Synthesis of 6,19-cyclopregnanes. Constrained analogues of steroid hormones†

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A procedure for the synthesis of 6,19-cyclopregnanes is described involving an intramolecular alkylation reaction of Δ^4-3-keto steroids with a 19-mesylate in the presence of KOH in isopropanol. Three 6,19-cyclopregnanes were prepared (**4**, **5**, **9**); in the rat, 6,19-cycloprogesterone (**4**) and its 21-hydroxy derivative **5** displaced [^{3}H]-dexamethasone from glucocorticoid receptors, the former compound being more active. Both compounds did not compete with [^{3}H]-aldosterone for kidney mineralocorticoid receptors nor with [^{3}H]-R5020 for uterus progesterone receptors.

Introduction

Glucocorticoids are a class of stress-induced, endogenously synthesized steroid hormone molecules. Synthetic glucocorticoids constitute an important group of compounds widely used for the treatment of inflammatory disorders, autoimmunity and cancer.[1] At the cellular level, they exert their activity upon binding to the glucocorticoid receptor (GR), a member of a protein superfamily of closely related intracellular receptors, which acts by activating or repressing the expression of target genes.[1–3] The structural similarity among some of these receptors originates cross-reactions and as a consequence, partial overlapping of biological and pharmacological properties of the respective steroidal ligands. Opposing conformational characteristics for glucocorticoids and mineralocorticoids have been described by Weeks *et al.*, who used X ray diffraction to demonstrate that optimal glucocorticoid properties could be obtained with steroids exhibiting a twisted A ring towards the alpha face of the steroid nucleus.[4] Previous work from our group has shown that 21-hydroxy-6,19-epoxyprogesterone (**1**) is a highly selective antiglucocorticoid devoid of mineralocorticoid and progestational activities (Fig. 1).[5] In Δ^4-steroids as **1**, the 6,19-epoxy bridge bends the steroid skeleton at the A–B ring junction stabilizing the quasi-*cis* conformation with an inverted ring A 1β half chair, a structural characteristic also present in the antiglucocorticoid and antiprogestagen RU-486 (mifepristone, **2**).[6]

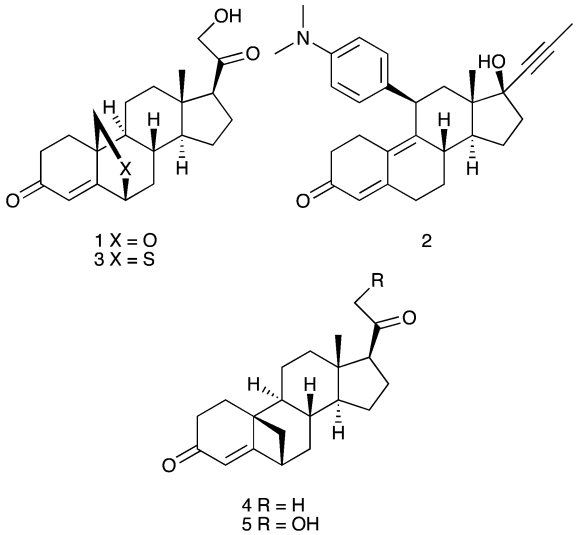

Fig. 1 Examples of antiglucocorticoids (**1–3**) and structures of 6,19-cyclopregnanes (**4**, **5**).

The crystal structure of the glucocorticoid receptor ligand binding domain in complex with RU-486, shows the latter molecule with its ring A exaggeratedly bent towards the alpha face, in a distorted conformation that closely matches that of compound **1**.[7] However, at variance with RU-486, which is a flexible molecule, 21-hydroxy-6,19-epoxyprogesterone has a rigid structure that locks the conformation of ring A. Replacement of oxygen with sulfur in the 6,19-bridge results in a slightly more flexible, less bent molecule, 6,19-sulfur-bridged pregnanes as in 21-hydroxy-6,19-epithioprogesterone (**3**) have antiglucocorticoid activities similar to **1**, although with an increased potency and selectivity.[8]

In the search for new conformationally restricted analogues with "specific" glucocorticoid or antiglucocorticoid activities, we prepared 6,19-cycloprogesterone (**4**) and its 21-hydroxy derivative **5**, anticipating that the increased tension introduced by the 4-membered ring would result in more bent and rigid structures.

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Table 1 Formation of 6,19-cyclopregnanes from 19-mesyloxy-Δ^4-3-ketopregnanes

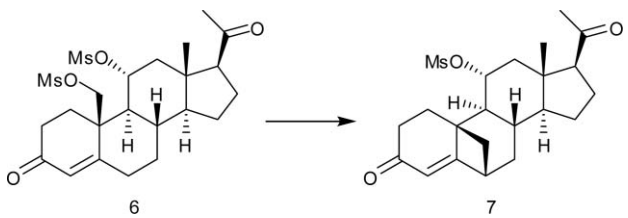
Entry	Steroid	Base (equiv.)	Time/h	Product (yield%)[a]
1	**6**	Na_2S–isopropanol (6)	8	**7** (30)
2	**6**	Na_2S–DMF (15)	24	**7** (20)
3	**6**	KOH–isopropanol (5)	3	**7** (53)
4	**10**	KOH–isopropanol (5)	1.5	**4** (74)
5	**11**	KOH–isopropanol (5)	2	**5** (60)

[a] Yields correspond to isolated products purified by flash chromatography on silica gel (ethyl acetate–hexane).

Results and discussion

Chemistry

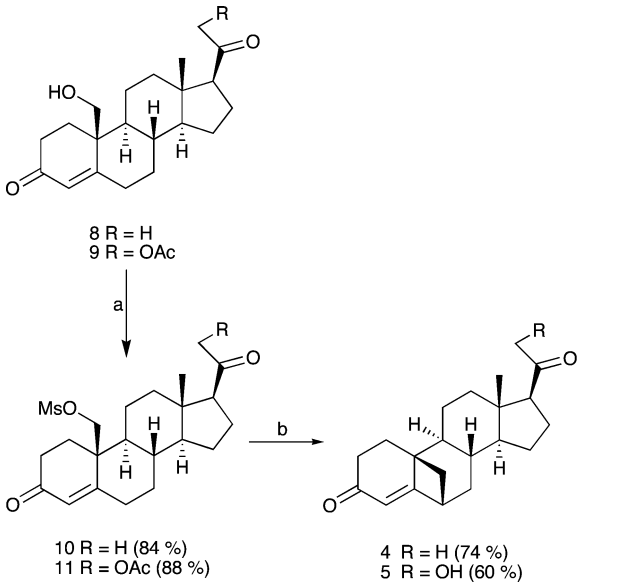
The strategy followed to obtain 6,19-cyclosteroids was based on our initial observation, while attempting to prepare an 11,19-epithiopregnane by reaction of dimesylate **6** with sodium sulfide in i-PrOH or DMF leading to 6,19-cyclopregnane **7** in low yield (Scheme 1; Table 1, entries 1 and 2). The 6,19-cyclopregnane **7** was formed, presumably, by the intramolecular alkylation of the thermodynamically controlled enolate derived from the Δ^4-3-keto moiety with the 19-mesylate. Use of KOH in i-PrOH as a base led to shorter reaction times and an increased yield of cyclized product (Table 1, entry 3).



Scheme 1 *Reagents and conditions:* see Table 1.

In the ^{1}H NMR of **7**, the absence of the low field AB quartet characteristic of the 19-CH_2OMs, indicated the loss of the 19-mesylate group of compound **6**. This was in agreement with the presence of a singlet at δ 3.01 corresponding to only one mesylate group (11-OMs). In the COSY 45 spectrum, the signal at δ 3.22 assigned to H-6 showed correlation peaks with resonances at δ 1.55 (H-19b), 2.13 (H-7β) and 2.54 (H-19a). Highly diagnostic for this structure were the correlations observed in the COSY LR spectrum which revealed long-range connectivities between H-4 (δ 5.65) and the hydrogens at positions 6 (δ 3.22), 19a (δ 2.54) and 2α (δ 2.29). The HSQC spectra showed correlation of the carbon at δ 32.3 (C-19) with H-19a (δ 2.54) and H-19b (δ 1.55) and of the carbon at δ 43.1 (C-6) with H-6 (δ 3.22).

Under similar cyclization conditions, mesylates **10** and **11** afforded cyclosteroids **4** and **5** respectively (Scheme 2; Table 1, entries 4 and 5). Mesylates were obtained by treatment of the corresponding 19-hydroxy steroids (**8** and **9**) with methanesulfonyl chloride in pyridine. Spectral data (1D and 2D NMR, HRFABMS) of compounds **4** and **5** were in agreement with the proposed structures. The structure of **4** was further confirmed by single crystal X-ray diffraction (Fig. 2). 6,19-Cycloprogesterone (**4**) crystallized in two well defined conformations with ring A as a 1β envelope, and differing slightly in the degree of bending at the



Scheme 2 *Reagents and conditions:* (a) MsCl, py, 3 h, 0 °C; (b) KOH, i-PrOH, 90 min, 25 °C.

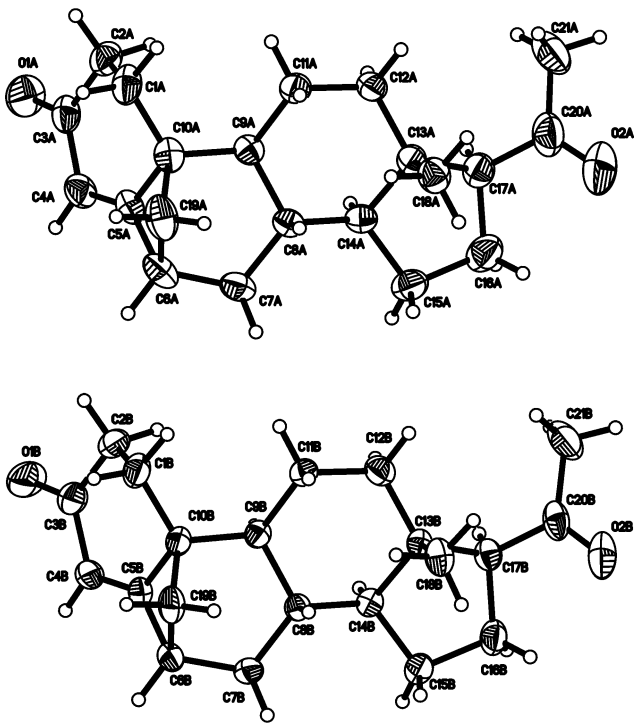


Fig. 2 Displacement ellipsoid diagrams (30% probability level) for both independent moieties of 6,19-cycloprogesterone (**4**), showing the numbering scheme used.

A–B ring junction. Interestingly, these two conformations were almost identical to those observed for 6,19-epoxyprogesterone[8] but at variance with this compound, AM1 calculations predicted the more bent structure of **4** to be more stable.

Receptor binding studies

Fig. 3 shows the binding properties of 6,19-cycloprogesterone (**4**) and its 21-hydroxy derivative **5**, to the glucocorticoid receptor from

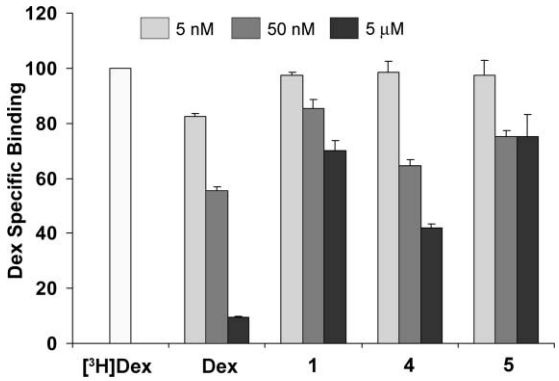


Fig. 3 [3H]-dexamethasone (Dex) displacement assays on glucocorticoid receptor from rat liver. Competition was measured by displacement of 5 nM [3H]-dexamethasone with unlabelled competitors: dexamethasone (Dex), compounds **4** and **5**. Data for 21-hydroxy-6,19-epoxyprogesterone (**1**) is included for comparison purposes. Each treatment was done in triplicate. Means ± SD from a representative experiment ($n = 3$) are shown.

rat liver compared to 21-hydroxy-6,19-epoxyprogesterone (**1**). No binding was observed in competition assays with [3H]-aldosterone for the mineralocorticoid receptor (from rat kidney) nor with [3H]-R5020 for the progesterone receptor (from rat uterus).[9,10] These results show that the selectivity towards the glucocorticoid receptor associated with the bent structure with an inverted A ring half chair is maintained.

Most interesting is the fact that while the 21-deoxy analogue of **1** (6,19-epoxyprogesterone) has no affinity for the glucocorticoid receptor,[5] the non-hydroxylated analogue **4** has higher activity than its 21-hydroxy counterpart **5**. This activity increase should originate in the structural characteristics of the 6,19-cyclo bridge (smaller steric bulk, increased bending of ring A) and not in mere changes in lipophilicity or hydrogen bond acceptor capacity associated with the removal of the oxygen bridging atom, as replacing the oxygen bridge in 6,19-epoxyprogesterone by a methylene group (*e.g.* 6,19-methanoprogesterone) did not increase the affinity for the glucocorticoid receptor.[11]

Experimental

Melting points were taken on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded in thin films using KBr disks on a Nicolet Magna IR 550 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 500 at 500.13 and 125.77 MHz in deuterochloroform. Chemical shifts are given in ppm downfield from TMS as internal standard, J values are given in Hz. Multiplicity determinations and 2D spectra were obtained using standard Bruker software. EIMS were collected on a VG Trio-2 mass spectrometer at 70 eV by direct inlet, HRFABMS were measured on a VG-ZAB mass spectrometer. Single crystal X-ray measurements were performed on a Bruker SMART CCD diffractometer, with graphite monochromated Mo K_α radiation. The structure was solved by direct methods with SHELXS97[12] and refined by full matrix least squares in F^2 using SHELXL97.[13] Hydrogen atoms were idealized at their expected positions (C–H: 0.93 Å; C–H_2: 0.97 Å; C–H_3: 0.96 Å) and allowed to ride. Molecular plots were drawn with XP, in the SHELXTL-NT package.[14] Crystallographic data (excluding structure factors) for the structure reported in this paper has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 622907.‡ Vacuum liquid chromatography (VLC) and column flash chromatography were carried out on Kieselgel 60-G (Merck) and Kieselgel S 0.040–0.063 mm respectively. TLC analysis was performed on silica gel 60 F254 (0.2 mm thick). The homogeneity of all compounds was confirmed by thin layer chromatography. Solvents were evaporated at reduced pressure and *ca.* 40–50 °C. 19-Hydroxyprogesterone (**8**) and 19-hydroxy-21-acetyloxyprogesterone (**9**) were obtained from pregnenolone acetate and 21-acetyloxypregnenolone respectively (Steraloids Inc.), following essentially the procedures described by Kirk *et al.* [15] Mesylate **6** was obtained from 11α-hydroxyprogesterone *via* 11α-acetyloxy-6,19-epoxyprogesterone[16] (see ESI†). In all cases, iodobenzene diacetate (DIB) was used instead of lead tetraacetate for functionalization of C-19.[17]

11-Methanesulfonyloxy-6,19-cyclopregn-4-ene-3,20-dione 7

Mesylate **6** (0.025 g, 0.05 mmol) was dissolved in a solution of KOH (0.014 g, 0.25 mmol) in isopropanol (2.0 cm^3). The reaction mixture was stirred at room temperature for 90 min and then concentrated to a third of its volume. Water was added to the residue and then extracted with dichloromethane. The organic layer was washed with water, dried with sodium sulfate and the solvent evaporated under vacuum. The resulting solid was purified by flash chromatography (silica gel, EtOAc–hexane 7 : 3) to give compound **7** (0.011 g, 53%) as a white solid: mp 145–146 °C (from methanol); ν_{max} (KBr) (cm^{-1}) 2939, 2876, 1701, 1655, 1339, 1173, 914; δ_H(500.13 MHz, $CDCl_3$; Me_4Si) 5.65 (1 H, s, 4-H), 5.10 (1 H, m, 11β-H), 3.22 (1 H, t, J 5.4, 6-H), 3.01 (3 H, s, 11-OMs), 2.85 (1 H, dd, J 12.0 and 4.9, 12β-H), 2.55 (1 H, m, 1α-H), 2.56 (1 H, t, J 9.2, 17-H), 2.54 (1 H, d, J 9.8, 19a-H), 2.45 (1 H, ddd, J 17.5, 14.5 and 4.3, 2β-H), 2.29 (1 H, dt, J 17.5 and 2.9, 2α-H), 2.21 (1 H, m, 16β-H), 2.13 (1 H, m, 7β-H), 2.13 (3 H, s, 21-H), 2.12 (1 H, m, 9-H), 2.08 (1 H, m, 8-H), 1.85 (1 H, dd, J 14.4 and 4.3, 1β-H), 1.80 (1 H, m, 16α-H), 1.74 (1 H, t, J 11.0, 12α-H), 1.68 (1 H, m, 15α-H), 1.59 (1 H, m, 7α-H), 1.55 (1 H, dd, J 9.8 and 5.4, 19b-H), 1.47 (1 H, m, 14-H), 1.29 (1 H, m, 15β-H), 0.78 (3 H, s, 18-H); δ_C(125.77 MHz, $CDCl_3$; Me_4Si) 208.0 (C-20), 199.0 (C-3), 178.6 (C-5), 112.2 (C-4), 79.5 (C-11), 62.3 (C-17), 53.7 (C-14), 52.2 (C-9), 50.3 (C-10), 46.9 (C-12), 45.0 (C-13), 43.1 (C-6), 39.8 (11-OMs), 34.6 (C-2), 32.6 (C-7), 32.3 (C-19), 32.0 (C-1), 31.5 (C-8), 31.2 (C-21), 23.3 (C-16), 23.0 (C-15), 14.7 (C-18); m/z (EI) 406 (M^+, 1), 311 (3), 310 (11), 267 (7), 43 (100); m/z (HRFAB) 407.1872 ($M^+ + H$) $C_{22}H_{31}O_5S$ requires 407.1892.

19-Methanesulfonyloxypregn-4-ene-3,20-dione 10

Methanesulfonyl chloride (0.191 g, 1.67 mmol) was added to a stirred solution of 19-hydroxyprogesterone **8** (0.100 g, 0.30 mmol) in dry pyridine (3.0 cm^3) at 0 °C under nitrogen. After 3 hours, the reaction mixture was acidified with 1 N HCl, and extracted with dichloromethane. The organic layer was washed with 5% aqueous $NaHCO_3$ and water, dried with sodium sulfate, and the solvent was evaporated. The residue was purified by flash chromatography

‡ CCDC reference number 622907. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b706828j

(ethyl acetate–hexane 7 : 3) to give **10** (0.102 g, 84%) as a white solid: mp 137–138 °C (from hexane–EtOAc); (found: C, 64.7; H, 7.9. $C_{22}H_{32}O_5S$ requires C, 64.7; H 7.9%); ν_{max}(KBr)/cm^{-1} 2941, 1699, 1668, 1355, 1174 and 957; δ_H(500.13 MHz, $CDCl_3$; Me_4Si) 5.97 (1 H, s, 4-H), 4.60 (1 H, d, *J* 9.8, 19a-H), 4.39 (1 H, d, *J* 9.8, 19b-H), 3.00 (3 H, s, 19-OMs), 2.12 (3 H, s, 21-H), 0.69 (3 H, s, 18-H); δ_C(125.77 MHz, $CDCl_3$; Me_4Si) 208.8 (C-20), 198.5 (C-3), 163.8 (C-5), 127.1 (C-4), 70.6 (C-19), 63.1 (C-17), 56.4 (C-14), 53.7 (C-9), 43.6 (C-13), 41.8 (C-10), 38.7 (C-12), 37.5 (19-OMs), 36.0 (C-8), 34.2 (C-2), 32.8 (C-6 or C-1), 32.7 (C-1 or C-6), 31.9 (C-7), 31.3 (C-21), 24.0 (C-15), 22.7 (C-16), 21.5 (C-11), 13.3 (C-18); *m/z* (EI) 408 (M$^+$, 21), 312 (12), 269 (5), 241 (2), 43 (100).

6,19-Cyclopregn-4-ene-3,20-dione 4

Cyclopregnane **4** was obtained from mesylate **10** (0.021 g, 0.05 mmol), following the procedure described for compound **7**. The resulting solid was purified by flash chromatography (EtOAc–hexane 7 : 3) to give **4** (0.012 g, 74%) as white crystals: mp 108–110 °C (from dioxane), ν_{max}(KBr)/cm^{-1} 2945, 2862, 1701, 1662, 1356, 1190; δ_H(500.13 MHz, $CDCl_3$; Me_4Si) 5.59 (1 H, s, 4-H), 3.20 (1 H, t, *J* 5.7, 6-H), 2.55 (1 H, t, *J* 6.8, 17-H), 2.45 (1 H, d, *J* 9.2, 19a-H), 2.26 (2 H, m, 2-H), 2.19 (1 H, m, 16β-H), 2.15 (1 H, m, 1α-H), 2.12 (1 H, m, 12β-H), 2.12 (3 H, s, 21-H), 2.09 (1 H, dd, *J* 12.8 and 6.5, 7β-H), 1.94 (1 H, m, 8-H), 1.85 (1 H, m, 1β-H), 1.80 (1 H, m, 9-H), 1.73 (1 H, m, 11α-H), 1.69 (1 H, m, 16α-H), 1.65 (1 H, m, 15α-H), 1.55 (1 H, t, *J* 12.8, 7α-H), 1.52 (1 H, m, 11β-H), 1.52 (1 H, t, *J* 11.0, 12α-H), 1.45 (1 H, dd, *J* 9.2 and 5.7, 19b-H), 1.39 (1 H, m, 14-H), 1.28 (1 H, m, 15β-H), 0.70 (3 H, s, 18-H); δ_C(125.77 MHz, $CDCl_3$; Me_4Si) 209.2 (C-20), 199.0 (C-3), 180.9 (C-5), 110.9 (C-4), 63.2 (C-17), 55.1 (C-14), 50.1 (C-10), 49.5 (C-9), 45.5 (C-13), 43.5 (C-6), 39.2 (C-12), 34.1 (C-2), 34.0 (C-8), 33.5 (C-7), 31.4 (C-21), 31.2 (C-19), 30.7 (C-1), 25.2 (C-11), 23.6 (C-15), 23.2 (C-16), 14.1 (C-18); *m/z* (EI) 312 (M$^+$, 32), 297 (3), 269 (20), 251 (5), 227 (16), 43 (100); *m/z* (HRFAB) 313.2128 (M$^+$ + H) $C_{21}H_{29}O_2$ requires 313.2168.

Crystallographic data and data collection parameters. Colorless prismatic crystals recrystallized from dioxane. $C_{21}H_{28}O_2$, $M = 312.43$, monoclinic, space group $P2_1$ (no 4); cell constants $a = 11.3312(12)$ Å, $b = 11.4371(12)$ Å, $c = 14.5628(16)$ Å; $\beta = 111.863(2)°$; $V = 1751.5(3)$ Å^3, D_c ($Z = 4$, $Z' = 2$) $= 1.185$ g cm^{-3}; $\mu = 0.074$ mm^{-1}; crystal dimensions $0.26 \times 0.18 \times 0.16$ mm, reflections measured: 13024, reflections unique (Friedel pairs merged): 4172, reflections observed ($I > 2\sigma(I)$): 2950; $R = 0.057$ and $R_w^2 = 0.090$. The structure is made up of two independent moieties (of the same chirality) in the asymmetric unit. The X-ray analysis only established the relative stereochemistry of this all-light-atom compound.‡

21-Acetyloxy-19-methanesulfonyloxypregn-4-ene-3,20-dione 11

Mesylate **11** was obtained from 21-acetyloxy-19-hydroxy-progesterone **9** (0.150 g, 0.386 mmol) and methanesulfonyl chloride (0.220 g, 1.93 mmol) following the procedure described for compound **10**. The product was purified by flash chromatography (ethyl acetate–hexane 6 : 4) to give **11** (0.158 g, 88%) as a white solid, mp 161–162 °C (from hexane–ethyl acetate); (found C 62.1, H 7.6. $C_{24}H_{34}O_7S$ requires C 61. 8, H 7.3%); ν_{max}/(KBr) (cm^{-1}) 2939, 2878, 1749, 1724, 1356, 1234, 1175, 959, 833; δ_H(500.13 MHz, $CDCl_3$; Me_4Si) 5.95 (1 H, s, 4-H), 4.70 (1 H, d, *J* 16.8, 21a-H), 4.59 (1 H, dd, *J* 9.8 and 0.7, 19a-H), 4.52 (1 H, d, *J* 16.8, 21b-H), 4.38 (1 H, d, *J* 9.8, 19b-H), 3.00 (3 H, s, 19-OMs), 2.17 (3 H, s, 21-H), 0.72 (3 H, s, 18-H); δ_C(125.77 MHz, $CDCl_3$; Me_4Si) 203.3 (C-20), 198.6 (C-3), 170.3 (CH_3COO), 163.7 (C-5), 127.3 (C-4), 70.4 (C-19), 69.0 (C-21), 58.9 (C-17), 56.4 (C-14), 53.7 (C-9), 44.5 (C-13), 41.8 (C-10), 38.5 (C-12), 36.1 (C-8), 34.4 (C-1), 32.9 (C-6 and C-2), 32.0 (C-7), 24.3 (C-15), 22.8 (C-16), 21.5 (C-11), 13.3 (C-18); *m/z* (EI) 466 (M$^+$, 4), 370 (4), 269 (19), 251 (5), 43 (100).

21-Hydroxy-6,19-cyclopregn-4-ene-3,20-dione 5

Cyclopregnane **5** was obtained from mesylate **11** (0.233 g, 0.5 mmol) following the procedure described for compound **7**. The resulting solid was purified by flash chromatography (silica gel, EtOAc–hexane 7 : 3) to give **5** (0.099 g, 60%) as a white solid, mp 153–154 °C (from methanol); ν_{max} (KBr)/(cm^{-1}) 2926, 2856, 1701, 1659, 1100; δ_H(500.13 MHz, $CDCl_3$; Me_4Si) 5.60 (1 H, s, 4-H), 4.23 (1 H, d, *J* 19.1 Hz, 21-H), 4.17 (1 H, d, *J* 19.1, 21-H), 3.27 (1 H, bs, OH), 3.22 (1 H, t, *J* 5.7 Hz, 6-H), 2.50 (1 H, t, *J* 9.2, 17-H), 2.45 (1 H, d, *J* 9.5, 19a-H), 2.28 (2 H, m, 2-H), 2.25 (1 H, m, 16β-H), 2.16 (1 H, m, 1α-H), 2.12 (1 H, m, 7β-H), 2.01 (1 H, m, 12β-H), 1.97 (1 H, m, 8-H), 1.86 (1 H, m, 1β-H), 1.81 (1 H, m, 16α-H), 1.80 (1 H, m, 9-H), 1.73 (1 H, m, 11α-H), 1.71 (1 H, m, 15α-H), 1.57 (1 H, m, 7α-H), 1.53 (1 H, m, 11β-H), 1.47 (1 H, m, 19b-H), 1.46 (1 H, m, 12α-H), 1.40 (1 H, m, 14-H), 1.38 (1 H, m, 15β-H), 0.76 (3 H, s, 18-H); δ_C(125.77 MHz, $CDCl_3$; Me_4Si) 210.1 (C-20), 199.1 (C-3), 180.8 (C-5), 110.9 (C-4), 69.3 (C-21), 58.6 (C-17), 55.1 (C-14), 50.0 (C-10), 49.3 (C-9), 46.2 (C-13), 43.4 (C-6), 38.8 (C-12), 34.0 (C-2), 33.9 (C-8), 33.4 (C-7), 31.1 (C-19), 30.6 (C-1), 25.1 (C-11), 23.6 (C-15), 23.2 (C-16), 14.2 (C-18); *m/z* (EI) 328 (M$^+$, 30), 297 (33), 269 (42), 251 (10), 241 (6), 227 (15), 41 (100); *m/z* (HRFAB) 329.2117 (M$^+$ + H) $C_{21}H_{29}O_3$ requires 329.2130.

Binding studies

Male Sprague Dawley rats, 21 days old, were adrenalectomized 48 h prior to experiments and maintained on Purina chow (diet 1), saline and fresh water *ad libitum*. Animals were sacrificed and bled by heart puncture. Liver was used as a source of glucocorticoid receptors. Tissue was homogenized with two volumes of TEGM buffer at pH 7.4 (0.1 M Tris-HCl, 10 mM EDTA, 10 mM β-mercaptoethanol, 20 mM Na_2MoO_4, 25% glycerol, 0.1 mM PMSF, 2.0 IU cm^{-3} aprotinin and 1 mg leupeptin (R buffer). The cytosolic fraction was prepared from the homogenate by differential centrifugation. After sedimentation of the nuclear fraction at 800 × g for 10 min, the cytosolic fraction was separated from mitochondria and microsomes by centrifugation at 105000 × g for 60 min. All steps were carried out at 4 °C. Binding was assayed in triplicate employing 400–600 μg cytosolic proteins and 1.2 nM [^{3}H]-dexamethasone in TEGM buffer. Binding was obtained by the displacement of [^{3}H]-dexamethasone with different concentrations of unlabelled dexamethasone (5, 50 and 5000 nM), or the corresponding competitor (**1**, **4** and **5**). All the incubations were carried out in a final volume of 0.5 cm^3 at 4 °C. After equilibrium was reached, unbound [^{3}H]-steroids were removed from the incubation with an equal volume of

charcoal–dextran (2 : 0.2%) in PBS, pH 7.4 during 20 min and subsequent centrifugation. Bound radioactivity was determined by scintillation counting.

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