Synthesis of 11β-(4-dimethylaminophenyl)-17β-hydroxy-17α-(3-methyl-1-butynyl)-4, 9-estradien-3-one and 11β-(4-acetophenyl)-17β-hydroxy-17α-(3-methyl-1-butynyl)-4, 9-estradien-3-one: two new analogs of mifepristone (RU-486)

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Abstract

From the structure activity relationship, two new analogs, 2 and 3, of the potent progesterone antagonist mifepristone 1 have been designed. The syntheses of these two analogs have been achieved in eleven steps through modified synthetic sequences and improved procedures starting from (1)-estrone. In comparison with mifepristone 1, the relative binding affinities of compound 2 for the progesterone receptor was found to be more, whereas that of compound 3 was less. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

In 1980, the French pharmaceutical company Roussel–Uclaf announced the discovery [1,2] of RU-486, now known by the generic name mifepristone (1). This was the first antiprogestin to be developed. Mifepristone (1), when used in combination with a prostaglandin, effectively and safely terminates early pregnancies. It is already in use [1,2] as an abortifacient in France, UK, Sweden, and China, has passed the clinical trials in USA, and is under clinical trials in India. It is also a potent antiglucocorticoid and shows promising activity in treating estrogen-dependent gynecological disorders and hormone deficient tumors.

Over the years, a number of mifepristone analogs have been synthesized [3–6] and were tested for their antiprogestin activity. Teutsch et al. [7] have synthesized a variety of 11β-substituted 19-norsteroids. Among these compounds, 11β-vinyl and 11β-phenyl compounds showed a high degree of binding affinity for the cytoplasmic uterine progesterone receptor, whereas the 11β-allyl and 11β-benzyl-19-norsteroids showed very low binding affinity. Thus, shifting the unsaturation by one carbon atom practically eliminates the receptor binding affinity. High binding affinity of the 11β-19-norsteroid led to the hypothesis that in the region corresponding to the 11β-position of the steroids, a pocket exists in the progesterone receptor, where hydrophobic interactions are probably involved (Scheme 1).

A very recent study by Spitz and Agranat [8] revealed that the 17α-substituent imparts higher binding affinity for the receptor and the substituent at 11β-position is responsible for its antagonistic action. It has also been reported [9] that replacement of the 11β-(dimethylaminophenyl) substituent with the 11β-(acetophenyl) moiety increases the relative binding affinity for the progesterone receptor in rat and lowers the relative binding affinity for the glucocorticoid receptor than that of mifepristone (1). In fact, the most active compound in this series, ORG
33628, has been claimed to be 32× as active as mifepristone in inducing abortions in rats, while being significantly less active as an antiglucocorticoid [8]. Compound ORG 33628, is a 17-spirofuran, 11β-(4-acetophenyl) analog of mifepristone.

In anticipation of changing the relative binding affinity for the receptor, we planned to incorporate an isopropyl group in place of methyl (e.g. 3-methyl-1-butynyl side chain), at 17α-position and successfully achieved the synthesis of a new analog (2) of mifepristone (1). Furthermore, with this new 17α-alkynyl moiety in compound (2), 4-acetophenyl group was introduced in the 11β-position to afford another new analog (3) of mifepristone (1). Modification of both the active sites (at 17α and 11β) are expected to bring about a cumulative effect on the receptor binding affinity as well as antiglucocorticoid activity of compound (3).

Herein, we wish to report, the syntheses of two new analogs, (2) and (3), of mifepristone (1), starting from (+)-estrone. Alteration of reported [6] synthetic sequences furnished the target compounds (2) and (3) in higher overall yields. Moreover, introduction of 3-methyl-1-butynyl moiety at 17α-position by nonhazardous modified method and a highly chemoselective epoxidation of 5(10)-olefin with a catalytic amount of hexafluoroacetone in H2O2 are the salient features of these syntheses. The structure of compound (2) was confirmed by a single crystal X-ray.

2. Experimental

All solvents and reagents used were of commercial grade. Dry tetrahydrofuran (THF) was freshly obtained by taking the liquid to reflux under nitrogen, in a recirculation still over sodium. Pyridine was purified by distillation and stored over KOH pellets. Reactions were monitored by TLC using TLC aluminum sheets, silica gel 60F254 precoated, Merck, Germany, and locating the spots spraying with ethanolic solution of phosphomolybdic acid followed by heating. Usual work-up means the organic extract was thoroughly washed with water and brine and finally dried over anhydrous sodium sulfate. -IR: Perkin–Elmer 599B. -1H NMR: Bruker AC 200 (200 MHz). For 1H NMR, CDCl3 as solvent and TMS as an internal standard and J values are given in Hz. -MS: Finnigan 1020C (70eV). -[α]D: JASCO-181 (4893 0 A). [α]D values are given in 10-1 deg cm2/g, m.p. (uncorrected): Yanaco Micro m.p. apparatus.

2.1. Estra-5 (10), 9(11)-dien-3-one, 17β-hydroxy-17α-(3-methyl-1-butynyl) cyclic 3-(1,2-ethanediyl acetal) (5)

In a 25-ml two-necked flask equipped with a septum and a nitrogen assembly, 3-methyl-1, 1-dibromobut-1-en (0.454 g, 2 mmol) in dry THF (5 ml) was placed under nitrogen. The solution was cooled to −78°C and to it was added n-BuLi (2 ml, 2 M solution in hexane). The reaction mixture was then slowly brought to −40°C and stirred at that temperature for 2 h. After this period, it was again cooled to −78°C and the 17-keto compound 4 (0.314 g, 1 mmol) in THF (3 ml) was added dropwise to it and was stirred for a further 8 h at 25°C. It was then quenched with ice-cold saturated ammonium chloride solution and the mixture was extracted with ethyl acetate (4 × 50 ml). After usual work up solvent was removed under vacuo to afford gum (0.432 g). Column chromatographic purification over silica gel by using hexane/ethyl acetate (9:1) as eluent furnished compound 5 as colorless foam (0.271 g, 71%), m.p. 56–58°C; [α]30 = +128.35° (c 0.79 in CHCl3); -IR (nujol) ν 3410 cm−1(OH); 1H NMR (CDCl3): δ = 0.85 (s, 3H, 18-H3), 1.15 (d, 6H, J = 6, isopropyl CH3), 2.46–2.75 (m, 2H, 3H, 17αCH3) Hz, 2.46–2.75 (m, 2H, 3H, 17αCH3).
2.2. *Estra-5α,10α-epoxy-9 (11)-en-3-one, 17β-hydroxy-17α-(3-methyl-1-butynyl) cyclic 3-(1,2-ethanediyl acetal) (6)*

Compound 5 (0.095 g, 0.25 mmol) was dissolved in CH₂Cl₂ (3 ml) and the solution was cooled to 0°C. Added to it was Na₂HPO₄ (0.090 g, 0.63 mmol), followed by hexafluoracetone trihydrate (0.013 g, 0.059 mmol) and 44.6% H₂O₂ (0.0076 g, 0.225 mmol). The reaction mixture was then slowly brought to 25°C and was stirred at that temperature for 3 h. It was then diluted with CH₂Cl₂ and to it was added 10% sodium thiosulfate solution (1 ml), stirred for an additional 30 min. The reaction mixture was extracted with CH₂Cl₂ (3 ml) and brine (25 ml), washed with water (3 ml) and dried over anhydrous sodium sulfate. Removal of solvent under vacuo afforded a gum (0.102 g) that was chromatographed over silica gel by purification over silica gel using ethyl acetate/hexane (2:8) as an eluent afforded compound 6 (0.048 g, 49%) as colorless crystalline solid, m.p. 178°C (from hexane/ethyl acetate); [α]²ο = -5.6 (c 0.56 in CHCl₃); -IR (nujol) γ 3410 cm⁻¹ (OH); -¹H NMR (CDCl₃): δ = 0.82 (s, 3H, 18-H), 1.16 (d, 6H, J = 6, isopropyl -CH₃), 2.41–2.65 (m, 2H, 4-H), 3.95 (m, 6H, ketal -CH₂), 6.1 (bd, 1H, 11-H); -MS (70 eV): m/z (%) 398 (M⁺), 383 (M⁺⁻CH₃), 337, 312, 297, 223, 141, 129, 59 (100%); -C₂₅H₃₄O₄ (398.55): calculated C 75.34, H 8.6; Found C 75.5, H 8.9.

2.3. *11β-[4-(N,N-dimethylamino)-phenyl]-17α-(3-methyl-1-butynyl)-estra-9-en-5α,17β-diol-3-one, cyclic 1,2-ethanediyl acetal (7)*

In a 25-ml two-necked flask equipped with a septum and a nitrogen assembly, Mg (0.053 g, 2.2 mmol) was placed to it 4-bromo-N,N-dimethylaniline (0.455 g, 2.27 mmol) in THF (1 ml) was introduced with a syringe. The mixture was heated at 45–50°C for 2 h, and during this period, all Mg disappeared, resulting a yellowish solution. In another two-necked flask, the α-epoxide 6 (0.074 g, 0.18 mmol) and CuI (0.0057 g, 0.03 mmol) in THF (4 ml) were placed under nitrogen and cooled to 0°C. To it the preformed Grignard reagent was added dropwise. The resulting reaction mixture was slowly brought to 25°C and was stirred at that temperature for a period of 6 h. It was then quenched with cold saturated NH₄Cl solution and extracted with diethyl ether (4 × 25 ml). The ether extract was worked up in the usual way. Removal of solvent under reduced pressure furnished a gum (0.103 g) that was chromatographed over silica gel to afford 7 (0.074 g, 77%) as a solid, m.p. 103°C (from hexane/ethyl acetate); -IR (nujol) γ 3460 cm⁻¹ (OH), 1510 (aromatic); -¹H NMR (CDCl₃): δ = 0.59 (s, 3H, 18-H), 1.19 (d, 6H, J = 7, isopropyl CH₃), 1.25 (m, 1H, isopropyl -CH), 2.90 (s, 6H, N, N-CH₃), 3.96 (m, 4H, ketel -CH₂), 4.26 (d, 1H, J = 5, 11-H), 6.66 (d, 2H, J = 10, Ar-H₂), 7.05 (d, 2H, J = 10, Ar-H₂); -C₂₃H₄₅NO₄ (519.70) calculated C 76.26, H 8.73; Found C 76.43, H 8.6.

2.4. *11β-[4-(N,N-dimethylamino)-phenyl]-17α-(3-methyl-1-butynyl)-estra-4,9-dien-17-one (2)*

The solution of the hydroxy ketal 7 (0.074 g, 0.142 mmol) in 70% AcOH (0.5 ml) was heated at 50°C for 2 h. It then was cooled and neutralized with cold aqueous NaHCO₃. Usual work up followed by removal of solvent under vacuum afforded a yellow gum (0.087 g) that was chromatographed over silica gel by using hexane/ethyl acetate (6:4) as an eluent to furnished 2 (0.045 g, 69%), as a solid, m.p. 178°C (from hexane/diethyl ether); [α]²ο = +126.25° (c 0.48 in CHCl₃); -IR (nujol) γ 3145 (OH), 1652 (-C = O), 1517 (aromatic); -¹H NMR (CDCl₃): δ = 0.56 (s, 3H, 18-H), 1.22 (d, 6H, isopropyl -CH₃), 2.95 (s, 6H, N, N-CH₃) 4.37 (d, 1H, J = 5, 11-H) 5.78 (s, 1H, 4-H), 6.67 (d, 2H, J = 8, Ar-H₂), 7.04 (d, 2H, J = 8, Ar-H₂); -MS (70 eV): m/z (%) 457 (M⁺), 439 (M⁺⁻H₂O), 121 (100%); -C₁₃H₁₇NO₂ (457.63); calculated C 81.36, H 8.59; Found C 81.3, H 8.7.

2.5. *11β-[2-methyl-2-phenyl-1,3-dioxalan]-17α-(3-methyl-1-butynyl)-estra-9-en-5α,17β-diol-3-one, cyclic 1,2-ethanediyl acetal (9)*

In a two-necked flask equipped with a septum and a nitrogen assembly, Mg (0.036 g, 1.5 mmol) was placed to it 2-methyl-2-(bromophenyl)-1,3-dioxalan 8 (0.318 g, 1.5 mmol) in THF (1 ml) was introduced. The reaction mixture was heated at 45 to 50°C for 2 h, and during this time, all Mg disappeared, resulting in a dark-colored solution. In another flask, compound 6 (0.120 g, 0.3 mmol) and CuI (0.0057 g, 0.03 mmol) in THF (4 ml) were placed under nitrogen and cooled to 0°C. To it the dark-colored solution of the Grignard reagent was added dropwise. The resulting reaction mixture was slowly brought to 25°C and was stirred at that temperature for a period of 6 h. It then was quenched with cold saturated NH₄Cl and extracted with diethyl ether (4 × 25 ml). Usual work up of the organic extract and removal of solvent under reduced pressure afforded a gum (0.318 g) that on column chromatographic purification over silica gel using ethyl acetate/hexane (2:8) as an eluent afforded compound 9 as solid (0.130 g, 77%); m.p. 173–174°C (from hexane/diethyl ether); [α]²ο = -46.1° (c 0.36 in CHCl₃); -IR (nujol) γ 3400 (-OH); -¹H NMR (CDCl₃): δ = 0.52 (s, 3H, 18-H), 1.2 (d, 6H, J = 6, isopropyl -CH₃), 1.65 (s, 3H, acetophenyl CH₃), 3.65–4.05 (m, 8H, ketal CH₂), 4.3 (bd, 1H, J = 5, 11H), 7.16 and 7.35 (AB pattern, J = 10, Ar-H); -MS (70eV): m/z (%) 562 (M⁺), 544 (100%), 434, 366, 191, 178, 133, 105; -C₂₃H₂₆O₂ (562.72); calculated C 74.7, H 8.7.
2.6. 11β-(4-acetophenyl)-17α-(3-methyl-1-butynyl)-estr-4, 9-dien-17β-ol-3-one (3)

A solution of the hydroxy ketal 9 (0.038 g, 0.067 mmol) in 70% AcOH (0.5 ml) was heated at 50 to 60°C for 2 h. The solution was then cooled and neutralized with cold aqueous NaHCO₃ solution. The resultant gummy mass was extracted with CH₂Cl₂ (3 × 25 ml), washed thoroughly with water (3 × 25 ml) and brine (2 × 25 ml), and was dried over anhydrous sodium sulfate. Removal of solvent afforded a yellowish gum (0.062 g) that was chromatographed over silica gel to get pure compound 3 (0.026 g, 84%) as foam, m.p. 97–100°C; [α]₅₀⁻⁵₁ 86.25° (c 0.26 in CHCl₃); -IR (nujol) cm⁻¹ 3459 (-OH), 1665 (-C=O); 1H NMR (CDCl₃) δ 0.49 (s, 3H, 18-H₃), 1.2 (d, 6H, J 6, isopropyl-CH₃), 2.6 (s, 3H, acetophenyl CH₃), 4.49 (bd, 1H, J = 5, 11-H), 7.3 and 7.9 (AB pattern, 4H, J = 10, Ar-H); -MS (70 eV): m/z (%) 439 (M⁺-OH), 413, 395, 346, 236, 147, 91, 67 (100%); C₃₁H₃₆O₃ (456.60): calculated C 81.54, H 7.95; Found C 81.3, H 8.1.

3. Results and discussion

Syntheses of our two new analogs 2 and 3 of mifepristone 1 start from estra-5 (10), 9 (11)-dien-3,17-dione, cyclic-3-(1,2-ethanediyl acetal) 4 [10,11] (Scheme 2). Introduction of a 3-methyl-1-butynyl side chain at 17α orientation of compound 4 was tried with 3-methylbut-1-yne [12] and n-butyl lithium in THF at temperatures ranging from −78°C to 25°C. The reaction furnished a complex mixture of products from which we failed to isolate the desired product 5. We then turned our attention at an efficient procedure for the generation of 3-methyl-1-butynyl-lithium in situ in the reaction mixture. This was accomplished by the reaction of 3-methyl-1, 1-dibromobut-1-en in THF with 2 equivalents of n-BuLi in hexane. 3-methyl-1, 1-dibromobut-1-en was prepared by the reaction of CBr₄ with isobutyaldehyde in the presence of triphenylphosphine by a reported [13] procedure. Alkynylation of 4 with 3-methyl-1-butynyl lithium proceeded smoothly to give the 17α-alkynylated compound 5 in 71% yield as a foam. Chemoselective epoxidation of the 5 [10] double bond of 5 was achieved with H₂O₂ and a catalytic amount of hexafluoroacetone trihydrate to get a mixture of 5, 10α-epoxide 6 and 5, 10β-epoxide in good yield and in the ratio of 4:1. The isomeric ratio was assigned from the integration of the 200 MHz ¹H NMR spectrum of the epoxides that showed two signals at 6.1 and 5.9 ppm for the C-11 hydrogen of the α and the β isomer respectively. The combination of hexafluoroacetone with H₂O₂ produces 2-hydroperoxyhexafluoro-2-propanol, a reactive oxidizing agent [14] possessing con-

Scheme 2.
siderably selectivity, particularly in the oxidation of sterically hindered olefins. Moreover, the byproduct of epoxidation, 2-hydroxyhexafluoro-2-propanol, readily disproportionates with H₂O₂ to regenerate 2-hydroxyhexafluoro-2-propanol, thereby implementing a simple catalytic cycle. The required 5, 10α-epoxide 6 was isolated by column chromatography over silica gel in 49% yield as a solid. The 11β-(4-dimethylyaminophenyl) substituent was introduced by SN₂ opening of the oxirane ring of compound 6 with the Grignard reagent prepared from 4-bromo-N.N-dimethyl aniline in presence of a catalytic amount of Cu(I) iodide. The 11β-substituted compound 7 was obtained as solid in 77% yield. 4-Bromo-N.N-dimethyl aniline was prepared [15] by the regioselective monobromination of N,N-dimethylaniline with 2, 4, 4, 6-tetrabromo-2, 5-cyclohexadien [16]. 11β Stereocchemistry at C-11 is evident from the large shielding effect in the proton resonance of the C-18 methyl group. In the 11-unsubstituted compound 6, the 18-methyl signal comes at 0.82 ppm whereas, introduction of 11β-aryl moiety, shields the 18-methyl signal to 0.59 ppm in compound 7. Deketalization and simultaneous dehydration of 7 with 70% AcOH gave the target compound 2 in 69% yield as a pale yellow crystalline solid. The structure of compound 2 was finally confirmed by single crystal X-ray analysis.

For the synthesis of 11β-(acetophenyl)-17β-hydroxy-17α-(3-methyl-1-butynyl)-esta-4, 9-dien-3-one 3, the 5, 10α-epoxide 6 was utilized (Scheme 2). 2-Methyl-2-(4-bromophenyl)-1, 3-dioxalan 8 was prepared from 4-bromoacetophenone by ketalization with ethylene glycol in the presence of catalytic amount of PTSA, which in turn was prepared from bromobenzene by Fridel Craft acylation with acetic anhydride [17]. The Grignard reagent prepared from 8 reacted with the 5, 10α-epoxide 6 in S_N_2 mode, in presence of a catalytic amount of CuL, to furnish after column chromatographic purification the 11β-substituted steroid 9 in 72% yield as a solid. Deketalization of 9 and concomitant dehydration with 70% AcOH afforded the 11β-(4-acetophenyl) substituted steroid 3 as a foam in 84% yield. This new mifepristone analog 3 was fully characterized by IR, ¹H NMR, and mass and elemental analysis.

3.1. Relative binding affinities of compounds 1, 2, and 3 for progesterone receptors

Competitive binding assay for progesterone receptors (PR) was performed using rabbit uterine cytosol and ¹H progesterone (radioligand) in the presence of unlabelled cortisol at 4°C. Competitor dilutions were prepared in DMF: Tris-HCl buffer pH 7.4 (1:1). Results calculated as percent relative binding affinity for PR, revealed that compound 2 was the most active. Compound 1 showed RBA (103%) almost equivalent to that of progesterone (100%; Table 1). Compound 2 showed an RBA of 175%. Compound 3 showed 35% RBA that is approximately 1/5 of that of compound 2. As compared to compound 1 i.e. mifepristone, compound 2 is more active whereas compound 3 is less active. The activity of progesterone is taken as 100%.

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