Synthesis of Nitro Esters of Prednisolone, New Compounds Combining Pharmacological Properties of Both Glucocorticoids and Nitric Oxide

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Glucocorticoids (GC) are widely used in therapy for their many pharmacological properties including antiinflammatory and immunosuppressive actions. However, their use over long periods is hampered by a number of severe side effects. Given the biological properties of nitric oxide (NO) and previous experience with nonsteroidal antiinflammatory agents, we synthesized new chemical entities combining both NO and GC properties. Here we report the synthesis of nitro esters of prednisolone obtained through the esterification, with different linkers, on the hydroxy group at C-21 position of the corticosteroid structure. The alkyl chain, as of the nitrooxy derivative (**2**), or aromatic linkers, as of *o*-, *m*-, and *p*-nitrooxymethylbenzoate derivatives (**3**−**5**), respectively, furnish stable compounds that release NO and inhibit the GC receptors in biological assays. To improve solubility we introduced a more water-soluble linker such as the nitrooxyalkylpiperidine or -piperazine group (**6**−**9**). Also these compounds retained properties of both NO and prednisolone. Compound **5** (NCX 1015) was selected for its better profile: enhanced antiinflammatory properties and reduced side effects compared with prednisolone. NCX 1015 is currently under preclinical development.

Introduction

Glucocorticoids (GC) have been used successfully for more than 50 years for their potent antiinflammatory and immunosuppressive action. Use of GC in therapy is limited by their side effects, which depend on the dose used and duration of treatment. The continued use of GC leads to an array of unwanted effects ranging from alteration of the endocrine homeostasis to a number of complications such as hypertension, hyperglycemia, fluid and electrolyte abnormalities, osteoporosis, gastrointestinal damage, increased susceptibility to infection, etc.[1,2]

Based on the knowledge of the mechanism of action of the GC, a medicinal chemistry effort was done in order to identify new compounds possessing potent antiinflammatory properties and reduced complications. Specifically, GC bind to a specific member of the nuclear receptor superfamily, the glucocorticoid receptor (GR), which has been cloned and more recently the crystal structure has been determined.[3] Upon ligand binding, the GR translocates to the nucleus where it interacts with specific DNA sequences. The net result is a regulation of gene transcription, through either activation or repression mechanisms. Currently, it is believed a better drug profile can be achieved through a degree of dissociation between transactivation and transrepression, for example, strong transrepression/low transactivation. Recent reviews illustrate the ongoing effort toward the realization of more selective glucocorticoid-related therapeutic agents.[2,4]

Prompted by the successful results with nitric oxide- (NO-) releasing nonsteroidal antiinflammatory agents (NSAIDs),[5−7] we identified a strong rationale to apply the same synthetic approach to glucocorticoids. More than a decade of intense research has demonstrated that NO exerts antiinflammatory effects, has cytoprotective actions on gastric mucosa, and is relevant to bone metabolism, to name a few of its biological actions.[8,9] Thus, we introduced a NO-releasing moiety into the GC structure so as to have new chemical entities that could release NO slowly over time, the first step being the ester bond cleavage. Structural changes were introduced in position 21 of the GC so as to avoid interference with molecular recognition of the GC.

Here, we describe a group of NO-releasing derivatives of prednisolone (**1**) that appear of interest as a new class of improved GC-related agents.

On the basis of previous experience with NO-releasing NSAIDs, we started with the introduction of the nitrooxybutyryl (**2**) and the isomers of nitrooxymethylbenzoyl groups (**3**−**5**) into the 21 position of prednisolone. The compounds displayed inhibitory activity on GR, therefore showing that they retain GC-related effects. As a further step, with the aim to improve water solubility, we synthesized the compounds **6**−**9** by linking the nitrooxy (-ONO_2) moiety to C-21 of prednisolone through heterocycles such as piperidine (**6** and **7**) and piperazine (**8** and **9**). To determine the effect of the nitrooxymethyl

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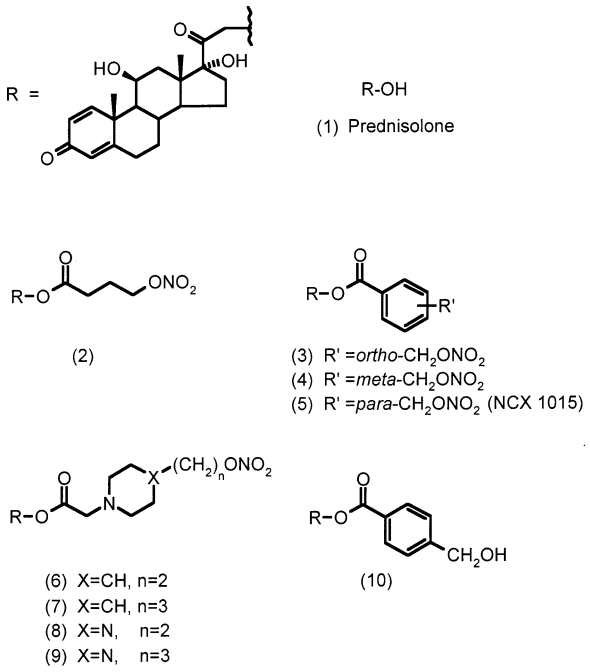
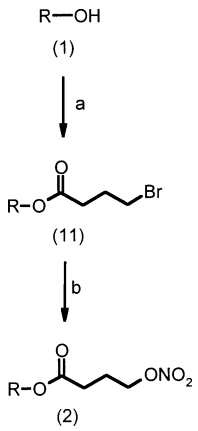


Figure 1. Structure of the different NO-releasing derivatives of prednisolone. Compound **5** (NCX 1015) was selected for further development.

Scheme 1[a]



[a] Reagents: (a) 4-bromobutyryl chloride, Et_3N, THF, rt, 4 h; (b) $AgNO_3$, CH_3CN/THF, reflux, 8 h.

moiety in derivative **5**, the corresponding hydroxymethyl derivative **10** has been also prepared (Figure 1).

Chemistry

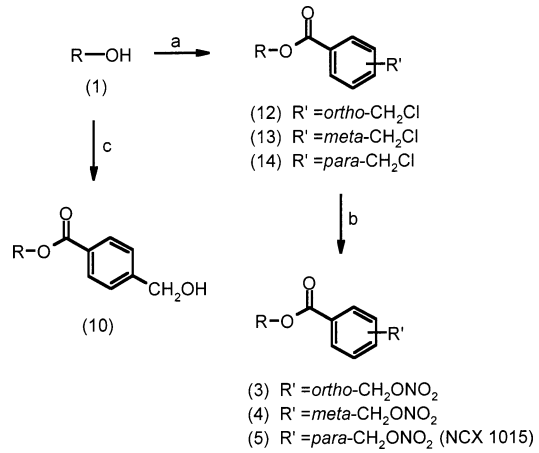
The synthesis of compound **2** is represented in Scheme 1.

The alkyl chain was introduced in the position 21 of prednisolone (**1**) by reaction with the commercially available 4-bromobutyryl chloride. The 21-bromobutyryl derivative (**11**) was converted into the final nitro ester (**2**) by treatment with silver nitrate ($AgNO_3$) in a refluxing mixture of acetonitrile/tetrahydrofuran as solvent.

The derivatives **3**, **4**, and **5**, characterized by the presence of an aromatic spacer, were prepared following a two-step procedure reported in Scheme 2.

The condensation of the ortho, meta, and para isomers of chloromethylbenzoyl chloride[10] with prednisolone (**1**) in tetrahydrofuran, in the presence of triethylamine as

Scheme 2[a]



[a] Reagents: (a) corresponding chloromethylbenzoyl chloride, Et_3N, THF, rt, 18 h; (b) $AgNO_3$, CH_3CN/THF, reflux, 4 h; (c) 4-hydroxymethylbenzoyl chloride, Et_3N, THF, rt, 1 h.

base, furnished the corresponding prednisolone 21-chloromethylbenzoate derivatives **12**, **13**, and **14**, respectively. These three chloro derivatives were converted into the target nitro ester compounds **3**, **4**, and **5** by treatment with silver nitrate in a refluxing mixture of acetonitrile/tetrahydrofuran as solvent.

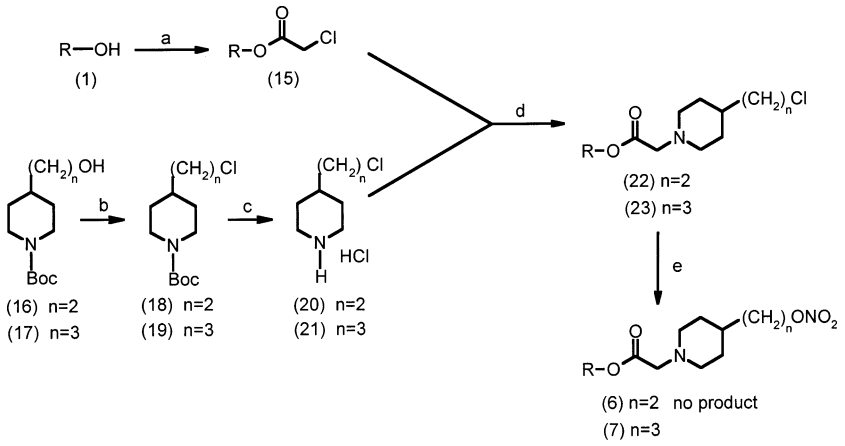
Synthesis of compounds **6**−**9**, characterized by the presence of a heterocyclic ring, was achieved from a common chloro intermediate (**15**). The prednisolone (**1**) was selectively acylated on its 21-position by chloroacetyl chloride in tetrahydrofuran, with triethylamine as base, to give compound **15**. Two different strategies were employed for the synthesis of piperidyl derivatives **6** and **7**. The first approach for the preparation of **6** and **7** is reported in Scheme 3.

The known 2-[4-(*N*-Boc-piperidyl)]-1-ethanol **16**[11] and 3-[4-(*N*-Boc-piperidyl)]-1-propanol **17**[12] were transformed into the corresponding chloro derivatives **18**[11] and **19**, respectively, by direct chlorination under standard conditions (carbon tetrachloride, triphenylphosphine). The subsequent removal of the *t*-Boc protecting group by anhydrous HCl in ethyl acetate furnished 4-(2-chloroethyl)piperidine **20**[11] and 4-(3-chloropropyl)-piperidine **21** as hydrochloride salts.

These compounds were condensed with **15** to give the piperidyl derivatives **22** and **23**, respectively, which were submitted to the subsequent reaction with silver nitrate. While the derivative **7** was obtained in acceptable yield, although after long time reaction (48 h) and using an excess of silver nitrate, the same reaction performed on compound **22** did not yield the desired product **6** but only the degradation of the starting material.

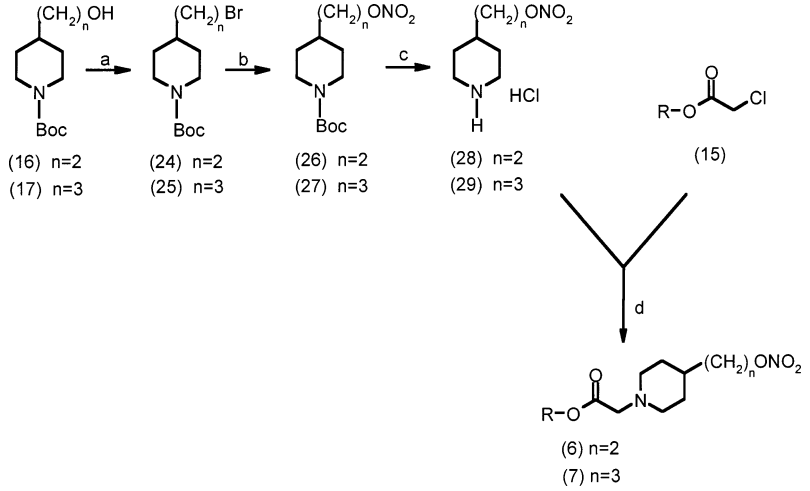
Due to the low versatility of this method, which depends on the reactivity of the chloro alkyl chain joined to the piperidine ring, an alternative convergent approach, reported in Scheme 4, was employed for the synthesis of derivatives **6** and **7**. In this way, the alcohols **16** and **17** were transformed into the corresponding bromo derivatives **24**[13] and **25**,[14] respectively, in good yields. The subsequent S_N2 reaction with silver nitrate in tetrahydrofuran proceeded in a short time (less than 1 h) to afford the compounds **26** and **27**, respectively. The *t*-Boc protecting group was removed

Scheme 3[a]



[a] Reagents: (a) $ClCOCH_2Cl$, Et_3N, THF, rt, 18 h; (b) CCl_4, PPh_3, DCM, rt, 18 h; (c) HCl/AcOEt, 0 °C, 3 h; (d) Et_3N, DMF, rt, 24 h; (e) $AgNO_3$, CH_3CN/THF, reflux, 48 h.

Scheme 4[a]



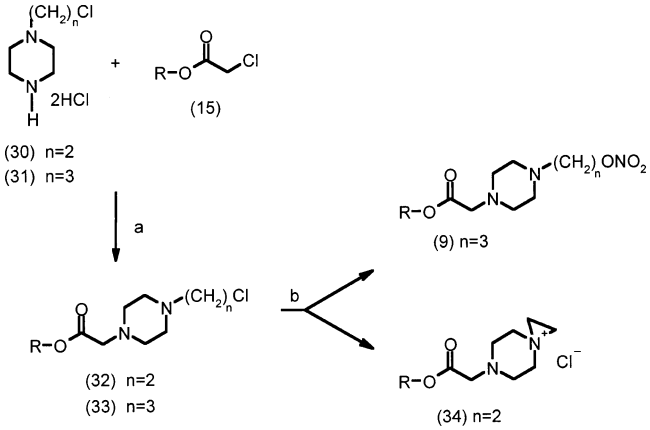
[a] Reagents: (a) CBr_4, PPh_3, THF, rt, 18 h; (b) $AgNO_3$, THF, rt, 1 h; (c) HCl/AcOEt, 0 °C, 3 h; (d) Et_3N, DMF, rt, 24 h.

with HCl in ethyl acetate to give the corresponding piperidines **28** and **29** (as hydrochloride salts). Finally, the condensation of the chloroacetyl prednisolone derivative **15** with **28** and **29** furnished in a good yield the compounds **6** and **7**, respectively, which were isolated as trifluoroacetic salts after purification by reversed-phase HPLC and immediate lyophilization.

Also for the preparation of piperazinyl derivatives **8** and **9**, two different strategies were employed. In the first route (Scheme 5), 1-(2-chloroethyl)piperazine **30**[15] and 1-(3-chloropropyl)piperazine **31**[16] (as bishydrochloride salts) were joined to **15** by use of triethylamine in *N*,*N*′-dimethylformamide to furnish the intermediates **32** and **33**, respectively. Only for the latter compound (**33**) did the reaction with silver nitrate in the standard conditions furnish the nitro ester (**9**) in a good yield, while for derivative **32** the reaction with silver nitrate did not furnish the desired product (**8**), due to a probable intramolecular S_N2 reaction of the chloroethylamino moiety, with the generation of a chemically reactive species (an aziridinium ion) corresponding to compound **34** (Scheme 5).

All the attempts to synthesize **8** under different reaction conditions failed; thus a new synthetic route, depicted in Scheme 6, was developed for the synthesis of **8**, along with the corresponding homologue **9**. The

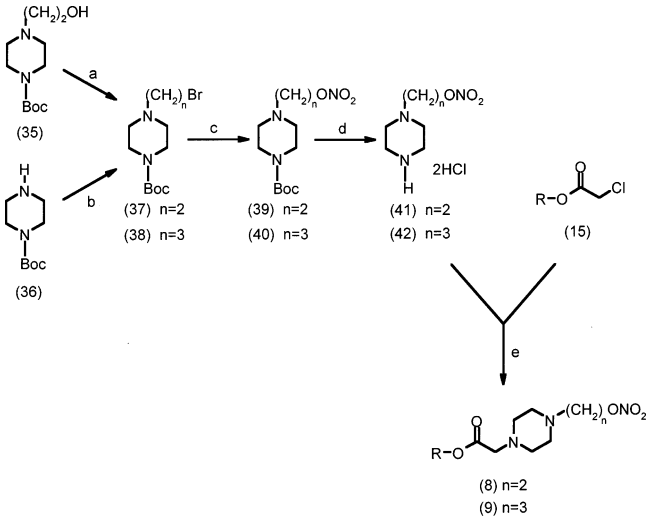
Scheme 5[a]



[a] Reagents: (a) Et_3N, DMF, rt, 24 h; (b) $AgNO_3$, CH_3CN/THF, reflux, 48 h.

bromo derivative **37** was prepared by direct bromination from the corresponding alcohol **35**[17] under standard conditions (carbon tetrabromide/triphenylphosphine with tetrahydrofuran as solvent), while **38** was synthesized by the reaction of *N*-Boc-piperazine **36**[18] with an equimolar amount of 1,3-dibromopropane. These bromoalkyl-*N*-Boc-piperazines **37** and **38** were subsequently trans-

Scheme 6[a]



[a] Reagents: (a) CBr_4, PPh_3, THF, rt, 18 h; (b) 1,3-dibromopropane, DCM, Et_3N, rt, 18 h; (c) $AgNO_3$, THF, rt, 2 h; (d) HCl/AcOEt, 0 °C, 4 h; (e) Et_3N, DMF, rt, 24 h.

formed into the corresponding nitro esters **39** and **40**, respectively, after treatment with an excess of silver nitrate in tetrahydrofuran at room temperature. In these latter compounds, the *t*-Boc protecting group was removed with anhydrous HCl in ethyl acetate to give **41** and **42**. These nitrooxy derivatives were condensed with **15** to yield **8** and **9**, respectively, isolated as trifluoroacetic salts after purification by reversed-phase HPLC.

To determine the effect of nitrooxy moiety, we synthesized the hydroxy derivative **10** (Scheme 2). Prednisolone (**1**) was condensed with the commercially available 4-hydroxymethylbenzoyl chloride in the presence of triethylamine to give the target compound **10**.

Results

The compounds synthesized (**2**−**10**) were stable either in buffer solution or in the standard vehicle used in the assays, whereas in the biological environment such as blood or cell cultures they were subject to cleavage of the ester bond. Binding affinity to the human GR was determined by the displacement of 50 nM [^{3}H]dexamethasone, a reference GR agonist, by a 1 μM concentration of test compounds **2**−**10** and compared with that of prednisolone (**1**). The results are shown in Table 1.

Thus, in the peripheral blood mononuclear cells (PBMC) assay, compounds **2**, **3**, and **5**−**10** displaced GR binding. The percent displaced of [^{3}H]dexamethasone binding ranged from 30% to 87%. The data show that all compounds retained the capacity to interact with the recognition site of GR. Differences in potency may well depend on the time to dissociate the prednisolone part from the nitro ester moiety. GR binding inhibition appeared to be independent of the presence of the nitrate group as shown by comparing compound **5** with **10**, the latter being the denitrated form of **5** (Figure 1, Table 1). Previous data on NSAIDs[19,20] showed that one key feature of nitro esters linked to the drug through the butyryl or benzoyl moiety is the release of NO slowly, whereas classic nitrovasodilators release NO more rapidly. Analysis of compound **5**, which was

Table 1. Effect of Prednisolone, Compounds **1**−**10** on Glucocorticoid Receptor Binding with [^{3}H]Dexamethasone as Ligand in Human Blood Mononuclear Cells

compound (at 1 μM)	[^{3}H]dexamethasone bound[a] (fmol/mL)	% displacement of [^{3}H]dexamethasone specific binding[a]
control	12.4 ± 1.5	
1	5.5 ± 2.3	56
2	1.6 ± 0.8	87
3	8.7 ± 0.6	30
4	19.8 ± 4.2	
5	6.6 ± 1.4	47
6	1.7 ± 0.4	86
7	2.3 ± 1.2	81
8	4.2 ± 0.8	66
9	2.1 ± 1.0	83
10	5.3 ± 1.7	57

[a] Binding data are expressed as mean ± SEM of $n = 3$.

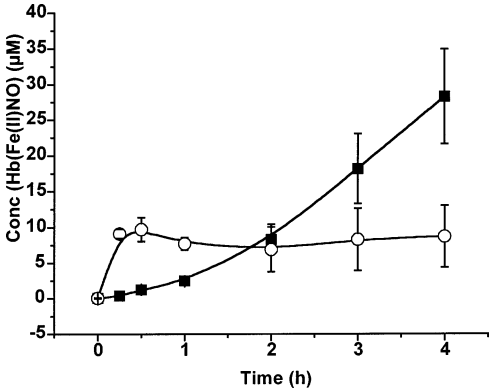


Figure 2. Kinetics of nitrosylhemoglobin formation in whole rat blood following treatment with *S*-nitroso-*N*-acetylpenicillamine (SNAP) (○) and (**5**, NCX 1015) (■). SNAP induced NO release rapidly with HbFe(II)NO formation, which was stable over 4 h, 9.1 ± 0.6 μM at 15 min and 8.7 ± 4.3 μM at 4 h. HbFe(II)NO formation from **5** was slow and ranged from 0.4 ± 0.3 μM at 15 min to 28.2 ± 6.6 μM at 4 h. Values are mean ± SEM of three independent experiments.

selected for further studies, clearly shows that NO is released slowly (Figure 2). The example of Figure 2 shows that a reference NO donor such as SNAP led to the formation of the bioactive NO form, detected as the complex NO−hemoglobin [HbFe(II)NO], which occurred rapidly (15 min) and was stable over 4 h (9.1 ± 0.6 μM at 15 min and 8.7 ± 4.3 μM at 4 h). Compound **5** released bioactive NO with a time-dependent formation of the paramagnetic adduct HbFe(II)NO, and the time course of HbFe(II)NO formation ranged from 0.4 ± 0.3 μM after 15 min of incubation to 28.2 ± 6.6 μM at 4 h.

Introduction of a lipophilic ester moiety into position 21 led, however, to the decrease of solubility. Thus, a further step was made by using spacers having different solubility properties. To achieve this we replaced the phenyl ring with piperidine or piperazine rings (compounds **6**−**9**). Compounds **6**−**9** showed good water solubility as salts with trifluoroacetic acid. The substitution of the phenyl (**5**, NCX-1015) with a piperidine (**6** and **7**) increased the solubility by 2 orders of magnitude, while the presence of a piperazine furnished more soluble derivatives (**8** and **9**), which are 10^4-fold more soluble than **5**. The buffer solubilities of NCX-1015 (**5**) and piperine/piperazine derivatives (**6**−**9**) were determined by the protocol reported by Casini et al.[21] (Table 2).

Table 2. Solubility Data of Prednisolone and Selected NO-Releasing Derivatives

compound	solubility[a] (mM)
1	0.27
5 (NCX 1015)	0.007
6	0.71
7	0.15
8	21.3
9	53.3

[a] In pH 7.4 buffer, at 25 °C.

Table 3. Antiinflammatory Properties of Prednisolone and Selected NO-Releasing Derivatives[a]

treatment[b]	PMN per mouse (10^6)	% inhibition
vehicle control	16.4 ± 1.2	
prednisolone	9.1 ± 0.6[c]	45
2	9.1 ± 1.0[c]	45
5 (NCX-1015)	4.1 ± 0.5[c,d]	75
8	9.0 ± 0.7[c]	46
9	7.8 ± 0.9[c]	52

[a] As measured on the inhibition of neutrophil (PMN) extravasation in the zymosan-induced peritonitis mouse model. [b] Equimolar doses of drugs (13.8 μmol/kg) or vehicle (0.25 mL) were given i.p. 1 h prior to zymosan (1 mg/peritoneal cavity). Neutrophil (PMN) influx into the cavities was measured at the 4 h time point. Data are mean ± SEM of $n = 5$ mice per group. [c] $P < 0.05$ vs vehicle control (one-way analysis of variance plus Bonferroni post-hoc analysis). [d] $P < 0.05$ vs prednisolone (one-way analysis of variance plus Bonferroni post-hoc analysis).

The compounds were also effective in vivo and displayed potent antiinflammatory properties. For instance, when tested on the intense recruitment of neutrophils provoked by intraperitoneal zymosan injection, all derivatives were able to produce significant inhibitory effects (Table 3). While prednisolone and compounds **2**, **8**, and **9** produced approximately 40−50% inhibition, compound **5** was significantly more effective, producing >70% inhibition of white blood cell recruitment (Table 3).

On the basis of different criteria ranging from in vitro to in vivo properties, compound **5**, NCX 1015, was selected for further investigation. In addition, this prednisolone derivative displayed higher potency than prednisolone in animal models of acute and chronic inflammation.[22,23] Interestingly, initial data showed that NCX 1015 can spare the bone compartment, possibly through the release of NO, thereby suggesting that treatment with this new class of compounds can be associated with a reduced risk of secondary osteoporosis.[24] Unlike prednisolone, prolonged NCX 1015 treatment did not appear to cause hypertension in normotensive animals (Dr. M. D'Amico, unpublished data) or gastrointestinal damage.[25] Other studies are ongoing. Interestingly, like prednisolone, NCX 1015 was effective when given orally.[22] Overall, the data with the lead compound (**5**) support the concept that NO-releasing glucocorticoids possess properties that make them of interest for their wider antiinflammatory properties and reduced side effects.

Conclusion

The attempts made to improve the overall profile of GC have led to the synthesis of a series of prednisolone derivatives that combine the biological properties of both GC and NO. Interestingly, one lead compound (**5**, NCX 1015) has shown greater antiinflammatory properties than prednisolone while it displayed lesser liability to induce side effects such as hypertension, gastrointestinal damage, and bone metabolism complications. This achievement provided a stimulus to extend the approach to other compounds, for example, dexamethasone or budesonide, whose activity profile is being characterized. Overall, NO-releasing GC derivatives offer a new opportunity to improve significantly the therapeutic benefits of the GC class of drugs.

Experimental Section

General Methods. All reactions were carried out under argon atmosphere, unless otherwise described. Standard syringe techniques were applied for transferring anhydrous solvents. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography on silica gel (precoated F_{254} Merck plates); the spots were examined with UV light and visualized with aqueous $KMnO_4$. ^{1}H NMR spectra were recorded in the given solvent with a Bruker AC 200 spectrometer. Chemical shifts are reported as δ values in parts per million. The splitting pattern abbreviations are as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), br (broad), and m (multiplet). Melting points (mp) were determined on a Buchi−Tottoli apparatus and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. Column chromatography were carried out on Merck silica gel (230−240 mesh). All compounds obtained commercially were used without further purification. Organic solutions were dried over anhydrous $MgSO_4$. Methanol was distilled from magnesium turnings, dioxane was distilled from calcium hydride, and anhydrous DMF was distilled from calcium chloride and stored over molecular sieves (3 Å). HPLC separations were conducted with a Waters Delta Prep 3000 A reversed-phase column (30 × 3 cm; 15 mm). The compounds were eluted with a gradient of 0−60% B in 25 min at a flow rate of 30 mL/min; the mobile phases were solvent A [10% (v/v) acetonitrile in 0.1% TFA] and solvent B [60% (v/v) acetonitrile in 0.1% TFA]. Analytical HPLC analyses were performed on a Bruker liquid chromatography (LC) 21-C instrument with a Vydac 218 TP 5415 C18 column (250 × 4 mm, 5 mm particle size) and equipped with a Bruker LC 313 UV variable-wavelength detector. Recording and quantification were accomplished with a chromatographic data processor coupled to an Epson computer system (QX-10).

Prednisolone 21-[4′-(Bromo)butyrate] (11). To a solution of prednisolone (**1**) (2.5 g, 7 mmol) in tetrahydrofuran (150 mL) were added triethylamine (3.9 mL, 28 mmol) and 4-bromobutyryl chloride (3.25 mL, 28 mmol). The reaction was stirred for 4 h at room temperature and the solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate and water. The two phases were separated and the organic phase was washed with brine (5 mL) and dried (Na_2SO_4) and the solvent was removed under reduced pressure. The crude product was purified by crystallization with *n*-hexane. Yield 82%; white solid, mp = 178−180 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (s, 3H), 1.45 (s, 3H), 0.90−1.15 (m, 2H), 1.30−1.60 (m, 2H), 1.60−1.80 (m, 3H), 1.80−2.20 (m, 5H), 2.35 (m, 1H), 2.50−2.63 (m, 4H), 3.35 (t, 2H), 4.35 (s, 1H), 4.76 (d, $J = 3.7$ Hz, 1H), 4.83 (d, $J = 17.5$ Hz, 1H), 5.15 (d, $J = 17.5$ Hz, 1H), 5.46 (s, 1H), 5.97 (s, 1H), 6.22 (dd, $J = 10.1$ and 1.6 Hz), 7.38 (d, $J = 10.1$ Hz, 1H).

Prednisolone 21-[4′-(Nitrooxy)butyrate] (2). To a solution of compound **11** (2.8 g, 5.5 mmol) dissolved in acetonitrile/tetrahydrofuran (150 mL, 4/1 v/v) was added silver nitrate (1.87 g, 11 mmol). The mixture was stirred at reflux in the dark for 8 h. The precipitate was filtered off, the solvent was evaporated under vacuum, and the residue was dissolved in ethyl ether and water. The organic phase was washed with water and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by silica gel chromatography with ethyl acetate/*n*-hexane (6/5 v/v) as eluent. Yield 41%; white solid, mp = 80−85 °C; ^{1}H NMR

(DMSO-d_6) δ 0.82 (s, 3H), 1.42 (s, 3H), 0.90−1.15 (m, 2H), 1.30−1.55 (m, 2H), 1.60−1.80 (m, 3H), 1.90−2.15 (m, 5H), 2.35 (m, 1H), 2.48−2.65 (m, 4H), 4.29 (s, 1H), 4.58 (t, J = 6.4 Hz, 2H), 4.74 (d, J = 3.8 Hz, 1H), 4.77 (d, J = 17.5 Hz, 1H), 5.11 (d, J = 17.5 Hz, 1H), 5.42 (s, 1H), 5.91 (s, 1H), 6.16 (dd, J = 10.1 and 1.6 Hz, 1H), 7.32(d, J = 10.1 Hz, 1H). Anal. Calcd for ($C_{25}H_{33}NO_9$): C, H, N.

General Procedure for the Synthesis of Compounds 12−14. A solution of **1** (721 mg, 2 mmol) in tetrahydrofuran (5 mL) was added to a mixture of chloromethylbenzoyl chloride (756 mg, 4 mmol) and triethylamine (0.56 mL, 4 mmol) cooled with an ice bath. The reaction was stirred for 18 h at room temperature and the solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate (20 mL) and water (5 mL). The two phases were separated and the organic phase was washed with brine (5 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified to furnish the derivatives **12**−**14**, used for the next step.

Prednisolone 21-[2′-(Chloromethyl)benzoate] (12). This compound was purified by flash chromatography with ethyl acetate/petroleum ether (6/4 v/v) as eluent. Yield 78%; yellow solid, mp = 88−90 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H), 0.89 (m, 1H), 1.02 (m, 1H), 1.35 (m, 2H), 1.35 (s, 3H), 1.66 (m, 3H), 2.02 (m, 3H), 2.29 (m, 1H), 2.44 (m, 2H), 4.28 (s, 1H), 4.55 (s, 2H), 4.70 (d, J = 3.6 Hz, 1H), 4.86 (d, J = 17.2 Hz, 1H), 5.14 (d, J = 17.2 Hz, 1H), 5.47 (s, 1H), 5.92 (s, 1H), 6.18 (dd, J = 10.0 and 1.2 Hz, 1H), 7.30 (d, J = 10.0 Hz, 1H), 7.43 (m, 4H).

Prednisolone 21-[3′-(Chloromethyl)benzoate] (13). This compound was purified by crystallization with *n*-hexane. Yield 82%; white solid, mp = 240−245 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (s, 3H), 1.41 (s, 3H), 0.85−1.15 (m, 2H), 1.25−1.60 (m, 2H), 1.60−1.80 (m, 3H), 1.80−2.10 (m, 3H), 2.29 (m, 1H), 2.48−2.56 (m, 2H), 4.33 (m, 1H), 4.77 (d, J = 3.8 Hz, 1H), 4.89 (s, 2H), 5.05 (d, J = 17.5 Hz, 1H), 5.36 (d, J = 17.5 Hz, 1H), 5.51 (s, 1H), 5.93 (s, 1H), 6.18 (dd, J = 10.1 and 1.6 Hz, 1H), 7.35 (d, J = 10.1 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 7.97 (d, J = 7.7 Hz, 1H), 8.08 (s, 1H).

Prednisolone 21-[4′-(Chloromethyl)benzoate] (14). This compound was purified by silica gel chromatography with dichloromethane/acetone (8/2 v/v) as eluent. Yield 97%; white solid, mp = 242−247 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (s, 3H), 1.41 (s, 3H), 0.85−1.15 (m, 2H), 1.25−1.60 (m, 2H), 1.60−1.80 (m, 3H), 1.80−2.10 (m, 3H), 2.29 (m, 1H), 2.48−2.55 (m, 2H), 4.33 (s, 1H), 4.76 (d, J = 3.6 Hz, 1H), 4.78 (s, 2H), 5.04 (d, J = 17.5 Hz, 1H), 5.35 (d, J = 17.5 Hz, 1H), 5.50 (s, 1H), 5.93 (s, 1H), 6.19 (dd, J = 10.1 and 1.7 Hz, 1H), 7.35 (d, J = 10.1 Hz, 1H), 7.62 (d, J = 8.2 Hz, 2H), 8.01 (d, J = 8.2 Hz, 2H).

General Procedure for the Synthesis of Compounds 3−5. A solution of **12**, **13**, or **14** (1 mmol) in acetonitrile/tetrahydrofuran (6 mL, 4/2 v/v) was treated with silver nitrate (2 mmol, 340 mg) at reflux in the dark for 4 h. The precipitate was filtered off, the solvent was evaporated under vacuum, and the residue was dissolved in ethyl acetate (10 mL) and water (2 mL). The organic phase was washed with water (3 × 2 mL) and brine (2 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product purified furnished compound **3**, **4**, or **5**).

Prednisolone 21-[2′-(Nitrooxymethyl)benzoate] (3). This compound was purified by flash chromatography with ethyl acetate/petroleum ether (1/1 v/v) as eluent. Yield 67%; amorphous white solid, mp = 158−160 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H), 0.89 (m, 1H), 1.02 (m, 1H), 1.35−1.60 (m, 2H), 1.41 (s, 3H), 1.66−1.76 (m, 3H), 1.99−2.08 (m, 3H), 2.29 (m, 1H), 2.50−2.54 (m, 2H), 4.33 (s, 1H), 4.78 (d, 1H), 4.79 (d, J = 17.4 Hz, 1H), 5.35 (d, J = 17.4 Hz, 1H), 5.50 (s, 1H), 5.92 (m, 3H), 6.19 (d, J = 10.1 Hz, 1H), 7.35 (d, J = 10.1 Hz, 1H), 7.62−7.73 (m, 2H), 7.90 (d, J = 7.75 Hz, 1H), 8.03 (d, J = 7.75 Hz, 1H). Anal. Calcd for ($C_{29}H_{33}NO_9$): C, H, N.

Prednisolone 21-[3′-(Nitrooxymethyl)benzoate] (4). This compound was purified by crystallization with *n*-hexane. Yield 83%; white solid, mp = 208−216 °C; ^{1}H NMR (DMSO-d_6) δ 0.83 (s, 3H), 1.40 (s, 3H), 0.83−1.15 (m, 2H), 1.25−1.60 (m, 2H), 1.60−1.80 (m, 3H), 1.80−2.10 (m, 3H), 2.29 (m, 1H), 2.48−2.56 (m, 2H), 4.31 (m, 1H), 4.76 (d, J = 3.8 Hz, 1H), 5.08 (d, J = 17.5 Hz, 1H), 5.31 (d, J = 17.5 Hz, 1H), 5.50 (s, 1H), 5.67 (s, 2H), 5.92 (s, 1H), 6.18 (dd, J = 10.1 and 1.6 Hz, 1H), 7.33 (d, J = 10.1 Hz, 1H), 7.66 (t, J = 7.6 Hz, 1H), 7.77 (d, J = 7.9 Hz, 1H), 8.04 (d, J = 7.9 Hz, 1H), 8.11 (s, 1H). Anal. Calcd for ($C_{29}H_{33}NO_9$): C, H, N.

Prednisolone 21-[4′-(Nitrooxymethyl)benzoate] (5). This compound was purified by crystallization with tetrahydrofuran/*n*-hexane. Yield 54%; white solid, mp = 233−234 °C; ^{1}H NMR (DMSO-d_6) δ 0.84 (s, 3H), 0.85−1.15 (m, 2H), 1.41 (s, 3H), 1.20−1.60 (m, 2H), 1.60−1.80 (m, 3H), 1.80−2.10 (m, 3H), 2.29 (m, 1H), 2.50−2.55 (m, 2H), 4.33 (s, 1H), 4.76 (s, 1H), 5.04 (d, J = 17.7 Hz, 1H), 5.35 (d, J = 17.7 Hz, 1H), 5.48 (s, 1H), 5.69 (s, 2H), 5.92 (s, 1H), 6.17 (d, J = 10.1 Hz, 1H), 7.34 (d, J = 10.1 Hz, 1H), 7.64 (d, J = 8.3 Hz, 2H), 8.04 (d, J = 8.3 Hz, 2H). Anal. Calcd for ($C_{29}H_{33}NO_9$): C, H, N.

Prednisolone 21-Chloroacetate (15). To a solution of prednisolone (**1**) (1 mmol, 360 mg) in tetrahydrofuran (10 mL) was added triethylamine (1.1 mmol, 153 μL). The mixture was cooled at 0 °C, and chloroacetyl chloride (1.1 mmol, 87 μL) was added dropwise. The mixture was stirred for 18 h at room temperature (TLC ethyl acetate/petroleum ether 7/3), diluted with ethyl acetate (10 mL), and then washed with water (5 mL) and brine (5 mL). The separated organic phase was dried (Na_2SO_4) and concentrated under reduced pressure, and the residue was precipitated with petroleum ether (10 mL). After filtration, the final product (**15**) (420 mg, 95% yield) was obtained as a pale yellow solid. This product was used without any purification for the next reaction: mp = 245−247 °C, ^{1}H NMR (DMSO-d_6) δ 0.79 (s, 3H), 0.89 (m, 1H), 0.99 (m, 1H), 1.33 (m, 2H), 1.38 (s, 3H), 1.66 (m, 3H), 2.01 (m, 3H), 2.29 (m, 1H), 2.49 (m, 4H), 4.28 (s, 1H), 4.74 (d, J = 3.8 Hz, 1H), 4.83 (d, J = 17.4 Hz, 1H), 5.18 (d, J = 17.4 Hz, 1H), 5.47 (s, 1H), 5.92 (s, 1H), 6.15 (dd, J = 10.0 and 1.2 Hz, 1H), 7.32 (d, J = 10.0 Hz, 1H).

***N*-Boc-4-(3-chloropropyl)piperidine (19).** A solution of (**17**) (729 mg, 3 mmol) and triphenylphosphine (2.36 g, 9 mmol) in dry dichloromethane (20 mL) at room temperature under argon was treated with freshly distilled carbon tetrachloride (0.45 mL, 4.5 mmol). After 18 h, the mixture was filtered, and the filtrate was washed with water (5 mL) and brine (5 mL), dried (Na_2SO_4), and concentrated at reduced pressure. The residue was purified by flash chromatography (ethyl acetate/petroleum ether 2/8 v/v) to give compound **19** as a yellow oil (668 mg, yield 85%). ^{1}H NMR ($CDCl_3$) δ 1.13 (m, 2H), 1.38 (m, 2H), 1.45 (s, 9H), 1.55 (m, 3H), 1.76 (m, 2H), 2.68 (t, J = 11.8 Hz, 2H), 3.53 (t, J = 8.4 Hz, 2H), 4.06 (br s, 2H).

4-(3-Chloropropyl)piperidine Dihydrochloride (21). Compound **19** (524 mg, 2 mmol) was dissolved in ethyl acetate (10 mL) and the solution was saturated with dry hydrogen chloride at 0 °C. The mixture was stirred for 3 h to complete the deprotection, and the solvent was evaporated to give a solid residue that was suspended in ethyl ether (30 mL) and stirred for 1 h at room temperature. After filtration, compound **21** was obtained as a crystalline white salt (mp = 160−161 °C) used without any purification for the next reaction.

Prednisolone 21-[4-(2-Chloroethyl)piperidin-1-yl]acetate (22). To a stirred solution of **15** (436 mg, 1 mmol) and **20** (201 mg, 1.1 mmol) in *N*,*N*′-dimethylformamide (5 mL) cooled at 0 °C was added triethylamine (0.3 mL, 2.2 mmol) dropwise. The mixture was stirred overnight at room temperature, the solvent was removed under vacuum, and the resulting residue was dissolved in a mixture of ethyl acetate (10 mL) and water (5 mL). The organic phase was washed with brine (2 mL), dried (Na_2SO_4), and then evaporated. Purification by flash chromatography (dichloromethane/methanol 9/1 v/v) furnished **22** as a white solid (350 mg, yield 64%); mp = 195−197 °C; ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 0.87 (m, 2H), 0.92 (m, 1H), 1.23 (m, 1H), 1.39 (s, 3H), 1.66 (m, 4H), 2.00 (m, 8H), 2.32 (m, 1H), 2.50 (m, 5H), 2.62 (t, J = 6.8 Hz, 2H), 3.33 (t, J = 4.6 Hz, 2H), 3.66 (t, J = 6.8 Hz, 2H), 4.28 (s, 1H), 4.73 (s, 1H), 4.78 (d, J = 13.2 Hz, 1H), 5.08 (d, J = 13.2 Hz, 1H), 5.43 (s, 1H), 5.92 (s, 1H), 6.18 (dd, J = 10.4 and 1.4 Hz, 1H), 7.32 (dd, J = 10.4 and 1.4 Hz, 1H).

Prednisolone 21-[4-(3-Chloropropyl)piperidin-1-yl]acetate (23). By following the same procedure reported for the synthesis of **22** but with **21** in place of **20**, compound **23** was obtained as a brown solid (yield 76%), mp = 170−171 °C. ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 0.86 (m, 2H), 1.08 (t, J = 7.4 Hz, 2H), 1.17 (m, 1H), 1.24 (m, 1H), 1.38 (s, 3H), 1.66 (m, 4H), 1,86 (m, 2H), 1,98 (m, 6H), 2.28 (m, 1H), 2.50 (m, 5H), 2.62 (t, J = 6.8 Hz, 2H), 3.39 (t, J = 4.6 Hz, 2H), 3.67 (t, J = 6.8 Hz, 2H), 4.28 (s, 1H), 4.73 (s, 1H), 4.77 (d, J = 13.2 Hz, 1H), 5.08 (d, J = 13.2 Hz, 1H), 5.46 (s, 1H), 5.92 (s, 1H), 6.15 (dd, J = 10.4 and 1.4 Hz, 1H), 7.33 (d, J = 10.4 Hz, 1H).

Prednisolone21-[4-(3-(Nitrooxy)propyl)piperidin-1-yl]-acetate Trifluoroacetic Salt (7). To a solution of **23** (270 mg, 0.5 mmol) in a mixture of acetonitrile (12 mL) and tetrahydrofuran (8 mL) was added silver nitrate (255 mg, 3 equiv, 1.5 mmol), and after 48 h at reflux the reaction mixture was cooled to room temperature, the salts were filtered off, and the solvent was evaporated under reduced pressure. The crude product was purified by preparative HPLC and submitted to immediate lyophilization to give **7** as a white crystalline solid (312 mg, yield 45.4%), mp = 120−121 °C. ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H), 0.91 (m, 1H), 1.23 (m, 3H), 1.39 (s, 3H), 1.66 (m, 8H), 1.97 (m, 7H), 2.34 (m, 1H), 2.49 (m, 4H), 3.49 (m, 3H), 3.51 (d, J = 7 Hz, 2H), 4.28 (m, 1H), 4.51 (t, J = 6.4 Hz, 2H), 4.75 (s, 1H), 4.81 (d, J = 17.4 Hz, 1H), 5.19 (d, J = 17.4 Hz, 1H), 5.46 (s, 1H), 5.92 (s, 1H), 6.16 (dd, J = 10.0 and 1.6 Hz, 1H), 7.34 (d, J = 10.0 Hz, 1H). Anal. Calcd for ($C_{33}H_{45}F_3N_2O_{11}$): C, H, N.

***N*-Boc-4-(2-nitrooxyethyl)piperidine (26).** A solution of **24** (873 mg, 3 mmol) and silver nitrate (765 mg, 4.5 mmol) in dry tetrahydrofuran (15 mL) was stirred at room temperature for 1 h. The salts were filtered off and the solvent was removed under reduced pressure. The crude product was dissolved in dichloromethane (15 mL), the organic phase was washed with water (5 mL) and dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (eluent ethyl acetate/petroleum ether 2/8 v/v) to afford **26** (641 mg, yield 78%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.16 (m, 2H), 1.46 (s, 9H), 1.66 (m, 5H), 2.69 (t, J = 11.6 Hz, 2H), 4.17 (br s, 2H), 4.51 (t, J = 6.4 Hz, 2H).

***N*-Boc-4-(3-nitrooxypropyl)piperidine (27).** By following the same procedure reported for the synthesis of **26** but with **25** in place of **24**, compound **27** was obtained as a yellow oil (708 mg, yield 82%). ^{1}H NMR ($CDCl_3$) δ 1.37 (m, 2H), 1.44 (s, 9H), 1.58 (m, 2H), 1.77 (m, 5H), 2.72 (m, 2H), 4.21 (br s, 2H), 4.44 (t, J = 6.6 Hz, 2H).

4-(2-Nitrooxyethyl)piperidine Hydrochloride (28). Compound **26** (548 mg, 2 mmol) was dissolved in a saturated solution of hydrogen chloride in ethyl acetate (10 mL) and stirred for 3 h at 0 °C. The solvent was removed by rotary evaporation at reduced pressure and the resulting residue was triturated with dry ethyl ether to give **28** as a white solid (630 mg, 100%); mp = 143−145 °C.

4-(3-Nitrooxypropyl)piperidine Hydrochloride (29). By following the same procedure reported for the synthesis of **28** but with **27** in place of **26**, compound **29** was isolated as a white solid (630 mg, 100%); mp = 150−152 °C.

Prednisolone 21-[4-(2-Nitrooxyethyl)piperidin-1-yl]acetate Trifluoroacetic Salt (6). To a solution of prednisolone 21-chloroacetate (**15**) (436 mg, 1 mmol) and **28** (231 mg, 1.1 mmol) in *N*,*N*′-dimethylformamide (5 mL) cooled to 0 °C was added triethylamine (0.31 mL, 2.2 mmol). The reaction mixture was stirred overnight at room temperature and evaporated to dryness. The residue was stirred with ethyl acetate (10 mL), insoluble material was filtered off, and the filtrate was evaporated to dryness. A final purification by preparative HPLC gave **7** as a white crystalline solid (358 mg, yield 52%), mp = 126−127°C. ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H), 0.92 (m, 1H), 1.39 (s, 3H), 1.48 (m, 3H), 1.66 (m, 6H), 1.89 (m, 7H), 2.34 (m, 1H), 2.51 (m, 4H), 3.12 (m, 2H), 3.49 (m, 1H), 3.51 (d, J = 7 Hz, 2H), 4.31 (m, 1H), 4.51 (t, J = 6.2 Hz, 2H), 4.75 (s, 1H), 4.92 (d, J = 17.4 Hz, 1H), 5.25 (d, J = 17.4 Hz, 1H), 5.47 (s, 1H), 5.92 (s, 1H), 6.17 (d, J = 10.0 Hz, 1H), 7.34 (d, J = 10.0 Hz, 1H). Anal. Calcd for ($C_{32}H_{43}F_3N_2O_{11}$): C, H, N.

Prednisolone 21-[4-(3-Nitrooxypropyl)piperidin-1-yl]-acetate Trifluoroacetic Salt (7). By following the same procedure reported for the synthesis of **6** but with **29** in place of **28**, compound **7** was isolated as a white crystalline solid (yield 45%).

Prednisolone 21-[4-(2-Chloroethyl)piperazin-1-yl]acetate (32). To a solution of **15** (436 mg, 1 mmol) and **30** (265 mg, 1.2 mmol) in *N*,*N*′-dimethylformamide (5 mL) cooled in an ice bath was added triethylamine (0.46 mL, 3.3 mmol); the mixture was stirred overnight and then concentrated. The residue was taken up in ethyl acetate (15 mL), washed with water (5 mL) and brine (5 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography (dichloromethane/methanol 9/1 v/v) furnished **32** as a brown solid (423 mg, yield 77%); mp = 218−220 °C. ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 0.87 (m, 2H), 0.92 (m, 1H), 1.23 (m, 1H), 1.39 (s, 3H), 1.66 (m, 5H), 2.00 (m, 6H), 2.32 (m, 1H), 2.50 (m, 5H), 2.62 (t, J = 6.8 Hz, 2H), 3.33 (t, J = 4.6 Hz, 2H), 3.66 (t, J = 6.8 Hz, 2H), 4.28 (s, 1H), 4.73 (s, 1H), 4.78 (d, J = 13.2 Hz, 1H), 5.08 (d, J = 13.2 Hz, 1H), 5.43 (s, 1H), 5.92 (s, 1H), 6.18 (dd, J = 10.4 and 1.4 Hz, 1H), 7.32 (dd, J = 10.4 and 1.4 Hz, 1H).

Prednisolone 21-[4-(3-Chloropropyl)piperazin-1-yl]acetate (33). By following the same procedure reported for the synthesis of **32** but with **31** in place of **30**, compound **33** was isolated as a white crystalline solid (yield 68%); mp = 228−230 °C. ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 0.83 (m, 2H), 0.91 (m, 1H), 1.26 (m, 1H), 1.43 (s, 3H), 1.68 (m, 6H), 1.81 (t, J = 7 Hz, 2H), 2.02 (m, 1H), 2.37 (t, J = 6.6 Hz, 4H), 2.49 (m, 8H), 3.33 (t, J = 4.8 Hz, 2H), 3.65 (t, J = 6.4 Hz, 2H), 4.28 (s, 1H), 4.73 (s, 1H), 4.78 (d, J = 17.6 Hz, 1H), 5.09 (d, J = 17.6 Hz, 1H), 5.42 (s, 1H), 5.92 (s, 1H), 6.18 (dd, J = 10.2 and 1.2 Hz, 1H), 7.31 (dd, J = 10.2 Hz, 1H).

Prednisolone 21-[4-(3-Nitrooxypropyl)piperazin-1-yl]-acetate Ditrifluoroacetic Salt (9). A solution of **33** (563 mg, 1 mmol) and silver nitrate (510 mg, 3 mmol) in a mixture of acetonitrile (12 mL) and tetrahydrofuran (8 mL) was refluxed in the dark for 48 h. The precipitate was filtered off, and the solvent was evaporated under vacuum. The crude product was purified by reversed-phase HPLC to give the desired product (425 mg, yield 52%) as a white crystalline powder after lyophilization; mp = 200−202 °C. ^{1}H NMR (DMSO-d_6) δ 0.78 (s, 3H), 0.86 (d, J = 10.4 Hz, 1H), 1.06 (m, 1H), 1.27 (m, 1H), 1.39 (s, 3H), 1.65 (m, 4H), 2.07 (m, 6H), 2.33 (m, 4H), 2.49 (m, 4H), 2.83 (m, 2H), 3.16 (m, 2H), 3.36 (m, 1H), 3.52 (t, J = 6. 2 Hz, 2H), 4.16 (m, 2H), 4.59 (t, J = 6 Hz, 2H), 4.74 (s, 1H), 4.81 (d, J = 17.6 Hz, 1H), 5.15 (d, J = 17.6 Hz, 1H), 5.45 (s, 1H), 5.92 (s, 1H), 6.16 (d, J = 10 Hz, 1H), 7.33 (dd, J = 10 Hz, 1H). Anal. Calcd for ($C_{34}H_{45}F_6N_3O_{13}$): C, H, N.

***N*-Boc-4-(2-bromoethyl)piperazine (37).** *N*-Boc-4-(2-hydroxyethyl)piperazine (**35**) (1.15 g., 5 mmol) was dissolved in dry tetrahydrofuran (20 mL), and carbon tetrabromide (1.82 g., 5.5 mmol) was added. Then a solution of triphenylphosphine (1.44 g, 5.5 mmol) in dry tetrahydrofuran (5 mL) was added dropwise over 2 h. The mixture was stirred at room temperature for 18 h,and then *n*-hexane (10 mL) was added to the mixture, which was washed with saturated aqueous $NaHCO_3$ (2 × 5 mL), water (5 mL), and brine (5 mL) and dried (Na_2SO_4). The organic phase was evaporated to give an oil that was purified by flash chromatography (ethyl acetate/petroleum ether 2/8) to afford **37** as an oil that slowly crystallized on storage (979 mg, yield 67%). ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 2.45 (t, J = 5.2 Hz, 4H), 2.79 (t, J = 7.2 Hz, 2H), 3.43 (m, 6H).

***N*-Boc-4-(3-bromopropyl)piperazine (38).** To a solution of *N*-Boc-piperazine (1 g, 5 mmol) and triethylamine (0.77 mL, 5.5 mmol) in dichloromethane (15 mL) cooled in an ice bath was added 1,3-dibromopropane (0.56 mL, 5.5 mmol), and the solution was stirred for 18 h at room temperature. The mixture was diluted with dichloromethane (5 mL), washed with a saturated aqueous solution of $NaHCO_3$ (5 mL), water (5 mL), and brine (5 mL), dried (Na_2SO_4), and then concentrated. The residue, purified by flash chromatography (ethyl acetate/petroleum ether 3/7) furnished **38** as an oil (1.18 g, yield 77%).

^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.94 (m, 2H), 2.38 (t, J = 5.0 Hz, 4H), 2.49 (t, J = 7.0 Hz, 2H), 3.43 (t, J = 5.0 Hz, 4H), 3.60 (t, J = 6.6 Hz, 2H).

***N*-Boc-4-(2-Nitrooxyethyl)piperazine (39).** To a solution of **37** (880 mg, 3 mmol) in dry tetrahydrofuran (10 mL) was added silver nitrate (765 mg, 4.5 mmol) in one portion. The mixture was stirred for 2 h at room temperature, the salts were filtered off, and then the filtrate was concentrated under reduced pressure. The crude product was dissolved in dichloromethane (20 mL), the organic phase was washed with water (5 mL) and dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (eluent ethyl acetate/petroleum ether 1/1) to give **39** as an oil (704 mg, yield 92%). ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 2.46 (t, J = 5 Hz, 4H), 2.73 (t, J = 5.6 Hz, 2H), 3.43 (t, J = 5.2 Hz, 4H), 4.58 (t, J = 5.6 Hz, 2H).

***N*-Boc-4-(3-nitrooxypropyl)piperazine (40).** By following the same procedure reported for the synthesis of **39** but with **38** in place of **37**, compound **40** was isolated as an oil (yield 95%). ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.90 (m, 2H), 2.36 (t, J = 5.0 Hz, 4H), 2.42 (t, J = 7.2 Hz, 2H), 3.41 (t, J = 5.0 Hz, 4H), 4.53 (t, J = 6.4 Hz, 2H).

1-(2-Nitrooxyethyl)piperazine Hydrochloride (41). Compound **39** (550 mg, 2 mmol) was dissolved in a 2 M solution of hydrogen chloride in ethyl acetate (20 mL) cooled in an ice bath and stirred for 4 h at room temperature. The solvent was removed at reduced pressure and the residue was triturated with dry ethyl ether to give **41** as a white solid (496 mg, yield 100%); mp = 180−181 °C. ^{1}H NMR (DMSO-d_6) δ 3.45 (m, 9H), 3.54 (t, J = 6.6 Hz, 2H), 4.04 (t, J = 6.6 Hz, 2H), 10.01 (br s, 2H).

1-(3-Nitrooxypropyl)piperazine Hydrochloride (42). By following the same procedure reported for the synthesis of **41** but with **40** in place of **39**, compound **42** was isolated as a white solid (yield 95%); mp = 180−181 °C, ^{1}H NMR (DMSO-d_6) δ 2.19 (t, J = 7 Hz, 4H), 3.22 (t, J = 7.4 Hz, 2H), 3.62 (m, 7H), 3.75 (t, J = 6.4 Hz, 2H), 9.78 (br s, 2H).

Prednisolone 21-[4-(2-Nitrooxyethyl)piperazin-1-yl]-acetate Bistrifluoroacetic Salt (8). To a stirred solution of **15** (436 mg, 1 mmol) and **41** (372 mg, 1.5 mmol) in *N*,*N*′-dimethylformamide (5 mL) cooled at 0 °C was added triethylamine (0.84 mL, 6 mmol) dropwise. The mixture was stirred overnight at room temperature, the solvent was removed under vacuum, and the resulting residue was dissolved in a mixture of ethyl acetate (15 mL) and water (5 mL). The organic phase was then washed with brine (2 mL), dried (Na_2SO_4), and then evaporated. Purification on reversed-phase HPLC furnished **8** as a white solid after lyophilization (370 mg, yield 46%); mp = 108−110 °C. ^{1}H NMR (DMSO-d_6) δ 0.79 (s, 3H), 0.88 (d, J = 11.6 Hz, 1H), 0.94 (m, 1H), 1.28 (m, 1H), 1.39 (s, 3H), 1.66 (m, 2H), 2.02 (m, 6H), 2.33 (m, 4H), 2.49 (m, 4H), 2.83 (m, 2H), 3.16 (m, 2H), 3.36 (m, 1H), 3.52 (m, 2H), 4.29 (m, 4H), 4.78 (s, 1H), 4.83 (d, J = 17.6 Hz, 1H), 5.16 (d, J = 17.4 Hz, 1H), 5.47 (s, 1H), 5.92 (s, 1H), 6.16 (d, J = 10 and 1.6 Hz, 1H), 7.32 (dd, J = 10 Hz, 1H). Anal. Calcd for ($C_{33}H_{43}F_6N_3O_{13}$): C, H, N.

Prednisolone 21-[4-(3-Nitrooxypropyl)piperazin-1-yl]-acetate Bistrifluoroacetic Salt (9). By following the same procedure reported for the synthesis of **8** but with **42** in place of **41**, compound **9** was isolated as a white solid (yield 47%).

Prednisolone 21-[4′-(Hydroxymethyl)benzoate] (10). To a solution of prednisolone (**1**) (14.8 g, 41 mmol) in tetrahydrofuran (400 mL) were added triethylamine (5.7 mL, 41 mmol) and 4-hydroxymethylbenzoyl chloride (2.8 g, 16 mmol). The reaction was stirred for 1 h at room temperature and the solvent was evaporated under vacuum. The residue was dissolved in chloroform and water. The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and with brine and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (*n*-hexane/chloroform/tetrahydrofuran 5/3/2 v/v/v) to afford **10**: yield 12%; white solid, mp = 228−235 °C. ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H), 0.85−1.15 (m, 2H); 1.41 (s, 3H); 1.20−1.60 (m, 2H); 1.60−1.90 (m, 3H); 1.90−2.15 (m, 4H); 2.30 (m, 1H); 2.50−2.60 (m, 2H); 4.32 (s, 1H); 4.76 (d, 1H), 4.86 (d, J = 3.8 Hz, 2H); 5.03 (d, J = 17.5 Hz, 1H); 5.34 (d, J = 17.5 Hz, 1H); 5.50 (s, 1H); 5.93 (s, 1H); 6.17 (dd, J = 10.1 and 1.6 Hz, 1H); 7.34 (d, J = 10.1 Hz, 1H); 7.62 (d, J = 8.2 Hz, 2H); 8.02 (d, J = 8.2 Hz, 2H). Anal. Calcd for ($C_{29}H_{34}O_7$): C, H.

Solubility Assays. The assay was carried out according to the method described by Casini et al.[21] A standard solution was prepared by dissolving a precisely weighed amount (generally 1 mg) of compound **5**−**9** in 10 mL of methanol. The UV absorption maximum of each compound was determined (with a PerkinElmer-Lambda 25 spectrophotometer), eventually diluting the solution (with MeOH) as necessary. A saturated solution of each compound was then prepared by stirring magnetically a small volume of phosphate buffer (pH 7.4) in the presence of excess compound for 3 h. The saturated solution was filtered to remove solid compound (through a Gelman GHP Acrodisc 0.45-μm filter) and scanned by UV at the wavelength of the absorption maximum previously determined. Total solubility was determined by the relationship $C' = A'C/A$, where C = concentration of standard solution (milligrams per milliliter), A = absorbance of standard solution, A' = absorbance of saturated solution, and C' = concentration of saturated solution (milligrams per milliliter).

Biological Assays: (A) Glucocorticoid Receptor Binding. Human peripheral blood mononuclear cells (PBMC) were isolated from whole blood by the histopaque density gradient method. Cells were counted and 1×10^6 cells were transferred to assay tubes in RPMI containing 1% glutamine. [^{3}H]Dexamethasone (50 nM) was added to each tube, followed immediately by test compounds at the concentrations stated, and tubes were vortexed and incubated for 1 h at 37 °C. Cells were then washed four times with 0.01 M ice-cold phosphate-buffered saline (PBS), and the amount of [^{3}H]dexamethasone was determined by liquid scintillation. Concentration of radioactive dexamethasone bound was calculated by subtracting nonspecific binding values from test values and multiplying by the molar/radioactivity ratio. Data (mean ± SEM) are expressed as femtomoles of [^{3}H]dexamethasone bound per milliliter of n = 3 determinations for each group. The compounds were dissolved in DMSO to prepare a 10 mM stock solution and diluted in buffer prior to experimentation. Control cells received vehicle alone (0.1% DMSO).

(B) Inflammation Models. The effect of selected compounds on the process of neutrophil extravasation into an inflammatory site was determined as described by Paul-Clark et al.[22] Briefly, compounds were administered at the anti-inflammatory dose of 13.8 μmol/kg (corresponding to 5 mg/kg prednisolone) given intraperitoneally 1 h prior to zymosan; the vehicle control group was injected with 100 μL of peanut oil. Zymosan (1 mg in 0.5 mL of saline) was given i.p. and the extent of cell recruitment determined 4 h later as reported.[22] Data (mean ± SEM of 5 mice/group) are reported as number of polymorphonuclear cells migrated per cavity as well as percentage of inhibition vs control migration (vehicle group).

Measurements of NO Release. We used an electron paramagnetic resonance (EPR) spectroscopic approach to measure bioactive NO as nitrosylhemoglobin [HbFe(II)NO] in rat whole blood. The method used is a modification of that of Carini et al.[20] Venous rat blood was taken by withdrawal with heparinized syringe from an abdominal vein and deoxygenated with a gas mixture of nitrogen (95%) and carbon dioxide (5%) for 30 min in a sealing glass purge system equipped with an inlet and outlet valve and a water warming jacket to maintain controlled temperature (37 °C). *S*-Nitroso-*N*-acetylpenicillamine (SNAP) was used as reference NO donor. Drugs were dissolved in DMSO. The drug was added to blood at a final concentration of 100 μM. The blank was prepared by adding the vehicle (final content 1% v/v).

Aliquots of blood samples (500 μL) were taken at fixed times and anaerobically transferred into EPR quartz tubes (3 mm i.d.). To increase both sensitivity and reproducibility, the plasma erythrocytes were directly separated by tube centrifu-

gation at 4 °C (3200 rpm, 10 min) for erythrocyte nitrosylhemoglobin analysis.

EPR measurements were carried out with a Bruker EMX spectrometer (X band) equipped with a high-sensitivity cylindrical cavity (ER4119HS; Bruker). EPR samples were stored in liquid nitrogen (77 K) and analyzed at 100 K. The spectrometer was operated at a microwave frequency of 9.33 GHz, microwave power of 20.1 mW, modulation frequency of 100 kHz, modulation amplitude of 5.0 G, scan number of 20, resolution of 1024 points, conversion time of 20.48 ms, time constant of 10.24 ms, sweep time of 20.97 s, center field of 3300 G, and sweep width of 1200 G. Recorded EPR data were manipulated and plotted with the EPR software (Bruker WINEPR system, version 2.11) and Origin for Windows, version 7.0 (Microcal, Northampton, MA).

The concentration of HbFe(II)NO, expressed as micromolar, was determined by double integration of the signal with $CuSO_4$−ethylenediaminetetraacetic acid (EDTA) as reference standard.[26] All spectra were subtracted for the vehicle and the final concentration of nitrosylhemoglobin in whole blood was determined by improvement for the plasma.

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