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Synthesis and $GABA_A$ receptor activity of 2,19-sulfamoyl analogues of allopregnanolone

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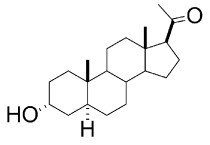
ABSTRACT

The synthesis of new analogues of allopregnanolone with a bridged sulfamidate ring over the β-face of ring A has been achieved from easily available precursors, using an intramolecular aziridination strategy. The methodology also allows the synthesis of 3α-substituted analogues such as the 3α-fluoro derivative. $GABA_A$ receptor activity of the synthetic analogues was evaluated by assaying their effect on the binding of [^{3}H]flunitrazepam and [^{3}H]muscimol. The 3α-hydroxy-2,19-sulfamoyl analogue and its *N*-benzyl derivative were more active than allopregnanolone for stimulating binding of [^{3}H]flunitrazepam. For the binding of [^{3}H]muscimol, both synthetic analogues and allopregnanolone stimulated binding to a similar extent, with the *N*-benzyl derivative exhibiting a higher EC_{50}. The 3α-fluoro derivative was inactive in both assays.

1. Introduction

Neuroactive steroids are positive allosteric modulators of the $GABA_A$ receptor that interact with a specific steroid recognition site on the receptor–ion channel complex.[1] The current interest in the therapeutic properties of these compounds results from their potential activity as anticonvulsants,[2–4] anaesthetics[5] and for the treatment of several neurological and psychiatric disorders.[6–9] Endogenous neuroactive steroids (neurosteroids) such as 3α-hydroxy-5α-pregnan-20-one (**1**) and its 5β isomer (**2**), are rapidly biotransformed when administered exogenously and several synthetic analogues of these compounds with improved bioavailability have been developed.[10] Structure–activity relationship studies have demonstrated that a saturated pregnane nucleus with either 5α- or 5β-stereochemistry, a 3α-hydroxyl group and a 20-ketone form the basic pharmacophore.[11] However, some variations to this core unit are well tolerated, such as relatively large, polar (for water solubility) substituents at the 2-position on the β-face that can include ethers, for example, minaxolone **3**[12,13] and amines, for example, Org 21465 **4**.[14]



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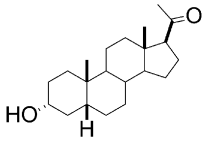

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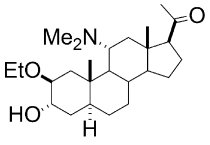

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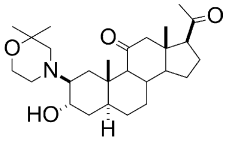

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The reported synthesis of 2β-aminopregnanes (such as **4**) starts from 2α,3α-epoxysteroids.[14] Opening of these conformationally rigid unsymmetrical epoxides by nucleophiles follows the Fürst-Plattner rule with exclusive formation of the diaxial product under kinetically controlled conditions,[15,16] that is, attack of the nucleophile on the β-face at C-2, giving rise simultaneously to 3α-hydroxy derivatives (a condition for central nervous system activity) having a C-2β substituent. We envisaged another approach to such analogues and focused again our attention on the intramolecular aziridination reaction of olefinic sulfamates.[17] We have previously shown[18] that treatment of a pregn-5-en-19-yl sulfamate (i.e., compound **5**) with iodosylbenzene and a copper salt catalyst, leads to

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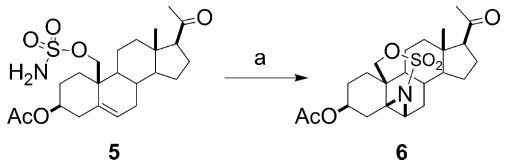

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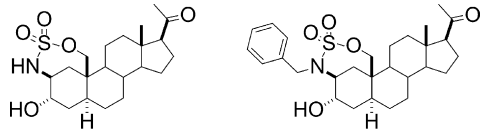
Scheme 1. Reagents and conditions: (a) PhI=O, $Cu(CH_3CN)_4PF_6$, CH_3CN, N_2, 25 °C, 20 h.

formation of the corresponding nitrene and consequent aziridination of the 5,6 double bond with total facial stereoselectivity on the β-face (compound **6**; Scheme 1). By analogy, in the case of a 2,3-pregnene C-19 sulfamoyl derivative, a 2β,3β,19-sulfamoyl aziridine derivative should be obtained. Subsequent regioselective opening of the aziridine ring at C-3 by nucleophiles (vide supra) would give access to analogues of allopregnanolone (**1**) having a 2β amino group as part of a cyclic sulfamidate moiety in their structure. These types of compounds are of interest *per se*, since the sulfamidate moiety may be considered as part of the pharmacophore which provides a certain degree of conformational rigidity to the molecules that could favor their eventual binding to the receptor. Alternatively, the sulfamidate group, after activation, serves as a point of entry for a variety of nucleophiles at C-19 thereby allowing further structural modifications.[17,19] Finally, this methodology gives easy access to modified 3α-fluoro derivatives (via nucleophilic opening of the aziridine ring with fluoride anion), recently shown to be an interesting new family of potentially active analogues of neurosteroids.[20,21] We report herein the synthesis and $GABA_A$ receptor activity of rigid analogues of allopregnanolone having a sulfamoyl moiety on the β-face at the C-2 position and either a pharmacologically relevant hydroxy group or fluoride atom at the 3α position (compounds **7**, **8** and **9**).



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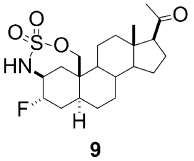

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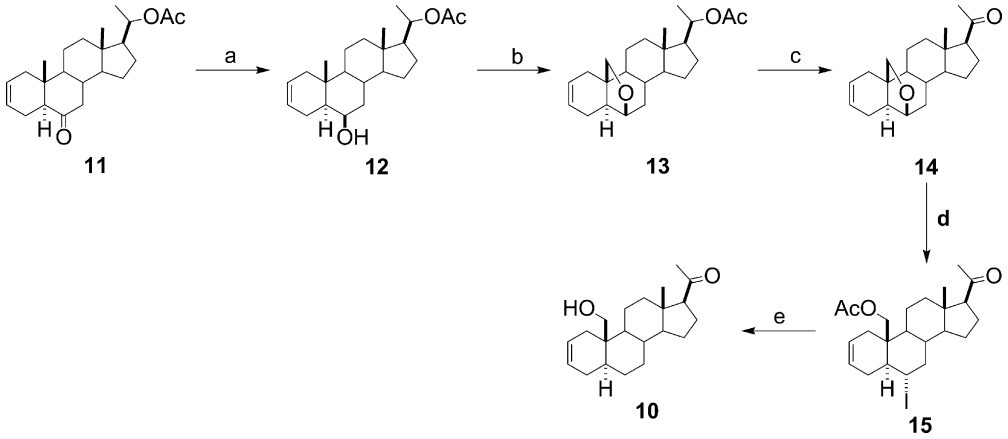
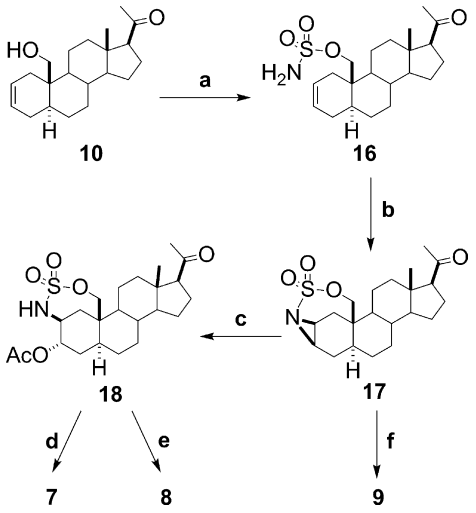
2. Chemistry

With these considerations in mind, we chose 19-hydroxypregn-2-en-20-one (**10**) as an appropriate precursor for our study. The published synthesis of such unsaturated pregnenes starts from pregnanolone acetate but gives only modest yields owing mainly to the poor regioselectivity of 3β-tosylate elimination in the 5αH-reduced pregnane.[22] We thus envisaged a different strategy starting from the 6-keto-derivative **11** (Scheme 2). This compound can be synthesized from cheap, commercially available pregnenolone acetate in seven steps following a procedure described in part by Mori and co-workers for the synthesis of brassinolides.[23] The keto group of compound **11** was first reduced stereoselectively to 6β-hydroxypregnane **12** using the bulky lithium tri-*tert*-butoxyaluminum hydride, which approaches from the less sterically hindered α face. The characteristic resonance and coupling pattern for the 6α-H in the ^1H NMR spectra of **12** (δ 3.85 ppm, J = 2.8 Hz) was indicative of the correct configuration at this position (6α-H in an equatorial orientation). Treatment of compound **12** with (diacetoxyiodo)benzene (DIB) and I_2 in the presence of light, generated the 6β-alkoxy radical resulting in remote functionalization of the 19-CH_3 group[24] to epoxysteroid **13** (75% yield). Regeneration of the carbonyl group at C-20 was accomplished using standard procedures. Thus, removal of the acetate group with LAH in THF afforded the 20β-hydroxy derivative which upon further oxidation with PCC provided compound **14** in good yield (73% over two steps). Opening of the 6,19-epoxy moiety in compound **14** was achieved using a reaction previously developed by us for the cleavage of ethers.[25] Thus, treatment of **14** with ZnI_2 in acetic anhydride gave 6α-iodo-19-acetyloxypregnane **15** in 74% yield. Removal of iodine in compound **15** was easily performed by a classical radical dehalogenation (tributyltin hydride, *hv*, toluene)[26] and further treatment of the product with potassium hydroxide in methanol afforded compound **10** in excellent yield (92% from **15**). Absence of the typical signal for *CH*I in the ^1H NMR spectrum of compound **10** confirmed the success of the radical reaction.

The synthesis of sulfamidates **7**, **8** and **9** is shown in Scheme 3. Reaction of 19-hydroxypregnane **10** with sulfamoyl chloride generated in situ,[27] then gave the polar, unsaturated steroidal sulfamate **16** as the sole isolable product in 66% yield. The presence of the sulfamidate group in **16** was confirmed by NMR and mass spectrometry. Compound **16** was then used for the direct intramolecular aziridination of the 2,3-double bond.[18] Thus, reaction of **16** with 1.5 equiv of iodosylbenzene and 10% $Cu(CH_3CN)_4PF_6$ in ethanol at room temperature for 18 h led stereospecifically to the sulfamoylaziridine **17** in 75% yield. No other products could be



Scheme 2. Reagents and conditions: (a) $LiAl(O\text{-}tBu)_3H$, THF, 0–25 °C, 1 h; (b) DIB, I_2, *hv*, 25 °C, 2 h; (c) (i) LAH, THF, 0–25 °C, 1 h; (ii) PCC, $BaCO_3$, MS 3 Å, Cl_2CH_2, 25 °C, 4 h; (d) ZnI_2/Ac_2O, N_2, 25 °C, 1 h; (e) (i) n-Bu_3SnH, N_2, 25 °C, 5 h; (ii) KOH, MeOH, N_2, 20 °C, 5 h.



Scheme 3. Reagents and conditions: (a) $HCO_2H/ClSO_2NCO$, DMA, 0–25 °C, 4 h; (b) PhI=O, $Cu(CH_3CN)_4PF_6$, CH_3CN, N_2, 25 °C, 18 h; (c) TBAA, THF, N_2, 0–25 °C, 1 h; (d) KOH, MeOH, N_2, 25 °C, 2 h; (e) (i) $PhCH_2Br$, 40% NaOH, Et_3PhCH_2NCl, Cl_2CH_2, 25 °C, 45 min; (ii) KOH, MeOH, N_2, 25 °C, 2 h; (f) TBAF, THF, N_2, 25 °C, 1 h.

detected by TLC and ^1H NMR analysis of the crude reaction mixture. The structure of compound **17** was confirmed by ^1H and ^{13}C NMR (proton decoupled and DEPT) spectroscopy. Treatment of compound **17** with tetrabutylammonium acetate afforded the cyclic sulfamate **18** resulting from regioselective opening of the aziridine at C-3 (79% yield), thereby providing the 3α-oxygenated moiety required for central nervous system (CNS) activity. The same reaction using cesium acetate as nucleophile resulted in a lower yield (71%). Smooth deacetylation of **18** with potassium hydroxide furnished the 3α-hydroxy derivative **7** in almost quantitative yield. The equatorial orientation of 3β-H in this compound was clearly established by its narrow resonance ($W_{1/2} \approx 9$ Hz) in the ^1H NMR spectrum. The overall yield of **7** starting from pregnenolone acetate (18 steps) was 7.2%.

To test the effect of substituents on the sulfamidate nitrogen on the biological activity, we synthesized the benzyl derivative **8**. Thus, treatment of **18** under the usual conditions of benzylation[28,29] (Scheme 3, step e), gave *N*-benzyl derivative in 96% yield. Smooth hydrolysis of the acetate group in C-3 with KOH in methanol at room temperature gave the analogue **8** in 7.3% yield from pregnenolone acetate.

Table 1
Effects of allopregnanolone (**1**) and sulfamidates **7**, **8** and **9** on binding of [^{3}H]flunitrazepam to membranes from rat cerebellum[a]

Concentration (nM)	% Stimulation			
	1	**7**	**8**	**9**
10	93 ± 10	104 ± 11	108 ± 2	n.d.
25	93 ± 7	113 ± 10	113 ± 6[b]	n.d.
50	92 ± 12	122 ± 9[b]	118 ± 2[c]	n.d.
100	97 ± 11	122 ± 16[b]	117 ± 3[c]	n.d.
250	122 ± 10[c]	126 ± 9[c]	122 ± 2[b]	n.d.
500	132 ± 14[c]	126 ± 11[b]	125 ± 5[c]	n.d.
800	140 ± 19[b]	170 ± 7[c]	134 ± 8[c]	98 ± 8
EC_{50} (nM)	265 ± 117	63 ± 45	81 ± 35	—

[a] Membranes were incubated with 1 nM [^{3}H]flunitrazepam in the absence or presence of **1**, **7**, **8** or **9.** The samples were filtered under vacuum through glass microfiber filters, and specific radioactivity bound to membranes was determined by liquid scintillation counting. Data shown represent mean values from four separate experiments performed in duplicate.
[b] p <0.05 versus controls with no steroid added.
[c] p <0.01 versus controls with no steroid added. n.d.: not determined.

Table 2
Effects of allopregnanolone (**1**) and sulfamidates **7**, **8** and **9** on binding of [^{3}H]muscimol to membranes from rat cerebellum[a]

Concentration (nM)	% Stimulation			
	1	**7**	**8**	**9**
20	95 ± 19	100 ± 11	119 ± 15	n.d.
50	100 ± 22	105 ± 18	121 ± 5[c]	n.d.
100	108 ± 25	108 ± 20	121 ± 8[c]	n.d.
200	106 ± 24	106 ± 19	117 ± 10[b]	n.d.
400	116 ± 26	118 ± 17	124 ± 13[b]	n.d.
800	165 ± 13[c]	170 ± 30[c]	124 ± 5[b]	96 ± 2
1000	173 ± 25[c]	176 ± 31[b]	141 ± 14[b]	n.d.
EC_{50} (nM)	658 ± 259	542 ± 145	1890 ± 617	—

[a] Membranes were incubated with 10 nM [^{3}H]muscimol in the absence or presence of **1**, **7**, **8** or **9.** The samples were filtered under vacuum through glass microfiber filters, and specific radioactivity bound to membranes was determined by liquid scintillation counting. Data shown represent mean values from four separate experiments performed in duplicate.
[b] p <0.05 versus controls with no steroid added.
[c] p <0.01 versus controls with no steroid added. n.d.: not determined.

With the aim of extending the scope of this methodology to the synthesis of 3α-fluoro derivatives,[20,21] we treated compound **17** with TBAF in THF at room temperature (Scheme 3, step f). As expected, regioselective opening of the aziridine ring gave the 3α-fluoro-2β,19-sulfamidate **9** in 7.7% overall yield (from pregnenolone acetate). The structure of **9** was confirmed by NMR and mass spectrometry. In the former, signals at δ_H 4.53 and δ_C 88.9 corresponding to H-3β and C-3, respectively, together with the values of the coupling constants of these atoms with the neighboring fluorine atom ($^2J_{H-F}$ 44.2 Hz and $^1J_{C-F}$ 175.0 Hz), provided adequate proof of structure.

3. $GABA_A$ receptor activity

$GABA_A$ receptor affinity was evaluated by assaying the effect of the synthetic analogues **7**, **8** and **9** on the binding of [^{3}H]flunitrazepam (a specific ligand for the benzodiazepine binding site of $GABA_A$ receptors) and [^{3}H]muscimol (a specific ligand for the GABA binding site) as compared with the effects of the typical neurosteroid allopregnanolone.[11] This compound is known to stimulate binding of [^{3}H]flunitrazepam and [^{3}H]muscimol by noncompetitive interactions of the steroid with these binding sites. Thus, as shown in Table 1 allopregnanolone (**1**) stimulated [^{3}H]flunitrazepam with a maximum binding in excess of 140% (EC_{50} 265 ± 117 nM). Compound **7** and its *N*-benzyl derivative **8**, were significantly more active in the 25–100 nM range, with a smaller EC_{50} (63 ± 45 nM and 81 ± 35 nM). For the stimulation of [^{3}H]muscimol binding (Table 2), allopregnanolone and the sulfamidates **7**

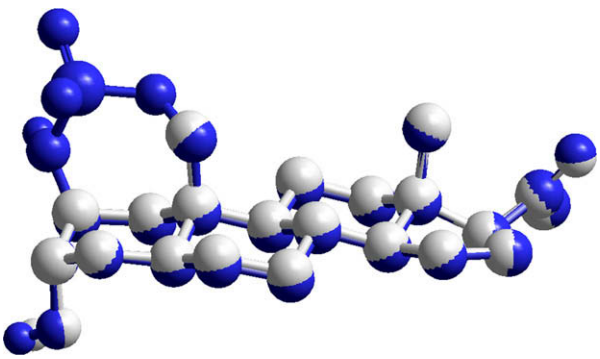


Figure 1. Superposition of calculated structures (HF/6-31G**) of sulfamidate **7** (blue) and allopregnanolone (**1**) (white). Overlay corresponds to best fit for O(3), C(3), C(17) and C(20) (RMS error 0.051 Å); O(3)–C(20) distances are **1**: 10.04 Å; **7**: 10.15 Å.

and **8**, had similar dose–response curves, with the *N*-benzyl derivative **8** exhibiting a higher EC_{50} (1890 ± 617 nM). On the other hand, the 3α-fluoro analogue **9** differing from **7** only in the substituent at C-3, showed no significant effects (compared to controls) on the binding of [^{3}H]flunitrazepam and [^{3}H]muscimol, when assayed at the highest concentration allowed by its solubility in the biological medium (800 nM).

Ab-initio calculations (HF/6-31G**) showed that introduction of the sulfamidate bridge in allopregnanolone does not affect significantly the steroid backbone conformation (Fig. 1). Thus, the relative positions and orientations of the C-17 side chain and the 3α-hydroxyl are maintained. The enhanced biological activity displayed by analogues **7** and **8** may then be related to favorable interactions of the sulfamidate moiety with the receptor, in agreement with observations that H-bond acceptors in the proximity of positions 2 and 3 increase activity.[30] Most interesting is the fact that introduction of the bulky benzyl substituent (**8**) is well tolerated; this is in line with results on 2- and 3-substituted analogues of allopregnanolone, that suggested the presence of a hydrophobic pocket in the receptor that could accommodate bulky groups in the neighborhood of ring A.[30,31]

The lack of activity of the 3-fluoro derivative **9** was somewhat disappointing, considering previous reports on activity of other 3α-fluoro steroids.[20,21] In this case, replacement of the 3α-hydroxyl by fluorine was highly deleterious to $GABA_A$ receptor activity, indicating that further work is needed to ascertain the effect of alternate substituents at position 3α of neuroactive steroids.

4. Experimental

4.1. General

Melting points (mp) were taken on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded in thin films using KBr disks on a Nicolet Magna 550 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured in a Bruker AC-200 (200.13 and 50.32 MHz) or a Bruker Avance II 500 (500.13 and 125.72 MHz) NMR spectrometer in deuteriochloroform unless indicated otherwise. Chemical shifts are given in ppm downfield from internal TMS; *J* values are given in hertz. Spectra were assigned by analysis of the DEPT, COSY 45, HETCOSY and HMBC obtained using standard Bruker software. The electron impact mass spectra (MS) were collected on a Shimadzu QP-5000 mass spectrometer at 70 eV by direct inlet. Vacuum liquid chromatography (VLC) was carried out on Kieselgel 60-G (Merck); column flash chromatography was carried out on Kieselgel S 0.040–0.063 mm or Florisil. Thin layer chromatography (TLC) analysis was performed on Silica Gel 60 F254 (0.2 mm thick). The plates were visualized by spraying with a 3.5% solution of phosphomolybdic acid in ethanol. All solvents were distilled and stored over 4 Å molecular sieves before use. The homogeneity of all compounds was confirmed by TLC. Solvents were evaporated at 45 °C under vacuum. High-resolution mass spectrometry and elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

20β-Acetyloxypregn-2-en-6-one (**11**) was obtained from 20β-acetyloxypregn-5-en-3β-ol following essentially the procedure described by Mori and co-workers.[23] The latter compound was obtained from pregnenolone acetate following the procedure described previously by us.[32] The geometries of compounds **1** and **7** were optimized using the ab-initio quantum chemistry program GAUSSIAN 03[33] and the HF/6-31G** basis set.

4.2. Syntheses

4.2.1. 20β-Acetyloxypregn-2-en-6β-ol (12)

To a stirred solution of 20β-acetyloxy-pregn-2-en-6-one **11** (1.44 g, 4.02 mmol) in dry THF (75 mL) was added lithium tri-*tert*-butoxyaluminum hydride (1.94 g, 7.63 mmol) under N_2 at 0 °C. After 1 h, acetone (10 mL) was added and when gas evolution ceased the reaction mixture was neutralized with 1 N HCl. The solvent was evaporated to one fifth of its original volume and the residue was diluted with dichloromethane (60 mL) and successively washed with a saturated solution of $NaHCO_3$ (20 mL), brine (20 mL) and water (20 mL). The organic layer was dried with sodium sulfate and evaporated in vacuo. Purification by flash chromatography gave 6β-hydroxy steroid **12** (1.38 g, 95%) as a white solid: mp 150–152 °C (from AcOEt–hexane); IR (KBr) 3502, 2937, 2872, 1724, 1454, 1375, 1075, 1047, 1022 cm^{-1}; ^{1}H NMR (200.13 MHz): 5.68 (1H, m, 3-H), 5.57 (1H, m, 2-H), 4.85 (1H, m, 20-H), 3.85 (1H, t, *J* = 2.8 Hz, 6α-H), 2.31 (1H, m, 4α-H), 2.02 (3H, s, acetate), 1.84–1.86 (4H, m, 4β-H, 7β-H, 12β-H and 1β-H), 1.78 (1H, m, 1α-H), 1.67–1.72 (3H, m, 15α-H, 8-H and 16β-H), 1.60 (1H, m, 17-H), 1.41–1.46 (3H, m, 11β-H, 5α-H and 11α-H), 1.17–1.25 (4H, 7α-H, 15β-H, 12α-H and 16α-H), 1.15 (3H, d, *J* = 6.2 Hz, 21-H), 1.08 (1H, m, 14-H), 0.77 (1H, m, 9-H), 0.76 (3H, s, 19-H), 0.67 (3H, s, 18-H); ^{13}C NMR (50.32 MHz): 170.4 (acetate), 125.6 (C-2 and C-3), 72.8 (C-20), 70.3 (C-6). 55.5 (C-14), 55.1 (C-17), 54.2 (C-9), 44.5 (C-5), 42.4 (C-13), 41.7 (C-1), 39.7 (C-7), 39.3 (C-12), 34.6 (C-10), 30.0 (C-8), 26.4 (C-4), 25.4 (C-16), 24.2 (C-15), 21.5 (acetate), 20.7 (C-11), 19.9 (C-21), 15.1 (C-19), 12.5 (C-18); MS (ESI): *m/z* 401 (M+H_2O+Na, 12), 383 (M+Na, 100), 283 (40), 243 (20); Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.22; H, 10.04.

4.2.2. 20β-Acetyloxy-6,19-epoxypregn-2-ene (13)

To a solution of alcohol **12** (1.00 g, 2.77 mmol) in dry dichloromethane (330 mL) in a water jacketed flask were added iodobenzene diacetate (0.45 g, 1.39 mmol) and iodine (0.70 g, 2.77 mmol) under N_2. The solution was irradiated with a 300 W tungsten lamp (5000 lm) while being stirred at 25 °C. Two aliquots of iodobenzene diacetate (0.45 g, 1.39 mmol) and iodine (0.70 g, 2.77 mmol) were added each 30 min. After 1 h, the reaction was finished (tlc) and a solution of saturated sodium thiosulfate (60 mL) was added. The organic layer was washed with brine (60 mL) and water (60 mL), dried with sodium sulfate and evaporated in vacuo. The oily mixture was chromatographed on neutral alumina with cyclohexane/AcOEt (95:5–8:2), to give epoxypregnene **13** (0.74 g, 75%) as a white solid: mp 147–149 °C (from AcOEt–hexane); IR (KBr) 3448, 2931, 2872, 1731, 1445, 1372, 1033, 1019, 943, 852 cm^{-1}. ^{1}H NMR (200.13 MHz): 5.67 (1H, m, 3-H), 5.57 (1H, m, 2-H), 4.85 (1H, m, 20-H), 3.97 (1H, d, *J* = 4.8 Hz, 6α-H), 3.78 (1H, d, *J* = 8.0 Hz, 19b-H), 3.53 (1H, d, *J* = 8.0 Hz, 19a-H), 2.01 (3H, s, acetate), 1.15 (3H, d, *J* = 6.1 Hz, 21-H), 0.70 (3H, s, 18-H); ^{13}C NMR (50.32 MHz): 170.3 (acetate), 124.9 (C-3), 123.8 (C-2), 81.5 (C-6), 72.8 (C-20), 70.9 (C-19), 54.9 (C-14), 54.8 (C-17), 53.9 (C-9), 46.1 (C-5), 42.9 (C-10 and C-13), 39.3 (C-12), 36.9 (C-7), 34.6 (C-8), 27.5 (C-1), 26.6 (C-4), 25.4 (C-16), 23.5 (C-15), 22.2 (C-11), 21.5 (acetate), 19.9 (C-21), 12.9 (C-18); MS (ESI): *m/z* 399 (M+H_2O+Na, 25), 381 (M+Na, 100), 299 (55); Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.08; H, 9.39.

4.2.3. 6,19-Epoxypregn-2-en-20-one (14)

To a stirred solution of compound **13** (1.00 g, 2.79 mmol) in dry THF (23 mL) was added lithium aluminum hydride (0.52 g, 13.84 mmol) under N_2 at 0 °C. After 1 h, acetone (5 mL) was added, and when gas evolution ceased the reaction mixture was neutralized with 1 N HCl. The solvent was evaporated to one third of its original volume, a solution of saturated sodium potassium tartrate (40 mL) was added and the mixture extracted with dichloromethane (2 × 20 mL); the organic layer was successively washed with a saturated solution of $NaHCO_3$ (15 mL), brine (15 mL) and water (15 mL), dried with sodium sulfate and evaporated under reduced pressure. Chromatography on silica gel using a gradient of cyclo-

hexane/AcOEt (9:1–6:4) gave the 20-alcohol (0.77 g, 87%): mp 155–157 °C (from AcOEt–hexane); IR (KBr) 3427, 2929, 2869, 1453, 1374, 1494, 1033, 737, 656 cm^{-1}; ^1H NMR (200.13 MHz): 5.68 (1H, m, 3-H), 3.98 (1H, d, J = 4.6 Hz, 6α-H), 3.80 (1H, d, J = 7.9 Hz, 19b-H), 3.74 (1H, m, 20-H), 3.54 (1H, d, J = 7.9 Hz, 19a-H), 2.56 (1H, m, 2-H), 1.14 (3H, d, J = 6.1 Hz, 21-H), 0.82 (3H, s, 18-H); ^{13}C NMR (50.32 MHz): 124.9 (C-3), 123.9 (C-2). 81.6 (C-6), 70.9 (C-19), 70.4 (C-20), 58.4 (C-17), 54.9 (C-14), 53.9 (C-9), 46.1 (C-5), 43.1 (C-13), 42.9 (C-10), 40.1 (C-12), 36.8 (C-7), 34.6 (C-8), 27.5 (C-1), 26.6 (C-4), 25.7 (C-16), 23.8 (C-15), 23.6 (C-21), 22.2 (C-11), 12.9 (C-18); MS (ESI) m/z 633 (2 M+Na^+, 100); Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.68; H, 10.09.

Pyridinium chlorochromate (0.95 g, 4.42 mmol), barium carbonate (0.65 g, 3.32 mmol) and 4 Å molecular sieves (0.30 g) in dry dichloromethane (25 mL) were vigorously stirred at 25 °C under N_2 for 15 min and then a solution of the alcohol obtained above (0.70 g, 2.21 mmol) in dry dichloromethane (25 mL) was added. After 4 h, the reaction mixture was diluted with diethyl ether (45 mL), and percolated through a silica gel pad with hexane/AcOEt 8:2. Evaporation of the solvent gave 20-ketosteroid **14** (0.58 g, 83%) as a white solid: mp 105–107 °C (from AcOEt–hexane); IR (KBr) 2927, 2873, 1704, 1437, 1356, 1222, 1167, 1017, 850, 758 cm^{-1}; ^1H NMR (500.13 MHz): 5.68 (1H, m, 3-H), 5.57 (1H, m, 2-H), 3.98 (1H, d, J = 4.8 Hz, 6α-H), 3.78 (1H, d, J = 8.0 Hz, 19bH), 3.55 (1H, d, J = 8.0 Hz, 19a-H), 2.52 (1H, t, J = 9.0 Hz, 17-H), 2.16–2.17 (2H, m, 4α-H and 16β-H), 2.11 (3H, s, 21-H), 2.05 (1H, dt, J = 12.4, 3.2 Hz, 12β-H), 1.95–1.99 (3H, m, 1α-H, 1β-H and 4β-H), 1.80 (1H, dt, J = 12.7, 5.2 Hz, 7β-H), 1.74 (1H, m, 8-H), 1.60–1.67 (4H, m, 5-H, 11α-H, 15α-H and 16α-H), 1.44 (1H, td, J = 12.7, 3.8 Hz, 12α-H), 1.27–1.30 (3H, m, 11β-H, 14-H and 15β-H), 1.12 (1H, t, J = 12.0 Hz, 7α-H), 1.05 (1H, td, J = 11.4, 2.7 Hz, 9-H), 0.69 (3H, s, 18-H); ^{13}C NMR (125.77 MHz): 209.4 (C-20), 125.0 (C-3), 123.7 (C-2), 81.5 (C-6), 70.9 (C-19), 63.6 (C-17), 55.7 (C-14), 53.8 (C-9), 46.1 (C-5), 44.7 (C-13), 42.9 (C-10), 39.1 (C-12), 36.7 (C-7), 34.8 (C-8), 31.4 (C-21), 27.5 (C-1), 26.6 (C-4), 23.7 (C-15), 22.9 (C-16), 22.3 (C-11), 13.8 (C-18); MS (ESI) m/z 369 (M+MeOH+Na^+, 100); Anal. Calcd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 80.00; H, 9.36.

4.2.4. 6α-Iodo-19-acetyloxypregn-2-en-20-one (15)

6,19-Epoxypregn-2-en-20-one (**14**) (0.70 g, 2.23 mmol) was added to freshly prepared zinc iodide (2.80 g, 17.8 mmol) and acetic anhydride (21.5 mL, 228.0 mmol) at 25 °C under N_2. After 10 min the reaction mixture was diluted with dichloromethane (70 mL) and a saturated solution of $NaHCO_3$ (30 mL) was added. The organic layer was separated and washed with aqueous sodium thiosulfate (30 mL), brine (30 mL) and water (30 mL), dried with sodium sulfate and the solvent evaporated in vacuo. The residue was chromatographed on Florisil with cyclohexane/AcOEt (95:5–8:2), to give iodosteroid **15** (0.69 g, 74%) as an amorphous solid; attempts to recrystallize this compound were unsuccessful, resulting in extensive decomposition. IR (KBr) 2940, 2880, 1742, 1703, 1439, 1364, 1039, 738, 672 cm^{-1}; ^1H NMR (500.13 MHz): 5.67 (1H, m, 3-H), 5.55 (1H, m, 2-H), 4.49 (1H, td, J = 12.0, 4.3 Hz, 6β-H), 4.23 (1H, d, J = 12.2 Hz, 19b-H), 4.15 (1H, d, J = 12.2 Hz, 19a-H), 2.67 (1H, br d, J = 16.2 Hz, 4α-H), 2.60 (1H, dt, J = 12.8, 3.9 Hz, 7β-H), 2.52 (1H, t, J = 9.0 Hz, 17-H), 2.17–2.21 (2H, m, 16β-H and 1β-H), 2.11 (3H, s, 21-H), 2.05 (3H, s, acetate), 2.04 (1H, m, 12β-H), 1.92 (2H, m, 5-H and 7α-H), 1.80–1.81 (2H, m, 1α-H and 4β-H), 1.64–1.74 (4H, m, 11α-H, 16α-H, 15α-H and 8-H), 1.39–1.47 (2H, m, 12α-H and 11β-H), 1.21 (1H, m, 15β-H), 1.15 (1H, m, 14-H), 1.07 (H, td, J = 11.6, 3.7 Hz, 9-H), 0.59 (3H, s, 18-H); ^{13}C NMR (50.32 MHz): 209.2 (C-20), 170.6 (acetate), 126.5 (C-3), 124.7 (C-2), 63.4 (C-19 and C-17), 56.5 (C-14), 53.2 (C-9), 50.3 (C-5), 47.2 (C-7), 44.1 (C-13), 41.3 (C-10), 39.5 (C-8), 38.8 (C-6), 38.7 (C-12), 36.2 (C-1), 32.9 (C-4), 31.4 (C-21), 24.2 (C-15), 22.8 (C-16), 21.5 (C-11), 21.2 (acetate), 13.3 (C-18); MS (ESI): m/z 485 (M+H, 5), 357 (M-I, 20), 297 (65).

4.2.5. 19-Hydroxypregn-2-en-20-one (10)

To a stirred solution of compound **15** (0.60 g, 1.44 mmol) in dry toluene (12.5 mL) was added tri-*n*-butyltin hydride (0.5 mL, 1.88 mmol) dropwise under N_2 over a 5 min period. The resulting solution was stirred at 25 °C for 5 h and then concentrated in vacuo. The residue was dissolved in acetonitrile (40 mL) and partitioned with hexane (3 × 10 mL). The acetonitrile layer was evaporated in vacuo and the residue was purified by flash chromatography on silica gel (gradient of hexane–AcOEt) to give 19-acetyloxypregn-2-en-20-one (0.48 g, 1.33 mmol, 92%). To a solution of the latter compound (312 mg, 0.87 mmol) in methanol (55 mL) was added KOH 10% (10 mL) under N_2 at 20 °C. After 5 h the reaction mixture was neutralized with 2 N HCl and evaporated to a fifth of its original volume. The resulting mixture was diluted with dichloromethane (50 mL) washed with brine, dried with sodium sulfate and the solvent evaporated. Column chromatography on silica gel using cyclohexane/AcOEt (8:2) as eluent gave compound **10** (265 mg, 0.84 mmol, 97%) as a white solid: mp 143–144 °C (from AcOEt–hexane); IR (KBr) 3477, 2911, 2845, 1701, 1447, 1357, 1216, 1043, 748, 668, 594 cm^{-1}; ^1H NMR (500.13 MHz): 5.60 (2H, m, 2-H and 3-H), 3.81 (1H, dd, J = 11.5, 5.4 Hz, 19b-H), 3.73 (1H, dd, J = 11.5, 3.5 Hz, 19a-H), 2.52 (1H, t, J = 9.1 Hz, 17-H), 2.40 (1H, br d, J = 16.4 Hz, 1β-H), 2.17 (1H, m, 16β-H), 2.11 (3H, s, 21-H), 2.00–2.03 (2H, m, 4β-H and 12β-H), 1.88 (1H, m, 4α-H), 1.62–1.69 (6H, m, 1α-H, 7β-H, 11α-H, 11β-H, 15α-H and 16α-H), 1.46–1.55 (3H, m, 6α-H, 8-H, and 5-H), 1.37 (1H, dd, J = 12.3, 5.1 Hz, 12α-H), 1.32 (1H, dd, J = 13.1, 3.9 Hz, 6β-H), 1.21 (1H, m, 15β-H), 1.12 (1H, m, 14-H), 0.95 (1H, m, 7α-H), 0.86 (1H, td, J = 11.3, 5.0 Hz, 9-H), 0.65 (3H, s, 18-H); ^{13}C NMR (50.32 MHz): 209.6 (C-20), 127.0 (C-3), 126.3 (C-2), 63.8 (C-17), 61.8 (C-19), 57.1 (C-14), 54.1 (C-9), 44.2 (C-13), 41.4 (C-5), 39.3 (C-12), 38.8 (C-10), 36.0 (C-8), 34.0 (C-1), 31.7 (C-7), 31.5 (C-21), 30.7 (C-4), 28.4 (C-6), 24.3 (C-15), 22.6 (C-16), 21.9 (C-11), 13.3 (C-18); MS (ESI): m/z 339 (M+Na^+, 100), 295 (5); Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 80.09; H, 10.02.

4.2.6. 19-Sulfamoyloxypregn-2-en-20-one (16)

Formic acid (0.143 mL, 3.77 mmol) was added dropwise to neat chlorosulfonyl isocyanate (0.33 mL, 3.79 mmol) at 0 °C with rapid stirring. Gas evolution was observed during the addition process. The resulting viscous suspension was stirred for 18 h at 25 °C. The reaction mixture was cooled to 0 °C, DMA (0.8 mL) was added and the solution was stirred for 5 min. A solution of compound **10** (200 mg, 0.63 mmol) in DMA (2.2 mL) was added dropwise, the resulting solution was allowed to warm to 25 °C and stirring continued until the reaction was complete, as determined by TLC (ca. 4 h). The reaction was quenched by the successive addition of AcOEt (15 mL) and brine (5 mL) and the mixture was poured into AcOEt (20 mL) and water (10 mL). The organic layer was collected and the aqueous layer was extracted with AcOEt (3 × 20 mL). The organic extracts were washed with brine (2 × 10 mL), dried over sodium sulfate and concentrated in vacuo. Purification of the residue by flash chromatography on Florisil (gradient of hexane–AcOEt) gave 19-sulfamoyloxypregn-2-en-20-one **16** (165.3 mg, 0.42 mmol, 66%) as a white solid: mp 163–165 °C (from AcOEt–hexane); IR (KBr) 3348, 3281, 2918, 2839, 1703, 1448, 1356, 1266, 1204, 985, 921, 744 cm^{-1}; ^1H NMR (500.13 MHz): 5.69 (1H, m, 3-H), 5.61 (1H, m, 2-H), 4.66 (2H, br s, SO_2NH_2), 4.41 (1H, d, J = 10.0 Hz, 19b-H), 4.18 (1H, d, J = 10.0 Hz, 19a-H), 2.51 (1H, t, J = 9.0 Hz, 17-H), 2.35 (1H, dd, J = 17.3, 5.0 Hz, 1β-H), 2.11 (3H, s, 21-H), 0.66 (3H, s, 18-H); ^{13}C NMR (125.77 MHz): 210.7 (C-20), 125.9 (C-3), 124.9 (C-2). 69.3 (C-19), 63.6 (C-17), 57.0 (C-14), 53.6 (C-9), 44.1 (C-13), 41.2 (C-5), 39.1 (C-12), 37.6 (C-10), 35.8 (C-8), 34.0 (C-1), 31.4 (C-7), 31.1 (C-21), 30.0 (C-4), 28.3 (C-6), 24.1 (C-15), 22.5 (C-16), 21.5 (C-11), 13.1 (C-18); MS (ESI): m/z 813 (M_2+Na, 60), 450 (M+MeOH+Na, 53), 353 (100); Anal. Calcd

for $C_{21}H_{33}NO_4S$: C, 63.77; H, 8.41; N, 3.54; S, 8.11. Found: C, 63.52; H, 8.23; N, 3.41; S, 7.96.

4.2.7. 2β,3β -Iminopregnan-20-one *N*,19-sultone (17)

To a solution of 19-sulfamate **16** (55.0 mg, 0.139 mmol) in anhydrous acetonitrile (2.0 mL) containing activated 3 Å molecular sieves (0.1 g), were added at 25 °C under N_2, iodosylbenzene (122.4 mg, 0.556 mmol) and $Cu(CH_3CN)_4PF_6$ (15.5 mg, 0.042 mmol). The reaction mixture was stirred at room temperature for 18 h, molecular sieves were removed by filtration and the filtrate was evaporated to dryness in vacuo. The residue was purified by flash chromatography on silica gel (hexane/AcOEt 6:4–3:7) to give aziridine **17** (41.0 mg, 75%) as a white solid: mp 141–143 °C (from AcOEt–hexane); IR (KBr) 3393, 2937, 2874, 1699, 1366, 1174, 1003, 975, 837, 794 cm^{-1}; ^1H NMR (500.13 MHz): 4.42 (1H, dd, J = 12.3, 1.7 Hz, 19b-H), 4.19 (1H, d, J = 12.3 Hz, 19a-H), 3.08 (1H, dd, J = 7.5, 5.5 Hz, 3β-H), 2.97 (1H, m, 2α-H), 2.80 (1H, dd, J = 15.3, 0.9 Hz, 1β-H), 2.48 (1H, t, J = 8.8 Hz, 17-H), 2.28 (1H, dd, J = 16.3, 11.8 Hz, 4α-H), 2.14 (1H, m, 16β-H), 2.10 (3H, s, 21-H), 2.04–2.07 (2H, m, 4β-H and 12α-H), 1.87 (1H, ddd, J = 15.3, 3.2, 1.7 Hz, 1α-H), 1.80 (1H, dd, J = 12.6, 3.5 Hz, 7β-H), 1.66–1.73 (3H, m, 11α-H, 15α-H and 16α-H), 1.60 (1H, m, 6α-H), 1.37–1.40 (4H, m, 5-H, 6β-H, 8-H and 12α-H), 1.18–1.22 (2H, m, 11β-H and 15β-H), 1.13 (1H, m, 14-H), 0.98 (1H, m, 7α-H), 0.87 (1H, m, 9-H), 0.60 (3H, s, 18-H); ^{13}C NMR (125.77 MHz, $CDCl_3$ + 5% CD_3OD): 209.9 (C-20), 72.3 (C-19), 63.2 (C-17), 56.4 (C-14), 53.9 (C-9), 43.7 (C-13), 40.4 (C-5), 40.2 (C-12), 39.6 (C-10), 38.7 (C-3), 37.9 (C-2), 35.9 (C-8), 35.5 (C-1), 31.1 (C-21), 30.9 (C-7), 26.9 (C-6), 24.7 (C-4), 23.9 (C-15), 22.5 (C-16), 20.7 (C-11), 13.1 (C-18); HRMS (MALDI) m/z [M+Na^+] 416.1854 (calcd for $C_{21}H_{31}NO_4SNa$ 416.1872).

4.2.8. 3α-Hydroxy-*S*,*S*-dioxo-19,2-(epoxythioimino)pregnan-20-one (7)

To a solution of aziridine **17** (17.0 mg, 0.043 mmol) in anhydrous THF (1.0 mL) was added TBAA (65.0 mg, 0.216 mmol) at 0 °C and the mixture was allowed to reach 25 °C under N_2. After 1 h, the THF was removed in vacuo and the oily residue was purified by preparative TLC (hexane/AcOEt 1:1) to give sulfamidate **18** (15.5 mg, 0.034 mmol, 79%) as a white solid: mp 138–140 °C (from AcOEt–hexane); IR (KBr) 3357, 2945, 2878, 1742, 1374, 1240, 1180, 1033, 980, 780 cm^{-1}; ^1H NMR (200.13 MHz): 4.78 (1H, m, 3β-H), 4.59 (1H, br s, SO_2NH), 4.51 (1H, d, J = 12.6 Hz, 19b-H), 4.30 (1H, d, J = 12.6 Hz, 19a-H), 3.55 (1H, m, 2α-H), 2.71 (1H, d, J = 14.8 Hz, 1β-H), 2.52 (1H, t, J = 9.0 Hz, 17-H), 2.12 (3H, s, acetate), 2.11 (1H, s, 21-H), 0.63 (3H, s, 18-H); ^{13}C NMR (50.32 MHz): 209.3 (C-20), 170.2 (acetate), 71.6 (C-19), 70.2 (C-3), 63.6 (C-17), 56.3 (C-14), 52.8 (C-9), 49.5 (C-2), 44.0 (C-13), 40.2 (C-10), 39.9 (C-5), 38.6 (C-12), 36.0 (C-8), 31.5 (C-21), 31.1 (C-7), 29.8 (C-4), 28.9 (C-1), 27.6 (C-6), 24.2 (C-15), 22.7 (C-16), 21.3 (C-11), 21.2 (acetate), 13.5 (C-18). Compound **18** (21.0 mg, 0.046 mmol) was dissolved in methanol and KOH 2.5 N in methanol (3.7 mL) added under N_2 at 0 °C. The reaction mixture was warmed to 25 °C and after 2 h the solution was neutralized with 2 N HCl (4.6 mL) and concentrated in vacuo to a fifth of its volume. The resulting mixture was diluted with AcOEt (15.0 mL) washed with brine (5.0 mL) and water (5.0 mL), dried with sodium sulfate and evaporated to dryness. The residue was purified by preparative TLC (hexane/AcOEt 1:1) to give sulfamidate **7** (19.0 mg, 99%) as a white solid: mp 195–197 °C (from AcOEt–hexane); IR (KBr); 3500, 3290, 2929, 2854, 1697, 1448, 1362, 1173, 1077, 963, 738 cm^{-1}; ^1H NMR (500.13 MHz): 4.85 (1H, br s, SO_2NH); 4.46 (1H, d, J = 12.7 Hz, 19b-H), 4.26 (1H, d, J = 12.7 Hz, 19a-H), 3.83 (1H, br s, $W_{1/2} \approx 9$ Hz, 3β-H), 3.50 (1H, m, 2α-H), 2.60 (1H, dd, J = 15.0, 2.2 Hz, 1β-H), 2.52 (1H, t, J = 8.9 Hz, 17-H), 2.16 (1H, m, 16β-H), 2.12 (3H, s, 21-H), 2.02–2.08 (2H, m, 4α-H and 12β-H), 1.80 (2H, m, 5-H and 11α-H), 1.63–1.71 (4H, m, 1α-H, 7β-H, 15α-H and 16α-H), 1.51 (1H, d, J = 14.7 Hz, 4β-H), 1.36–1.40 (2H, m, 6β-H and 12α-H), 1.26–1.31 (2H, m, 8-H and 11β-H), 1.17–1.22 (2H, m, 14-H and 15β-H), 1.08 (1H, m, 6α-H), 0.97–0.99 (2H, m, 7α-H and 9-H), 0.62 (3H, s, 18-H); ^{13}C NMR (125.77 MHz): 209.5 (C-20), 71.7 (C-19), 68.5 (C-3), 63.6 (C-17), 56.3 (C-14), 52.8 (C-9), 52.3 (C-2), 44.1 (C-13), 40.3 (C-10), 38.8 (C-5), 38.7 (C-12), 36.1 (C-8), 31.6 (C-4), 31.5 (C-21), 31.1 (C-7), 28.9 (C-1), 27.9 (C-6), 24.2 (C-15), 22.7 (C-16), 21.4 (C-11), 13.5 (C-18); HRMS (MALDI) m/z [M+Na^+] 434.1958 (calcd for $C_{21}H_{33}NO_5SNa$ 434.1977).

4.2.9. *N*-Benzyl-3α-hydroxy-*S*,*S*-dioxo-19,2-(epoxythioimino)-pregnan-20-one (8)

To a solution of sulfamidate **18** (13.0 mg, 0.029 mmol) in dichloromethane (0.5 mL) were added NaOH 40% (0.2 mL), triethylbenzylammonium chloride (1.0 mg, 0.004 mmol) and benzyl bromide (17 μL, 0.143 mmol) at 25 °C with vigorous stirring. After 45 min dichloromethane (10.0 mL) and water (3.0 mL) were added. The organic layer was separated, washed with brine (3.0 mL), dried with sodium sulfate and evaporated in vacuo. The residue was purified by preparative TLC (hexane/AcOEt 7:3) to give the *N*-benzyl pregnane (15.0 mg, 0.027 mmol, 96%): ^1H NMR (200.13 MHz): 7.32–7.44 (5H, m, Ph-H), 4.97 (1H, m, 3β-H), 4.83 (1H, d, J = 16.1 Hz, C***H***H-Ph), 4.61 (1H, d, J = 12.5 Hz, 19b-H), 4.46 (1H, d, J = 12.5 Hz, 19a-H), 4.44 (1H, d, J = 16.1 Hz, CH*H*-Ph), 3.27 (1H, m, 2α-H), 2.60 (1H, d, J = 15.4 Hz, 1β-H), 2.47 (1H, t, J = 9.2 Hz, 17-H), 2.10 (3H, s, 21-H), 2.04 (3H, s, acetate), 0.59 (3H, s, 18-H). KOH 2.5 N in methanol (1.1 mL) was added at 25 °C under N_2 to a solution of the above compound (14.0 mg, 0.026 mmol) in methanol (6.4 mL). After 2 h the mixture was neutralized with HCl 2 N (1.3 mL) and concentrated in vacuo to a fifth of its volume. The resulting mixture was diluted with dichloromethane (15.0 mL) washed with brine (5.0 mL) and water (5.0 mL), dried with sodium sulfate and the solvent evaporated in vacuo. The oily residue was purified by preparative TLC (hexane/AcOEt 4:6) to give the *N*-benzyl sulfamidate **8** (12.6 mg, 0.025 mmol, 96%) as a white solid: mp 147–149 °C (from AcOEt–hexane); IR (KBr); 3500, 3290, 2929, 2854, 1697, 1448, 1362, 1173, 1077, 963, 738 cm^{-1}; ^1H NMR (500.13 MHz): 7.31–7.39 (5H, m, Ph-H), 4.63 (1H, d, J = 15.9 Hz, C*H*H-Ph), 4.60 (1H, d, J = 12.1 Hz, 19b-H), 4.46 (1H, d, J = 12.1 Hz, 19a-H), 4.45 (1H, d, J = 15.9 Hz, CH*H*-Ph), 3.83 (1H, m, 3β-H), 3.22 (1H, m, 2α-H), 2.60 (1H, d, J = 15.1 Hz, 1β-H), 2.48 (1H, t, J = 9.0 Hz, 17-H), 2.11–2.15 (2H, m, 4α-H and 16β-H), 2.10 (3H, s, 21-H), 2.03 (1H, m, 12β-H), 1.73–1.77 (4H, m, 1α-H, 5-H, 11α-H, and 7β-H), 1.65–1.67 (2H, m, 15α-H and 16α-H), 1.35–1.40 (5H, m, 4β-H, 6α-H, 6β-H, 11β-H and 12α-H), 1.19–1.23 (2H, m, 8-H and 15β-H), 1.07 (1H, m, 14-H), 0.94–0.99 (2H, m, 7α-H and 9-H), 0.59 (3H, s, 18-H); ^{13}C NMR (125.77 MHz): 209.2 (C-20), 136.3 (Ph C-1′), 128.8 (Ph C-3′,5′), 128.3 (Ph C 4′), 128.0 (Ph C-2′,6′), 72.1 (C-19), 66.9 (C-3), 63.5 (C-17), 57.8 (C-2), 57.0 (C-14), 53.5 (***C***H_2-Ph), 52.8 (C-9), 43.8 (C-13), 40.0 (C-10), 38.7 (C-12), 38.5 (C-5), 35.9 (C-8), 32.9 (C-4), 31.6 (C-7), 31.4 (C-21), 31.0 (C-1), 27.4 (C-6), 24.2 (C-15), 22.7 (C-16), 21.0 (C-11), 13.4 (C-18); MS (ESI): m/z 524 (M+Na, 100), 363 (10); HRMS (MALDI) m/z [M+Na^+] 524.2451 (calcd for $C_{28}H_{39}NO_5SNa$ 524.2447).

4.2.10. 3α-Fluoro-*S*,*S*-dioxo-19,2-(epoxythioimino)pregnan-20-one (9)

To a solution of aziridine **17** (20.0 mg, 0.051 mmol) in anhydrous THF (1.0 mL) was added TBAF (29.0 mg, 0.127 mmol) at 0 °C and the mixture was allowed to reach 25 °C under N_2. After 1 h the THF was removed in vacuo and the oily residue was purified by preparative TLC on silica gel (hexane/AcOEt 4:6), to give the 3α-fluoro derivative **9** (17.7 mg, 0.043 mmol, 84%) as a white solid: mp 188–190 °C (from AcOEt–hexane); IR (KBr); 3553, 3276, 2934, 2877, 1697, 1448, 1362, 1176, 1059, 967, 738 cm^{-1};

^{1}H NMR (500.13 MHz): 4,75 (1H, br s, SO_2NH), 4.53 (1H, br d, J_{H-F} = 44.2 Hz, 3β-H), 4.50 (1H, d, J = 12.5 Hz, 19b-H), 4.33 (1H, d, J = 12.5 Hz, 19a-H), 3.70 (1H, m, 2α-H), 2.71 (1H, br d, J = 15.7 Hz, 1β-H), 2.52 (1H, t, J = 8.9 Hz, 17-H), 2.17 (1H, m, 16β-H), 2.11 (3H, s, 21-H), 2.06–2.08 (2H, m, 4α-H and 12β-H), 1.74–1.79 (4H, m, 4β-H, 5-H, 7β-H and 11α-H), 1.65–1.69 (3H, m, 1α-H, 15α-H and 16α-H), 1.35–1.43 (3H, m, 6β-H, 11β-H and 12α-H), 1.16–1.27 (4H, m, 6α-H, 8-H, 14-H and 15β-H), 0.97–1.03 (2H, m, 7α-H and 9-H), 0.62 (3H, s, 18-H); ^{13}C NMR (125.77 MHz): 209.2 (C-20), 88.9 (d, J_{C-F} = 175.0 Hz, C-3), 71.8 (C-19), 63.5 (C-17), 56.5 (C-14), 52.9 (C-9), 49.8 (d, J_{C-F} = 31.4 Hz, C-2), 44.0 (C-13), 40.4 (C-10), 39.2 (C-5), 38.7 (C-12), 36.0 (C-8), 31.5 (C-21), 31.2 (C-7), 30.0 (d, J_{C-F} = 19.7 Hz, C-4), 29.9 (C-1), 27.5 (C-6), 24.2 (C-15), 22.8 (C-16), 21.3 (C-11), 13.5 (C-18); HRMS (MALDI) m/z [M+Na$^+$] 436.1956 (calcd for $C_{21}H_{32}FNO_4SNa$ 436.1934).

4.3. GABA$_A$ receptor activity assays

4.3.1. Membrane preparation

Whole cerebella from male Sprague-Dawley rats (200–250 g) were homogenized in 5 vol (v/w) of ice-cold 0.32 M sucrose, using a Teflon-glass homogenizer at 1200 rpm. The homogenate was centrifuged at 1000g for 10 min at 4 °C. The supernatant was carefully decanted and centrifuged at 15,000g for 20 min at 4 °C. The pellet (P_2) was washed twice with 50 mM Tris–HCl buffer (pH 7.4) followed by centrifugation at 15,000g for 20 min at 4 °C. The final pellet was suspended in 1.2 mL of the same buffer and frozen at −20 °C. On the days of the assay, membranes were thawed, centrifuged at 15,000g for 20 min at 4 °C and the pellet was washed twice with 30 vol of the incubation ice-cold buffer followed by centrifugation (15,000g, 20 min). The final pellet was resuspended in the incubation buffer (50 mM Tris–Hcl, pH 7.1, in the case of [^{3}H]flunitrazepam and 50 mM Tris–acetate, pH 7.4, in the case of [^{3}H]muscimol) to a protein concentration of approximately 1 mg/mL.

4.3.2. [^{3}H]Flunitrazepam binding assays

The effects of allopregnanolone (**1**) and sulfamidates **7**, **8** and **9** on [^{3}H]flunitrazepam binding were evaluated in cerebellum P_2 homogenates using essentially the protocol described previously.[34] Briefly, 100 μL aliquots of cerebellum membrane preparation P_2 were incubated with 1 nM [^{3}H]flunitrazepam (85.2 Ci/mmol) in the presence or absence of different concentrations of the steroids (10–800 nM). All steroids were dissolved in DMSO (Sigma–Aldrich Corp., St. Louis, MO) and diluted with the incubation buffer (1:1000) immediately before use. Diazepam (1 μM) was used to determine non-specific binding. Assays were carried out at 4 °C for 90 min and terminated by rapid filtration through glass microfiber filters (Micro 96 Harvester, Molecular Devices). Filter bound radioactivity was quantified by liquid scintillation spectrophotometry.

4.3.3. [^{3}H]Muscimol binding assays

The effects of allopregnanolone (**1**) and sulfamidates **7**, **8** and **9** on [^{3}H]muscimol binding were evaluated in cerebellum P_2 homogenates using essentially the protocol described previously.[34] Briefly, 100 μL aliquots of cerebellum membrane preparation P_2 were incubated with 10 nM [^{3}H]muscimol (18.0 Ci/mmol) in the presence or absence of different concentrations of the steroids (20–1000 nM). All steroids were dissolved in DMSO (Sigma–Aldrich Corp., St. Louis, MO) and diluted with the incubation buffer (1:1000) immediately before use. GABA (10 μM) was used to determine non-specific binding. Assays were carried out at 4 °C for 60 min and terminated by rapid filtration through glass microfiber filters (Micro 96 Harvester, Molecular Devices). Filter bound radioactivity was quantified by liquid scintillation spectrophotometry.

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Supplementary data

Supplementary data (^{1}H and ^{13}C NMR spectra of compounds **7**, **8**, **9**, **15**, **17** and **18**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.08.008.

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