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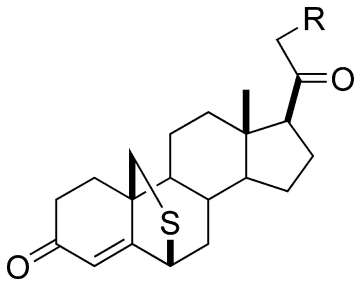
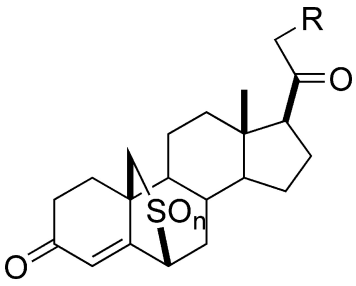



**3a R = H**
**3b R = OH**




**9a R = H, n=1**
**9b R = OH, n=1**
**10a R = H, n=2**
**10b R = OH, n=2**

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# 6,19-Sulfur-Bridged Progesterone Analogues with Antiimmunosuppressive Activity[1]

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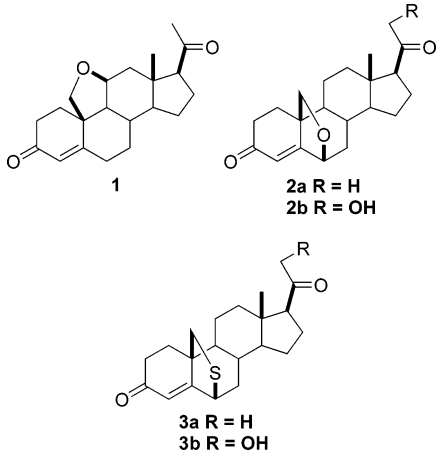
Sulfur-bridged pregnanes 6,19-epithioprogesterone, 21-hydroxy-6,19-epithioprogesterone, and the corresponding sulfoxides and sulfones were synthesized and tested as blockers of the immunosuppresive activity of dexamethasone in rat thymocytes. A new one-pot procedure is described for the preparation of 6,19-epithioprogesterone and related compounds by iodocyclization of a 19-sulfanylpregn-5-ene. Antiimmunosuppresive activity was evaluated by the ability of the different steroids to block dexamethasone-mediated apoptosis in thymocytes and dexamethasone-mediated inhibition of the NF$\kappa$-B transcription factor activity. DNA fragmentation and annexin V-FITC positive cells were taken as parameters of apoptosis whereas NF$\kappa$-B activity was tested by the expression of the reporter vector $\kappa$B-luciferase by TNF-$\alpha$ in Hela cells. 21-Hydroxy-6,19-epithioprogesterone *S*,*S*-dioxide had improved activity in both parameters, while 21-hydroxy-6,19-epithioprogesterone had improved activity only in blocking dexamethasone-induced programmed cell death.

## Introduction

Glucocorticoids exert a wide range of antiinflammatory and other immunosuppressive activities after binding to the glucocorticoid receptor. Today synthetic derivatives are widely used in the treatment of inflammatory disorders, autoimmunity and cancer.[2]

Opposing conformational characteristics for glucocorticoids and mineralocorticoids have been described by Weeks et al., who used X-ray diffraction to demonstrate that optimal glucococorticoid properties could be obtained with corticoids exhibiting a torsioned A ring toward the alpha face.[3] Previous work from our group has shown that structural modifications that flatten the pregnane skeleton increase mineralocorticoid activity.[4] In this respect we have used oxygen bridges involving selected carbons of the steroid nucleus to obtain conformationally restrained analogues of progesterone. Thus, the introduction of a 11,19-epoxy bridge gives a flat molecule, 11,19-epoxyprogesterone (**1**), shown to be a potent mineralocorticoid comparable to aldosterone in vivo, despite the lack of "typical" groups associated with corticoid activity as the 21-hydroxyl and/or a functionalized C-18.[5−7] This compound remains to this date as one of the few synthetic agonists of the mineralocorticoid receptor, its activity being associated primarily with the flatness introduced by the 11,19-epoxy bridge. In contrast, the introduction of a 6,19-epoxy bridge in the progesterone molecule gives rise to a bent structure in which the A ring has an inverted chair conformation. Although 6,19-epoxyprogesterone (**2a**) with its ring A highly torsioned toward the $\alpha$-face is devoid of glucocorticoid activity, introduction of a hydroxyl group at C-21 gives rise to a highly selective antiglucocorticoid, 21-hydroxy-6,19-epoxyprogesterone (**2b**), which at variance with most of the known antiglucocorticoids (e.g. mifepristone, 11$\beta$-(4-(dimethylamino)phenyl)-17$\beta$-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one) is completely devoid of antiprogestagen activity[8] and is not abortifacient.[9] Recently, this compound has been used to explore the mechanism of cortisol/progesterone antagonism in the regulation of 15-hydroxyprostaglandin dehydrogenase activity.[10]

These basic findings strongly suggested novel applications for an antiglucocorticoid without gestagenic or antigestagenic properties, which should neither be abortive nor otherwise affect ovarian or endometrial

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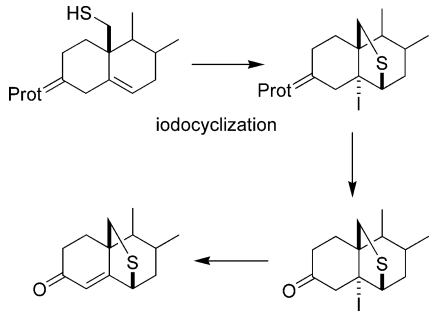
**Scheme 1**




functions. According to these considerations and using epoxypregnane **2b** as a lead, we initially focused our attention on the characteristics of the 6,19-bridge and the possibility of achieving an optimal degree of bending that would maximize the antiglucocorticoid activity maintaining the lack of antigestagenic properties, a highly desirable combination of properties from the medical standpoint.

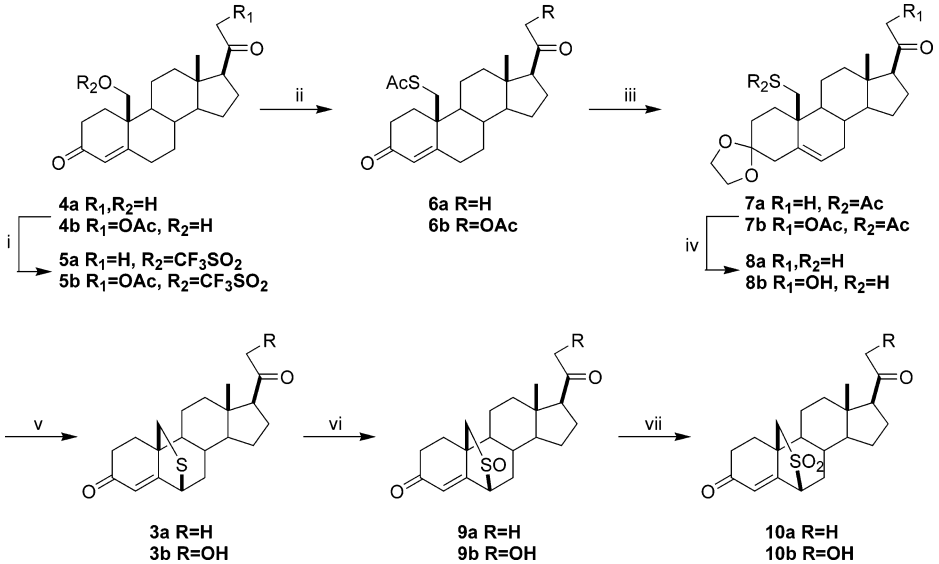
6,19-Sulfur bridges are an attractive alternative to generate analogues of 6,19-epoxyprogesterone and related compounds. The longer C−S bonds in the 6,19-epithio isosteres **3a** and **3b** should give rise to a less tensioned structure, which semiempirical calculations (AM1) predicted to be less bent than the oxygen-bridged analogues. The possibility of evaluating the effect of small changes in the degree of bending can be envisaged. Furthermore, the sulfur bridge allows for wider structural variations (e.g. sulfoxide and sulfone derivatives) exhibiting different lipophilicities and increased steric and polar interactions on the $\beta$-face of the steroid molecule.

## Chemistry

At variance with 6,19-epoxy bridges, 6,19-sulfur bridges cannot be introduced directly via hypoiodite type reactions.[11] The strategy followed in this work as outlined in Scheme 1, was to introduce a thiol group at C-19 and form the S-heterocycle by an iodine induced addition to the 5,6-double bond, presumably proceeding via the sulfenyl iodide.[12] The resulting iodine at C-5 can then be used to regenerate the 4,5 double bond in the final product.

19-Hydroxyprogesterone (**4a**) was used as starting material for the synthesis of 6,19-epithioprogesterone (**3a**) and its oxidized analogues **9a** and **10a** (Scheme 2, **a** series). Thus the 19-hydroxy group was converted to the triflate[13] and displaced with potassium thioacetate in acetone to give the 19-thioacetate **6a**. Throughout these reactions it was important that the double bond be conjugated with the C-3 carbonyl, as a nonconjugated 5,6-double bond would react intramolecularly with the 19-triflate giving the useless 5,19-cyclosteroid. Compound **6a** was converted to the ethyleneketal derivative[14] (**7a**) in which the double bond migrated to the 5,6-position as required for the cyclization and the 19-thioacetate was hydrolyzed with potassium hydroxide in methanol to give the free thiol (**8a**). Several reaction conditions summarized in Table 1 were tried for the iodocyclization of the latter compound. Entry 1 corresponds to the conditions used by Nicolau et al.[12] that in our case gave rise to a complex mixture of products. Increasing the amount of iodine (entry 2) and the reaction temperature, gave the cyclized 5-iodo-3-ketone **11** (with hydrolysis of the ethylene ketal), together with its elimination product, 6,19-epithioprogesterone (**3a**); dimer **12** was also formed, probably arising from oxidation of the thiol by iodine. Dimer **12** was also the major product when the thiol **8a** was treated with iodine and triethylamine in that order (entry 3); however, when the order of addition was reversed, direct cyclization to ketal **13** was observed (entry 4). Reducing the amount of iodine and adding the steroid to the mixture of triethylamine and iodine resulted in the one-pot conversion of 19-thiol **8a** into 6,19-epithioprogesterone (**3a**) without isolation of intermediates in 46% yield (entry 5). Formation of the oxidized derivatives **9a** and **10a** was accomplished by oxidation with potassium hydrogen monoperoxysulfate (Oxone) in aqueous methanol at room temperature.[15] Short reaction times gave sulfoxide

**Scheme 2**[a]



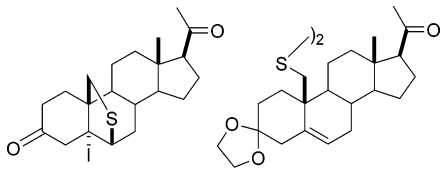

[a] Reagents. i) $(F_3CSO_2)_2O$, py; ii) KSAc, acetone; iii) ethylene glycol, $(EtO)_3CH$, $H_2SO_4$; iv) KOH, MeOH; v) $I_2$, $Et_3N$, $Cl_2CH_2$; vi) $KHSO_5$, MeOH, 30 min; vii) $KHSO_5$, MeOH, 24 h.

**Table 1.** Iodocyclization of Thiols **8a** and **8b**

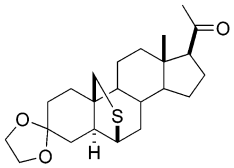
| entry | steroid | base | steroid/iodine/base | conditions | products (% yield)[a] |
|---|---|---|---|---|---|
| 1 | **8a** | $K_2CO_3$ | 1:2:5.6 | −78 °C, 3 h | complex mixture |
| 2 | **8a** | $K_2CO_3$ | 1:4:5.6 | −10 °C, 1.5 h | **3a** (14%), **11** (7%), **12** (7%) |
| 3 | **8a** | $Et_3N$ | 1:4:1.1 | 0 °C, 30 min; 25 °C, 2 h | **12** (40%)[b] |
| 4 | **8a** | $Et_3N$ | 1:4:1.1 | 0 °C, 30 min; 25 °C, 2 h | **13** (45%)[c] |
| 5 | **8a** | $Et_3N$ | 1:2.1:1.1 | 0 °C, 30 min; 25 °C, 2 h | **3a** (46%)[d] |
| 6 | **8b** | $Et_3N$ | 1:2.4:1 | 0 °C, 10 min; 25 °C, 3 h | **3b** (40%)[d] |

[a] See Supporting Information for NMR spectral data of compounds **11**−**13**; compound **11** decomposed to give **3a** upon standing in solution. [b] Iodine and then $Et_3N$ added to steroid solution. [c] $Et_3N$ and then iodine added to steroid solution. [d] Steroid added to $Et_3N$ and iodine solution.

**9a** (single stereoisomer), while longer reaction times gave the sulfone **10a**.



**11** **12**



**13**

Following essentially the above procedure, the sulfur isostere of 21-hydroxy-6,19-epoxyprogesterone **3b** and the oxidized derivatives **9b** and **10b** were obtained using 21-acetoxy-19-hydroxyprogesterone (**4b**) as starting material (Scheme 2, **b** series). 21-Hydroxy-6,19-epithioprogesterone (**3b**) was obtained in an overall yield of 13%. Chemoselective oxidation of **3b** with Oxone afforded the sulfoxide **9b** and the sulfone **10b** depending on the reaction time as indicated above, without affecting the α-ketolic side chain.

**Conformation of 6,19-Epithioprogesterone and Analogues.** As mentioned above, semiempirical calculations (AM1) predicted that the longer C−S bonds would give rise to a less tensioned structure for 6,19-epithioprogesterone (**3a**) compared to its oxygen bridged analogue, resulting in a less bent A ring. This was confirmed by single-crystal X-ray diffraction studies of both 6,19-epoxyprogesterone (**2a**) and 6,19-epithioprogesterone (**3a**). 6,19-Epoxyprogesterone crystalized with two very similar but independent molecules per asymmetric unit that differed slightly in the bending of the A ring, while 6,19-epithioprogesterone had a single form with a less bent ring A (see Supporting Information for displacement elipsoid diagrams). Superposition of the X-ray structures of the sulfur and oxygen bridged steroids (Figure 1) shows the difference in ring A bending.

As mentioned above, both sulfoxides **9a** and **9b** were obtained as single isomers. In both cases, the $^1H$ NMR spectra showed a ca. 0.3 ppm downfield shift for H-4 when compared to the reduced analogues (**3a** and **3b**) and almost no difference when compared with the corresponding sulfones (**10a** and **10b**). These data indicated almost identical environments for H-4 in

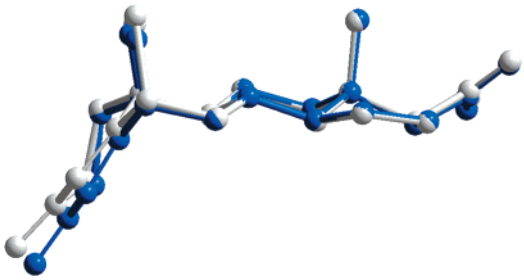


**Figure 1.** Superposition of the crystal structures of 6,19-epoxyprogesterone (**2a**) (blue) and 6,19-epithioprogesterone (**3a**) (white). Angles between the A-ring plane (defined by atoms C-3, C-4, C-5, C-10) and the B−C−D plane (defined by atoms C-5 to C-17) are 59.2° for **2a** and 54.2° for **3a**. The RMS error for overlay of atoms C-7−C-9 and C-11−C-18 is 0.0333 Å; distance O-3(**2a**)/O-3(**3a**) 0.66 Å.

6,19-sulfoxides and 6,19-sulfones, suggesting that the sulfoxide oxygen is oriented toward the A ring in **9a** and **9b**.

## Biological Activity

**Antiglucocorticoid Activity on Dexamethasone-Mediated Apoptosis.** Apoptosis is essential for the development and maintenance of the immune system.[2] Glucocorticoids induce apoptosis in nucleated cells of the vascular system playing a central antiinflammatory role.[16] Thus, apoptosis of thymocytes has been repeatedly employed as a representative parameter of glucocorticoid-mediated immunosuppression.[17,18] In vivo treatment with glucocorticoids induces 90% of rat thymic cells death, being mainly immature cells which localize in the thymus cortex.[19] Early studies have reported that the apoptotic pattern of DNA fragmentation appeared rapidly (within 2 h) in rat thymocytes after the injection of dexamethasone, it occurred prior to loss of cellular viability, was maximal at 12−24 h and disappeared 48−72 h after treatment.[20] In this work, the DNA fragmentation was clearly visualized 2 h after hormone treatment (data not shown).

Thus, antiimmunosuppresive activity was evaluated by the ability of the different steroids to block dexamethasone-mediated apoptosis in thymocytes. Dose−response experiments were performed in order to test the apoptosis-blocking capacities of the different sulfur-bridged steroids (Figure 2). The results show that the 21-hydroxy derivatives **3b** and **10b** were equally effective at $10^{-4}$ M to prevent dexamethasone-induced DNA fragmentation (compare lane 5 and 9 vs lane 2). On the other hand, the sulfoxide **9b** was ineffective at both concentrations tested (lanes 6 and 7). The antiapoptotic activity of the respective 21-deoxy analogues was also assayed with negative results except for the sulfone **10a** which showed marginal activity at $10^{-4}$ M (data not

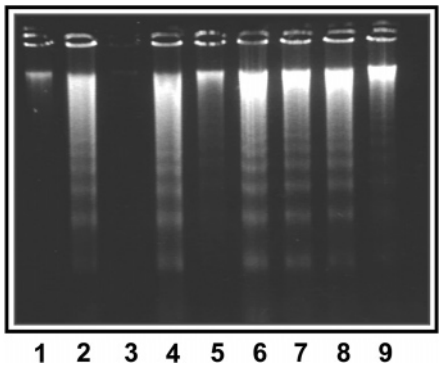



**Figure 2.** Inhibition of dexamethasone $10^{-8}$ M induced apoptosis (DNA fragmentation) in isolated thymocytes by 21-hydroxyepithiopregnanes (**3b**, **9b** and **10b**). Lane 1: control (no steroids added); lanes 2−9: dexamethasone $10^{-8}$ M; lane 3: mifepristone $10^{-6}$ M; lane 4: **3b** $10^{-5}$ M; lane 5: **3b** $10^{-4}$ M; lane 6: **9b** $10^{-5}$ M; lane 7: **9b** $10^{-4}$ M; lane 8: **10b** $10^{-5}$ M; lane 9: **10b** $10^{-4}$ M.

shown). None of the 21-hydroxy derivatives attained the potency of the antiglucocorticoid RU-486 (lane 3 vs lanes 4−9) in this assay.

Upon induction of apoptosis, rapid alterations in the organization of membrane phospholipids occur leading to exposure of phosphatidylserine (PS) to the external cell surface.[21] Recognition of PS by phagocytes in vivo results in the removal of cells programmed to die. In vitro detection of externalized PS can be achieved through interaction with the anticoagulant annexin V.[22] In the present work we used a fluorescein isothiocyanate (FITC) conjugate of annexin V to allow the detection of apoptosis by flow cytometry. Thymocytes were incubated in the presence of dexamethasone ($10^{-8}$ M) and the sulfur-bridged steroids at different concentrations. Table 2 summarizes the results obtained in this assay, fold induction of apoptosis was calculated as the ratio between positive annexin cells observed in each treatment with respect to the controls (no steroids added). Dexamethasone $10^{-8}$ M increased apoptosis 2-fold, this effect was almost completely blocked upon coincubation with mifepristone (RU-486) $10^{-6}$ M. Similar inhibition was obtained upon coincubation with 21-hydroxy-6,19-epithioprogesterone (**3b**) $10^{-6}$ M and $10^{-7}$ M and with the 21-hydroxy-sulfone **10b** in the $10^{-7}$−$10^{-5}$ M range, both compounds resulting more active than the 6,19-epoxy analogue **2b**. The sulfoxide **9b** was less active at all concentrations tested ($10^{-7}$−$10^{-5}$ M). Only compound **10b** inhibited the dexamethasone effect in a dose-dependent manner in the concentration range tested. The 21-deoxy analogues **3a** and **10a** were less active than the corresponding 21-hydroxy steroids in agreement with the DNA fragmentation results.

**Antiglucocorticoid Activity on Dexamethasone Ability To Inhibit TNFα-Mediated Activation of NFκB Transcription Factor.** The transcription factor, nuclear factor-kappa-B (NFκB), can be induced by pro-inflammatory cytokines and is important in immunological and inflammatory processes.[23] Glucocorticoids have also been known as repressors of NFκB activity. Antiimmunosuppresive activity was also evaluated by the ability of the different steroids to reverse dexamethasone-mediated inhibition of TNFα activity. Hela cells were transfected with a reporter vector expressing luciferase enzyme under the control of the κB response element. The results summarized in Figure 3 show that the 21-hydroxy derivatives **3b** and **9b** were marginally effective at $10^{-5}$ M to prevent dexamethasone inhibition (Figure 3, D and E respectively), while the sulfone derivative **10b** had a strong blocking effect on glucocorticoid activity (Figure 3, F). In fact, TNFα-mediated luciferase expression was potentiated by this compound.

**Table 2.** Inhibition of Dexamethasone-Induced Apoptosis in Thymocites by Epithiopregnanes **3a**, **3b**, **9a**, **9b**, **10a**, and **10b**[a]

| treatment | apoptosis (fold induction)[b] |
|---|---|
| dexamethasone $10^{-8}$ M + **3a** $10^{-7}$ M | 1.68 ± 0.24 (2) |
| dexamethasone $10^{-8}$ M + **3a** $10^{-6}$ M | 1.60 ± 0.11 (2) |
| dexamethasone $10^{-8}$ M + **3a** $10^{-5}$ M | 1.42 ± 0.29 (2) |
| dexamethasone $10^{-8}$ M + **9a** $10^{-7}$ M | 1.41 ± 0.04 (2) |
| dexamethasone $10^{-8}$ M + **9a** $10^{-6}$ M | 1.30 ± 0.08 (2) |
| dexamethasone $10^{-8}$ M + **9a** $10^{-5}$ M | 1.71 ± 0.03 (2) |
| dexamethasone $10^{-8}$ M + **10a** $10^{-7}$ M | 1.50 ± 0.08 (2) |
| dexamethasone $10^{-8}$ M + **10a** $10^{-6}$ M | 1.50 ± 0.04 (2) |
| dexamethasone $10^{-8}$ M + **10a** $10^{-5}$ M | 1.53 ± 0.27 (2) |
| dexamethasone $10^{-8}$ M + **3b** $10^{-7}$ M | 1.19 ± 0.20 (3)[c] |
| dexamethasone $10^{-8}$ M + **3b** $10^{-6}$ M | 1.18 ± 0.18 (3)[c] |
| dexamethasone $10^{-8}$ M + **3b** $10^{-5}$ M | 1.45 ± 0.12 (3) |
| dexamethasone $10^{-8}$ M + **9b** $10^{-7}$ M | 1.59 ± 0.14 (3) |
| dexamethasone $10^{-8}$ M + **9b** $10^{-6}$ M | 1.56 ± 0.19 (3) |
| dexamethasone $10^{-8}$ M + **9b** $10^{-5}$ M | 1.78 ± 0.03 (3) |
| dexamethasone $10^{-8}$ M + **10b** $10^{-7}$ M | 1.22 ± 0.14 (3) |
| dexamethasone $10^{-8}$ M + **10b** $10^{-6}$ M | 1.11 ± 0.13 (3)[c] |
| dexamethasone $10^{-8}$ M + **10b** $10^{-5}$ M | 1.03 ± 0.13 (3)[d] |
| dexamethasone $10^{-8}$ M | 1.94 ± 0.10 (4) |
| dexamethasone $10^{-8}$ M + mifepristone $10^{-6}$ M | 1.31 ± 0.24 (4)[c] |
| dexamethasone $10^{-8}$ M + **2b** $10^{-7}$ M | 1.77 ± 0.07 (2) |
| dexamethasone $10^{-8}$ M + **2b** $10^{-6}$ M | 1.63 ± 0.08 (2) |

[a] Thymocytes were incubated during 4 h at 37 °C. A fluorescein isothiocyanate (FITC) conjugate of annexin V was used to detect apoptosis by flow cytometry. Positive annexin V cells were analyzed as described in Experimental Section. Inhibition by mifepristone and **2b** is presented for comparison purposes. [b] Results are expressed as the mean fold induction relative to controls ± SD ($n$); control = 1. [c] $P < 0.05$ vs dexamethasone. [d] $P < 0.01$ vs dexamethasone.

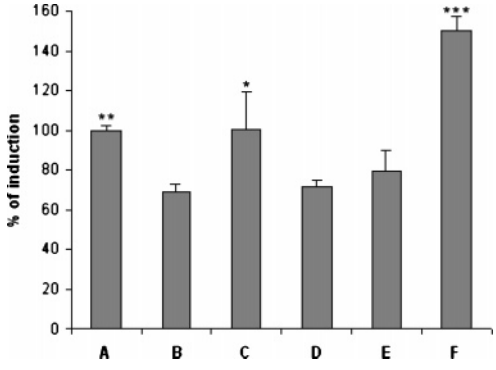



**Figure 3.** Blocking effects of 21-hydroxyepithiopregnanes **3b** (D), **9b** (E) and **10b** (F) at $10^{-5}$ M final concentration, on the inhibition of TNFα-mediated luciferase/β-galactosidase ratio by dexamethasone in Hela cells. A: TNFα 10 ng/mL (no steroids added); B−F: dexamethasone $10^{-7}$ M; C: mifepristone $10^{-6}$ M. Results are expressed as mean ± SD of three independent experiments. *: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.001$ vs B.

**Antiprogestin Activity.** This was assayed in Cos-1 cells transfected with the progesterone receptor. The inhibiting effect of the sulfur derivatives **3b**, **9b**, and **10b** was evaluated on the stimulation of the luciferase/β galactosidase ratio by progesterone $10^{-8}$ M.[24] Figure 4 shows the results when the different steroids were co-incubated with progesterone. The Type II antiprogestin

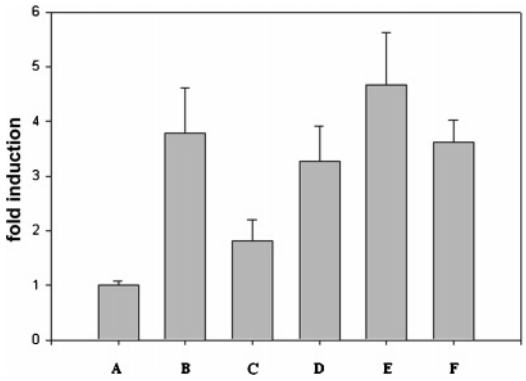



**Figure 4.** Inhibiting effects of 21-hydroxyepithiopregnanes **3b** (D), **9b** (E) and **10b** (F) at $10^{-6}$ M final concentration, on the stimulation of the luciferase/$\beta$-galactosidase ratio by progesterone in Cos-1 cells transfected with the progesterone receptor. A: control (no steroids added); B−F: progesterone $10^{-8}$ M; C: mifepristone $10^{-6}$ M. Results are expressed as mean ± SD of three independent experiments.

mifepristone, inhibited 52% of the progesterone response (column C; however, none of the steroids assayed (**3b**, **9b** and **10b**) inhibited progesterone action, suggesting that these compounds are not progesterone antagonists.

## Discussion

Besides sensitivity and lack of toxicity, one of the main goals for an efficient "specific" or "pure" antiglucocorticoid should be its lack of overlapping or cross-activation with other structure and receptor related steroid families exhibiting quite different hormonal properties (e.g. the antagonists of mineralocorticoids, androgens or progestagens), a pitfall usually described as "promiscuity".

A second goal intends to suppress or inhibit medically unfavorable effects within the vast network of the pleiotropic glucocorticoid response itself. This goal refers to pathologies in which certain glucocorticoid effects are overinduced, or situations in which even normal levels of these effects, in contrast to other glucocorticoid responses, become medically undesirable. A typical example of the first type are Cushing's disease and syndrome of diverse aethiology, including iatrogenic Cushing. Those of the second type comprise diverse immunodeficiency syndromes in which immunosuppression of any origin, including that inherent to glucocorticoids, should be held to a minimum while other glucocorticoid functions such as, for example, metabolic anti-stress responses (i.e. glyconeogenic responses) should be maintained. Most desirable, although difficult to achieve, is, in this category, the development of drugs counteracting certain antigen-specific immunosuppressions while not affecting the initial, unspecific immunosuppressive phenomenon of antiinflammation.

At this point it seems pertinent to discuss our present, as yet incomplete knowledge on how glucocorticoids regulate physiological processes. Their potent immunosuppressive activity is, according to present ideas, derived from the receptor's ability to repress the transcription of many immunocompetent molecules by interfering with transcription factors such as NF-$\kappa$B, AP-1, NF-AT, CREB and members of the Stat family. It is believed that certain of these factors are predominantly involved in inflammation (innate immunity), while others are so mainly in acquired immunity, but specific differences are still a matter of discussion.[2] Independently from the issues of this discussion however, glucocorticoids are also known to control thymus programmed cell death through modulation of a few genes such as members of the *bcl-2* family.[25−27] Even if mechanisms for the hormone dependent activation of these particular genes are still poorly understood, they are known to include DNA-binding-dependent activation of gene expression, transcriptional repression and cross-talk between signaling pathways at the cytosolic level.[2]

Several years ago we showed the antiglucocorticoid properties of 21-hydroxy-6,19-epoxyprogesterone (**2b**) and its lack of affinity at the receptoral level for both mineralocorticoid and progesterone receptors.[8] This drug has recently been shown to lack abortifacient properties.[9] The synthetic procedure described above allowed us now, for the first time, to prepare a series of 6,19-sulfur-bridged analogues of **2b** only with improved potency but also as first steps toward specificity for determined glucocorticoid-dependent mechanisms.

Thus 21-hydroxy-6,19-epithioprogesterone (**3b**) exhibited improved antiapoptotic activity in comparison to the oxygen-bridged analogue, while the oxidation of the sulfur atom to a sulfone function (**10b**) had a marked effect on both antiapoptosis and the ability to revert dexamethasone-mediated-inhibition of NF$\kappa$B activity. Hence, the reduced analogue **3b**, blocks dexamethasone-induced apoptosis selectively. Interestingly, none of these steroids were able to block dexamethasone-induced tyrosine aminotransferase (TAT) activity in rat hepatoma cells in the concentration range $10^{-7}$−$10^{-5}$ M (data not shown).

## Experimental Section

**General.** 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 measured in Bruker AC-200 or AM-500 NMR spectrometers in deuteriochloroform (using TMS as internal standard). The *J* values are given in Hz. Spectra were assigned by analysis of the DEPT, COSY 45 and HETCOSY spectra and by comparison with those of progesterone.[28]

Single-crystal X-ray measurements were performed on an *R*3*m* Siemens four-circle diffractometer, with graphite monocromated Mo Kα radiation. The structures were solved by direct methods with SHELXS97[29] and refined by full matrix least squares in $F^2$ using SHELXL97.[30] Hydrogen atoms were placed at calculated positions and allowed to ride onto their host carbon atoms. Molecular plots were drawn with XP, in the SHELXLTL-PC package.[31]

The electron impact mass spectra (EI) were measured in a Shimadzu QP 5000 mass spectrometer at 70 eV by direct inlet. Electron impact high-resolution mass spectra (HRMS) were obtained in a VG ZAB BEQQ mass spectrometer. All solvents used were reagent grade. Solvents were evaporated at ca. 45 °C under vacuum. Vacuum liquid chromatography (VLC) and column chromatography were carried out on Kieselgel 60-G (Merck) and Kieselgel S 0.040−0. 063 mm, respectively. TLC analysis was performed on silicagel 60 F254 (0.2 mm thick). The homogeneity of all compounds was confirmed by thin-layer chromatography.

19-Hydroxyprogesterone (**4a**) and 6,19-epoxyprogesterone (**2a**) were obtained from pregnenolone acetate, and 21-acetoxy-19-hydroxyprogesterone (**4b**) was obtained from 21-acetoxypregnenolone (Steraloids Inc.), following essentially the procedures described by Kirk et al.[32,33] Mifepristone was purchased from Sigma-Aldrich.

**19-(Acetylsulfanyl)-3,3-ethylenedioxypregn-5-en-20-one (7a).** A solution of 19-hydroxyprogesterone (**4a**) (522 mg, 1.58 mmol) in cold pyridine (5.2 mL) was added dropwise to a stirred solution of trifluoromethanesulfonic anhydride (0.7 mL, 4.16 mmol) in cold pyridine (3.6 mL) under nitrogen. The solution was allowed to warm to room temperature, and after 1 h, dichloromethane (50.0 mL) was added. The reaction mixture was washed with 1 M sulfuric acid, 5% aqueous sodium bicarbonate solution and water, dried and evaporated to dryness, yielding crude triflate **5a** as an orange solid (680 mg, 100%) which was used immediately in the following step; $^1$H NMR (200.13 MHz) $\delta$ 0.68 (3H, s, 13-$CH_3$), 2.12 (3H, s, 20-$CH_3$), 2.50 (1H, t, $J$ = 8.0 Hz, H-17), 4.68 (1H, d, $J$ = 10.0 Hz, 19a-H), 4.85 (1H, d, $J$ = 10.0 Hz, 19b-H), 5.99 (1H, s, H-4); $^{13}$C NMR (50.32 MHz) $\delta$ 209.0 (C-20), 197.6 (C-3), 165.0 (C-5), 127.9 (C-4), 78.5 (C-19), 63.2 (C-17), 56.3 (C-14), 53.9 (C-9), 44.0 (C-13), 42.0 (C-10), 38.8 (C-12), 36.5 (C-1), 34.0 (C-8), 32.8 (C-7), 32.6 (C-6), 32.0 (C-2), 31.3 (C-21), 24.1 (C-15), 22.9 (C-16), 21.6 (C-11), 13.3 (C-18).

A mixture of the 19-triflate **5a** obtained above (680 mg, 1.58 mmol) and potassium thioacetate (680 mg, 6.0 mmol) in acetone (35.0 mL), was stirred at room temperature for 20 h under nitrogen. The reaction mixture was diluted with dichlorometane, filtered and evaporated to dryness affording crude 19-(acetylsulfanyl)pregnane **6a** (614 mg, 100%); $^1$H NMR (200.13 MHz) $\delta$ 0.70 (3H, s, 13-$CH_3$), 2.12 (3H, s, 20-$CH_3$), 2.32 (3H, s, 19-$CH_3$COS), 2.55 (1H, t, $J$ = 8.7 Hz, H-17), 3.19 (1H, d, $J$ = 13.6 Hz, 19a-H), 3.49 (1H, d, $J$ = 13.6 Hz, 19b-H), 5.88 (1H, s, 4-H); $^{13}$C NMR (50.32 MHz) $\delta$ 208.9 (C-20), 198.7 (C-3), 194.4 ($CH_3$*C*OS), 166.7 (C-5), 126.1 (C-4), 63.3 (C-17), 56.1 (C-14), 54.7 (C-9), 43.9 (C-13), 41.9 (C-10), 38.7 (C-12), 35.8 (C-8), 34.2 (C-1), 33.6 (C-2), 33.0 (C-7), 32.7 (C-6), 32.7 (C-19), 31.3 (C-21), 30.6 (*C*$H_3$−COS), 24.2 (C-15), 22.9 (C-16), 21.6 (C-11), 13.4 (C-21); EIMS *m/z* 388 (2) $[M]^+$, 345 (7), 300 (6), 43 (100).

To a solution of compound **6a** (614 mg, 1.58 mmol) in ethylene glycol (0.85 mL, 15.4 mmol) were added ethyl orthoformate (1.26 mL, 7.2 mmol) and *p*-toluenesulfonic acid (52.0 mg, mmol). The mixture was stirred for 2 h at room temperature under nitrogen, poured over saturated aqueous $NaHCO_3$ and extracted with dichloromethane. Column chromatography on silica gel with ethyl acetate−hexane as eluant yielded compound **7a** (256 mg, 63%); mp 151−153 °C (from EtOAc−hexane);$^1$H NMR (200.13 MHz) $\delta$ 0.65 (3H, s, 13-$CH_3$), 1.62 (1H, m, 7$\alpha$-H), 2.11 (3H, s, 20-$CH_3$), 2.31 (3H, s, 19-$CH_3$-COS), 2.10 (1H, m, 7$\beta$-H), 2.53 (1H, t, $J$ = 8.8 Hz, H-17), 3.03 (1H, d, $J$ = 14.4 Hz, 19a-H), 3.37 (1H, d, $J$ = 14.4 Hz, 19b-H), 3.94 (4H, m, ketal), 5.53 (1H, brd, $J$ = 5.0 Hz, 6-H); $^{13}$C NMR (50.3 MHz) $\delta$ 209.0 (C-20), 195.4 ($CH_3$*C*OS), 136.3 (C-5), 124.9 (C-6), 108.9 (C-3), 64.4−64.5 (O*C*$H_2$*C*$H_2$O), 63.5 (C-17), 57.6 (C-14), 50.2 (C-9), 44.0 (C-13), 41.7 (C-4), 39.5 (C-10), 38.9 (C-12), 35.6 (C-1), 32.8 (C-8), 31.2 (C-7), 31.2 (C-2), 31.2 (C-19), 31.2 (C-21), 30.9 (*C*$H_3$COS), 24.3 (C-15), 22.8 (C-16), 22.1 (C-11), 13.4 (C-18). EIMS *m/z* 432 (4) $[M]^+$, 389 (5), 343 (5), 99 (100). Anal. Calcd. for $C_{25}H_{36}O_4S$: C, H, S.

**6,19-Epithiopregn-4-ene-3,20-dione (3a).** Thioacetate **7a** (64 mg, 0.16 mmol) was dissolved in dry methanol (1.8 mL) and the solution deoxygenated by bubbling dry nitrogen through it for 15 min. A solution of KOH (14.5 mg, 0.26 mmol) in methanol (0.10 mL) was added, and the mixture was stirred at room temperature for 15 min. The reaction mixture was neutralized with 1N HCl, diluted with water, concentrated and extracted with dichloromethane. Evaporation of the solvent afforded the 19-sulfanyl derivative **8a** (45 mg, 77%) which was used immediately in the following step; $^1$H NMR (200.13 MHz) $\delta$ 0.70 (3H, s, 13-$CH_3$), 1.25 (1H, dd, $J$ = 4.7 and 8.5 Hz, 19-HS), 1.63 (1H, m, 7$\alpha$-H), 2.12 (1H, m, 7$\beta$-H), 2.77 (1H, t, $J$ = 8.5 Hz, 17-H), 2.56 (1H, dd, $J$ = 11.1 and 8.5, 19a-H), 3.07 (1H, dd, $J$ = 11.1 and 4.7, 19b-H), 3.94 (4H, m, ketal), 5.66 (1H, br d, $J$ = 5.0 Hz, H-6); $^{13}$C NMR (50.32 MHz) $\delta$ 209.4 (C-20), 135.3 (C-5), 126.1 (C-6), 108.9 (C-3), 64.3−64.4 (O*C*$H_2$*C*$H_2$O), 63.6 (C-17), 58.0 (C-14), 50.6 (C-9), 44.2 (C-13), 41.9 (C-4), 40.4 (C-10), 39.1 (12), 35.9 (C-1), 33.0 (C-8), 31.3 (C-2), 31.3 (C-21), 30.8 (C-7), 26.2 (C-19), 24.3 (C-15), 22.9 (C-16), 22.0 (C-11), 13.4 (C-18).

To a solution of triethylamine (0.017 mL, 0.12 mmol) and iodine (63 mg, 0.25 mmol) in dry dichloromethane (32 mL) cooled to 0 °C was added a solution of the thiol **8a** (45 mg, 0.12 mmol) in dichloromethane (20 mL), and the mixture stirred at 0 °C for 30 min and then 2 h at room temperature. Saturated aqueous sodium thiosulfate was added until a colorless mixture was obtained and the reaction mixture extracted with dichloromethane. Evaporation of the solvent followed by column chromatography on silica gel with ethyl acetate as eluant yielded 6,19-epithiopregn-4-ene-3,20-dione (**3a**, 19 mg, 46%); mp 183−185 °C (from EtOAc−hexane); $\lambda_{max}$(MeOH) 241 nm; $\nu_{max}$ (KBr)/cm$^{-1}$ 2945, 1712, 1667, 1362, 1191, 742; $^1$H NMR (200.13 MHz) $\delta$ 0.74 (3H, s, 13-$CH_3$), 1.62 (1H, m, 7$\alpha$-H), 1.99 (1H, m, 7$\beta$-H), 2.12 (3H, s, 20-$CH_3$), 2.50 (1H, t, $J$ = 8.0 Hz, 17-H), 2.57 (1H, d, $J$ = 10.5 Hz, 19a-H), 3.05 (1H, d, $J$ = 10.5 Hz, 19b-H), 3.89 (1H, dd, $J$ = 2.2 and 3.6, 6-H), 5.80 (1H, s, 4-H); $^{13}$C NMR (50.32 MHz) $\delta$ 208.9 (C-20), 198.9 (C-3), 172.2 (C-5), 115.4 (C-4), 63.3 (C-17), 54.8 (C-14), 50.9 (C-9), 49.3 (C-6), 46.4 (C-10), 44.5 (C-13), 42.4 (C-7), 38.4 (C-12), 35.8 (C-19), 33.7 (C-8), 33.5 (C-2), 31.4 (C-21), 30.3 (C-1), 24.0 (C-15), 23.1 (C-16), 23.1 (C-11), 13.7 (C-18); EIMS *m/z* 344 (3) $[M]^+$, 254 (5), 149 (5), 84 (50), 49 (100). Anal. Calcd for $C_{21}H_{28}O_2S$: C, H, S.

**6,19-Epithiopregn-4-ene-3,20-dione *S*-Oxide (9a).** To a solution of crude compound **3a** (20.0 mg, 0.06 mmol) in methanol (1.9 mL) at 0 °C was added a solution of Oxone (56.9 mg, 0.18 mmol) in water (1.26 mL). After being stirred 30 min at room temperature, the mixture was diluted with saturated aqueous sodium bisulfite, concentrated and extracted with dichloromethane. Purification by prep TLC ($CH_2Cl_2$−MeOH 20:1) afforded sulfoxide **9a** (9.5 mg, 45%); mp 180−181 °C (from EtOH); $\lambda_{max}$(MeOH) 242 nm; $\nu_{max}$ (KBr)/cm$^{-1}$ 2938, 1705, 1669, 1362, 1177, 1035, 735; $^1$H NMR (200.13 MHz) $\delta$ 0.70 (3H, s, 13-$CH_3$), 2.11 (3H, s, 20-$CH_3$), 2.50 (1H, t, $J$ = 8.0 Hz, 17-H), 3.75 (1H, d, $J$ = 24 Hz, 19a-H), 3.98 (1H, d, $J$ = 24 Hz, 19b-H), 3.83 (1H, bt, $J$ = 2.2 and 3.6, 6-H), 6.07 (1H, s, 4-H); $^{13}$C NMR (50.32 MHz) $\delta$ 208.4 (C-20), 197.0 (C-3), 167.5 (C-5), 123.2 (C-4), 68.5 (C-6), 63.4 (C-19), 63.0 (C-17), 55.0 (C-14), 49.5 (C-9), 47.5 (C-10), 44.3 (C-13), 38.0 (C-12), 35.1 (C-8), 35.0 (C-7), 33.3 (C-2), 31.3 (C-21), 30.0 (C-1), 23.8 (C-15), 23.0 (C-16), 22.8 (C-11), 13.7 (C-19); EIMS *m/z* 360 $[M]^+$ (1.3), 345 (1), 344 (3), 312 (1), 297 (6), 255 (5), 43 (100); HRMS *m/z* found $[M]^+$ 360.1765 ($C_{21}H_{28}O_3S$ requires 360.1759).

**6,19-Epithiopregn-4-ene-3,20-dione *S*,*S*-Dioxide (10a).** To a solution of crude compound **3a** (20.0 mg, 0.06 mmol) in methanol (1.9 mL)) at 0 °C was added a solution of Oxone (56.4 mg, 0.18 mmol) in water (1.3 mL). After being stirred 24 h at room temperature, the mixture was diluted with saturated aqueous sodium bisulfite, concentrated and extracted with dichlorometane. Purification by prep TLC ($CH_2Cl_2$−MeOH 20:1) afforded sulfone **10a** (10.0 mg, 44%); mp 186−187 °C (from EtOH); $\lambda_{max}$(MeOH) 241 nm; $\nu_{max}$ (KBr)/cm$^{-1}$ 2945, 1697, 1312, 1134, 735; $^1$H NMR (500.13 MHz) $\delta$ 0.73 (3H, s, 13-$CH_3$), 2.12 (3H, s, 20-$CH_3$), 2.50 (1H, t, $J$ = 8.0 Hz, 17-H), 2.98 (1H, d, $J$ = 13.3 Hz, 19a-H), 3.46 (1H, d, $J$ = 13.3 Hz, 19b-H), 3.83 (1H, t, $J$ = 2.6, 6-H), 6.09 (1H, s, 4-H); $^{13}$C NMR (125.77 MHz) $\delta$ 208.5 (C-20), 195.7 (C-3), 162.9 (C-5), 123.7 (C-4), 64.9 (C-6), 63.5 (C-19), 62.9 (C-17), 55.2 (C-14), 51.2 (C-9), 46.7 (C-10), 44.5 (C-13), 38.1 (C-12), 36.3 (C-7), 33.5 (C-8), 33.3 (C-2), 31.3 (C-21), 30.5 (C-1), 23.8 (C-15), 23.5 (C-11), 23.0 (C-16), 13.8 (C-18); EIMS *m/z* 376 $[M]^+$ (4), 358 (1), 343 (1), 344 (1) 329 (1), 312 (13), 279 (1.5); HRMS *m/z* found $[M]^+$ 376.1703 ($C_{21}H_{28}O_4S$ requires 376.1708).

**21-Hydroxy-6,19-epithiopregn-4-ene-3,20-dione (3b).** A solution of 21-acetoxy-19-hydroxyprogesterone (**4b**) (1 g, 2.58 mmol) in cold pyridine (5.0 mL) was added dropwise to a stirred solution of trifluoromethanesulfonic anhydride (1.0 mL, 5.94 mmol) in cold pyridine (3.5 mL) under nitrogen. The solution was allowed to warm to room temperature, and after 1 h, dichloromethane (98.0 mL) was added. The reaction mixture was washed with 1 M sulfuric acid, 5% aqueous sodium bicarbonate solution and water, dried and evaporated

to dryness, yielding crude **5b** as an orange solid (1.34 mg, 100%). $^1$H NMR (200.13 MHz) $\delta$ 0.68 (3H, s, 13-$CH_3$), 2.13 (3H, s, 21-$CH_3$CO), 2.50 (1H, t, $J$ = 8.0 Hz, 17-H), 4.67 (1H, d, $J$ = 10.0 Hz, 19a-H), 4.64 (1H, d, $J$ = 10.0 Hz, 21a-H), 4.73 (1H, d, $J$ = 10.0 Hz, 21b-H), 4.83 (1H, d, $J$ = 10.0 Hz, 19b-H), 5.97 (1H, s, 4-H); $^{13}$C NMR (125.77 MHz) $\delta$ 203.2 (C-20), 197.8 (C-3), 170.2 ($CH_3$*C*OO), 161.9 (C-5), 127.8 (C-4), 78.5 (C-19), 69.1 (C-21), 59.2 (C-17), 56.3 (C-14), 53.7 (C-9), 44.3 (C-13), 42.0 (C-10), 38.3 (C-12), 36.1 (C-1), 34.0 (C-8), 32.7 (C-7), 32.5 (C-6), 31.9 (C-2), 24.2 (15), 22.7 (C-16), 21.5 (C-11), 20.4 (*C*$H_3$COO), 13.1 (C-18).

A mixture of crude 19-triflate **5b** obtained above (1.34 g, 2.58 mmol) and potassium thioacetate (1.34 g, 11.83 mmol) in acetone (75.0 mL) was stirred at room temperature for 20 h under nitrogen. The reaction mixture was diluted with dichlorometane, filtered and evaporated to dryness. Vacuum liquid chromatography on silica gel with ethyl acetate−hexane as eluant yielded 19-(acetylsulfanyl)pregnane **6b** (714 mg, 62%); $^1$H NMR (200.13 MHz) $\delta$ 0.74 (3H, s, 13-$CH_3$), 2.16 (3H, s, 21-$CH_3$CO), 2.32 (3H, s, 19-$CH_3$COS), 2.50 (1H, t, $J$ = 8.0 Hz, 17-H), 3.18 (1H, d, $J$ = 13.7 Hz, 19a-H), 3.47 (1H, d, $J$ = 13.7 Hz, 19b-H), 4.50 (1H, d, $J$ = 16.8 Hz, 21a-H), 4.70 (1H, d, $J$ = 16.8 Hz, 21b-H), 5.87 (1H, s, 4-H); $^{13}$C NMR (125.77 MHz) $\delta$ 203.3 (C-20), 198.7 (C-3), 194.4 ($CH_3$*C*OS), 170.1 ($CH_3$*C*OO), 166.6 (C-5), 126.1 (C-4), 69.0 (C-21), 58.9 (C-17), 56.2 (C-14), 54.6 (C-9), 44.6 (C-13), 41.9 (C-10), 38.4 (C-12), 35.8 (C-8), 34.3 (C-1), 33.6 (C-2), 33.5 (C-7), 32.9 (C-6), 32.7 (C-19), 30.6 (*C*$H_3$COS), 24.3 (C-15), 22.8 (C-16), 21.5 (C-11), 20.4 (*C*$H_3$COO), 13.3 (C-18); EIMS *m/z* 446 (2) $[M]^+$, 403 (7), 358 (5), 43 (100).

To a solution of compound **6b** (714 mg, 1.60 mmol) in ethylene glycol (0.85 mL, 15.38 mmol) were added ethyl orthoformate (1.24 mL, 7.46 mmol) and *p*-toluenesulfonic acid monohydrate (60.6 mg, 0.32 mmol). The mixture was stirred for 3 h at room temperature under nitrogen, poured over saturated aqueous $NaHCO_3$ and extracted with dichloromethane. Column chromatography on silica gel with ethyl acetate−hexane as eluant yielded ethylene ketal **7b** (461 mg, 59%); $^1$H NMR (200.13 MHz) $\delta$ 0.70 (3H, s, 13-$CH_3$), 2.31 (3H, s, 19-$CH_3$COS), 2.50 (1H, t, $J$ = 8.4 Hz, 17-H), 3.03 (1H, d, $J$ = 14.0 Hz, 19a-H), 3.36 (1H, d, $J$ = 14.0 Hz, 1H), 3.94 (4H, m, ketal), 4.50 (1H, d, $J$ = 16.7 Hz, 21a-H), 4.70. (1H, d, $J$ = 16.7 Hz, 21b-H), 5.53 (1H, br d, $J$ = 2.7 Hz, 6-H); EIMS *m/z* 490 (3) $[M]^+$, 447 (6), 402 (6), 99 (69), 43 (100).

Thioacetate **7b** (461 mg, 0.94 mmol) was dissolved in dry methanol (14 mL) and the mixture deoxygenated by bubbling dry nitrogen through it for 15 min. A solution of KOH (103 mg, 1.95 mmol) in methanol (0.72 mL) was added, and the mixture was stirred at room temperature for 15 min. The reaction mixture was neutralized with 1 N HCl, diluted with water, concentrated and extracted with dichloromethane. Evaporation of the solvent afforded the 19-sulfanyl derivative **8b** (377 mg, 89%), $^1$H NMR (200.13 MHz) $\delta$ 0.74 (3H, s, 13-$CH_3$), 1.25 (1H, dd, $J$ = 5.0 and 8.5 Hz, 19-SH), 2.16 (3H, s, 21-$CH_3$CO), 2.58 (1H, dd, $J$ = 11.0 and 8.5, 19a-H), 2.60 (1H, t, $J$ = 8.5 Hz, 17-H), 3.09 (1H, dd, $J$ = 11.0 and 5.0, 19b-H), 3.94 (4H, m, ketal), 4.50 (1H, d, $J$ = 16.8 Hz, 21a-H), 4.70 (1H, d, $J$ = 16.8 Hz, 21b-H), 5.65 (1H, br d, $J$ = 5.0 Hz, H-6).

To a solution of triethylamine (0.116 mL, 0.84 mmol) and iodine (518 mg, 2.04 mmol) in dry dichloromethane (240 mL) cooled to 0 °C was added a solution of thiol **8b** (363 mg, 0.84 mmol) in dichloromethane (200 mL), and the mixture stirred at 0 °C for 10 min and 3 h at room temperature. Saturated aqueous sodium thiosulfate was added until a colorless mixture was obtained, and the reaction mixture was extracted with dichloromethane. Evaporation of the solvent followed by column chromatography on silica gel with ethyl acetate as eluant yielded 21-hydroxy-6,19-epithiopregn-4-ene-3,20-dione (**3b**, 122 mg, 40%) mp 150−151 °C (from EtOH); $\lambda_{max}$(MeOH) 240 nm; $\nu_{max}$ (KBr)/cm$^{-1}$ 3465, 2938, 1712, 1676, 1070, 735; $^1$H NMR (500.13 MHz) $\delta$ 0.76 (3H, s, 13-$CH_3$), 2.46 (1H, t, $J$ = 9.3 Hz, 17-H), 2.57 (1H, d, $J$ = 10.7, 19a-H), 3.04 (1H, d, $J$ = 10.7, 19b-H), 3.90 (1H, dd, $J$ = 1.0 and 1.5 Hz, H-6), 4.16 (1H, d, $J$ = 11.0 Hz, 21a-H), 4.22 (1H, d, $J$ = 11.0 Hz, 21b-H), 5.79 (1H, s, H-4); $^{13}$C NMR (125.77 MHz) $\delta$ 209.9 (C-20), 198.9 (C-3), 172.1 (C-5), 115.5 (C-4), 69.3 (C-21), 58.8 (C-17), 54.8 (C-14), 50.7 (C-9), 49.2 (C-6), 46.3 (C-10), 45.2 (C-13), 42.3 (C-7), 38.1 (C-12), 35.8 (C-19), 33.7 (C-8), 33.4 (C-2), 30.2 (C-1), 23.0 (C-16), 23.0 (C-11), 24.1 (C-15), 13.8 (C-18); EIMS *m/z* 360 (32) $[M]^+$, 344 (16), 329 (54), 301 (100), 153 (34), 91 (43), 43 (99). Anal. Calcd for $C_{21}H_{28}O_3S$: C, H, S.

**21-Hydroxy-6,19-epithiopregn-4-ene-3,20-dione *S*-Oxide (9b).** To a solution of crude compound **3b** (47.5 mg, 0.13 mmol) in methanol (4.3 mL) at 0 °C was added a solution of Oxone (127.4 mg, 0.40 mmol) in water (2.8 mL). After being stirred 30 min at room temperature, the mixture was diluted with saturated aqueous sodium bisulfite, concentrated and extracted with dichlorometane. Purification by prep TLC ($CH_2Cl_2$−MeOH 20:1) afforded sulfoxide **9b** (24.0 mg, 48%); mp 198−199 °C (from EtOH); $\lambda_{max}$(MeOH) 245 nm; $\nu_{max}$ (KBr)/cm$^{-1}$ 3458, 2938, 1719, 1667, 1077, 1041; $^1$H NMR (500.13 MHz) $\delta$ 0.68 (3H, s, 13-$CH_3$), 2.50 (1H, t, $J$ = 8.0 Hz, 17-H), 3.72 (1H, d, $J$ = 20.0 Hz, 19a-H), 3.88 (1H, d, $J$ = 20.0 Hz, 19b-H), 3.83 (1H, bt, 6-H), 4.18 (2H, bs, 21-H), 6.08 (1H, s, 4-H); $^{13}$C NMR (125.77 MHz) $\delta$ 209.6 (C-20), 197.2 (C-3), 162.7 (C-5), 123.8 (C-4), 69.3 (C-21), 68.4 (C-6), 63.3 (C-19), 58.4 (C-17), 55.0 (C-14), 49.4 (C-9), 47.5 (C-10), 45.0 (C-13), 37.7 (C-12), 35.0 (C-8), 34.8 (C-7), 33.3 (C-2), 29.2 (C-1), 23.9 (C-15), 22.9 (C-16), 22.6 (C-11), 13.7 (C-18); EIMS *m/z* 376 (12) $[M]^+$, 359 (38), 345 (6), 313 (62), 159 (38), 91 (55), 55 (100), 41 (99). Anal. Calcd for $C_{21}H_{28}O_4S$: C, H, S.

**21-Hydroxy-6,19-epithiopregn-4-ene-3,20-dione *S*,*S*-Dioxide (10b).** To a solution of crude compound **3b** (47.5 mg, 0.13 mmol) in methanol (4.3 mL) at 0 °C was added a solution of Oxone (190.4 mg, 0.60 mmol) in water (4.3 mL). After being stirred 24 h at room temperature, the mixture was diluted with saturated aqueous sodium bisulfite, concentrated and extracted with dichlorometane. Purification by prep TLC ($CH_2Cl_2$−MeOH 20:1) afforded sulfone **10b** (26.0 mg, 50%); mp 230−231 °C (from EtOH); $\lambda_{max}$(MeOH) 241 nm; $\nu_{max}$ (KBr)/cm$^{-1}$ 3465, 2945, 1712, 1676, 1305, 7420; $^1$H NMR (500.13 MHz) $\delta$ 0.75 (3H, s, 13-$CH_3$), 2.50 (1H, t, $J$ = 8.0 Hz, 17-H), 3.45 (1H, d, $J$ = 13.5 Hz, 19a-H), 3.98 (1H, d, $J$ = 13.5 Hz, 19b-H), 3.82 (1H, br s, 6-H), 4.19 (2H, bs, 21-H), 6.09 (1H, s, 4-H); $^{13}$C NMR (125.77 MHz) $\delta$ 209.6 (C-20), 195.7 (C-3), 162.7 (C-5), 123,8 (C-4), 69.2 (C-21), 64.8 (C-6), 63.5 (C-19), 58.5 (C-17), 51.0 (C-9), 55.2 (C-14), 46.7 (C-10), 45.2 (C-13), 37.3 (C-12), 36.2 (C-7), 33.5 (C-8), 33.3 (C-2), 30.5 (C-1), 23.8 (C-15), 23.3 (C-16), 23.0 (C-11), 13.9 (C-18); EIMS *m/z* 392 (1) $[M]^+$, 361 (29), 333 (11), 267 (23), 253 (15), 91 (43), 55 (77), 43 (100). Anal. Calcd for $C_{21}H_{28}O_5S$: C, H, S.

**Crystallographic Data and Data Collection Parameters. 6,19-Epoxypregn-4-en-3,20-dione (2a).** Colorless rhombic plates recrystallized from acetone: mp 142−143 °C. $C_{21}H_{28}O_3$, $M$ = 328.43, orthorhombic, space group $P2_12_12_1$ (No 19); cell constants $a$ = 10.405(2) Å, $b$ = 16.508(3) Å, $c$ = 20.850(4) Å; $V$ = 3581.3(12) Å$^3$, $D_c$ ($Z$ = 8, two very similar but independent molecules per asymmetric unit) = 1.218 g cm$^{-3}$; crystal dimensions 0.45 × 0.40 × 0.18 mm, reflections measured: 3885 reflections unique: 3541, reflections observed ($I > 2\sigma(I)$): 1863; $R$ = 0.057 and $R_w^2$ = 0.090.

**6,19-Epithiopregn-4-ene-3,20-dione (3a)**. Colorless hexagonal plates recrystallized from acetone: mp 183−185 °C. $C_{21}H_{28}O_2S$, $M$ = 344.49, orthorhombic, space group $P2_12_12_1$ (No 19); cell constants $a$ = 11.153(2) Å, $b$ = 12.650(3) Å, $c$ = 12.940(4) Å; $V$ = 1825.6(8) Å$^3$, $D_c$ ($Z$ = 4) = 1.253 g cm$^{-3}$; crystal dimensions 0.35 × 0.30 × 0.05 mm, reflections measured: 2007 reflections unique: 1856, reflections observed ($I > 2\sigma(I)$): 1004; $R$ = 0.057 and $R_w^2$ = 0.100.

**Biological Activity Assays. DNA Fragmentation Assay.** Sprague Dawley rats weighing 70−80 g were the source of thymocytes; 72 h after adrenalectomy thymuses were freed from adjacent tissue, minced, suspended in RPM 1640 medium and filtered through 8 to 10 sheets of gauze. Cells were then suspended in 2 mL of medium per thymus and counted in a Neubauer chamber. Viability was analyzed by Trypan blue exclusion. 2 × 10$^7$ Cells were incubated on 24-well plastic dishes in 1 mL of RPMI 1640 medium containing 10% charcoal stripped-fetal calf serum and concanavalin A (2 $\mu$g/mL).

Treatments were performed by adding 1 $\mu$L of steroid stock solutions in DMSO. Cells were incubated at 37 °C during 2.5 h in a water bath under a normal atmosphere. After the incubation, cells were gently resuspended, placed in a 1.5 mL tube and centrifuged at 2000 rpm during 5 min at room temperature.

Apoptotic DNA from thymocytes was isolated according to the protocol described by Herrmann et al.[34] Briefly, cells were gently resuspended in 0.1 mL of lysis buffer (50 mM Tris, pH 7.5, 20 mM EDTA and 1% Nonidet P-40) during 40 s to 1 min. After centrifugation at 2000 rpm during 5 min at room temperature, the apoptotic DNA, remaining in the supernatant, was collected in another tube. The supernatants were then treated with 6 $\mu$L of 100 mg/mL RNAse A and 13 $\mu$L of 10% SDS plus 6 $\mu$L of water and incubated at 56 °C during 2 h. After RNAse treatment, 35 $\mu$L of 20 mg/mL proteinase K were added to each sample and digested at 37 °C overnight. DNA was precipitated by adding 75 $\mu$L of 10 M ammonium acetate and 450 $\mu$L of absolute ethanol. After precipitation, DNA was resuspended in 50 $\mu$L water and 10% of the sample was electrophoresed in 1.6% agarose gels containing 0.5 $\mu$g/mL ethidium bromide and visualized under UV light.

**Annexin V-FITC Apoptosis Assay.** Thymocytes were obtained from CF-1 21 days old male mice. Cells were incubated as described above during 4 h at 37 °C. An annexin V-FITC apoptosis detection kit from Clontech Inc. (Palo Alto, CA) was used and fluorescence was detected according to the RAPID protocol recommended by the manufacturer. Briefly, after incubation, cells were centrifugated at 2000 rpm for 5 min, the media removed and resuspended in 200 $\mu$L of binding buffer (approx $1 \times 10^6$ cells). Propidium iodide (5 $\mu$L) and annexin V-FITC (10 $\mu$L) were added and cells incubated for 15 min at room temperature in the dark. Samples were analyzed by flow cytometry in a Cytoron Absolute cytometer (Ortho Diagnostic Systems). Data were analyzed with Wimdi 2.7 software.

**Cell Culture and Transient Transfection.** Cos-1 and Hela cells were cultured at 37 °C under humified atmosphere with 5% $CO_2$ in DMEM supplemented with 10% fetal calf serum (FCS) containing penicillin (100 IU/mL), streptomycin (100 $\mu$g/mL) and glutamine (2 mM) in p100 plates. For transient transfections, $5 \times 10^5$ cells were plated in 60 mm plates and transfected by the lipofectin method according to the manufacter protocol (Lipofectine Plus, Gibco Inc.). Analysis of the PR activity were performed by cotransfecting 1 $\mu$g of phPR expressing human isoform B of the progesterone receptor[35] and 3 $\mu$g of pMMTV-luc plasmid which expresses luciferase enzyme under the control of Mouse Mammary Tumor Virus promoter containing several HRE elements;[36] 3 $\mu$g of pRSV-LacZ (Clontech Inc., Palo Alto, CA) was also introduced as control of transfection. Eighteen hours after transfection, the medium was replaced by new one containing 10% charcoal-stripped FCS and antibiotics. Cells were then incubated during 36 h under the different treatments. Incubations were stopped by aspirating the medium and washing the cells twice with phosphate saline buffer (PBS).

The immunosuppressive effect of GR was tested by the ability of the receptor to inhibit TNFα activity. Thus, Hela cells were transfected with 1 $\mu$g of p$\kappa$B-luc plasmid (kindly supplied by Dr. O. A. Coso, University of Buenos Aires, Argentina) which expresses luciferase enzyme under the control of $\kappa$B elements from HIV promoter; 3 $\mu$g of pRSV-LacZ (Clontech Inc., Palo Alto, CA) was also introduced as control of transfection. Six hours after transfection, the medium was replaced by new one containing antibiotics and the different steroids were added. Sixteen hours later, TNFα was added to a final concentration of 10 ng/mL. Cells were then incubated during 8 h. Incubations were stopped by aspirating the medium and washing the cells twice with phosphate saline buffer (PBS). Cells were then harvested in lysis buffer, and luciferase activity was measured with a luciferase kit according to the manufacturer protocol (Promega Inc.). $\beta$-Galactosidase activity was measured as previously described.[23] Steroids were applied from 1000-fold stock solutions in DMSO. The following hormone concentrations were used: dexamethasone $10^{-7}$ M; progesterone $10^{-8}$ M; mifepristone and the different sulfur derivatives, $10^{-5}$ M.

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**Supporting Information Available:** Complete crystallographic data (in cif format) and displacement elipsoid diagrams for compounds **2a** and **3a**. NMR spectral data for compounds **11**−**13** and elemental analysis data for compounds **3a**, **3b**, **7a**, **9b** and **10b**. This material is available free of charge via de Internet at http://pubs.acs.org.

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