA). Six replicates were measured for each concentration of each compound tested, from which the IC50 growth inhibition values for all compounds were determined.

2.8.1. 3-Dimensional spheroid culture

T47D cell spheroids were prepared using the liquid overlay method (Offner et al., 1993). Briefly, a T47D cell suspension of 2.5×10^5 cells/ml in 100 μL of RPMI 1640 medium supplemented with 10% FBS was plated on 1% agarose-coated 96-well culture plates. After 5 days of incubation at 37 °C in a humidified atmosphere with 5% CO_2, the cells formed spheroids that were then used to analyze the antiproliferative activity of the test compounds compared with known antiprogestins.

2.8.2. Gene expression studies

For gene expression analyses, RNA was isolated from T47D cells treated with progesterone and antiprogestins using the RNeasy kit (no. 74104; QIAGEN, Valencia, CA) and was reverse transcribed using SuperScript III (no. 18080-093; Invitrogen, Carlsbad, CA). Real-time PCR and subsequent analyses were carried out with 0.25% Sybr Green using an ABI Prism 7700 Sequence Detector System (PE Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The expression of PR-regulated genes and housekeeping genes was detected as described earlier. Melting curve analysis was performed after each real-time PCR to ascertain PCR product specificity. Relative expression was determined using the formula 2_DDCt. Real-time PCR assays were performed in duplicate per experiment, and each experiment was repeated at least three times.

2.9. In vivo antiprogestational activity

In vivo antiprogestational activity was measured by an anti-nidation assay in which the antiprogestin test compounds were screened in Sprague–Dawley rats. Briefly, three control rats (vehicle treated, s.c.), three rats treated (s.c.) with a known progestin antagonist and three rats treated (s.c.) with the test compound (3 mg/day) were studied. The female rats were placed with male rats for three to four days, and their vaginas were examined for sperm plugs every morning. The presence of a sperm plug indicated day one of pregnancy. Pregnant rats were treated daily with 3 mg of the antiprogestin compounds beginning on day five of pregnancy. On day nine, the rats were euthanized, and their nidation sites were counted.

2.10. Cell cycle analysis

T47D cells were synchronized to G_0–G_1 phase by serum deprivation for three days in 0.5% dextran-coated charcoal-treated serum-containing medium, then released into the cell cycle by the addition of 10^{-8} mol/L E2 in 10% fetal bovine serum–containing medium (Balasenthil et al). Flow cytometry was performed to analyze the cell cycle progression.

2.11. Protein analysis

The expression of proteins was determined using Western blot analysis. Total protein was isolated from the cervical tissues by homogenization in lysis buffer. Equal amounts of protein (60 μg) from representative samples were separated on a denaturing polyacrylamide gel and transferred to a nylon membrane. Nonspecific binding of antibodies was blocked by incubation (overnight, 4 °C) in TBS containing 0.1% Triton X-100 (TBST) and 5% nonfat dry milk. The membranes were then incubated with their respective primary antibodies in TBST and 5% milk overnight at 4 °C. The specific binding of antibodies to target proteins was visualized using species-specific IgG followed by chemiluminescent detection and exposure to enhanced chemiluminescencehyperfilm (Amersham, Piscataway, NJ). The antibody for cyclin D1 was purchased from NeoMarkers (Fremont, CA), whereas p21 and beta-actin antibodies were procured from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

2.12. Statistical analysis

All experiments were carried out in triplicate, with the results are expressed as the means ± SEM where applicable. A Student's *t*-test was used to determine the validity of the differences between control and treatment data sets. A *P*-value of less than 0.05 was considered significant.

3. Results and discussion

3.1. Chemistry

The field of progesterone receptor antagonists (antiprogestins) has progressed in two directions since the first compound of this class, mifepristone (RU-486), was discovered by Teutsch et al [17]. Full receptor antagonists have been investigated for applications in post-coital contraception, abortion and breast cancer, whereas mixed agonist/antagonists have been considered for gynecological indications such as endometriosis and uterine fibroids [18].

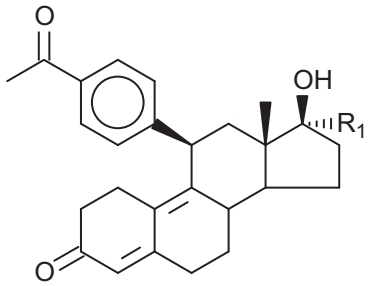
Over the years, knowledge about the structure/activity relationship of antiprogestins has grown considerably [19,20]. Mifepristone's use is limited by its strong antiglucocorticoid activity.

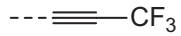
Replacement of the 17-propynyl group in mifepristone with 3-hydroxypropyl [21], 17-acetoxy [22] or 17,17 spiro ether [23] gave rise to potent antiprogestins with significantly reduced antiglucocorticoid activity, as shown in Scheme 1.

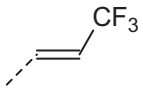
Recently, 17-perfluoralkyl derivatives have been reported that exhibit very strong antiprogestational activity and minimal antiglucocorticoid activity. The compound ZK230 211 was selected from this class of compounds based on its extraordinary potency and its highest receptor selectivity observed to date [24].

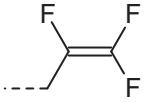
All these compounds exhibit strong antiprogestational activity with only marginal agonistic activity. Asoprisnil, however, is the first representative of a new class of antiprogestins with a dual antagonistic/agonistic profile [25].

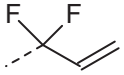
We report here the synthesis and biological characterization of a new class of antiprogestins derived from ZK230 211. The high potency and selectivity of the latter compound is predominantly attributed to its pentafluoro side chain [24]. To investigate the influence of partially fluorinated 17 sidechains on antiprogestin potency and selectivity, the following new antiprogestins were synthesized:

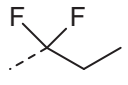


1a (EC301) R_1 = 

1b (EC315) R_1 = 

1c (EC308) R_1 = 

1d EC(304) R_1 = 

1e (EC305) R_1 = 

The syntheses of EC301 and EC315 are presented in Scheme 2.

The intermediate **2** may be synthesized according to a previously reported protocol [32]. Treatment of 2-bromo-3,3,3-trifluoropropene with 2 eqs of LDA at −78 °C generated 3,3,3-trifluoropropynyllithium, which was added to **2** to give the desired product, **3**. Red-Al reduction of **3** at −78 °C selectively yielded the trans-double bond. Upon hydrolysis, compounds **3** and **4** yielded **1a** (EC301) and **1b** (EC315), respectively.

The syntheses of EC304 and EC305 are presented in Scheme 3.

Dehydration of the 17-keto derivative (**2**) was achieved by treatment with excess of acetic anhydride in pyridine at 70 °C for 30 h to afford **5**. Addition of difluoroallyllithium to **5** at −100 °C generated the addition product, which upon acid hydrolysis yielded **1d** (EC304). The side chain double bond at C-17 can be selectively hydrogenated using 10% Pd/C under a hydrogen atmosphere to provide **1e** (EC305).

The synthesis of EC308 is presented in Scheme 4.

The intermediate **6** may be synthesized according to a previously reported protocol [33]. Ring-opening of the epoxide **6** with trifluorovinyllithium generated from 1,1,1,2-tetrafluoroethane and n-BuLi at −78 °C in the presence of boron trifluorideetherate afforded **7**. Subsequent epoxidation, conjugate Grignard addition and hydrolysis yielded **1c**.

3.2. Biological characterization

3.2.1. EC compounds showed potent antiprogestational activity and inhibited growth of T47D cells

We sought to determine the agonist and antagonistic activity of the EC compounds before testing their cytotoxicity in breast cancer cells, as the most promising currently known antiprogestins exhibit progestational and antiglucocorticoid activity. GeneBLAzer® beta-lactamase reporter technology was used to detect receptor agonism or antagonism induced by the compounds. Our results show that three of the initial five newly synthesized antiprogestins have significant antiprogestational activity, without significant progesterone agonist activity or glucocorticoid antagonist activity. The strong antiprogestational effect was confirmed by antinidation assays in rats (Fig. 5). Moreover, several of our test compounds were superior to the known antiprogestins, ZK230211 (internal code EC300) or RU-486 (Tables 1 and 3). Based on the cytotoxicity profiles and progestational/antiglucocorticoid activity results, we have selected EC304 as one of the lead candidate antiprogestins for more extensive study.

T47D cell 3D spheroids treated with EC304 exhibited a dose-dependent inhibition of spheroid formation after 6 days in culture. Treatment with EC304 dissociated the 3D cell spheroids, which were rendered cytostatic. Cell cycle analysis revealed that the cytostatic cells were arrested in G1 phase upon 24 h treatment with EC304. The percentage of gated cells in the untreated control T47D cells was 40.45%, whereas 50.21 and 54.22%, respectively, of cells treated with 1 nM and 10 nM EC304 were arrested in G1 phase (Fig. 1B). Mechanistically, the protein levels of the cyclin-dependent kinase inhibitor p21 were increased with EC304 treatment, further confirming that G1 phase arrest gives rise to the cytostatic effect (Fig. 1C).

3.3. Functional activity for progesterone receptor (PR) in breast cancer cells by MMTV-Luc reporter gene assay

To determine the relative agonist and antagonist activities of the EC compounds with respect to PR-mediated transcriptional activation, the MMTV-Luciferase (Luc) reporter gene assay was utilized. T47D human breast cancer cells that express PR (both A and B isoforms) and are responsive to progesterone (P4) were transduced with an adenoviral vector expressing MMTV-Luc containing progesterone response elements (PREs) in the promoter and LTR of the MMTV gene [1,2]. When cells were treated with EC compounds over a wide concentration range (5–500 nM), no induction of MMTV-Luc was detectable with EC304. This finding contrasts with P4, which dramatically induced expression of MMTV-Luc with an EC_{50} of 0.43 nM (Fig. 2A). Cells were also treated in the antagonists with a single dose of P4 (5 nM) in the absence orpresence of increasing concentrations of EC compounds. As exemplified with EC304, all EC compounds inhibited P4 induction of MMTV-Luc expression in a dose-dependent manner (Fig. 2B), with calculated IC_{50s} between 0.034 and 0.259 nM (Table 2). A direct comparison with the PR antagonist RU-486 revealed that ZK 230211 (EC300) and EC304 had lower IC_{50s} than RU-486 (0.124 nM), whereas EC315 had a higher IC_{50} (Table 2). Thus, under these conditions, the EC compounds are all PR antagonists either comparable to or more potent than RU-486.

3.4. Effects on endogenous PR target gene expression

To examine the relative agonist and antagonist activities of EC-compounds with endogenous PR target genes, the RNA expression of a panel of 27 known PR targets in T47D cells was assayed by the Quantigene branched DNA multiplex bead technology [3]. The genes were selected from previous microarray studies in the literature as well-characterized, robust targets of PR in breast cancer cells [2,4–11]. Cells were treated for 24 h with P4 or EC compounds alone or with P4 in the presence of a single concentration of EC compound. The single dose concentration of each EC compound that exhibit PR antagonism was chosen from the MMTV-Luc assay as the lowest concentration that gave maximal inhibition of induction by P4 (Fig. 2). Using a 2-fold increase in RNA expression as the criteria for an induced target gene, P4 induced expression of 22 of the 27 genes analyzed (Fig. 3, Table 4). Genes (RGS2, E2F1, CCND1, MYC) that were not significantly increased by P4 were early response genes that are not likely to be maintained at induced levels after 24 h of treatment [2,9,10]. The effects of the EC compounds compared with RU-486 in the agonist and antagonist mode for four representative genes (FKBP5, SGK, HSD11β2 and mRas) are shown in Fig. 3. None of the EC compounds alone significantly induced expression of these endogenous target genes, whereas two compounds, EC300 and EC304, completely inhibited induction by P4 (Fig. 3). Similar to the results obtained with MMVT-Luc assays, EC315 was a slightly less potent antagonist and did not completely inhibit P4 induction of target genes such as FKBP5, HSD11β2 and SGK. Table 4 summarizes the results with all 27 targets, and highlights the effects of EC304 and P4 as single compound, and of EC304 vs RU-486 as P4 antagonists. EC304 exhibited very little agonist activity with this set of target genes, with the exception of a 2.9 fold induction of IGF-I and a 6.6 fold induction of ZBTB16. However, ZBTB16 expression was induced 660-fold by P4, indicating that EC304 has less than 1% the activity of P4 on this gene. In the antagonist mode, EC304 inhibited P4 induction in a manner that was indistinguishable from that of RU-486 for each target gene (Table 4).

3.5. Down-regulation and phosphorylation of PR

A hallmark of progesterone agonists is to down-regulate PR protein upon prolonged treatment, whereas the antagonist RU-486 stabilizes PR protein in the cell [11,15,34]. To test how EC compounds affect PR down-regulation, T47D cells were treated for 24 h with P4, the synthetic progestin R5020, or each of the EC compounds, and steady-state PR protein levels were monitored by immunoblot assay of whole cell lysates with an antibody to both the PR-A and PR-B isoforms. As expected, substantial down-regulation of PR was observed with P4 and R5020 compared with vehicle

controls (EtOH, DMSO). Similar to results with RU-486, PR protein was stabilized and was not down-regulated by treatment with the EC compounds (Fig. 4, top panel). Progesterone binding in cells leads to a rapid increase of PR phosphorylation on several serine residues including serine 294, a residue that is predominantly phosphorylated on the PR-B isoform [35]. Although the role of PR phosphorylation is not well defined, data indicates that phosphorylation modulates nuclear translocation, PR protein turnover and PR-mediated transcriptional regulation in a target gene-specific manner [30,36]. The antagonist RU-486 has been shown previously to stimulate phosphorylation of PR on the same sites as are stimulated by the hormone agonist, whereas Onapristone (ZK98299) fails to increase PR phosphorylation [29,31,37]. Thus, RU-486 does not exert its antagonist action by interfering with or altering PR phosphorylation. By immunoblotting with an antibody that recognizes phospho-PR serine 294 [34], it was determined that the EC compounds permitted PR phosphorylation of serine 294 and thus affected phosphorylation in a manner similar to that of RU-486 (Fig. 4).

4. Conclusion

Substitution of the 17 pentafluor ethyl group of the steroid molecule ZK230 211 with partially fluorinated unsaturated side chains gave rise to compounds with significantly higher cytotoxicity in the T47D cell line model. EC304 was selected for further studies based on its satisfactory differentiation between progesterone and glucocorticoid binding. EC304 was shown to degrade PR receptors through BRCA1-mediated ubiquitination and subsequent proteasomaldegradation. In experiments with endogenous PR target genes, EC304 showed similar effects as RU-486.

Ongoing studies are focused on developing EC304 as a chemotherapeutic agent for PR-positive breast cancer patients, as well as a chemopreventive for BRCA1- and p53-mutated patients.

Acknowledgements

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