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Synthesis and biological evaluation of partially fluorinated antiprogestins and mesoprogestins

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ABSTRACT

A series of antiprogestins have been synthesized by partially fluorinating the steroid molecule in positions relevant for receptor binding. By introducing fluorine at the exo-methylene of the 17 spirofuran ring, we obtained partial agonists (mesoprogestins) with significant applications for antiproliferative and antiovulatory treatment strategies in gynecological therapy such as uterine fibroids, endometriosis and heavy menstrual bleeding. Compared to the standard drug RU486, our synthesized compounds exhibited considerable dissociation between antiprogestational and antiglucocorticoid PR receptors. Furthermore, our studies have shown that pure antiprogestins can be generated by partially fluorinating the 17 propenyl and propynl group or by substituting the 4' acetyl phenyl group in the 11 position using trifluromethyl group.

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1. Introduction

1.1. SARs considerations for choice of compounds (PRMs) to be synthesized

Progesterone antagonists of the RU486 (Mifepristone) family comprise pharmacodynamically different compounds. These pharmacodynamic differences result from compound-specific interactions with nuclear receptors apart from the PR (progesterone receptor) for example the GR (glucocorticoid receptor). Furthermore, the respective compounds also differ in terms of their biological effects at the PR, due to the relative amount of PRantagonistic and PR-agonistic properties as well as the mechanism of type I and type II antagonists [1]. Up until now, progesterone receptor antagonists (PRMs) have been clinically developed by different strategies ever since Mifepristone (RU486) was first discovered by Teutsch et al. [2]. The first approach deals with "PRantagonists" for the purpose of investigating different types of fertility control and cancer indications, such as post-coital contraception, therapeutic pregnancy termination (Mifepristone, CDB-2914), and breast cancer (Onapristone, ZK-230211). The second approach requires a partial agonistic profile as was first observed with Asoprisnil for the treatment of gynecological indications, such as endometriosis and fibroids [3,4]. The beneficial implications of both PR antagonists and partial agonists (mesoprogestins) have resulted in efforts to better understand the structure–activity relationships [5,6] and have led to the synthesis of new antiprogestins depicted in Fig. 1.

SARs lead to the understanding that the substitution pattern at 17 position of the steroid establishes binding to the PR and/or GR receptor. The 17-alpha propinyl group of Mifepristone leads to equal strong binding to both receptors therefore limiting its long term application. Substitution of the 17-propinyl group with a 3-hydroxypropyl, (Z)-3-hydroxyprop-1-enyl group [7], a 17,17-spiro-oxazole group [8], a 17-acetoxyprogesterone side chain [9], or the 17,17 spiro ether group [10] and most recently, ZK-230211 [11] a perfluoralkyl steroid leads to potent antiprogestins with significantly reduced antiglucocorticoid activity.

In contrast substitution at the 11 position influences the ratio of agonistic to antagonistic activity of the steroid molecule. Mesoprogestins were first observed with Asoprisnil [3] and later with other derivatives [13,14]. Partial agonistic profile can be explained by crystal structure investigations of the progesterone receptor binding domain complexed with Asoprisinil and the nuclear receptor corepressor and SMRT [12].

The concept of partial fluorination was applied on different moieties in the 11 and 17 positions of the steroid molecule in order to accomplish two objectives, the first aim was to acquire antiprogestins with reduced binding to the GR receptor and our second



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Fig. 1. Significant RU486 derivatives.



Fig. 2. Compound design.



Fig. 3. Synthesis of compound 1a (EC301).

aim was to synthesize pure antiprogestins and mesoprogestins (refer to Fig. 2). The paper covers basic molecular studies pertaining to partially fluorinated steroids and a preliminary first qualitative in vivo assay which permits a corresponding distinction (Fig. 3).

1.2. Pharmacodynamic concept

SERMs (specific estrogen receptor modulators) are known to generate a tissue-dependent mosaic of ER-agonistic and/or antagonistic effects [15,16]. The blend of estrogenic and antiestrogenic effects is advantageous for the use of SERMs (Tamoxifen and Raloxifen) for prevention and treatment of estrogen-dependent mammary gland tumors in human. Moreover, SERMs have the capability to partially abolish the negative effects of endogenous and exogenous estrogens because the blend of effects induced by SERMs varies in tissues and is sharply different compared to low doses of estrogen. By analogy to SERMs at the progesterone receptor, PRMs or SPRMs (selective progesterone receptor modulators) can induce a unique blend of PR-agonistic (e.g., kind of secretory transformation) and PR-antagonistic changes (e.g., thick-walled spiral arteries) in the human endometrium [17]. This has lead to ongoing studies to modulate PR-agonistic PRMs by improving the ratio of PR-agonist over PR-antagonistic effects. For example, one significant compound that does exhibit higher PR agonist over PR antagonistic response is RU486. RU486 is not a pure antagonist at the PR [1,3,18] and has shown remarkable PR-agonistic activity in the guinea pig model. The PR-agonistic activity due to RU486 may explain lower labor conditioning effects when combined with a prostaglandin; and lower labor inducing properties when administered without a prostaglandin as compared to pure PR antagonist Onapristone [19].

From a clinical perspective, "pure" antagonists may lead to superior PRMs for the induction of labor. While on the other hand, mixed PR-agonist/antagonists, in particular those with prominent PR-agonistic properties, appear to be an improved option for chronic uses in women with uterine disorders, or as an oral contraceptive. As previously stated, Asoprisnil the first observed compound with prominent PR agonistic effects [3,4], was used in a treatment study for women with fibroid disease and resulted in preventing endometrial antiproliferative effects in all subjects. In separate studies, women treated with Asoprisnil showed inhibition of menstruation irrespective of ongoing ovarian hormone secretion and ovulation, which demonstrates that the endometrium is the site of this antiproliferative effect [20]. More detailed studies revealed an arrest of endometrial mitotic activity [17], stressing the fact that the arrest of menstrual bleedings was only seen at antiovulatory doses of RU486. These two examples of using Asoprisnil and RU486 in clinical applications beyond pregnancy highlight the need to build up antiprogestins which can be modulated by shifting the pharmacodynamic ratio to produce desired agonistic and/or antagonistic effects.

2. Experimental

2.1. Chemistry

Nuclear magnetic resonance spectra were recorded on a Bruker ARX (300 MHz) spectrometer as deuterochloroform (CDCl₃) solutions 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, NJ. Thin-layer chromatography (TLC) analyses were carried out on silica gel GF (Analtech) glass plates (2.5 cm × 10 cm with 250 µM layer and pre-scored). Most chemicals and solvents were analytical grade and used without further purification. Commercial reagents were purchased from Aldrich Chemical Company (Milwaukee, WI). All experiments were carried out in oven dried glassware under inert atmosphere using dry nitrogen or argon gas.

2.1.1. 3,3-Ethylenedioxy- 5α ,17 β -dihydroxy-17-(3,3,3-trifluoro-1-propynyl)-11 β -{4'-[1',1'-ethylenedioxy)-ethyl]phenyl}-estr-9-ene (**4**)

n-Butyl lithium (55 mL, 2.5 N, 137.5 mmol) was added dropwise to a solution of di-isopropylamine (21.6 mL, 154 mmol) in THF (40 mL) at -78 °C under argon and the resulting LDA solution was stirred at -78 °C for 30 min. Separately, a solution of 2-bromo-3,3,3-trifluoropropene (12 g, 68.5 mmol) in THF (80 mL) was made, cooled to -78 °C and above prepared LDA was slowly added over a period of 20 min. After stirring for 15 min, a solution of 3,3-ethylenedioxy- 5α -hydroxy- 11β -{4'-[1',1'-(ethylenedioxy)-ethyl]phenyl}estra-9-ene-17-one (3) (6 g, 12.1 mmol) [21] in THF (80 mL) was added dropwise and stirred at -78 °C for 1 h and slowly allowed to warm to room temperature over 15 h. Reaction mixture was quenched with saturated aqueous ammonium chloride solution (50 mL) and extracted with ethyl acetate (3 \times 100 mL). The combined organic layers were washed with water and brine, dried over sodium sulfate, and evaporated in vacuo to afford the crude product. Purification was performed on a silica gel column using 25% ethyl acetate in hexane to afford 4 (5.0 g, 70%).

¹H 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).

¹³C 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.

2.1.2. 11β-(4'-Acetylphenyl)-17β-hydroxy-17-(3,3,3-trifluoro-1propynyl)-estra-4,9-diene-3-one (1a, EC301)

A solution of 3,3-ethylenedioxy- 5α ,17 β -dihydroxy-17-(3,3,3-trifluoro-1-propynyl)-11 β -{4'-[1',1'-(ethylenedioxy)-ethyl]phenyl}estr-9-ene (**4**) (3.5 g, 6 mmol) in methanol (35 mL) at 0 °C was treated dropwise with 50% sulfuric acid (2.2 mL) and was allowed to stir at room temperature for 2 h. The reaction mixture was carefully quenched with sodium bicarbonate solution (15 mL) and extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with water and brine, dried over sodium sulfate and evaporated *in vacuo* to afford the crude product. Purification was carried out on a silica gel column using 25% ethyl acetate in hexane to afford **1a**, EC301 (2.5 g, 87%).

¹H 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).

¹³C 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.

2.1.3. 3,3-*E*thylenedioxy- 5α -hydroxy- 17β -cyano- 17α -trimethylsilyloxy- 11β - $\{4'-[1',1'-(ethylenedioxy)-2'2'2-trifluoroethyl]phenyl\}$ -estr-9-ene (**6**)

Under argon, a few crystals of iodine were added to magnesium turnings (0.28 g, 11.5 mmol) in dry THF (11 mL) and the mixture was stirred until it turned colorless. A solution of 4'-bromo-trifluoroacetophenone ethylene glycol ketal [19] (3.32 g, 11.2 mmol) in dry THF (20 mL) was added and the reaction was refluxed for 1 h. The resulting Grignard solution was cooled in an ice bath and solid CuCl (0.33 g, 3.3 mmol) was added. After stirring at 0 °C for 30 min, a solution of 3,3-ethylenedioxy-5α,10α-epoxy-17β-cyano-17 α -trimethylsilyloxyestra-9(11)-ene (5) (2.45 g, 5.7 mmol) in dry THF (20 mL) was added and the mixture stirred at 0 °C for 1 h and at room temperature overnight. The reaction mixture was quenched with saturated ammonium chloride solution and extracted with ethyl acetate $(3 \times)$. The combined organic layers were washed once with saturated ammonium chloride solution, dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was purified on a silica column using 20% ethyl acetate in hexanes to afford 6 (2.8 g, 75%).

¹H NMR (δ , 300 MHz) 0.245 (s, 9H), 0.49 (s, 3H), 3.9–4.3 (m, 8H), 4.39 (d, *J* = 6 Hz, 1H), 4.44 (s, 1H), 7.24 (d, *J* = 9 Hz, 2H), 7.49 (d, *J* = 9 Hz).

2.1.4. 3,3-Ethylenedioxy- 5α -hydroxy- 11β -{4'-[1',1'-(ethylenedioxy)-2'2'2-trifluoroethyl]phenyl}-estr-9-en-17-one (**7**)

A solution of the Grignard adduct (6) (4.0 g, 6.2 mmol) in THF (15 mL) was treated dropwise with a solution of tetrabutylammonium fluoride in THF (1 M, 15.5 mL, 15.5 mmol) and the reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with water (50 mL) and extracted with ethyl acetate $(2\times)$. The combined organic fractions were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. The residue was dissolved in 20 mL of 10% aqueous methanol and 10 mL of CH₂Cl₂ and cooled in an ice bath. A solution of sodium hydroxide (2 M, 10 mL) was added and the reaction mixture stirred at 0 °C for 2 h. The reaction was diluted with water and extracted with methylene chloride $(2 \times)$ and the combined organic fractions were washed with water, dried over sodium sulfate and concentrated in vacuo. The residue was purified on a silica gel column using 30% ethyl acetate in hexanes followed by trituration with diisopropyl ether to give 7 (2.8 g, 82%).

¹H NMR (δ , 300 MHz) 0.45 (s, 3H), 3.96–4.39 (m, 9H), 7.24 (d, *J* = 9 Hz, 2H), 7.48 (d, *J* = 9 Hz).

2.1.5. 17β -Hydroxy- 17α -pentafluoroethyl- 11β -{4'-[1',1'-(ethylenedioxy)-2'2'2-trifluoroethyl]phenyl}-estrr-4,9-dien-3-one (**1b**, EC306)

Pentafluoroiodoethane (2.5 g, 10 mmol) was condensed into a solution of the 17-ketone (**7**) (0.49 g, 0.9 mmol) in dry toluene (18 mL) at -78 °C. A solution of methyl lithium lithium bromide in ether (1.5 M, 6 mL, 9 mmol) was added and the reaction mixture was stirred at -78 °C for 1 h and at 0 °C for another hour. The reaction was quenched with saturated ammonium chloride solution and extracted with ethyl acetate. The combined organic fractions were washed with water, brine, dried over sodium sulfated and concentrated *in vacuo*. The residue was taken up in methanol (10 mL), cooled to 0 °C and 50% sulfuric acid (0.4 mL) was added. The reaction was stirred at room temperature for 1 h, neutralized, extracted with ethyl acetate, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified over silica gel using 30% ethyl acetate in hexanes followed by crystallization from methanol to give **1b**, EC306 (0.38 g, 70%).

¹H NMR (δ , 300 MHz) 0.58 (s, 3H), 4.05–4.29 (m, 4H), 4.47 (d, *J* = 9 Hz, 1H), 5.79 (s, 1H), 7.20 (d, *J* = 7.5 Hz, 2H), 7.52 (d, *J* = 7.5 Hz).

2.1.6. 3,3-Ethylenedioxy-17 β -hydroxy-17-(2,3,3-trifluoroprop-2-enyl)-5(10),9(11)-estradiene (**9**)

A solution of 1,1,1,2-tetrafluoroethane (820 mg, 8 mmol) in ether (10 mL) was cooled to -78 °C as a 2.5 M solution of *n*-BuLi (2.4 mL, 6.1 mmol) was introduced over a period of 10 min and allowed to stir for 1 h at -78 °C. A solution of spiro-2'-(1'-oxacyc1opropane)-17(*S*)-[3,3-(ethylenedioxy)-5(10),9(11)-estradiene] **8** (1 g, 3.04 mmol) in ether (7 mL) was introduced, followed by boron trifluoride etherate (0.38 mL, 3.04 mmol) dropwise. The reaction mixture was stirred at -78 °C for 1 h and allowed to warm to room temperature over a period of 1 h. The reaction was quenched with sodium bicarbonate solution (20 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layer was washed further with water and brine, dried over sodium sulfate and evaporated *in vacuo* to afford the crude product. Purification was carried out on a silica gel column using 20% ethyl acetate in hexane to afford **9** (430 mg, 35%).

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

¹³C 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).

2.1.7. 3,3-*Ethylenedioxy-5α*,10*α*-epoxy-17*β*-hydroxy-17-(2,3,3-trifluoroprop-2-enyl)-estr-9(11)-ene (**10**)

Hydrogen peroxide (0.18 mL, 30%, 1.6 mmol) was added to an ice-cold solution of hexafluoroacetone trihydrate (350 mg, 1.6 mmol) in dichloromethane (3 mL). Solid Na₂HPO₄ (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-hy-droxy-17-(2,3,3-trifluoroprop-2-enyl)-5(10),9(11)-estradiene (9) (410 mg, 1 mmol) in dichloromethane (3 mL) was added and the 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) and washed with 10% sodium sulfite solution (15 mL), water, dried over sodium sulfate and concentrated under vacuum to obtain the mixture of crude epoxides. Separation of the isomeric epoxides was carried out on a silica gel column using 20% ethyl acetate in hexane to afford **10** (240 mg, 56%) of pure α -isomer.

 ^{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).

2.1.8. 3,3-Ethylenedioxy- 5α ,17 β -dihydroxy-17-(2,3,3-trifluoroprop-2-enyl)-11 β -{4'-[1',1'-(ethylenedioxy)-ethyl]phenyl}-estr-9-ene (**11**)

Magnesium turnings (220 mg, 9 mmol) in THF (10 mL) containing a crystal of iodine was heated to reflux for 10 min to become colorless. A solution of 2-(4-bromophenyl)-2-methyl-1,3-dioxolane (2.1 g, 8.5 mmol) in THF (5 mL) was introduced over a period of 5 min and allowed to reflux for 1 h. Reaction mixture was cooled under ice and solid CuCl (150 mg, 1.5 mmol) was added to it and continued to stir at 0 °C for 30 min. Finally a solution of 3,3-ethylenedioxy-5 α ,10 α -epoxy-17 β -hydroxy-17-(2,3,3-trifluoroprop-2-

envl)-estr-9(11)-ene (**10**) (730 mg, 1.7 mmol) in THF (5 mL) was added into the cuprate solution and allowed to stir for 2 h at 0 °C. The reaction was quenched with aqueous ammonium chloride solution (30 mL) and extracted with ethyl acetate (3×25 mL). The combined organic layers were washed further with water and brine, dried over sodium sulfate and evaporated *in vacuo* to afford the crude product. Purification was carried out on a silica gel column using 25% ethyl acetate in hexane to afford **11** (810 mg, 80%).

¹H 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).

2.1.9. 11β -(4'-Acetylphenyl)-17 β -hydroxy-17-(2,3,3-trifluoroprop-2enyl)-estra-4,9-diene-3-one (**1c**, EC308)

Compound **1c** was prepared by following exactly the procedure outlined for compound **1a** where the hydrolysis of **11** (1.5 g) was carried out using 50% sulfuric acid in methanol to give after work-up and purification **1c**, EC308 (1.1 g).

¹H 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).

¹³C 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.

2.1.10. 3,3-Ethylenedioxy-17β-hydroxy-17-(1,1,2,2,2-

pentafluoroethyl)-estra-5(10),9(11)-diene (**13**)

Pentafluoroiodoethane (4 g, 16 mmol) was condensed into a solution of 3,3-ethylenedioxyestra-5(10),9(11)-diene-17-one (**12**) (2.5 g, 8 mmol) in toluene (32 mL) at -78 °C, and stirred for 10 min. Methyl lithium lithium bromide solution in ether (1.5 M, 9.8 mL) was introduced dropwise and continued to stir at -78 °C for 1 h. Reaction mixture was warmed to 0 °C and stirred at that temperature for 1 h before quenching with saturated ammonium chloride solution. Extracted with ethyl acetate, the combined organic layer was washed once with brine, dried over sodium sulfate, concentrated under reduced pressure to obtain **13** (3.0 g, 85%), which was used for epoxidation without further purification.

¹H NMR (δ , 300 MHz) 0.93 (s, 3H), 1.2–2.8 (m, 18H), 3.98 (s, 4H), 5.6 (bs, 1H).

2.1.11. 3,3-*Ethylenedioxy*- 5α , 10α -epoxy- 17β -hydroxy-17-(1,1,2,2,2-pentafluoroethyl)-estra-9(11)-ene (**14**)

Following the procedure outlined for the synthesis of compound **10**, the epoxidation of **13** (3.0 g) was carried out using hydrogen peroxide and hexafluoroacetone in dichloromethane and gave after workup and purification **14** (1.8 g).

¹H NMR (*δ*, 300 MHz) 0.92 (s, 3H), 1.1–2.8 (m, 18H), 3.70–4.00 (m, 4H), 6.0 (bs, 1H).

¹³C NMR (75 MHz) 15.8, 22.8, 25.1, 25.2, 28.1, 31.8, 34.4, 35.4 (dd, *J* = 3.6, 8.1 Hz), 38.4, 40.3, 48.8, 49.0, 49.1, 60.1, 61.7, 64.1, 64.4, 84.2 (dd, *J* = 25.1, 21.5 Hz), 107.1, 117 (m), 122 (m), 126.86, 126.89, 135.1.

2.1.12. 3,3-Ethylenedioxy-5α,17β-dihydroxy-17-(1,1,2,2,2-

pentafluoroethyl)-11 β -[4'-(tert-butyldimethylsilyloxymethyl)phenyl]estr-9-ene (**15**)

Following the procedure outlined for the synthesis of compound **11**, the Grignard reaction of **14** (230 mg) was carried out using 4-(t-butyldimethylsilyloxymethyl) bromobenzene and magnesium and gave after workup and purification the required product **15** (340 mg).

¹H NMR (δ , 300 MHz) 0.08 (s, 6H), 0.53 (s, 3H), 0.93 (s, 9H), 1.2– 2.5 (m, 18H), 3.8–4.1 (m, 4H), 4.4 (bd, 1H), 4.70 (s, 2H), 7.0–7.2 (m, 4H).

2.1.13. 3,3-Ethylenedioxy- 5α ,17 β -dihydroxy-17-(1,1,2,2,2pentafluoroethyl)-11 β -[4'-(hydroxymethyl) phenyl]-estr-9-ene (**16**)

A solution of 3,3-ethylenedioxy-5 α ,17 β -dihydroxy-17-(1,1,2,2,2-pentafluoroethyl)-11 β -[4'-(*tert*-butyldimethylsilyloxymethyl)phenyl]-estr-9-ene (**15**) (340 mg) in THF (3 mL) was treated with TBAF (1.0 M, 1.3 mL) and stirred at r.t. for 2 h. Solvents were removed under reduced pressure and directly purified on a silica gel column using 60% ethyl acetate in hexane to afford **16** (210 mg, 75%).

2.1.14. 3,3-Ethylenedioxy- 5α , 17β -dihydroxy-17-(1,1,2,2,2-pentafluoroethyl)- 11β -(4'-formylphenyl)-estr-9-ene (**17**)

A suspension of 3,3-ethylenedioxy- 5α ,17 β -dihydroxy-17-(1,1,2,2,2-pentafluoroethyl)-11 β -[4'-(hydroxymethyl) phenyl]estr-9-ene (**17**) (210 mg), NMO (66 mg) and powdered 4 Å molecular sieve (190 mg) in dichloromethane (8 mL) was stirred at r.t., as TPAP (7 mg) was introduced as solid at once and stirred for 2 h. Pure product was obtained by directly passing the reaction mixture through a silica gel column using 30% ethyl acetate in hexane to afford 200 mg of **17**.

¹H NMR (δ , 300 MHz) 0.51 (s, 3H), 1.0–2.8 (m, 18H), 3.8–4.1 (m, 4H), 4.2–4.4 (m, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.78 (d, *J* = 8.3 Hz, 2H), 9.96 (s, 1H).

2.1.15. 17β -Hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)- 11β -{4'-[1'-hydroxy-2'2'2-trifluoroethyl] phenyl}-estra-4,9-dien-3-one (**18**)

A solution of the benzaldehyde substrate (17) (1.7 g, 3.05 mmol) in dry THF (6 mL) at 0 °C was treated with trifluoromethyl trimethylsilane (1.8 mL, 12.2 mmol) followed by a few drops of tetrabutylammonium fluoride in THF (1 M). The reaction was stirred from 0 °C to room temperature for 1 h, and then cooled back to 0 °C. A 10% H₂SO₄ solution (10 mL) was carefully added and the reaction was stirred overnight at room temperature. The reaction was neutralized with bicarbonate solution and extracted with dichloromethane. The combined organic extracts were dried over sodium sulfate and concentrated in vacuo. The residue was dissolved in THF (10 mL), treated with tetrabutylammonium fluoride in THF (1 M, 6 mL, 6 mmol) and stirred at room temperature for 1 h. The reaction was quenched with the addition of saturated ammonium chloride solution and extracted with ethyl acetate. After concentration in vacuo, the residue was purified over silica gel using 30% ethyl acetate in hexanes to give 18 (1.0 g, 58%).

¹H NMR (δ , 300 MHz) 0.58 (s, 3H), 4.49 (d, *J* = 9 Hz, 1H), 5.01 (m, 1H), 5.67 (s, 1H), 7.22 (d, *J* = 9 Hz, 2H), 7.38 (d, *J* = 9 Hz).

2.1.16. 17β -Hydroxy- 17α -(1,1,2,2,2-pentafluoroethyl)- 11β -4'-(2'2'2-trifluoracetyl)phenyl-estra-4,9-dien-3-one (**1d**, EC316)

A solution of the alcohol (**18**) (1 g, 1.77 mmol) in dichloromethane (15 mL) was cooled to 0 °C and treated successively with triethylamine (2.5 mL, 18 mmol) and a solution of pyridine sulfur trioxide complex (2.3 g, 14.4 mmol) in DMSO (8.6 mL). The reaction was allowed to warm to room temperature and stirred overnight. The reaction was cooled to 0 °C and quenched with the addition of saturated ammonium chloride (30 mL). The pH of the mixture was adjusted to ~4.0 with 10% H₂SO₄ and the mixture extracted with methylene chloride. The organic fractions were washed with saturated sodium bicarbonate solution, water and brine, combined, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified over silica gel using 30% ethyl acetate in hexanes followed by trituration with diisopropyl ether to give **1d** EC316 (0.9 g).

¹H NMR (δ , 300 MHz) 0.58 (s, 3H), 4.52 (d, *J* = 9 Hz, 1H), 5.82 (s, 1H), 7.40 (d, *J* = 6 Hz, 2H), 8.01 (d, *J* = 6 Hz).

2.1.17. 3,3-Ethylenedioxy-21,21-difluoro-17,23-epoxy-19,24-dinor-17α-chola-5(10),9(11),20-triene (**20**)

To a solution of diisopropylamine (0.95 mL, 6.8 mmol) in THF (10 mL) at -78 °C, n-BuLi (2.7 mL, 2.5 M, 6.8 mmol) was added and stirred for 30 min. A solution of diethyl difluoromethylphosphonate (1.1 mL, 6.8 mmol) in THF (10 mL) was added and stirred for 1 h at -78 °C. Finally, a solution of 3,3-ethylenedioxy-4',5'-dihydrospiro[estra-5(10),9(11)-diene-17_β,2'(3'H)-furan]-3'-one [1] (19, 1.0 g, 2.7 mmol) in THF (10 mL) was added dropwise, stirred for 30 min at -78 °C, slowly warmed to room temperature over a period of 1 h and allowed to reflux for 15 h. Reaction mixture was guenched with water (30 mL) and extracted with ethyl acetate $(3 \times 25 \text{ mL})$. The combined organic layers were washed once with brine, dried over sodium sulfate and evaporated in vacuo to afford the crude product. Purification was carried out on a silica gel column using 10% ethyl acetate in hexane to afford 400 mg (37%) of the required product (20).

¹H NMR (δ, 300 MHz) 0.83 (s, 3H), 1.1–2.9 (m, 20H), 3.60–3.95 (m, 2H), 3.96 (s, 4H), 5.49 (bs, 1H).

¹³C NMR (75 MHz) 13.8, 23.9, 24.6, 27.6, 28.4, 31.2, 31.4, 33.02, 36.9, 38.6, 41.3, 46.79, 46.82, 64.4, 64.5, 65.1, 93.6 (t, *J* = 3.9 Hz), 95.1 (dd, *J* = 17.9, 19.5 Hz), 108.1, 117.6, 126.1, 130.3, 136.5, 150.9 (dd, *J* = 282, 280 Hz).

2.1.18. 3,3-Ethylenedioxy-21,21-difluoro-5α,10α;17,23-bisepoxy-19,24-dinor-17α-chola-9(11),20-diene (**21**)

Hydrogen peroxide (0.18 mL, 30%, 1.6 mmol) was added to an ice-cold solution of hexafluoroacetone trihydrate (350 mg, 1.6 mmol) in dichloromethane (3 mL). Solid Na₂HPO₄ (180 mg, 1.3 mmol) was introduced and the reaction mixture was stirred for 1 h at 0 °C. An ice-cold solution of (**20**) (400 mg, 1 mmol) in dichloromethane (3 mL) was added and the 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) and washed with 10% sodium sulfite solution (15 mL), water, dried over sodium sulfate and concentrated under vacuum to obtain the mixture of crude epoxides. Separation of isomeric epoxides was carried out on a silica gel column using 10% ethyl acetate in hexane to afford 230 mg (55%) of pure α -isomer (**21**).

¹H NMR (δ, 300 MHz) 0.85 (s, 3H), 1.1–2.9 (m, 20H), 3.6–4.0 (m, 6H), 5.8–6.0 (m, 1H).

2.1.19. 3,3-Ethylenedioxy-21,21-difluoro- 5α -hydroxy- 11β -{4'-[1',1'- (ethylenedioxy)-ethyl]phenyl-} 17,23-epoxy-19,24-dinor- 17α -chola-9(10),20-diene (**22a**)

Magnesium turnings (85 mg, 3.5 mmol) in THF (5 mL) containing a crystal of iodine was heated to reflux for 10 min to become colorless. A solution of 2-(4-bromophenyl)-2-methyl-1,3-dioxolane (835 mg, 3.5 mmol) in THF (5 mL) was added to the reaction mixture and was allowed to reflux for 1 h. Reaction mixture was cooled under ice and solid CuCl (100 mg, 1.0 mmol) was added to it and continued to stir at 0 °C for 30 min. Finally a solution of (21) (480 mg, 1.15 mmol) in THF (5 mL) was added into the cuprate solution and allowed to stir for 2 h at 0 °C. guenched with saturated aqueous ammonium chloride solution (30 mL) and extracted with ethyl acetate $(3 \times 25 \text{ mL})$. The combined organic layers were washed further with water and brine, dried over sodium sulfate and evaporated in vacuo to afford the crude product. Purification was carried out on a silica gel column using 25% ethyl acetate in hexane to afford 350 mg (52%) of the required product (22a).

¹H NMR (δ , 300 MHz) 0.45 (s, 3H), 1.0–2.9 (m, 24H), 3.6–4.1 (m, 10H), 4.20 (d, *J* = 7.4 Hz, 1H), 4.39 (s, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 7.12 (d, *J* = 8.2 Hz, 2H), 7.26–7.32 (m, 2H).

¹³C NMR (75 MHz) 14.3, 23.2, 23.9, 24.1, 27.4, 28.2, 32.57, 32.60, 35.0, 38.2, 39.0, 39.4, 40.8, 47.3, 48.0 (t, J = 1.9 Hz), 49.48, 49.52, 64.04, 64.3, 64.4, 64.5, 64.6, 65.2, 70.2, 93.8 (t, J = 3.9 Hz), 94.4 (dd, J = 17.1, 19.8 Hz), 108.7, 108.8, 114.9, 125.1, 126.6, 126.9, 133.9, 134.3, 140.3, 146.5, 150.8 (t, J = 282 Hz), 156.1.

2.1.20. 21,21-Difluoro-11 β -(4'-acetyl)phenyl-17,23-epoxy-19,24-dinor-17 α -chola-4,9,20-triene (**2a**, EC307)

A solution of **22a** (350 mg, 0.6 mmol) in methanol (5 mL) at 0 °C was treated with 50% sulfuric acid (0.35 mL) and allowed to stir at room temperature for 2 h. The reaction mixture was carefully quenched with sodium bicarbonate solution (5 mL) and extracted with dichloromethane (3×15 mL). The combined organic layers were washed further with water and brine, dried over sodium sulfate and evaporated *in vacuo* to afford the crude product. Purification was carried out on a silica gel column using 25% ethyl acetate in hexane to afford 250 mg (88%) of the required product (**2a**, EC307).

¹H NMR (δ , 300 MHz) 0.52 (s, 3H), 1.1–2.9 (m, 19H), 2.57 (s, 3H), 3.70–4.0 (m, 2H), 4.37 (d, *J* = 7.2 Hz), 5.77 (s, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.87 (d, *J* = 8.1 Hz, 2H).

¹³C NMR (75 MHz) 14.6, 23.6, 25.9, 26.6, 27.5, 28.4, 31.05, 32.7, 32.8, 36.8, 39.1, 40.79, 40.84, 48.4, 49.67, 49.72, 65.4, 93.6 (t, *J* = 3.9 Hz), 94.7 (dd, *J* = 17, 20 Hz), 123.4, 127.3, 128.8, 130.0, 135.1, 144.4, 150.6, 151.0 (t, *J* = 282 Hz), 156.2, 197.6, 199.2.

2.1.21. 3,3-Ethylenedioxy-21,21-difluoro- 5α -hydroxy-11 β -[4'- (dimethylamino)-phenyl]-17,23-epoxy-19,24-dinor-17 α -chola-9(10),20-diene (**22b**)

Following the procedure outlined for the synthesis of compound (**22a**), the Grignard reagent prepared from 4-bromodimethylaniline was reacted with compound (**21**) and CuCl in THF to give after workup the required product (**22b**).

¹H NMR (δ , 300 MHz) 0.54 (s, 3H), 1.10–2.85 (m, 21H), 2.91 (s, 6H), 3.7–4.2 (m, 7H), 4.33 (s, 1H), 6.64 (d, *J* = 8.7 Hz, 2H), 7.02 (d, *J* = 8.6 Hz, 2H).

2.1.22. 21,21-Difluoro-11 β -[4'-(dimethylamino)phenyl]-17,23-epoxy-19,24-dinor-17 α -chola-4,9,20-triene (**2b**, EC310)

Following the procedure outlined for the synthesis of compound (**2a**), the Grignard adduct (**22b**) was hydrolyzed in 50% sulfuric acid to give after workup the required product (**2b**, EC310).

¹H NMR (δ , 300 MHz) 0.62 (s, 3H), 1.0–2.9 (m, 19H), 2.93 (s, 6H), 3.7–4.0 (m, 2H), 4.27 (d, *J* = 6.9 Hz, 1H), 5.76 (s, 1H), 6.66 (d, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 8.7 Hz, 2H).

2.1.23. 3,3-Ethylenedioxy-21,21-difluoro- 5α -hydroxy- 11β -(4'-isopropenylphenyl)-17,23-epoxy-19,24-dinor- 17α -chola-9(10),20-diene (**22c**)

Following the procedure outlined for the synthesis of compound (**22a**), the Grignard reagent prepared from 4-bromoisopropenylbenzene was reacted with compound (**21**) and CuCl in THF to give after workup the required product (**22c**).

¹H NMR (δ , 300 MHz) 0.52 (s, 3H), 1.0–3.0 (m, 21H), 2.14 (s, 3H), 3.6–4.0 (m, 6H), 4.23 (d, *J* = 7.1 Hz, 1H), 4.34 (s, 1H), 5.04 (d, *J* = 1.2 Hz, 1H), 5.38 (s, 1H), 7.14 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.3 Hz, 2H).

2.1.24. 21,21-Difluoro-11 β -(4'-isopropenylphenyl)-17,23-epoxy-19,24-dinor-17 α -chola-4,9,20-triene (**2c**, EC311)

Following the procedure outlined for the synthesis of compound (**2a**), the Grignard adduct (**22c**) was hydrolyzed in 50% sulfuric acid to give after workup the required product (**2c**, EC311). ¹H NMR (δ , 300 MHz) 0.58 (s, 3H), 1.0–2.9 (m, 19H), 2.14 (s, 3H), 3.18 (d, *J* = 30 Hz, 1H), 3.7–4.0 (m, 3H), 4.33 (d, *J* = 7.0 Hz, 1H), 5.06 (d, *J* = 1.3 Hz, 1H), 5.37 (s, 1H), 5.77 (s, 1H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.39 (d, *J* = 8.3 Hz, 2H).

2.1.25. 3,3-Ethylenedioxy-21,21-difluoro- 5α -hydroxy- 11β -(4'-bromophenyl)-17,23-epoxy-19,24-dinor- 17α -chola-9(10),20-diene (**22**f)

Following the procedure outlined for the synthesis of compound (**22a**), the Grignard reagent prepared from 1,4-dibromobenzene was reacted with compound (**21**) and CuCl in THF to give after workup the required product (**22f**).

¹H NMR (δ , 300 MHz) 0.50 (s, 3H), 1.0–2.9 (m, 21H), 3.7–4.0 (m, 7H), 4.17 (d, *J* = 7 Hz, 1H), 4.33 (s, 1H), 7.06 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H).

2.1.26. 21,21-Difluoro-11 β -(4'-bromophenyl)-17,23-epoxy-19,24-dinor-17 α -chola-4,9,20-triene (**2f**)

Following the procedure outlined for the synthesis of compound (**2a**), the Grignard adduct (**22f**) was hydrolyzed in 50% sulfuric acid to give after workup the required product (**2f**).

¹H NMR (δ , 300 MHz) 0.55 (s, 3H), 1.0–2.9 (m, 19H), 3.7–4.0 (m, 3H), 4.27 (d, *J* = 7.1 Hz, 1H), 5.77 (s, 1H), 7.03 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H).

¹³C NMR (75 MHz) 14.7, 23.6, 25.9, 27.5, 28.4, 29.2, 31.0, 32.8 (d, J = 3 Hz), 36.8, 39.0, 40.2, 40.8, 48.4, 49.72, 49.77, 53.5, 65.4, 93.7 (t, J = 4 Hz), 94.73 (dd, J = 17, 20 Hz), 119.7, 123.4, 128.8, 129.9, 131.8, 143.8, 144.6, 151.0 (t, J = 281 Hz), 156.2, 199.2.

2.1.27. 21,21-Difluoro-11β-[4'-(3'-pyridyl)phenyl]-17,23-epoxy-19,24-dinor-17α-chola-4,9,20-triene (**2d**, EC312)

One hundred milligram (0.2 mmol) of (**2f**), 3-pyridinylboronic acid (60 mg, 0.5 mmol), potassium carbonate (40 mg, 0.3 mmol), bis(triphenylphosphine)palladium(II) chloride (7 mg, 0.01 mmol) and triphenylarsine (7 mg, 0.02 mmol) were dissolved in a mixture of dioxane (5 mL) and water (1 mL) under a nitrogen atmosphere. The reaction mixture was stirred for 3 h at 100 °C and then cooled to room temperature. Water (15 mL) was added and the mixture was extracted twice with EtOAc (3×10 mL). The combined organic layers were washed with brine, dried (MgSO₄) and evaporated to dryness. Purification by column chromatography using 50% ethyl acetate in hexane afforded 75 mg (75%) of the required product (**2d**, EC312).

¹H NMR (δ , 300 MHz) 0.58 (s, 3H), 1.2–2.9 (m, 19H), 3.7–4.0 (m, 2H), 4.39 (d, *J* = 7.1 Hz), 5.77 (s, 1H), 7.1–7.4 (m, 3H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.8–7.9 (m, 1H), 8.5–8.6 (m, 1H), 8.82 (d, *J* = 1.7 Hz, 1H).

¹³C NMR (75 MHz) 14.6, 23.6, 24.7, 25.9, 27.5, 28.4, 31.1, 32.7 (d, *J* = 2.7 Hz), 36.7, 36.8, 39.0, 40.4, 40.7, 48.4 (t, *J* = 1.9 Hz), 49.7, 49.8, 65.3, 93.7 (t, *J* = 3.9 Hz), 94.7 (dd, *J* = 17, 20 Hz), 123.3, 123.6, 127.3, 127.8, 129.7, 134.2, 135.3, 136.1, 144.8, 145.0, 148.1, 148.3, 150.9 (t, *J* = 282 Hz), 156.3, 199.3.

2.1.28. 21,21-Difluoro-11β-[4'-(3'-furanyl)phenyl]-17,23-epoxy-19,24-dinor-17α-chola-4,9,20-triene (**2e**, EC313)

Following the procedure outlined for the synthesis of compound (**2d**), (**2f**), 3-furanylboronic acid, potassium carbonate, bis(triphenylphosphine)palladium (II) chloride and triphenylarsine were reacted in a mixture of dioxane and water to give, after purification, the required product (**2e**).

¹H NMR (δ , 300 MHz) 0.58 (s, 3H), 1.1–2.9 (m, 19H), 3.6–4.0 (m, 2H), 4.34 (d, *J* = 7.1 Hz, 1H), 5.77 (s, 1H), 6.67 (t, *J* = 0.9 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 2H), 7.45 (t, *J* = 1.7 Hz, 1H), 7.70 (s, 1H).

¹³C NMR (75 MHz) 14.6, 23.6, 25.8, 27.5, 28.3, 31.0, 32.7 (d, *J* = 2.6 Hz), 36.8, 39.0, 40.4, 40.7, 48.4 (t, *J* = 1.9 Hz), 49.7, 49.8,

65.3, 93.7 (t, *J* = 3.9 Hz), 94.7 (dd, *J* = 17, 19.7 Hz), 108.7, 123.1, 126.0, 126.1, 127.4, 129.5, 130.0, 138.4, 143.4, 143.7, 145.3, 150.1 (t, *J* = 281.5 Hz), 156.4, 199.3.

2.2. Biological assays

2.2.1. Molecular studies

In vitro studies were performed by Invitrogen Corporation, Madison. Nuclear receptor studies use beta-lactamase cDNA under transcriptional control of an upstream activator sequence (UAS). Details of the respective screenings are as follows.

Test compounds were supplied at $1000 \times \text{ of the desired starting}$ concentration (usually 100 nM) in 100% DMSO. The $1000 \times \text{ test}$ compounds were serially diluted (10 point ½-log increments) in 100% DMSO. An aliquot of the serial dilution is diluted 1:100 in Assay Media to a $10 \times \text{ concentration}$. The $10 \times \text{ concentration}$ is then added to the assay plate where the addition of other assay reagents dilutes the compounds to a final concentration of $1 \times$ in the assay with a final DMSO.

2.2.2. PR-agonist screen

PR-UAS-bla HEK 293T cells are thawed and resuspended in assay media (DMEM phenol red free, 2% CD-treated FBS, 0.1 mM NEAA, 1 mM sodium pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 468,750 cells/mL. Four microliters of a 10× serial dilution of R5020 (control agonist starting concentration, 100 nM) or compounds are added to appropriate wells of a 384well TC-treated assay plate. Thirty-two microliters of cell suspension (15,000 cells) is added to each well. Four microliters of assay media is added to all wells to bring the final assay volume to 40 µL. The plate is incubated for 16–24 h at 37 °C/5% CO₂ in a humidified incubator. Eight microliters of 1 µM substrate loading solution is added to each well and the plate is incubated for 2 h at room temperature. The plate is read on a fluorescence plate reader.

2.2.3. PR-antagonist screen, activated by R5020

PR-UAS-bla HEK 293T cells are thawed and prepared as described above for the agonist screen. Four microliters of a 10× serial dilution of RU-486 (control antagonist starting concentration, 100 nM) or compounds are added to appropriate wells of a TC-treated assay plate. Thirty-two microliters of cell suspension is added to the wells and pre-incubated at 37 °C/5% CO₂ in a humid-ified incubator with compounds and control antagonist titration for 30 min. Four microliters of 10× control agonist R5020 at the pre-determined EC80 concentration is added to wells containing the control antagonist or compounds. The plate is incubated for 16–24 h at 37 °C/5% CO₂ in a humidified incubator. Eight microliters of 1 μ M substrate loading solution is added to each well and the plate is incubated for 2 h at room temperature. The plate is read on a fluorescence plate reader.

2.2.4. GR antagonist screen activated by dexamethasone

GR-UAS-bla HEK 293T cells are thawed and resuspended in assay media (DMEM phenol red free, 2% CD-treated FBS, 0.1 mM NEAA, 1 mM sodium pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. Four microliters of a 10× serial dilution of RU486 (control antagonist starting concentration, 10 nM) or compounds are added to appropriate wells of a TC-treated assay plate. Thirty-two microliters of cell suspension is 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. Four microliters of 10× control agonist dexamethasone at the pre-determined EC80 concentration is added to wells containing the control antagonist or compounds. The plate is incubated for 16–24 h at 37 °C/5% CO₂ in a humidified incubator. Eight microliters of 1 µM substrate loading solution is added to each well and the plate is incubated for 2 h at room temperature. The plate is read on a fluorescence plate reader.

2.2.5. The guinea pig model for the assessment of PR-agonistic and antagonistic properties of PRMs

The animal studies were performed in Dr. Robert Garfield Lab, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA, after approval (no. 369) by the local ethical committee. Dunkin-Hartley guinea pigs (400–500 g body weight) were purchased from Charles River Laboratory. Animals were kept in an automatically climatized (21 °C) and illuminated (12:12 light–dark cycle) facilities. Tap water was available ad libitum from sipper tubes; the provided pelleted food was fortified with vitamin C and supplemented with fruits (oranges).

The studies were performed in cycling guinea pigs for the specific assessment of both PR-agonistic and PR-antagonistic properties and the interaction of corresponding properties. The studies were performed in the second half of the guinea pig cycle which is about 16 days long. The treatment was from cycle day 10–17 by daily s.c. injection of test compounds in 0.2 mL of the vehicle (0.2 mL benzylbenzoate/castor oil, ratio 1:4, v/v). Control animals were treated with 0.2 mL vehicle. The time of autopsy (day 18) is 24–48 h after the expected ovulation of the next cycle. This timing permits the study of the effects of the tested compounds on the ovulation and on the functional state of the old corpora lutea. Fresh corpora lutea confirm normal ovulation.

Typically, "pure" PR-antagonists lead to a proliferation and cornification of the vaginal epithelium. An advanced stage of shedding of the cornified layers of the vaginal epithelium prevails in ovulating controls at this stage of cycle (metestrus).

2.2.5.1. Assessment of unopposed estrogenic effects. Vaginal proliferation and cornification reflect the unopposed effects of the basal ovarian estrogen secretion of the ovary-intact animals. Uterine growth may occur, but is sometimes missing under such pure antagonists. These indicators of estrogen dominance may occur despite the presence of very high levels of progesterone in the circulation, which is brought about by the maintenance of corpora lutea ("antiluteolytic effect" of pure PR-antagonists) [25].

2.2.5.2. Assessment of PR-agonistic properties. Corresponding properties lead to an interference with the proliferation and cornification of the vaginal epithelium and induce a morphological change, the mucification of vaginal epithelium.

Marginal PR-agonistic effects: Reduction or inhibition of the vaginal cornification in presence of an ongoing proliferation of the basal squamous vaginal epithelium. *Pronounced effects:* absence of cornification, reduction or complete inhibition of epithelial proliferation. Simultaneously, a columnar epithelium of typical mucified cells appears. This is seen as a marker of a PR-mediated agonistic effect. The mucified epithelium may cover the surface of an abnormally branching layer of ceratinized cells. Similarly, the upper layer of squamous (non-cornifying) epithelium may be covered by a layer of mucified cells. As ultimate inhibitory step in this direction, a complete arrest of proliferation in the vaginal epithelium may be seen. This may then consist of a single layer of columnar mucified epithelial cells (see Figs. 8–10).

2.2.5.3. Effects on ovulation. Antiovulatory effects, absence of fresh CL on cycle day 19, may result from both PR-agonistic and PR-antagonistic properties.

2.2.5.4. Effects on old corpora lutea. The complete abolition of luteal regression is a reliable indicator of "pure" PR-antagonism; (undisturbed) degeneration of CL and no formation of new CL indicate a potential mesoprogestin.

2.3. Evaluation of uterine weight changes and bio-statistical analysis

Effects on uterine weights of hormonal active substances were compared to vehicle controls. Whereas a significant fold-change of weight could be demonstrated for RU486, EC306, and EC308, the uterine weights observed for all other substances were in the range of the vehicle control. Data were log-transformed for analysis. Effects are expressed as fold-change on the original scale. Multiple comparisons to the vehicle group were based on an ANOVA and the Bonferroni–Holm adjustment [26] controlling the experiment-wise error level <5%.

2.4. Screening data

In a screening study, substances were compared to a vehicle control. This experiment should help distinguish between substances that do increase uterine weight and those that do not. The data show heterogenous variances; therefore, we decided to perform all analyses on log-scale of weight, thereby achieving more homogenous variances. Axes in plots show log-scale. Sample sizes are in a range of 3–11, with the largest size used for the vehicle control.

3. Results and discussion

3.1. Chemistry

Compound **1a** was synthesized according to Fig. 4 starting from intermediate **3** [21]. Treatment of 2-bromo-3,3,3-trifluoropropene

with 2 equiv. of LDA generated the required 3,3,3-trifluoropropynyllithium at -78 °C which was then added to **3** to obtain **4**. Acid hydrolysis of **4** affords compound **1a** (EC301, Fig. 4).

As shown in Fig. 5, reaction of intermediate **5** [22] with the Grignard reagent generated from the ketal of α , α , α -trifluoro-4-bro-moacetophenone gave the adduct **6**. Fluoride ion removal of the TMS ether along with base hydrolysis of the intermediate cyanohydrin gave intermediate **7**. Acid hydrolysis of **7** affords compound **1b** (EC306).

Opening of epoxide **8** [23] with trifluorovinyllithium (generated from 1,1,1,2-tetrafluoroethane and *n*-BuLi at -78 °C) in presence of boron trifluoride etherate afforded **9**. Subsequent epoxidation, conjugate Grignard addition, and hydrolysis yielded **1c** (EC308) (Fig. 6).

As shown in Fig. 7, addition of pentafluoroethyl lithium to compound **12** was carried out to give compound **13**. Subsequent epoxidation and aryl Grignard addition yielded **15**. Removal of silyl protecting group using TBAF, followed by oxidation using TPAP/ NMO resulted in the required benzaldehyde **17**. Nucleophilic trifluoromethylation using trifluoromethyl trimethylsilane followed by removal of the TMS protecting group gave **18**. Oxidation using pyridine sulfur trioxide yielded **1d** (EC316).

The intermediate 17-spirodihydrofuran-3'(2'H)-one **19** was synthesized following the procedure of Jiang et al. [24]. Subsequent Wittig–Horner reaction with the reagent prepared from difluoromethyldiphenylphosphine oxide gave the exocyclic-difluoromethylene intermediate **20**. Subsequent epoxidation, conjugate Grignard addition, and hydrolysis gave the targeted compounds **2a–2c** (EC307, EC310, and EC311). Palladium-mediated Suzuki



Fig. 4. Synthesis of compound 1b (EC306).



Fig. 5. Synthesis of compound 1c (EC308).



Fig. 7. Syntheses of compounds 2a, 2b, 2c, 2d and 2e (EC307, EC310, EC311, EC312, and EC313).

coupling of compound **2f** with the appropriate boronic acid yielded **2d** (EC312) and **2e** (EC313).

3.2. Biological assays

3.2.1. In vitro studies

For the determination of the dissociation between antiglucocorticoid and antiprogestational activity transactivation studies were performed. RU486 served as standard substance for the antiglucocorticoid and antiprogestational activity and R5020 was used as standard for the progestational activity. Data are reported in relative % compared to standard (see Table 1).

All compounds with the 17-difluoro exomethylene tetrahydro furan moiety (307, 310, 311, 312, 313) showed a very high activity in the antiprogestational test exceeding the activity of RU486 up to nearly 4-fold. In addition, all these compounds exhibit a rather low

Table 1

Progesterone and glucocorticoid receptor profiling.

CMPD	Invitrogen receptor profiling						
	Progesterone	Glucocorticoid					
	Agonist (Rel to R5020)	Antagonist (Rel to RU486)	Antagonist (Rel to RU486)				
EC301	<2.85%	<6.45%	ND				
EC306	<2.85%	20.87%	11.66%				
EC307	<2.85%	243.4%	<5.83%				
EC308	<2.85%	20.67%	<5.83%				
EC310	<0.28%	383.7%	14.1%				
EC311	ND	165.4%	ND				
EC312	<0.28%	243.9%	26.6%				
EC313	<0.28%	79.1%	6.4%				
EC316	ND	16.13%	ND				

activity in the antiglucocorticoid assay, indicating a 10-fold better dissociation between antiprogestational and antiglucocorticoid activity. Fluorination in other positions of the steroid led to significantly lower activity in the antiprogestational assay compared to RU486. The dissociation between antiprogestational and antiglucocorticoid binding was also less pronounced.

3.2.2. In vivo characterization

3.2.2.1. PR-Ranking. Ranking of PRM-compounds concerning their ratio of PR-antagonistic and PR-agonistic properties was done by an orienting testing using a single very high dose (10.0 mg/ani-mal/day s.c. days 10–17, and autopsy day 18) in cycling guinea pigs: Table 2 summarizes the results of the test compound.

The evaluation of a single high dose of the test compounds led in all cases to distinct and compound-specific results, reflecting the ratio of PR-agonistic and antagonistic in the plateau of the dose-response curve.

3.2.2.2. Histology of the ovaries, corpora lutea. Of eight vehicle-treated control animals, seven had fresh corpora lutea (CL) in their ovaries on day 18 of the treatment cycle, which confirms reliable control of the cycle in the laboratory and normal ovulation in these animals.

Table 2

Molecular properties of new PRM and orientating study concerning the ratio of PR-agonistic and antagonistic properties at a single high dose (10.0 mg/day s.c.) in cycling guinea pigs.

Code of substance	Rate of ovulation	ut weight mean (g)	ER-PR-balance: proliferation, cornification versus mucification of vagina epithelium	Ovary/corpora lutea	Classification
EC301	0/3	1.3	prol ++ corn + muc (+)	(lf CL)	Almost pure antiprogestin
EC306	1/3	2.16*	prol+ corn absent muc +	lf CL	State of vagina, uterus and CL inconsistent-mesoprogestin
EC307	0/8	1.26	prol (+) corn absent muc+	deg CL	Mesoprogestin
EC308	0/3	1.8*	prol ++ corn (+)	(lf CL)	Mesoprogestin
EC310	1/3	1.37	prol + corn (+)	deg CL	Mesoprogestin
EC311	0/3	0.94	prol ++ corn+	deg CL	Blunted antiprogestin
EC312	0/8	1.03	prol absent corn absent	deg CL	Mesoprogestin, complete suppression of proliferation
EC313	0/8	1.13	prol (+) corn absent	deg CL	Mesoprogestin, almost complete suppression of proliferation
EC316	0/3	2.35*	prol ++ corn++ muc absent	(lf CL)	Almost pure antiprogestin
CDB2914	0/3	1.17	prol ++ corn+ muc (+)	(lf and deg CL)	Blunted antiprogestin
CDB4124	1/3	1.41	prol ++ corn ++ muc absent	(lf and deg CL)	Almost pure antiprogestin
RU486	0/3	1.83*	prol ++ corn ++	(lf CL)	Blunted antiprogestin
Onapr.	0/3	0.98	prol ++ corn ++	lf CL	Pure antiprogestin
controls	7/8	1.05	nuc absent prol + corn absent (metestrus) muc absent	fresh CL	-

Abbreviations: CL, corpora lutea; lf, large and functional; deg, degenerating.

Vag, vaginal epithelium; prol, proliferation of basal layers; corn, cornification; muc, mucification of upper layer; ut, uterus; n.t., not tested; (+)-++ indicate presence and dominance degree of feature in the vaginal histology.

* statistical significant difference.

With the exception of single animals, all tested compounds prevented ovulation. Old CL showed a substance-specific histological appearance. Some compounds led to the maintenance of large and apparently functional CL (Onapristone, EC306). Under other substances, a degeneration of CL or its complete disappearance took place, which indicates the completion of luteolysis as in the normal ovulatory cycle (see Table 2).

3.2.2.3. Uterine weight. Some compounds elevated the uterine weight dramatically. This increase versus controls was statistically significant (p < 0.05) for EC306, EC308, EC316, and RU486. Significant reductions of the uterine weight were not recorded. In extended studies (data not shown) including lower doses, uterine weight tended to be lower at higher doses of the following substances: EC307, EC312, and EC313. However, these differences were not statistically significant.

3.2.3. Histology of the vaginal mucosa (see Table 2 and Figs. 8-10)

Very different states of the vaginal mucosa and compound-wise transitions from ER- to PR-dominance were seen. *Pure PR-antago*-

nists: Strong proliferation of the basal squamous cell layer and a thick cornified upper without any signs of mucification were seen after treatment with EC316, CDB4124, and Onapristone. RU486 induces strong proliferation and cornification of the vaginal epithelium; however, the mucification of the upper layers of the vaginal epithelium indicates a disturbance of the cornification process by PR-agonistic activity. RU486 may thus not be classified as "pure" antagonist in this animal model.

3.2.3.1. Diminished ER-dominance. Compounds EC301, EC308, EC310, EC311, and CDB2914 led to a reduced and/or atypical cornification of the vaginal epithelium. The response varies among the animals; e.g., one animal of the EC308 group lacks vaginal cornification (see Fig. 11), contrary to the others in this group. After treatment with EC306, EC307, EC312, EC313, no induction of an estrogen dominated vagina, specifically no induction of cornification of the upper layers of the epithelium, which is typical of antiprogestins, is seen.

Marginal estrogenic effects prevail under most of these compounds, as indicated by basal layers of proliferating squamous cells

Fig. 8. Histology of vagina, day 18 of treatment cycle, dose cycle day 10–17, reference substances; (a) RU486 and (b) CDB2914, 10.0 mg/animal/day s.c.: proliferation of basal and cornification of upper epithelial layers indicate largely unopposed indirect estrogenic effects. Mucified cells on the surface of epithelium probably reflect an interference of PR-agonistic properties of both compounds.

Fig. 9. Histology of the vagina in cycling guinea pigs on day 18 of the treatment cycle, dose cycle day 10–17: 10.0 mg/day s.c.: (a) EC316, full unopposed indirect estrogenic effect with proliferation and cornification of the upper cell layers. Gradually blunted estrogenic effects in case of (b) EC306, (c) EC301, and (d) EC310. *Note:* Compound-related reduction of cornified cell layer compared to (a) and the appearance of mucified epithelial cells as upper layer (c and d).



Fig. 10. Histology of the vagina in cycling guinea pigs on day 18 of the treatment cycle, dose cycle day 10–17: (a) EC308, 10.0 mg/day s.c. and (b) EC313, 10.0 mg/day s.c.: mucification, no cornification, almost complete suppression of basal proliferation; (c) EC312 10.0 mg/day s.c.: no cornification, complete suppression of basal cell proliferation, formation of a mucified epithelium; (d) control at metestrous, epithelium after desquamation of cornified layers.

under a superficial layer of mucified epithelial cells (see Figs. 9 and 10).

Under EC308 and EC313 this basal proliferation was largely suppressed, under EC312 a complete suppression of basal proliferation prevails (EC312 see Fig. 10).

4. Discussion

Antiprogestins abolish inhibitory functions of the PR by acting on the proliferation inducing effects of estrogens in the genital tract. This inhibition is sufficient to induce massive estrogenic effects in the vagina leading to proliferation and the ceratinization of the vaginal epithelium reaching or exceeding the degree of stimulation which is normally seen at proestrus. Within the uterus a proliferation of uterine tissues and uterine growth may occur (see Tables 2 and 3). In the presence of the ovaries, despite the inhibition of follicular maturation and ovulation, a basal ovarian estrogen secretion may be sufficient for these growth events (see Table 4).

With regard to the therapeutic intention to block ovulation for all kinds of indications, fibroid disease, endometriosis, contraception, the issue of endometrial safety in humans has to be considered seriously. Considering the powerful estrogenic effects of pure PR-antagonists in some animal species and those of RU486 in some clinical studies [27] there appears to be an urgent need to shape PRMs in such a way, that the proliferation inducing potential of this class of compounds, via the ER, is controlled by the introduction of a PR-agonistic component. PRMs with substantial PR-agonistic properties for the arrest of endometrial proliferation, like EC312 and EC313, fulfill all criteria of such a mesoprogestin. By definition a mesoprogestin should be characterized by a reduced or absent abortifacient potential when compared to RU486. These studies remain to be done for the selected clinical candidates.

PRM for labor induction: It was shown in prior studies that the elevated responsiveness of the myometrium in pregnancy to pros-



Uterus weights and standard deviations (*Comment:* EC313 (EC303?) and EC 315 missing; check Meister's data).



Table 4

Statistical evaluation of uterus weights: multiple comparisons of the substances to the vehicle control. Raw and adjusted *p*-values are displayed, with sig = 1 indicating significant differences. Non-adjusted confidence intervals are provided for the fold-change of weight in comparison to the control group.

Substance	p Value	p-Adjust	Sign	l.CL	Fold	Up. CL
CDB.2914	0.42468	1.00000	0	0.80	1.20	1.60
CDB.4124	0.06528	0.84859	0	1.00	1.40	2.00
EC.301	0.16090	1.00000	0	0.90	1.30	1.80
EC.306	0.00096	0.01535	1	1.30	1.90	2.60
EC.307	0.72662	1.00000	0	0.70	1.10	1.50
EC.308	0.00280	0.03926	1	1.20	1.70	2.50
EC.310	0.11283	1.00000	0	0.90	1.30	1.90
EC.311	0.40999	1.00000	0	0.70	0.90	1.20
EC.312	0.88308	1.00000	0	0.80	1.00	1.30
EC.316	0.00005	0.00089	1	1.50	2.20	3.10
RU486	0.00160	0.02403	1	1.30	1.80	2.60
ZK98.299	0.74701	1.00000	0	0.70	1.00	1.30

taglandins results in an immediate effect. Otherwise inactive doses of a prostaglandin may lead to an immediate onset of labor and expulsion of the conceptuses [19]. Without prostaglandin supplementation, the onset of labor is inconsistent, and may be delayed by several days. The onset of labor in the latter case is likely a spontaneous event secondary to the compromise of the feto-placental unit. Pure PR antagonists being far superior to RU486 with respect to labor induction in combination with and without a prostaglandin. RU486 and other clinically tested substances appear not optimal for corresponding pregnancy-related indications.

5. Conclusion

The objective of the research described in this paper is an attempt to differentiate new PR-modulators with respect to the presence or absence of PR-agonistic properties. A continuum may be established of compounds from pure PR-agonist, via graded mixed agonists/antagonists to pure PR-antagonists. The current efforts focused on the mixed agonist/antagonists and antagonists. Two beneficial clinical applications are for seen. First, pure PR antagonists being far superior to RU486 with respect to labor induction in combination with a prostaglandin (**18**). Secondly, agonistic PRMs that inhibit ovulation are promising agents for the treatment of various gynecological disorders and perhaps for an estrogen-and bleedingfree approach to hormonal fertility control.

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