measured by GC (split injector, fused silica capillary column coated with RSL 160 BP, flame ionization detector).

Biology. Evaluation of antifilarial activities was carried out by following the previously described protocols,[3,7] which are briefly summarized below.

In Vivo Screening. Rodents *P. oris* of both sexes were infected by injection of 200 infective larvae, obtained by dissection of the mosquitoes *Aedes aegypti*. After control of the microfilaremia, they were treated between 150 and 180 days after infectation. Compounds were administered subcutaneously in 1% (carboxymethyl)cellulose suspension to two rodents for 5 consecutive days. Microfilaremia was controlled for 6 weeks. At the end, adult filariae were counted at the autopsy. A compound would present a statistically significant ($p = 0.12$) macrofilaricidal effect if not a single male and not more than 1.6 female filaria were found.

In Vitro Screening. Infective larvae isolated from the intermediate host were maintained in a biphasic culture medium composed of a cell feeder layer (L 929) with RPMI (90 mL), fetal calf serum (10 mL), penicillin (1000 IU/mL medium), and streptomycin (100 IU/mL medium). Twenty larvae were put in the medium (1.5 mL) after decontamination in a sterile medium.

Compounds were dissolved in DMSO (100 μL) at several concentrations, and 10 μL of each solution was added to 20 larvae. Each run was duplicated. The percentage of survival larvae corrected by control was determined.

Registry No. **2aT**, 110380-88-6; **2aC**, 110417-67-9; **2bT**, 110380-87-5; **2bC**, 110381-01-6; **2cT**, 110380-91-1; **2cC**, 110417-68-0; **2dT**, 110417-69-1; **2eT**, 110380-92-2; **2fT**, 110380-93-3; **2gT**, 110380-94-4; **2hT**, 110380-89-7; **2iT**, 110417-70-4; **2jT**, 110380-90-0; **2kT**, 110417-71-5; **2lT**, 110417-72-6; **2mT**, 110417-73-7; **2nT**, 110417-74-8; **2oT**, 110417-75-9; **2pT**, 110417-76-0; **2qT**, 110417-77-1; **2rT**, 110380-97-7; **2sT**, 110380-96-6; **2sC**, 110417-78-2; **3a**, 35427-68-0; **3b**, 39542-27-3; **3c**, 3967-02-0; **3d**, 110417-46-4; **3e**, 110381-05-0; **3f**, 110381-06-1; **3g**, 110381-07-2; **3h**, 98069-37-5; **3i**, 51270-39-4; **3j**, 22457-12-1; **4a**, 56631-61-9; **4b**, 17299-32-0; **4c**, 110417-47-5; **4d**, 110417-48-6; **4e**, 110417-49-7; **5a**, 110381-00-5; **5b**, 110380-99-9; **5c**, 110417-50-0; **5d**, 110417-51-1; **6a**, 110417-52-2; **6b**, 110381-12-9; **6c**, 110417-53-3; **6d**, 110417-54-4; **7** (R = Ph), 52147-97-4; **7** (R = 4-$O_2NC_6H_4$), 52148-00-2; **8** (R = Ph, NR′ = Cl), 55129-82-3; **8b**, 110417-59-9; **8c**, 110417-60-2; **8c**·3,5-$(O_2N)_2C_6H_3CO_2H$, 110417-80-6; **9**, 78-88-6; **10**, 110417-57-7; **11**, 110417-58-8; **12a**, 73281-91-1; **12b**, 110417-55-5; **12c**, 110417-56-6; **13c**, 110417-62-4; **13T**, 110417-63-5; **14bT**, 17299-25-1; **14cT**, 110417-65-7; **15b**, 110417-64-6; **15c**, 110417-66-8; **16b**, 779-53-3; **16c**, 41298-85-5; **16c**·$HClO_4$, 110417-79-3; **17b**, 110-91-8; **17c**, 109-01-3; CH_2Br_2, 74-95-3; $BrCH_2SO_3H$·Na, 34239-78-6; $BrCH_2SO_2Cl$, 10099-08-8; $ClCH_2SO_2Cl$, 3518-65-8; $PhCH_2Br$, 100-39-0; $PhCO_2Me$, 93-58-3; PhCOMe, 98-86-2; PhCHO, 100-52-7; $H_2C{=}C(Cl)CH_2SO_3H$·Na, 55947-45-0; $H_2C{=}C(Cl)CH_2SO_2Cl$, 40644-59-5; $MeSO_2NMe_2$, 918-05-8; 4-ClC_6H_4CHO, 104-88-1; 3,4-$Cl_2C_6H_3CHO$, 6287-38-3; 2-MeC_6H_4CHO, 529-20-4; 3-MeC_6H_4CHO, 620-23-5; 4-$H_3C(CH_2)_5C_6H_4CHO$, 49763-69-1; 4-MeC_6H_4CHO, 104-87-0; MeCHO, 75-07-0; 1-propylpiperazine, 21867-64-1; 1-phenylpiperazine, 92-54-6; 1-methylpiperazine, 109-01-3; pyrrolidine, 123-75-1; 1-piperazinecarboxylic acid ethyl ester, 120-43-4; 3-piperazinyl-1-(trifluoromethyl)benzene, 15532-75-9; phenylacyl bromide, 70-11-1; 1-(methylsulfonyl)-4-methylpiperazine, 59039-17-7; *N*-(methylsulfonyl)morpholine, 1697-34-3; *N*-(1-(2-chloro-2-propenyl)sulfonyl)morpholine, 110417-61-3.

Synthesis of New Antiinflammatory Steroidal 20-Carboxamides: (20*R*)- and (20*S*)-21-(*N*-Substituted amino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene

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The synthesis and antiinflammatory activities of new steroidal 20-carboxamides, (20*R*)- and (20*S*)-21-(*N*-substituted amino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (**5–8**) are described. These compounds were prepared from the respective isomer of 20-dihydroprednisolonic acid, (20*R*)- and (20*S*)-11β,17,20-trihydroxy-3-oxo-1,4-pregnadien-21-oic acid (**4a** and **4b**), by coupling with primary amines after the activation of the steroid acid with N,N^1-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole. Confirmation of the configurational assignment at C-20 of the 20-carboxamides was achieved by reduction of methyl (20*R*)- and (20*S*)-11β,17,20-trihydroxy-3-oxo-1,4-pregnadien-21-oate (**3a** and **3b**) to the known stereochemistry at C-20 of (20*R*)- and (20*S*)-11β,17,20,21-tetrahydroxy-3-oxo-1,4-pregnadiene (**2a** and **2b**). The topical antiinflammatory activities of these steroidal 20-carboxamides were assessed by the croton oil induced ear edema assay and their local and systemic antiinflammatory activities by the cotton pellet granuloma bioassay. Results of these investigations suggest a structure–activity relationship where carboxamide derivatives with the 20(*R*)-hydroxy configurations exhibit higher potency than those with the 20-(*S*)-hydroxy configurations. The amides of steroidal 21-oic acids with high local antiinflammatory potency exhibited systemic activities unlike the corresponding esters of steroidal 21-oic acids, which are devoid of systemic activities.

The limiting factor in the use of corticosteroids for the chronic treatment of inflammatory conditions has been their adverse systemic side effects.[1] Therapeutic approaches such as dosage forms for local application, alternate-day administration, and concomitant "protective" therapy have been employed to reduce the adverse systemic effects of potent steroids. As a synthetic approach to the design of safer antiinflammatory steroids, we have introduced the antedrug concept. The term antedrug can be applied to an active synthetic derivative that is inactivated by the first metabolic step upon entry into the circulation. Thus, a true antedrug acts only locally and undergoes only one predictable metabolic step to an inactive metabolite.[2,3] Compounds that have at least one active intermediary metabolite can be considered as partial antedrugs. It has recently been established that methyl 11β,17-dihydroxy-3,20-dioxo-1,4-pregnadien-21-oate and the isomers of methyl (20*R*)- and (20*S*)-11β,17,20-trihydroxy-3-oxo-1,4-pregnadien-21-oate (**3a** and **3b**) retain significant local antiinflammatory activity, but are devoid of prednisolone-like side effects, such as pituitary adrenal suppression and thymus involution.[2–4] It has been sug-

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Table I. Physical Properties of 20-Carboxamide Derivatives

no.[a]	R	yield, %	mp, °C	R_f[c]	$[\alpha]^{20}_D$, deg	formula	anal.
5a	CH_3	37	173–175[b]	0.44	+58.1[d]	$C_{22}H_{31}NO_5$	C, H, N[g]
5b	CH_3	40	208–210[b]	0.41	−20.1	$C_{22}H_{31}NO_5$	C, H, N
6a	C_2H_5	52	232–233[e]	0.51	+55.7	$C_{23}H_{33}NO_5$	C, H, N
6b	C_2H_5	54	232–234.5[b]	0.45	−29.3	$C_{23}H_{33}NO_5$	C, H, N
7a	C_3H_7	72	251–253[e]	0.55	+54.2	$C_{24}H_{35}NO_5$	C, H, N
7b	C_3H_7	80	267–268[b]	0.51	−32.6	$C_{24}H_{35}NO_5$	C, H, N
8a	CH_2Ph	80	264–266[b]	0.59	+16.9	$C_{28}H_{35}NO_5$	C, H, N
8b	CH_2Ph	85	203.5–204.5[f]	0.54	−25.4	$C_{28}H_{35}NO_5$	C, H, N,

[a] See Scheme I and the Experimental Section for structural formulas and nomenclature. [b] Recrystallized from acetone. [c] Chloroform–methanol (85:15) used as a developing solvent. [d] Measured in methanol solution at a concentration of ca. 1%. [e] Recrystallized from acetone–hexane. [f] Recrystallized from chloroform. [g] Anal. Calcd: C, 67.84%. Found: C, 66.97%.

gested in accord with the antedrug concept that the absence of the systemic activity of these corticosteroids was due to their rapid hydrolysis to the inactive steroidal 21-oic acids upon entry into the circulatory system.[2,3,5] The mechanism of cellular action of these new steroids is similar to conventional steroids since they bind to hepatic glucocorticoid receptors,[6,9] and inhibit cell migration and liberation of prostaglandins and lysosomal enzymes.[7]

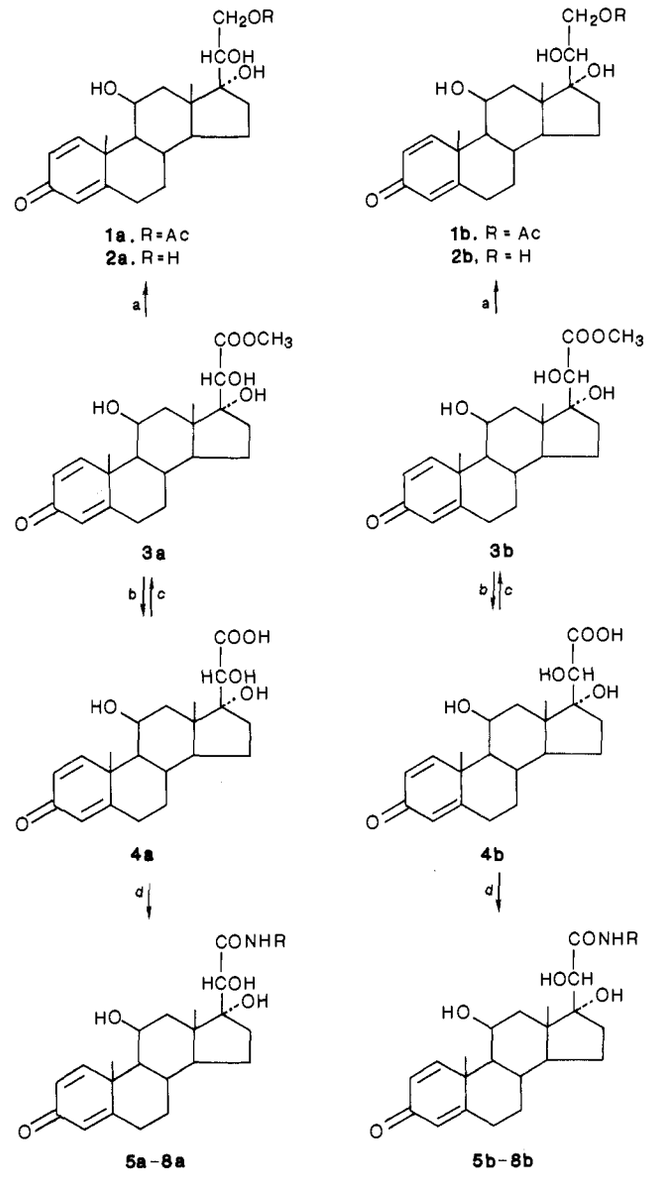
In continuing investigations of steroidal antedrugs we have included amide derivatives. Although steroidal carboxamides are expected to be hydrolyzed more slowly than the corresponding esters, the relationship between hydrolysis rates and therapeutic indices of the steroids with respect to local vs systemic activity has not been established. The antiglucocorticoid activities shown with a series of 17β-carboxamide derivatives of dexamethasone in cultured rat hepatoma cells (HTC) suggest that the carboxamide side chain plays an important role in determining potency as well as agonist/antagonist activity of the steroidal carboxamides.[8] In preliminary communications, the *N*-propyl (20*R*)- and (20*S*)-20-dihydroprednisolonamide epimers were reported to retain antiinflammatory activity in the cotton pellet granuloma bioassay and to bind to cytoplasmic receptors of rat liver and thymus.[6,9]

We now report the synthesis, local antiinflammatory activities, systemic effects, topical activity, and glucocorticoid receptor binding of the isomers of steroidal 20-carboxamides with different N-substituents.

Results and Discussion

Chemistry. An isomeric mixture of methyl (20*R*)- and (20*S*)-20-dihydroprednisolonate (**3a** and **3b**) was prepared by prolonged reaction of prednisolone with methanolic copper acetate as described by Lewbart and Mattox.[10] A complete separation of each isomer was achieved by semipreparative HPLC. As shown in Scheme I, each isomer of methyl 20ξ-dihydroprednisolonate was saponified with methanolic sodium hydroxide solution to yield the respective isomer of 20(*R* and *S*)-dihydroprednisolonic acid, (20*R*)- and (20*S*)-11β,17,20-trihydroxy-3-oxo-1,4-pregnadien-21-oic acid (**4a** and **4b**). The 20*R* isomeric carboxamide series of compounds (**5a–8a**) were successfully prepared in 40–85% yields by using a two-step procedure previously described by König and Geiger,[11] by activating

Scheme I[a]



[a] Reagents used: (a) $NaBH_4$–MeOH, (b) NaOH–MeOH, (c) CH_2N_2, (d) DCC, 1-hydroxybenzotriazole, amines–THF, CH_2Cl_2. Naming system: **a** for 20*R* configuration, **b** for 20*S* configuration.

the steroidal carboxylic acid **4a** with DCC and 1-hydroxybenzotriazole followed by coupling with primary amines. The 20*S* series (**5b–8b**) were obtained from **4b** by using the same procedure, in 40–85% yields. An attempt to prepare the carboxamides from steroidal car-

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boxylic acid and amines by condensing with DCC alone was unsuccessful due to the formation of stable *N*-acylureas, as suggested by Formstecher et al.[12] The structures of carboxamide products were confirmed by IR, ^{1}H NMR, and mass spectroscopy. The physical data and structural formulas are shown in Table I. All of the 20*R* isomeric carboxamides (**5a–8a**) showed positive optical rotation in contrast to negative rotation exhibited by the corresponding 20*S* isomers (**5b–8b**).

In order to confirm the configurational assignment at C-20 of methyl (20*R*)- and (20*S*)-dihydroprednisolonate (**3a** and **3b**), a reaction sequence designed to interrelate the methyl esters with steroidal glycerols of known stereochemistry at C-20 was performed as follows: Saponification of "delta-triol acetate" (**1a**), a compound of 20*S* orientation, afforded **2a** in 81% yield. Treatment of **3a** with an 8 molar excess of sodium borohydride in refluxing methanol afforded a product in 43% yield, which was identical with **2a** in all respects, including HPLC analysis. None of the above reactions were associated with configurational alteration at C-20. It is reported that an ester can be reduced to an alcohol with excess sodium borohydride in methanol, probably via methoxyborohydride, a more powerful reducing agent, and that this reaction is assisted by the proximal hydroxyl or ketone in the molecule.[13,14] An attempt to reduce the ester with lithium aluminum hydride failed mainly due to the many complex side products, which may include A-ring-reduced compounds. An analogous reaction scheme was carried out with the 20*S* compounds. It is well known that reduction of steroid-20-keto-21-acetate with sodium borohydride in aqueous dimethylformamide provides predominantly the 20(*R*)-ol.[15,16] When this reaction was applied to prednisolone 21-acetate, (20*R*)-21-acetoxy-11β,17,20-trihydroxy-3-oxo-1,4-pregnadiene (**1b**) was obtained in 63% yield. Saponification of **1b** afforded (20*R*)-11β,17,20,21-tetrahydroxy-3-oxo-1,4-pregnadiene (**2b**). Reduction of **3b** with a 12-fold molar excess of sodium borohydride in refluxing methanol gave a product identical with **2b** in 15% yield. The lower yield of **2b** compared with **2a** obtained from **3a** suggests that the configurational orientation of C-20 hydroxy group may play an important role in the reduction of methyl steroidal 21-oate.

Antiinflammatory Activity. The topical antiinflammatory activity of the 20-carboxamide derivatives was evaluated with the croton oil induced ear edema bioassay in the rat.[17] The results obtained with 0.75 mg of steroid applied to each ear are shown in comparison with prednisolone in Table II. The topical antiinflammatory activity of all the carboxamide derivatives was less than that of prednisolone with the benzyl derivative **8a** being the most active followed by the *n*-propyl (**7a**), ethyl (**6a, 6b**), and methyl substituted (**5a, 5b**) 20-carboxamides. For each pair of the N-substituted carboxamides, the *R* isomer was more active than the corresponding *S* isomer.

Since topical antiinflammatory activity of steroids as measured in the croton oil induced ear edema assay is known to correlate well with lipophilicity of compounds, octanol–water partition coefficients were determined for the 20-carboxamide derivatives (Table V). Almost a fourfold difference in partition coefficients is noted for compounds of this series and correlates well with topical antiinflammatory activity. An obvious discrepancy is noted with **8b**, however, where factors such as intrinsic activity and metabolism, which are not reflected in partitioning studies, also play roles in the antiinflammatory effectiveness of steroids.

Table II. Topical Antiinflammatory Activity of 20-Carboxamide Derivatives in the Croton Oil Ear Edema Assay

treatment	mean change,[a] mm (× 10^2)	% inhibition
control	46.2 ± 4.7	
prednisolone	14.5 ± 2.8***	68.6
5a[b]	30.7 ± 4.0*	33.5
5b	34.9 ± 4.6	24.5
6a	30.2 ± 3.4*	34.6
6b	30.5 ± 6.2*	33.8
7a	31.1 ± 3.8*	31.9
7b	35.3 ± 5.0	23.6
8a	26.5 ± 3.1**	42.6
8b	36.1 ± 3.0	21.9

[a] Following initial ear thickness measurements, all steroids were administered at a dose of 0.75 mg/ear to both ears, followed 30 min later by 50 μL of 5% croton oil in acetone. Five hours later, final thicknesses were measured, and mean changes ± SEM for five rats were calculated. The Student's *t* test was used to determine significant differences from vehicle treated controls at $p < 0.05$(*), $p < 0.01$(**), or $p < 0.005$ (***). [b] See Scheme I and the Experimental Section for structural formulas and nomenclature.

To simultaneously determine the local and systemic antiinflammatory effects of the carboxamide derivatives, the cotton pellet granuloma bioassay was employed. Inhibition of formation of granulation tissue around the pellet treated with steroids indicates local antiinflammatory action while the extent of granuloma formation around the contralateral pellet indicates systemic antiinflammatory action. Results of a study with 2-mg/treated pellets are shown in Table III. Three carboxamides (**5a, 7a,** and **8a**) exhibited higher local as well as systemic antiinflammatory activity than prednisolone. In agreement with the topical antiinflammatory activities, the 20*R* isomers were more potent that the corresponding *S* isomers.

Parameters of undesirable systemic actions include decreases in body weight gain, decreases in relative thymus and adrenal weights, and suppression of plasma corticosterone. These parameters were assessed in the granuloma bioassay and are shown in Table IV. Those derivatives that exhibited the most potent antiinflammatory activity also show the most significant undesirable systemic effects. However, **6a** shows antiinflammatory activity virtually identical with prednisolone, but has significantly less impact upon thymic involution and plasma corticosterone than does prednisolone.

The relative binding of the 20-carboxamides to rat hepatic cytosolic receptors is depicted in Table V. In comparison with prednisolone, high concentrations of the carboxamides were required to inhibit dexamethasone binding. However, as expected from their antiinflammatory activities, the *R* isomers showed higher receptor affinities than their corresponding *S* isomers. The highest binding affinities were seen with **8a** and **8b**, while the lowest affinities were exhibited by **6b** and **5b**. In general, the compounds with the larger substituents (**8a** and **7a**) had higher binding affinities than those with smaller substituents (**5b** and **6b**); however, exceptions to this trend were observed with **5a**, which also exhibited higher than expected local antiinflammatory activity.

Recently, a series of 17β-carboxamide derivatives of dexamethasone were shown to have antiglucocorticoid

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Table III. Effect of Locally Administered Steroidal 20-Carboxamides on Cotton Pellet Induced Granuloma Formation

	treated pellet		untreated pellet	
treatment	net dry wt, mg	% inhibn	net dry wt, mg	% inhibn
vehicle	77.9 ± 2.6[a]		79.2 ± 3.9	
prednisolone	30.7 ± 5.2***	60.6	58.2 ± 6.8*	26.6
5a[b]	18.9 ± 1.3***	75.5	40.8 ± 5.4***	48.6
5b	61.6 ± 10.1	21.1	82.8 ± 4.4	−4.5
6a	36.4 ± 5.0***	53.3	58.1 ± 5.0***	26.7
6b	59.9 ± 5.3**	23.1	83.1 ± 5.6	−4.9
7a	23.9 ± 5.6***	69.3	46.6 ± 9.6**	41.2
7b	33.4 ± 4.2***	57.1	48.3 ± 4.5***	39.1
8a	24.0 ± 4.7***	69.3	48.4 ± 6.9***	38.9
8b	61.6 ± 4.2**	20.9	75.2 ± 5.9	5.2

[a] Table values are the mean ± SEM for six animals. The Student's t test was used to determine significant differences from the vehicle-treated group at $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.005$ (***). [b] See Scheme I and the Experimental Section for structural formulas and nomenclature.

Table IV. Systemic Effects of Locally Administered Steroidal 20-Carboxamides

treatment	relative thymus wt, mg/100 g	relative adrenal wt, mg/100 g	body wt gain, g	plasma corticosterone, ng/mL
control	255.5 ± 12.9[a]	14.2 ± 0.8	56.1 ± 2.0	439.4 ± 38.6
prednisolone	110.2 ± 16.6***	12.1 ± 0.7*	55.0 ± 12.9	130 ± 42.7***
5a[b]	116.4 ± 11.5***	14.0 ± 0.6	46.0 ± 5.2*	321.2 ± 78.8
5b	247.0 ± 15.2	16.7 ± 0.8	55 ± 2.1	290.6 ± 71.3*
6a	204.9 ± 20.4*	15.7 ± 0.9	45.7 ± 4.3*	280.5 ± 51.2*
6b	256.8 ± 20.9	12.9 ± 0.8	54.7 ± 2.4	288.1 ± 36.2
7a	88.5 ± 11.5***	13.1 ± 1.0	30.2 ± 5.1***	235.5 ± 39.4***
7b	161.7 ± 21.7***	14.0 ± 0.5	56.7 ± 5.0	164 ± 15.3***
8a	167.1 ± 12.8***	12.4 ± 0.9	42.0 ± 1.9***	101.1 ± 46.2***
8b	264.2 ± 21.8	11.8 ± 0.8*	55.7 ± 2.8	297.7 ± 55.5*

[a] Values are the mean ± SEM for six animals. The Student's t test was used to determine significant differences from the vehicle-treated group at $p < 0.05$ (*) and $p < 0.005$ (***). [b] See Scheme I and the Experimental Section for structural formulas and nomenclature.

Table V. Relative Binding and Partition Coefficients of 20-Carboxamide Derivatives

treatment	IC_{50}[a]	octanol–water partition coefficient[b]
prednisolone	196.3 nM	30.0
5a[c]	274.8 μM	10.0
5b	574.0 μM	8.6
6a	493.2 μM	17.5
6b	868.9 μM	16.3
7a	251.2 μM	26.7
7b	336.5 μM	16.3
8a	53.6 μM	32.5
8b	75.2 μM	30.0

[a] IC_{50} represents the concentration of steroid required to inhibit the binding of 28 nM [^{3}H]dexamethasone by 50% to hepatic cytosolic receptors. [b] Values are the averages of two experiments, each carried out in duplicate. [c] See Scheme I and the Experimental Section for structural formulas and nomenclature.

effects in cultured rat hepatoma cells (HTC). The order of potency of these inhibitors was consistent with their affinities for the HTC glucocorticoid receptor. The *N*-benzyl 17β-carboxamide analogue of dexamethasone, which was the most potent antiglucocorticoid in cultured rat HTC, had the highest receptor affinity followed by the propyl and methyl derivatives.[8] A good correlation between pharmacological activities of glucocorticoids and their in vitro binding affinities to cytoplasmic receptors has been previously observed.[18] Interestingly, the *N*-benzyl 17β-carboxamide showed an additive suppression of in vitro phytohaemagglutinin-induced blastogenesis of normal human peripheral lymphocytes.[19]

Consistent with the steroidal 17-carboxamide results, the 20-carboxamides with bulkier N-substituent groups exhibited higher binding affinities and higher in vitro local antiinflammatory activities. In addition, in the present studies, the *R* isomers have higher binding affinities and higher in vitro antiinflammatory activities than their corresponding *S* isomers.

These results indicate that the size of the N-substituents at the steroidal C-21 and the orientation of the hydroxyl group at C-20 influence cytosolic receptor binding and antiinflammatory activities. The presence of undesirable systemic side effects suggests that, in contrast to the esters derived from the isomers of 20(*R*)-dihydroprednisolonic acid, the amides may not be rapidly hydrolyzed to inactive acids upon entry into the systemic circulation.

Experimental Section

Prednisolone and "delta-triol acetate", (20*S*)-21-acetoxy-11β,17,20-trihydroxy-3-oxo-1,4-pregnadiene, were obtained from the Upjohn Co. (Kalamazoo, MI). Melting points were determined on a Thomas capillary melting point apparatus and are uncorrected. IR spectra were determined as KBr pellets with a Perkin-Elmer Model 1430 spectrophotometer and were in agreement with the assigned structures. ^{1}H NMR spectra were obtained with a Bruker HX-270 instrument, and the chemical shifts were reported in parts per million (δ) downfield from tetramethylsilane as an internal standard. Mass spectra were recorded on a Finnigan 4510 GC/MS with a 70-eV source. The HPLC system (Waters Associates, Millford, MA) consisted of a C-18 reverse-phase analytical column (250 × 4.6 mm) or semipreparative 250 × 21 mm i.d. stainless steel column packed with Zorbax, and the detector wavelength was set at 254 nm. The flow rate was 0.5 mL/min for analytical separation with methanols water (60:40) as a mobile phase. Silica gel (Merck, 70–230 mesh) was used for column chromatographic separations. The homogeneity of the intermediates and products was determined by TLC on Merck 60F-254 plates with visualization under UV light. Ultraviolet absorbance data for the partition coefficient study were obtained on a Beckman UV 5260 spectrophotomer. Elemental analysis was performed by Galbraith Laboratories, Inc., Knoxville, TN.

Methyl (20*R*)-11β,17,20-Trihydroxy-3-oxo-1,4-pregnadien-21-oate (3a) and Methyl (20*S*)-11β,17,20-Trihydroxy-

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3-oxo-1,4-pregnadien-21-oate (3b). To a solution of prednisolone (6 g, 17 mmol) in methanol (250 mL) was added a solution of cupric acetate (1.5 g, 7.5 mmol) in methanol (300 mL). The mixture was allowed to stand for 30 min and subsequently bubbled with air at room temperature for 7 days. The reaction was stopped by the addition of 1% sodium bicarbonate solution (300 mL) containing Na_2EDTA (3 g). The methanol was removed by evaporation under vacuo at 35 °C. The remaining aqueous solution was extracted with ethyl acetate and washed with 1% sodium bicarbonate solution and water. The extract was dried (Na_2SO_4), and the solvent was evaporated to yield a dry residue (4.1 g). This crude mixture was dissolved in a small volume of acetone and placed on top of a silica gel column (850 × 28 mm). Elution with acetone–dichloromethane–hexane (3:2:5) gave a mixture of **3a** + **3b** (2.2 g, 33%), R_f 0.55 in chloroform–methanol (85:15). HPLC analysis showed a mixture of **3a** to **3b** in the ratio of 1:1.1. To separate each isomer, the above mixture was dissolved in methanol–water (60:40) and subjected to semipreparative HPLC with the above solvent mixture as a mobile phase at a flow rate of 6 mL/min (retention time of **3a** was 38 min and 57 min for **3b**). After the fraction containing **3a** was collected, methanol was evaporated in vacuo, and the aqueous phase was extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated to give platelets. Recrystallization from methanol afforded **3a** as platelets (750 mg): mp 254–255 °C; retention time (HPLC) 21.8 min; $[\alpha]^{20}_D$ +38.4°; ^1H NMR (Me_2SO-d_6) δ 1.05 (s, 3 H, 13-CH_3), 1.40 (s, 3 H, 10-CH_3), 3.62 (s, 3 H, 21-OCH_3), 4.06 (s, 1 H, 20-H), 4.21 (m, 1 H, 11-H), 5.91 (s, 1 H, 4-H), 6.15 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.32 (d, 1 H, J = 10 Hz, 1-H); MS, m/e (relative intensity) 390 (1.38, M^+), 121 (100). Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.56; H, 7.80.

The 20*S* isomer **3b** was obtained with the same procedure. Recrystallization from acetone–hexane mixture afforded **3b** (718 mg) as white prisms: mp 171–173 °C; retention time (HPLC) 31.2 min; $[\alpha]^{20}_D$ +17.7°; ^1H NMR ($CDCl_3$) δ 1.15 (s, 3 H, 13-CH_3), 1.45 (s, 3 H, 10-CH_3), 3.81 (s, 3 H, 21-OCH_3), 4.36 (s, 1 H, 20-H), 4.43 (m, 1 H, 11-H), 6.01 (s, 1 H, 4-H), 6.28 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.28 (d, 1 H, J = 10 Hz, 1-H); MS, m/e (relative intensity) 390 (2, M^+), 121 (100). Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 68.01; H, 7.67.

(20*R*)-11β,17,20-Trihydroxy-3-oxo-1,4-pregnadien-21-oic Acid (4a) from 3a. To a solution of **3a** (1 g, 2.56 mmol) in methanol (70 mL) was slowly added 2 N sodium hydroxide solution (4 mL). After being stirred for 3 h at room temperature, the reaction mixture was diluted with water (1 L), and the unreacted compound was removed by extracting with ethyl acetate. The remaining aqueous phase was acidified to pH 1.5 with 3 N hydrochloric acid, and the acidic portion was extracted twice with ethyl acetate (500 and 250 mL). The combined organic extracts were washed with water and dried (Na_2SO_4). The solvent was evaporated to dryness. Crystallization twice from methanol afforded **4a** (740 mg, 77%) as white prisms: mp 213–214 °C; $[\alpha]^{20}_D$ +34.7°; ^1H NMR (Me_2SO-d_6) δ 1.03 (s, 3 H, 13-CH_3), 1.39 (s, 3 H, 10-CH_3), 3.94 (s, 1 H, 20-H), 4.19 (m, 1 H, 11-H), 5.90 (s, 1 H, 4-H), 6.13 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.31 (d, 1 H, J = 10 Hz, 2-H), 8.30 (s, 1 H, 20-COOH). Anal. Calcd for $C_{21}H_{28}O_6$: C, 67.0; H, 7.50. Found: C, 66.51; H, 7.58.

(20*S*)-11β,17,20-Trihydroxy-3-oxo-1,4-pregnadien-21-oic Acid (4b) from 3b. Saponification of **3b** (1 g, 2.56 mmol) was carried out as in the preparation of **4a** from **3a**. Recrystallization from aqueous acetone gave **4b** (608 mg, 63%) as hexagonal prisms: mp 165–167 °C; $[\alpha]^{20}_D$ +18.2°; ^1H NMR (Me_2SO-d_6) δ 1.06 (s, 3 H, 13-CH_3), 1.42 (s, 3 H, 10-CH_3), 4.07 (s, 1 H, 20-H), 4.15 (m, 1 H, 11-H), 5.89 (s, 1 H, 4-H), 6.15 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.33 (d, 1 H, J = 10 Hz, 1-H), 8.31 (s, 1 H, 20-COOH). Anal. Calcd for $C_{21}H_{28}O_6$: C, 67.0; H, 7.50. Found: C, 66.47; H, 7.61.

General Synthetic Procedures of Carboxamides 5a–8a and 5b–8b. All procedures for carboxamide synthesis were virtually the same. To an ice-cooled solution of **4a** (500 mg, 1.33 mmol) for synthesis of **5a–8a** or **4b** for **5b–8b** in tetrahydrofuran (8 mL) and dichloromethane (40 mL) were added dicyclohexylcarbodiimide (301 mg, 1.46 mmol) and 1-hydroxybenzotriazole (200 mg, 1.46 mmol) in tetrahydrofuran (7 mL). The reacton mixture was stirred at 4 °C for 24 h. After the precipitated dicyclohexylurea was removed by suction filtration, the respective amines (1.4 mmol) were added to the filtrate, and the reaction was continued at 4 °C. After 10–24 h, the reaction mixture was diluted with dichloromethane (500 mL) and washed with 0.1 N hydrochloric acid and water. The organics were dried over anhydrous sodium sulfate and evaporated under vacuo to dryness. After being dissolved in a small amount of methanol, the residue was subjected to silica gel column chromatography (70 × 1.5 cm). Elution was performed with chloroform–methanol (9:1) as an eluent. Fractions (15 mL) were collected and examined by TLC. The fractions containing carboxamide were combined, and the organics were evaporated under vacuo to dryness. Recrystallizing solvent and physical data are shown in Table 1.

(20*R*)-21-(Methylamino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (5a): ^1H NMR (Me_2SO-d_6) δ 0.99 (s, 3 H, 13-CH_3), 1.37 ns, 3 H, 10-CH_3), 2.59 (d, 3 H, J = 4.5 Hz, NHCH_3), 3.78 (d, 1 H, J = 7 Hz, 20-H), 4.16 (m, 1 H, 11-H), 5.89 (s, 1 H, 4-H), 6.12 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.30 (d, 1 H, J = 10 Hz, 1-H), 7.77 (m, 1 H, NH); MS, m/e (relative intensity) 389 (5, M^+), 121 (92), 89 (100).

(20*S*)-21-(Methylamino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (5b): ^1H NMR (Me_2SO-d_6) δ 0.99 (s, 3 H, 13-CH_3), 1.38 (s, 3 H, 10-CH_3), 2.57 (d, 3 H, J = 4.5 Hz, NHCH_3), 3.93 (d, 1 H, J = 8 Hz, 20-H), 4.18 (m, 1 H, 11-H), 5.88 (s, 1 H, 4-H), 6.13 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.30 (d, 1 H, J = 10 Hz, 1-H), 7.61 (m, 1 H, NH); MS, m/e (relative intensity) 389 (4, M^+), 121 (86), 89 (100).

(20*R*)-21-(Ethylamino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (6a): ^1H NMR (Me_2SO-d_6) δ 1–1.07 (m, 6 H, 13-CH_3 and $NHCH_2CH_3$), 1.38 (s, 3 H, 10-CH_3), 3.15 (m, 2 H, $NHCH_2$), 3.78 (d, 1 H, J = 5 Hz, 20-H), 4.16 (m, 1 H, 11-H), 5.89 (s, 1 H, 4-H), 6.12 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.29 (d, 1 H, J = 10 Hz, 1-H), 7.77 (m, 1 H, NH); MS, m/e (relative intensity) 403 (4, M^+), 121 (81), 103 (100).

(20*S*)-21-(Ethylamino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (6b): ^1H NMR ($CDCl_3$) δ 1.14–1.20 (m, 6 H, 13-CH_3 and $NHCH_2CH_3$), 1.47 (s, 3 H, 10-CH), 3.34 (m, 2 H, $NHCH_2$), 4.09 (d, 1 H, J = 5 Hz, 20-H), 4.43 (m, 1 H, 11-H), 6.01 (s, 1 H, 4-H), 6.19 (m, 1 H, NH), 6.24 (dd, J = 10 and 2 Hz, 2-H), 7.26 (d, 1 H, J = 10 Hz, 1 H); MS, m/e (relative intensity) 403 (4, M^+), 121 (76), 103 (100).

(20*R*)-21-(*n*-Propylamino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (7a): ^1H NMR ($CDCl_3$) δ 0.94 (t, 3 H, J = 6 Hz, $NHCH_2CH_2CH_3$), 1.15 (s, 3 H, 13-CH_3) 3.2 (m, 2 H, $NHCH_2$), 4.06 (s, 1 H, 20-H), 4.39 (m, 1 H, 11-H), 6.0 (s, 1 H, 4-H), 6.25 (dd, 1 H, J = 10 and 2 Hz, 2-H), 6.9 (m, 1 H, NH), 7.31 (d, 1 H, J = 10 Hz, 1-H); MS, m/e (relative intensity) 417 (8, M^+), 121 (64), 117 (100).

(20*S*)-21-(*n*-Propylamino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (7b): ^1H NMR ($CDCl_3$) δ 0.94 (t, 3 H, J = 6 Hz, $NHCH_2CH_2CH_3$), 1.14 (s, 3 H, 13-CH_3), 1.46 (s, 3 H, 10-CH_3), 3.26 (m, 2 H, $NHCH_2$), 4.10 (d, 1 H, J = 5 Hz, 20-H), 4.44 (m, 1 H, 11-H), 6.02 (s, 1 H, 4-H), 6.19 (m, 1 H, NH), 6.26 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.26 (d, 1 H, J = 10 Hz, 1-H); MS, m/e (relative intensity) 417 (3, M^+), 121 (43), 117 (100).

(20*R*)-21-(Benzylamino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (8a): ^1H NMR (Me_2SO-d_6) δ 1.04 (s, 3 H, 13-CH_3), 1.37 (s, 3 H, 10-CH_3), 3.91 (d, 1 H, J = 5 Hz, 20-H), 4.14 (m, 1 H, 11-H), 4.20–4.46 (m, 2 H, CH_2Ph), 5.89 (s, 1 H, 4-H), 6.13 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.18–7.34 (m, 6 H, 1-H and Ph), 8.08 (m, 1 H, NH); MS, m/e (relative intensity) 465 (2, M^+), 121 (33), 91 (100).

(20*S*)-21-(Benzylamino)-11β,17,20-trihydroxy-3,21-dioxo-1,4-pregnadiene (8b): ^1H NMR (Me_2SO-d_6) δ 1.02 (s, 3 H, 13-CH_3), 1.39 (s, 1 H, 10-CH_3), 4.01 (d, 1 H, J = 5 Hz, 20-H), 4.15–4.32 (m, 3 H, 11-H and CH_2Ph), 5.89 (s, 1 H, 4-H), 6.13 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.22–7.36 (m, 6 H, 1-H and Ph), 8.08 (m, 1 H, NH); MS, m/e (relative intensity) 465 (2, M^+), 121 (33), 91 (100).

(20*R*)-21-Acetoxy-11β,17,20-trihydroxy-3-oxo-1,4-pregnadiene (1b). To a solution of prednisolone 21-acetate (400 mg, 1 mmol) in dimethylformamide (15 mL) was added sodium borohydride (50 mg) in water (1 mL). After 3 h at room temperature, excess 10% acetic acid was added. The reaction mixture was diluted with water (400 mL) and extracted twice with ethyl acetate (300 and 200 mL). The extracts were combined and washed with water, followed by drying (Na_2SO_4). The solvent was evaporated to give white prisms. Recyrstallization from acetone afforded **1b**

(235 mg) as prisms: mp 208–209 °C; $[\alpha]^{20}_D$ +6.0°; ^{1}H NMR ($CDCl_3$) δ 1.08 (s, 3 H, 13-CH_3), 1.46 (s, 3 H, 10-CH_3), 2.10 (s, 3 H, 21-$OCOCH_3$), 4.0–4.25 (m, 3 H, 20-H and 20-CH_2O), 4.40 (m, 1 H, 11-H), 6.02 (s, 1 H, 4-H), 6.26 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.27 (d, 1 H, J = 10 Hz, 2-H); MS, m/e (relative intensity) 404 (0.89, M^+), 121 (100); Anal. Calcd for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.26; H, 8.01.

(20*S*)-11β,17,20,21-Tetrahydroxy-3-oxo-1,4-pregnadiene (2a) from 1a. To a solution of **1a** (100 mg, 0.25 mmol) in methanol (25 mL), was added 2 N sodium hydroxide solution (3 mL). After 10 min at room temperature, the reaction mixture was diluted with water (350 mL) and extracted with ethyl acetate. The organics were washed with 0.5 N hydrochloric acid and water, followed by drying over anhydrous sodium sulfate. Evaporation of the solvent afforded a white residue, which was crystallized from acetone–methanol mixture to give **2a** (72 mg) as needles (mp 233–236 °C). The analytical sample was obtained by recrystallization from acetone as colorless needles: R_f 0.35 in chloroform–methanol (85:15); retention time (HPLC) 16.5 min; mp 234.5–236 °C; $[\alpha]^{20}_D$ +7.6°; MS, m/e (relative intensity) 362 (2, M^+), 121 (100); Anal. Calcd for $C_{21}H_{30}O_5$: C, 69.59; H, 8.34. Found: C, 69.50; H, 8.44.

(20*R*)-11β,17,20,21-Tetrahydroxy-3-oxo-1,4-pregnadiene (2b) from 1b. The saponification procedure of **1b** (150 mg) was similar to the preparation of **2a** from **1a**. After they were extracted with ethyl acetate, the organics were dried (Na_2SO_4). The solvent was evaporated to dryness, and the residue was subjected to silica gel column chromatography (70 × 1.5 cm). Elution with chloroform–methanol (9:1) and evaporating the solvent gave **2b** (103 mg) as a noncrystalline white foam (determined pure by TLC and HPLC): R_f 0.35 in chloroform–methanol (85:15); retention time (HPLC) 18.2 min; $[\alpha]^{20}_D$ +2.0°; ^{1}H NMR ($CDCl_3$) δ 1.10 (s, 3 H, 13-CH_3), 1.46 (s, 3 H, 10-CH_3), 3.80 (m, 3 H, 20-H and 20-CH_2O), 4.43 (m, 1 H, 11-H), 6.01 (s, 1 H, 4-H), 6.26 (dd, 1 H, J = 10 and 2 Hz, 2-H), 7.27 (d, 1 H, J = 10 Hz, 1-H); MS, m/e (relative intensity) 362 (5, M^+), 121 (100); Anal. Calcd for $C_{21}H_{30}O_5$: C, 69.59; H, 8.34. Found: C, 69.27; H, 8.40.

(20*S*)-11β,17,20,21-Tetrahydroxy-3-oxo-1,4-pregnadiene (2a) from 3a. To a solution of **3a** (300 mg, 0.77 mol) in methanol (25 mL) was slowly added sodium borohydride (230 mg). After an initial vigorous reaction subsided, the reaction mixture was refluxed for 45 min. The solution was cooled, water (75 mL) was added, and some of the methanol was evaporated under vacuo. The mixture was extracted with dichloromethane (300 mL), and the organics were washed with water, dried, and evaporated to dryness. Crystallization from the acetone–hexane mixture afforded colorless platelets (120 mg). Recrystallization from acetone gave the analytical sample as colorless needles, which were identical with **2a** in all respects.

(20*R*)-11β,17,20,21-Tetrahydroxy-3-oxo-1,4-pregnadiene (2b) from 3b. To a solution of **3b** (300 mg) in methanol (25 mL) was slowly added sodium borohydride (345 mg). After it was refluxed for 2 h, the reaction mixture was cooled and methanol was evaporated to give the concentrate. The residue was diluted with water (300 mL) and extracted twice with dichloromethane (300 and 100 mL). The combined organics were washed with 0.5 N acetic acid and water. After the organic extracts were dried (Na_2SO_4), the solvent was evaporated to dryness. The constituents of the residue were separated by silica gel column (70 × 1.5 cm) chromatography with chloroform–methanol (9:1) as an eluent. The first eluate, which was the less polar product, gave the starting material **3b** (165 mg). The second eluate was evaporated to give a noncrystalline white foam (42 mg), which was identical with **2b**.

Ear Edema Bioassay. The relative topical antiinflammatory potencies were measured with a modification of the croton oil induced ear edema assay.[17] Male Sprague–Dawley rats (100–200 g) were maintained on a standard diet with water ad libitum and under controlled lighting conditions (light on 600–1800 h) for 1 week prior to use. On the day of the experiment, ear thickness was measured with a dial thickness gauge, after which 25 μL of vehicle (methanol) or drug solution was applied to each surface of both ears for a total of 50 μL/ear. Thirty minutes later, 50 μL/ear of 5% croton oil in acetone was applied in the same manner. After 5 h, at the peak of the inflammatory response, the ear thicknesses were remeasured, and blood was drawn by cardiac puncture. Plasma was immediately separated and frozen until corticosterone assays could be performed.

Cotton Pellet Granuloma Bioassay. Animals were maintained as described above. Prior to implantation of cotton pellets (35 ± 1 mg), 2 mg of steroid dissolved in acetone was applied to the treated pellet, while the untreated pellet received an equal volume of vehicle. Two untreated pellets were implanted in control animals, and one treated (right) pellet and one untreated (left) pellet were implanted in test animals as previously described.[20] Animals were sacrificed 7 days later, and cotton pellets and surrounding granulation tissue were recovered, dried at 60 °C for 48 h, and weighed. Cardiac-puncture blood was drawn, and plasma was prepared for subsequent corticosteroid assays. Thymus tissue and adrenal gland weights as well as initial and final body weights were recorded.

Steroid Binding Assays. Rat liver cytosolic preparations and steroid binding studies were carried out as previously described.[6] Briefly, livers from bilaterally adrenalectomized rats were homogenized in 4 volumes of TTES buffer (10 mM TES, 12 mM thioglycerol, 1.5 mM EDTA, and 0.25 M sucrose, pH 7.4) containing 20 mM sodium molybdate. The homogenate was centrifuged for 1 h at 4 °C and the supernatant used as the cytosolic receptor preparation.

Receptor binding of the amide derivatives of 20(*R*)- and 20(*S*)-dihydroprednisolonic acid was carried out by incubating 28 nM [^{3}H]dexamethasone (New England Nuclear, Boston, MA; 35 Ci/mmol specific activity) with various concentrations of the competitors and 0.1 mL of liver cytosol for 5 h at 4 °C. Bound and free steroids were separated by adding 0.1 mL of a suspension of 10% charcoal and 1% dextran in 10 mM Tris (pH 8.0), mixing, centrifuging for 5 min at 3000*g*, and counting 0.1 mL of supernatant in a scintillation counter as previously described.[6]

Plasma Corticosterone Measurement. Plasma corticosterone was quantitated by radioimmunoassay by the procedure recommended in the RSL Rat Corticosterone (^{3}H) Kit (Radioassay Systems Laboratories, Inc., Carson, CA).

Partition Coefficient Measurement. Octanol–water partition coefficients were measured by an application of the procedure of Alhaider et al.[21] Briefly, octanol and phosphate buffer, 5 mL of each, were placed in a screw-cap culture tube and gently mixed. The 100 μL of steroid solution (0.5 mg steroid in 100 μL of DMSO) was added to each tube. The two phases were vigorously mixed (vortexed) for exactly 2 min followed by centrifugation at 2000 rpm for 1 h. The absorbance at 244 nm of each separated phase was then read on a Beckman UV 5260 spectrophotometer against the appropriate blank (buffer or 1-octanol). The partition coefficient P was calculated as P = C-octanol/C-aqueous, where C-octanol is the concentration of the drug in the octanol phase, and C-aqueous is the concentration of the drug in the aqueous phase.

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Registry No. **1a**, 2871-71-8; **1b**, 96346-38-2; **2a**, 2299-46-9; **2b**, 15847-24-2; **3a**, 97232-42-3; **3b**, 97274-84-5; **4a**, 54023-88-0; **4b**, 98102-80-8; **5a**, 110417-81-7; **5b**, 110417-82-8; **6a**, 110417-83-9; **6b**, 110417-84-0; **7a**, 97142-22-8; **7b**, 97142-21-7; **8a**, 104008-74-4; **8b**, 104008-75-5; prednisolone, 50-24-8; prednisolone 21-acetate, 52-21-1.

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