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Nitrosothiol Esters of Diclofenac: Synthesis and Pharmacological Characterization as Gastrointestinal-Sparing Prodrugs[†,‡]

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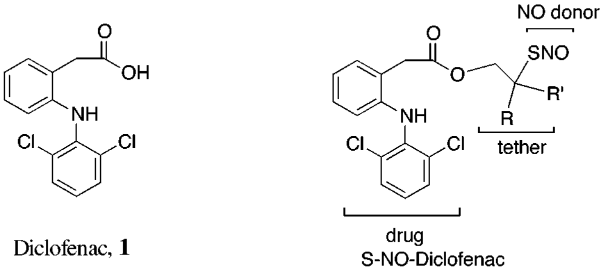
Despite its widespread use, diclofenac has gastrointestinal liabilities common to nonsteroidal antiinflammatory drugs (NSAIDs) that might be reduced by concomitant administration of a gastrointestinal cytoprotectant such as nitric oxide (NO). A series of novel diclofenac esters containing a nitrosothiol (−S-NO) moiety as a NO donor functionality has been synthesized and evaluated in vivo for bioavailability, pharmacological activity, and gastric irritation. All S-NO-diclofenac derivatives acted as orally bioavailable prodrugs, producing significant levels of diclofenac in plasma within 15 min after oral administration to mice. At equimolar oral doses, S-NO-diclofenac derivatives (**20a**−**21b**) displayed rat antiinflammatory and analgesic activities comparable to those of diclofenac in the carrageenan-induced paw edema test and the mouse phenylbenzoquinone-induced writhing test, respectively. All tested S-NO-diclofenac derivatives (**20a**−**21b**) were gastric-sparing in that they elicited markedly fewer stomach lesions as compared to the stomach lesions caused by a high equimolar dose of diclofenac in the rat. Nitrosothiol esters of diclofenac comprise a novel class of NO-donating compounds having therapeutic potential as nonsteroidal antiinflammatory agents with an enhanced gastric safety profile.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever, and inflammation, particularly arthritis.[1−3] Among the most popular NSAIDs, diclofenac (**1**) (Chart 1) has been approved in 120 countries since its introduction 25 years ago[4] and is ranked 30th among the top 200 drugs with respect to new prescriptions and refills in the United States alone.[5]

The pharmacological activity of NSAIDs is related to the suppression of prostaglandin H_2 (PGH_2) biosynthesis from arachidonic acid by inhibiting the activity of the enzyme cyclooxygenases (COXs).[6] Recently, it was discovered that COX exists in two isoforms, COX-1 and COX-2, which are regulated differently.[7] COX-1 provides cytoprotection in the gastrointestinal (GI) tract, whereas inducible COX-2 mediates inflammation.[8] Since most of the NSAIDs in the market show greater selectivity for COX-1 than COX-2,[9] chronic use of NSAIDs, including diclofenac, may elicit appreciable GI irritation, bleeding, and ulceration[2,3] with side effects including nausea, vomiting, abdominal pain, dyspepsia, and diarrhea.[10−12] The incidence of clinically significant GI side effects due to NSAIDs is high (over 30%) and causes some patients to abandon NSAID therapy.[5] NSAID-related GI adverse side effects account for more than 70 000 hospitalizations and 7000 deaths annually in the United States.[13] GI damage from NSAIDs is generally attributed to two factors: local GI irritation by the carboxylic acid moiety common to most NSAIDs ("topical effect") and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining GI health and homeostasis.[3,5,14]

Chart 1. Diclofenac and S-NO-Diclofenac



Diclofenac, **1**

S-NO-Diclofenac

Synthetic approaches based upon NSAID chemical modification have been taken with the aim of improving the NSAID safety profile. One such approach has been to mask the NSAID carboxylic acid moiety in NSAID ester prodrugs that hydrolyze in vivo to release the active parent NSAID.[5,14,15] Such NSAID derivatives are indeed gastroprotective in rodent models of acute NSAID-induced damage.[5,14,15] By itself, though, masking the carboxylic acid moiety is considered unlikely to ameliorate the severe GI complications of chronic NSAID use, since such prodrugs reduce only the topical irritancy effect without remediating the NSAID-induced tissue deficit of beneficial prostaglandins.

Highly selective COX-2 inhibitors have recently been developed and marketed as promising gastroprotective

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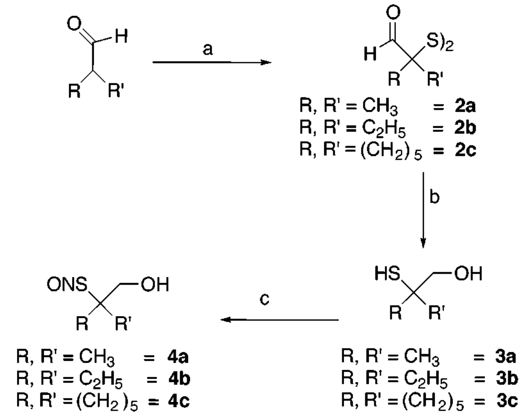
agents.[16] Two of the compounds, celecoxib (Celebrex) and rofecoxib (Vioxx), have been extensively studied and show exceptional antiinflammatory properties with reduced GI toxicity.[17] Despite the initial enthusiasm surrounding selective COX-2 inhibitors, questions remain regarding their ultimate benefit and safety, since in certain circumstances COX-2 may be important to homeostasis.[18] Some potential limitations of (long-term) COX-2 inhibitor therapy include ulcer exacerbation in high-risk patients, delayed gastroduodenal ulcer healing, thrombosis due to prostacyclin deficiency, and kidney toxcity.[18,19] Chronic use of COX-2 inhibitors may affect regulation of female reproduction, bone formation, and renal functions, since disruption of the gene for COX-2 impaired those functions in mice.[19,20] Thus, COX-2 inhibitors have not eliminated the need for improved drugs in the NSAID area.

A more recent strategy for devising a gastric-sparing NSAID, studied by Wallace and colleagues, involves chemically coupling a nitric oxide (NO)-releasing moiety to the parent NSAID.[21−25] Along with prostaglandins, NO appears to play an important cytoprotective role in GI homeostasis and defense by helping to maintain mucosal blood flow, optimizing mucus gel secretion, and inhibiting activation of proinflammatory cells.[21−25] Thus, NO may counteract the detrimental effect of COX inhibition.

Syntheses of NO-releasing analogues of several NSAIDs (aspirin, diclofenac, naproxen, ketoprofen, flurbiprofen) have been reported. These compounds are formed by coupling a nitrooxybutyl moiety to the respective parent NSAID through a short-chain ester linkage.[21−26] Some of these NSAID-derived organic nitrate esters display comparable antiinflammatory activity to the respective parent NSAIDs in acute rodent models, but with reduced GI toxicity.[21−26] Furthermore, in contrast to COX-2 inhibitors and standard NSAID, a NO-releasing NSAID derivative and a NO donor have shown existing ulcer-healing properties in rats.[23] A potential limitation of this class of NO donor NSAIDs arises from their intrinsic nature as indirect sources of NO. Organic nitrates require metabolic conversion by as yet ill-defined, likely enzyme-mediated reductive catabolism in order to produce NO under physiological conditions.[27] Furthermore, tolerance issues may restrict the therapeutic applicability and efficacy of organic nitrates.[27]

A synthetic approach toward NO-tethered diclofenac prodrugs distinct from organic nitrate esters of NSAID is depicted in Chart 1. To this intent, we have introduced a nitrosothiol (−S-NO) moiety into diclofenac through an ester linkage. S-Nitrosothiols are considered as biological sources of NO and serve as NO sources or donors without the need for metabolic transformation.[28,29] Although nitrosothiol catabolism is incompletely understood, transition metal-dependent redox processes and/or enzyme-catalyzed decompositions likely predominate biological pathways for NO release from nitrosothiols in vivo. Furthermore, S-nitrosothiols can directly modulate cell physiology without generating NO as the effector molecule through S-transnitrosation reactions, by which the NO group is effectively transferred from the S-nitrosothiol to the thiol of a target biomolecule in exchange for a hydrogen.[30,31] In terms of their NO-donating functionality, crystalline tertiary S-nitrosothiols are particularly stable as compared to primary and secondary S-nitrosothiols, facilitating their synthesis and profiling.[32,33]

Scheme 1[a]



[a] Reagents and conditions: (a) S_2Cl_2/CCl_4, 55 °C; (b) LAH, THF; (c) *t*-BuONO, CH_2Cl_2.

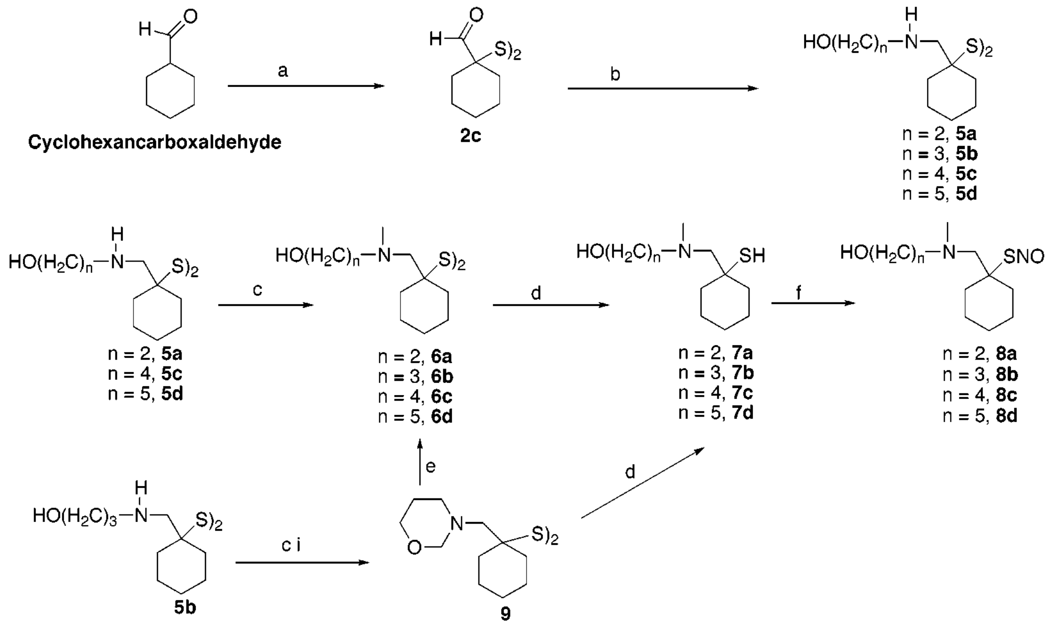
We report the synthesis and biological evaluation of a series of novel diclofenac ester derivatives bearing tertiary nitrosothiols and demonstrate their effectiveness as GI-sparing, analgesic, and antiinflammatory prodrugs.

Chemistry

Our initial approach to the S-NO-diclofenac target compounds involved synthesis of nitrosylated tethers with the aim of coupling them directly to diclofenac through an ester linkage. To this intent, the three different classes of NO-containing tethers presented in Schemes 1−3 were synthesized. Aliphatic aldehydes with an enolizable proton react with sulfur monochloride (S_2Cl_2) to give disulfide−dialdehydes that can be reduced to tertiary thio alcohols.[34,35] We chose this route to synthesize tertiary thio alcohols **3a**−**3c** starting from commercially available aldehydes such as 2-methylpropanal, 2-ethylbutanal, and cyclohexanecarboxaldehyde (Scheme 1). We first prepared disulfides **2a**−**2c** in high yields by reacting the above aldehydes with S_2Cl_2 in CCl_4 at 55 °C.[34] (Upon study of this sulfenation reaction, we found that *tert*-butyl methyl ether could substitute for the CCl_4 to form disulfide **2c** in 89% yield.) Disulfides **2a**−**2c** were reduced with $LiAlH_4$ to afford thio alcohols **3a**−**3c** in high yields. Subsequently, each thio alcohol was nitrosylated with *tert*-butyl nitrite (*t*-BuONO) in dichloromethane to give nitrosothiol tethers **4a**−**4c** as green oils in moderate-to-good yields.

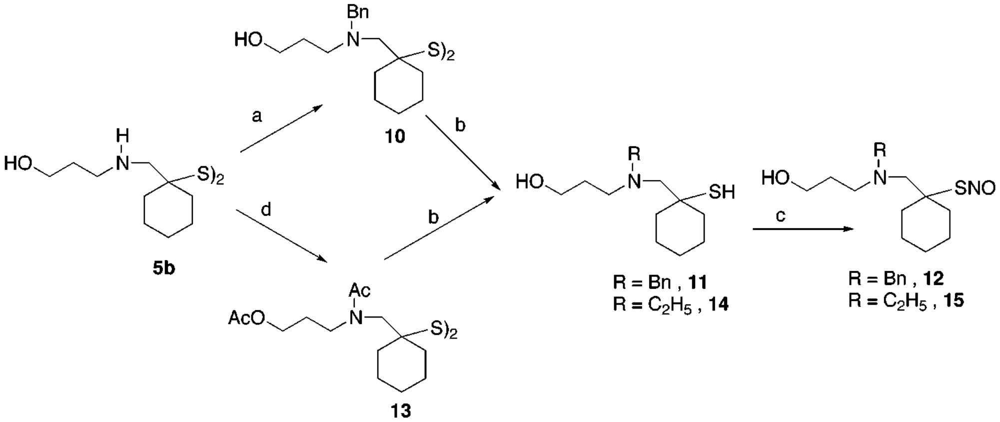
Next, we synthesized a series of nitrosylated tethers containing a tertiary amine to allow for subsequent salt formation (Scheme 2). Reductive amination of **2c** with primary amino alcohols of varying chain lengths (n = 2, 3, 4, 5) formed secondary amino disulfides **5a**−**5d** in good-to-moderate yields. Compound **5a** was readily methylated using aqueous formaldehyde followed by addition of $NaBH_4$ in methanol to obtain **6a**. Reduction of the disulfide bond in **6a** was accomplished with $LiAlH_4$ at room temperature to afford **7a**. Thiol **7a** was nitrosylated with *t*-BuONO in methanol in the presence of concentrated HCl to afford nitrosylated tether **8a**. In a similar fashion, nitrosylated tethers **8c**

Scheme 2[a]



[a] Reagents and conditions: (a) S_2Cl_2/CCl_4; (b) i. $NH_2(CH_2)_nOH$, $CHCl_3$, reflux, 6 h, ii. $NaBH_4$, MeOH, rt; (c) i. 38% CH_2O, MeOH, ii. $NaBH_4$; (d) LAH, THF; (e) $NaBH_4$, HOAc, rt; (f) *t*-BuONO, MeOH, HCl.

Scheme 3[a]



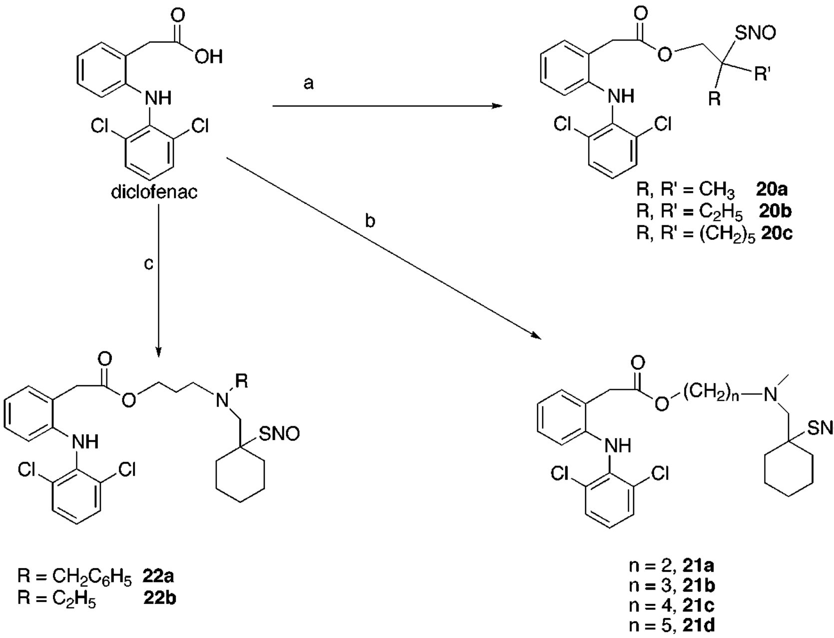
[a] Reagents and conditions: (a) BnBr acetone, K_2CO_3, rt, 12 h; (b) LAH, THF, reflux, 12 h; (c) *t*-BuONO, CH_2Cl_2; (d) Ac_2O, pyridine, rt.

and **8d** with carbon chain lengths 4 and 5 were synthesized, starting with **5c** and **5d,** respectively. Addition of aqueous formaldehyde to a methanolic solution of **5b** afforded oxazine **9** as a crystalline solid. Oxazine **9** was opened with $NaBH_4$ in glacial HOAc at room temperature to give the acyclic disulfide **6b**, which was reduced with $LiAlH_4$ in THF or powdered zinc in HOAc at room temperature to give the amino thiol **7b** in good yield. The amino thiol **7b** was nitrosylated with *t*-BuONO to give **8b** in high yield (Scheme 2).

Finally, nitrosothiol tethers **12** and **15** were synthesized by replacing the methyl group of **8b** with a benzyl or ethyl group, respectively (Scheme 3). Alkylation of **5b** with benzyl bromide in acetone in the presence of K_2CO_3 gave the benzylated disulfide **10**. Reduction and nitrosylation were carried out as before to give **12** as an oil. The *N*-ethyl derivative was prepared by acetylation of **5b** with acetic anhydride in pyridine to give **13.** Treatment of **13** with excess $LiAlH_4$ cleaved the *O*-acetate, reduced the acetamide to the *N*-ethyl moiety, and cleaved the disulfide to give **14**. Nitrosylation of **14** with acidic *t*-BuONO gave **15** as an oil.

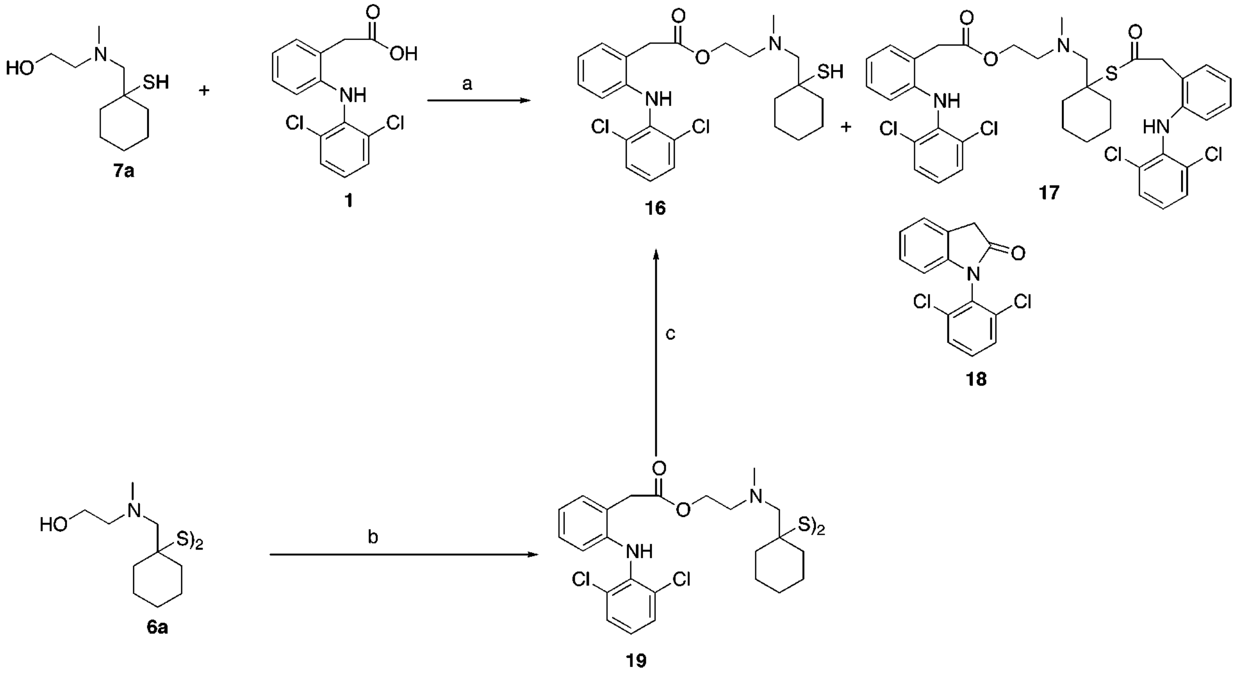
The nitrosylated tether could be acylated directly by diclofenac using DCC/DMAP through careful, dropwise addition of a solution of DCC in CH_2Cl_2 to a mixture of diclofenac, nitrosylated tether, and DMAP in CH_2Cl_2 so as to minimize the lactam **18** formation (Scheme 5). The couplings were usually complete within 30–40 min at 0 °C, and the products **20a**–**20c**, **21a**–**21d**, and **22a** and **22b** were obtained in moderate-to-good yield (Scheme 4). On a large scale at room temperature with longer reaction times, considerable product decomposition occurred during the coupling reaction, with evolution of gaseous nitrogen oxides. Therefore, the reaction temperature was carefully controlled to maximize the yield of desired coupled product. Compounds **20a**–**20c** were obtained as crystalline solids after chromatographic purification. Among the series **21a**–**21d**, only **21b** was obtained as a crystalline solid, whereas **21a**, **21c**, **21d**, **22a**, and **22b** were green oils readily converted by HCl to amorphous powders. HPLC analysis showed that crystalline nitrosothiol esters of diclofenac such as **20b**, **20c**, and **21b** were stable at room temperature in closed vials for more than 1 year.

Scheme 4[a]



[a] Reagents and conditions: (a) **4a**–**4c**, DCC, DMAP, CH_2Cl_2, 0 °C, 30 min; (b) **8a**–**8d**, DCC, DMAP, CH_2Cl_2, rt, 30 min; (c) **12**, **15**, DCC, DMAP, CH_2Cl_2, 0 °C, 30 min.

Scheme 5[a]



[a] Reagents and conditions: (a) DCC, CH_2Cl_2; (b) diclofenac, DCC, CH_2Cl_2; (c) Zn, HOAc, rt.

However, most of the amorphous nitrosothiols as HCl salts decomposed to the corresponding sulfhydryl and disulfide on standing at room temperature in closed vials for 2–3 months.

In some cases, the limited stability of the –S-NO functionality on the tethers or the desire to prepare the free thiol-containing derivatives required a different strategy. In such cases, we designed alternative synthetic schemes involving coupling of diclofenac to a nonnitrosylated, thiol tether followed by nitrosylation of the free thiol as the final step. Acylation of free thiol-containing tether **7a** with diclofenac using DCC/DMAP produced varying amounts of *O*,*S*-bisacylated **17** and lactam **18** as byproducts (Scheme 5). However, this problem was eliminated by acylation of disulfide **6a** with diclofenac and subsequent reduction using powdered zinc in acetic acid to afford thiol **16** as the major product in high yield.

Pharmacology

Biological characterization of the novel diclofenac nitrosothiol esters was conducted with the aim of establishing their bioavailability, analgesic and antiinflammatory activities, and gastric tolerance relative to the sodium salt of the parent NSAID, diclofenac (**1**). In

Table 1. Relative Bioavailability of S-NO-NSAIDs as Plasma Diclofenac in Mice

compound[a]	peak diclofenac (nmol/mL) ± SEM ($n = 7$)	relative plasma diclofenac AUC$_{0-2\,h}$ (mean %)
diclofenac sodium[b]	23.9 ± 2.9	100
20a[b]	19.8 ± 2.9	80
20b[b]	13.4 ± 2.1	66
20c[b]	15.3 ± 2.3	80
diclofenac sodium[c]	19.8 ± 2.5	100
21a[c]	5.5 ± 1.0	37
21b[c]	7.4 ± 2.3	46
21c[c]	5.9 ± 1.3	42
21d[c]	2.4 ± 0.3	20
22a[c]	6.8 ± 1.8	35
22b[c]	4.5 ± 2.2	35

[a] All compounds administered to mice at an oral dose of 20 μmol/kg. [b] Vehicle: 0.5% methocel/5% PEG-400. [c] Vehicle: 0.5% methocel/50% PEG-400. All values are mean ± SEM ($n = 5-10$, number of animals).

all cases, rodent models that are well-established pharmacological systems were used for in vivo NSAID profiling.

Relative Bioavailability Studies. After administration of diclofenac nitrosothiol esters orally to mice, the level of diclofenac in plasma was measured and compared to the level produced by oral administration of diclofenac sodium. This end-point would also indicate the prodrug nature of these compounds: i.e., whether the ester bond of the S-NO-diclofenac derivatives is hydrolyzed in vivo to liberate diclofenac. Diclofenac levels were determined by HPLC analysis of blood plasma extracts obtained from 30 min to 2 h postdosing. The plasma diclofenac area under the curve (AUC) from 0 to 2 h postdosing was determined for each compound. Because of the range of solubilities encountered among the diclofenac derivatives studied, vehicles containing either 5% PEG or 50% PEG (by volume) were used such that all compounds could be administered orally in the same dosage state, i.e., as a fine suspension.

Within the S-NO-diclofenac series **20a**−**20c** administered in aqueous 0.5% methocel−5% PEG, there was no significant difference in mouse plasma diclofenac levels when the dimethyl moiety (**20a**) was replaced with a cyclohexyl group (**20c**) (Table 1). Equimolar doses of either **20a** or **20c** resulted in the same level of plasma diclofenac which was 80% of that from an equimolar dose of diclofenac sodium (100%). An ethyl moiety (**20b**) reduced the apparent relative bioavailability slightly (80% vs 66% relative to diclofenac sodium).

The extent of in vitro hydrolysis of diclofenac−morpholine prodrugs incubated in plasma has been reported to reflect the carbon chain length between the ester and the tertiary amine function.[5] Specifically, diclofenac−morpholine esters with an even number of carbons in the chain yielded diclofenac more efficiently than did prodrugs with an odd number of carbons.[5] These data led us to investigate the potential influence of chain length variations of the alkyl spacer in our S-NO-diclofenac esters on relative diclofenac bioavailability in the mouse. Varying the chain length of the alkyl spacer in the series **21a**−**21d** altered relative diclofenac bioavailability over an approximately 2-fold range when the compounds were administered in 0.5% methocel−50% PEG (Table 1). Increasing the chain length from ethyl (**21a**) to propyl (**21b**) increased the mean relative bioavailability slightly, whereas no significant difference was noted between propyl (**21b**) and butyl (**21c**). Lowest relative bioavailability (20%) was observed for **21d**, which had the longest (i.e., pentyl) carbon chain studied. These relative diclofenac bioavailability differences among S-NO-diclofenac derivatives of varying ester chain length may reflect several factors operating in vivo: e.g., the ability of the S-NO-diclofenac esters to interact with the active sites of hydrolases contributing to their hydrolysis and altered pK_a and lipophilicity of the prodrugs with change in carbon chain length.[5] Since our relative bioavailability data were obtained in vivo, alteration of spacer chain length in the S-NO-diclofenac derivatives might also have influenced compound absorption, tissue uptake/compartmentalization, and metabolism which in turn would affect the appearance of diclofenac from them in the plasma. Slightly lower relative diclofenac bioavailability was observed with compounds **22a** and **22b** which were synthesized by replacing the *N*-methyl group of **21b** with benzyl and ethyl substitutions, respectively, as compared to that observed with **21b** (Table 1).

In summary, oral administration of all the novel S-NO-diclofenac esters delivered diclofenac in the plasma to varying degrees, likely depending upon some salient structural features identified above, particularly chain length of the alkyl spacer and substituent at the tertiary amine group. In all cases, however, plasma diclofenac peaked within 30 min following oral adminstration, as would be expected if the S-NO-diclofenac esters were NSAID prodrugs in vivo.

Analgesic Activity. The phenylbenzoquinone (PBQ)-induced writhing model in the mouse[36] was used to assess the analgesic activity of diclofenac and selected S-NO-diclofenac derivatives (**20a**−**20c**, **21a, 21b**) (Table 2). At an oral dose of 100 μmol/kg, all of the S-NO-diclofenac compounds evidenced analgesic activity, and diclofenac sodium itself at this dose inhibited writhing by 84%. The order of potency is **20b** ≥ diclofenac sodium > **21a** > **20c** = **21b** > **20a**. These data indicate that our synthetic diclofenac analogues are analgesics.

Antiinflammatory Activity. Injection of carrageenan into the rat foot pad causes local inflammation and edema, evident by an increase in paw volume of 1.2 mL at 3 h postinjection.[28] An NSAID administered orally just prior to the carrageenan injection acts as an antiinflammatory agent and attenuates the increase in paw volume.[37] A relatively high equimolar oral dose (100 μmol/kg) of either diclofenac sodium or S-NO-diclofenac derivatives (**21a**, **21b**) elicited a comparable (≈58%) suppression of carrageenan-induced paw inflammation (Table 2). These results indicate that after oral administration, esters **21a** and **21b** are antiinflammatory agents with similar efficacy to the parent NSAID, diclofenac.

Gastric Lesion Test. To characterize the GI safety profiles of our S-NO-diclofenac compounds, experiments were performed with fasted male rats to measure the extent of NSAID-induced stomach lesions.[38,39] Diclofenac sodium at a high oral dose (100 μmol/kg) caused significant stomach lesions with a lesion score of >40 mm, whereas equimolar oral doses of **20a**−**20c**, **21a**, and **21b** caused negligible lesions (Table 3, entries 3−5, 9, 10). Likewise, at lower doses (5, 25, or 50 μmol/kg),

Table 2. Analgesic and Antiinflammatory Effects of S-NO-NSAIDs

	analgesic effect in PBQ-induced writhing test (mice)		antiinflammatory effect in carrageenan-induced paw edema test (rat)	
compound[a]	% inhib of writhing	activity ratio vs diclofenac[b]	% inhib of paw volume increase	activity ratio vs diclofenac[b]
vehicle[c]	0 (27)			
diclofenac[c]	84[e] (18)	1.0		
20a[c]	32[e] (9)	0.4	ND[f]	
20b[c]	95[e] (9)	1.1	ND	
20c[c]	58[e] (9)	0.7	ND	
vehicle[d]	0 (27)		0 (20)	
diclofenac[d]	84[e] (26)	1.0	53[e] (10)	1.0
21a[d]	70[e] (27)	0.8	55[e] (15)	1.0
21b[d]	53[e] (9)	0.6	58[e] (5)	1.1

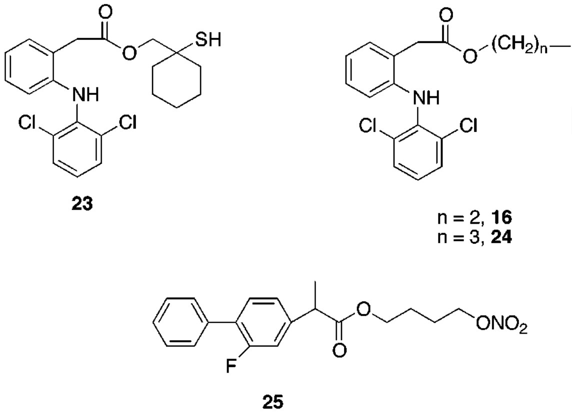
[a] All compounds administered at an oral dose of 100 μmol/kg. [b] Activity ratio = (% inhibition with the compound at 100 μmol/kg, po)/(% inhibition with diclofenac at 100 μmol/kg, po). [c] Vehicle: 0.5% methocel/5% PEG-400. [d] Vehicle: 0.5% methocel/50% PEG-400. [e] Difference statistically significant from respective vehicle ($p < 0.05$). All values are mean ± SEM (the number of animals in parentheses). [f] ND, not determined.

Table 3. Gastric Toxicity Studies in Rats

entry	compound[a]	gastric lesion score (mm)
1	vehicle[b]	0
2	diclofenac sodium[b]	41.3 ± 5.4
3	**20a**[b] (SNO)	0.7 ± 0.4
4	**20b**[b] (SNO)	0.4 ± 0.2
5	**20c**[b] (SNO)	2.0 ± 0.8
6	vehicle[c]	0
7	diclofenac sodium[c]	54.3 ± 3.9
8	**20c**[c] (SNO)	0.8 ± 0.5
9	**21a**[c] (SNO)	1.1 ± 0.9
10	**21b**[c] (SNO)	0.7 ± 0.4
11	**23**[c] (SH)	6.7 ± 1.1
12	**16**[c] (SH)	10.2 ± 2.8
13	**24**[c] (SH)	28.3 ± 4.2

[a] All compounds administered at an oral dose of 100 μmol/kg to 5–10 rats/group. [b] Vehicle: 0.5% methocel/5% PEG-400. [c] Vehicle: 0.5% methocel/50% PEG-400.

Chart 2. Sulfhydryls **23** and **24** and Flurbiprofen Nitrooxybutyl Ester **25**



diclofenac still produced appreciable stomach lesions, whereas our S-NO-diclofenac esters were devoid of apparent GI toxicity (data not shown).

The mechanism of gastroprotection by NSAIDs functionalized with NO-donating groups is poorly defined but likely complex and multifactorial, given that several aspects of GI physiology are influenced or regulated by NO.[21−25] The limited published data in this regard come mainly from studies on NSAID nitrooxybutyl esters. Masking the carboxylic acid group of diclofenac as an ester prodrug may itself attenuate the topical irritation component of NSAID-induced gastropathy and elicit an acute GI-sparing effect by reducing the local intracellular accumulation of irritant NSAID in stomach tissue as the free acid.[5,14,15] However, a flurbiprofen nitrooxybutyl ester **25** (Chart 2) was gastroprotective even after systemic administration as compared to the parent NSAID itself, suggesting that the GI-sparing property of the NO-releasing derivative was not completely dependent upon a tissue-contact-related mechanism.[21] This observation further suggests that the flurbiprofen nitrooxybutyl ester containing organic nitrate was not acting solely as a “prodrug.” Given evidence that NSAIDs, whether derivatized to NO-donating nitrooxybutyl esters or not, comparably inhibit PG synthesis in the gastric mucosa,[21] current focus is on the potential for bioactive NO (or NO-derived metabolites) to account for the acute GI-sparing effects of NO-releasing NSAIDs.

An attempt was made to gain some pharmacological insight into the role of the NO moiety in the gastroprotection afforded by the nitrosothiol diclofenac derivatives. To this intent, GI lesion scores were compared in rats 18 h after a single oral dose (100 μmol/kg) of either diclofenac (Table 3, entry 7), the S-NO derivatives **20c**, **21a**, and **21b** (Table 3, entries 8−10), or their respective des-NO analogues **23**, **16**, and **24** (Chart 2) (Table 3, entries 11−13). All of these sulfhydryl (SH) derivatives induced fewer gastric lesions than did diclofenac but more gastric lesions than each respective nitrosothiol derivative. The limited, NO-independent gastroprotection afforded by the sufhydryl derivatives may reflect a “prodrug effect” (i.e., intact absorption) and/or GI cytoprotection by the free nucleophilic thiol group. Sulfhydryls with gastric-sparing properties have been identified.[40]

NO itself may support or promote several endogenous GI defense mechanisms involving increased mucus and bicarbonate secretion, increased mucosal blood flow, and prevention of neutrophil adherence to the vascular endothelium, all of which are believed capable of minimizing chemically induced gastric injury.[21−26] Very recently, it has been suggested that prevention of gastric damage by a nitrooxybutyl ester of aspirin may reflect, at least in part, an NO-mediated suppression of the NSAID-induced upregulation of gastric cysteine proteases in the caspase cascade, which modulates programmed cell death (apoptosis).[41]

Conclusions

A series of novel diclofenac ester derivatives bearing tertiary nitrosothiols has been synthesized. These compounds are NO-releasing diclofenac prodrugs that yield the parent NSAID in vivo and have good antiinflammatory and analgesic activities in rodent models. At equimolar concentrations, the S-NO-diclofenac derivatives were gastric-sparing relative to diclofenac sodium in rats. The relative diclofenac bioavailability of the S-NO-diclofenac esters in vivo varied among the structures examined, with the carbon chain length of the alkyl tether a particular influence. Our data suggest that S-NO prodrugs may represent a safer chemical alternative to diclofenac for the treatment of inflammatory diseases and pain. The molecular mechanism of gastroprotection by the S-NO-diclofenac derivatives likely involves the action of NO and is multifactorial and thus awaits elucidation. Ultimately, any potential advantage of S-NO-NSAIDs over standard NSAIDs or COX-2 inhibitors would need to be demonstrated in the clinic.

Experimental Section

General Methods. All reagents and dry solvents were obtained from commercial sources and used without further purification. Flash chromatography[42] was performed on silica gel (Merck, 230−400 mesh). ^{1}H and ^{13}C NMR were recorded on a Bruker AMX-300 instrument. HPLC was performed with a Beckman liquid chromatograph equipped with a variable-wavelength spectrophotometric detector and System Gold software. The purity of some testing compounds was checked by HPLC with two diverse solvent systems: system A − linear gradient solvent system of 0.1% TFA in water/CH_3CN from 50/50 to 5/95 in 20 min and flow rate 1 mL/min; system B − 0.1% TFA in water/MeOH from 50/50 to 5/95 in 20 min and flow rate 1 mL/min ($\lambda = 254$ nm on a μ−Bondapak C18 (3.9 × 150 mm) column). Low-resolution mass spectra were recorded on a Perkin-Elmer API-150EX spectrometer with atmospheric pressure turbo ion spray. High-resolution mass spectra (HRMS) were obtained from the Auburn Mass Spectra Center, Auburn, AL. Elemental analyses were obtained from Robertson Microlit Laboratories, Madison, NJ.

1-[(Formylcyclohexyl)disulfanyl]cyclohexanecarbaldehyde (2c). (a) To a stirred solution of cyclohexanecarboxaldehyde (100 g, 89 mmol) in CCl_4 (100 mL) was added sulfur monochloride (36.4 mL, 91 mmol) dropwise at 50 °C. After a short lag (15 min), evolution of HCl gas began. After HCl evolution ceased, the mixture was stirred at 55 °C for 1 h, then cooled to room temperature. The CCl_4 was evaporated to produce a yellow solid, and the solid was placed in a sintered glass funnel and washed with hexane (3 × 100 mL) to give **2c** (114 g, 89%) as a white solid: mp 85−88 °C.

(b) Sulfur monochloride (2 mL, 25 mmol) and cyclohexanecarboxaldehyde (6 mL, 49.6 mmol) were dissolved in *tert*-butyl methyl ether (30 mL). The flask was placed in an oil bath at 55 °C, and the solution was stirred for 2 h. The flask was removed from the oil bath and set at room temperature for 3 h, until crystals formed. The flask, containing a layer of crystals, was placed in the freezer overnight, and the mother liquor was then decanted off. The crystals were washed with several small portions of hexane (15 mL) until the hexane wash did not turn yellow. The crystals were then dried in vacuo to give **2c** (6.3 g, 89%) as a white solid: mp 85−88 °C; ^{1}H NMR ($CDCl_3$) δ 1.24−1.33 (m, 6 H), 1.42−1.46 (m, 6 H), 1.62−1.69 (m, 4 H), 1.94−1.99 (m, 4 H), 8.94 (s, 2 H); ^{13}C NMR δ 23.0, 25.1, 30.3, 60.8, 194.3. Anal. ($C_{14}H_{22}O_2S_2$) C, H, N, S.

2-Methyl-1-hydroxypropane-2-thiol (3a). To a stirred solution of **2a** (15.0 g, 85.7 mmol) in THF (100 mL) was added dropwise 1 M $LiAlH_4$ in THF (85.7 mL, 85.7 mmol), and the reaction mixture was stirred at room temperature under N_2 for 1 h. The mixture was poured onto ice, treated with 3 N HCl (150 mL), and extracted with EtOAc (2 × 200 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated to give **3a** (12.8 g, 71%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 6 H), 1.63 (s, 1 H), 2.25 (brs, 1 H), 3.44 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 28.3, 46.3, 73.29; HRMS *m*/*z* calcd for $C_4H_{11}OS$ (M + 1) 107.0530, found 107.0525. Compounds **3b** and **3c** were synthesized in a manner similar to the synthesis of **3a**. Spectroscopic data are reported elsewhere.[34,35]

2-Methyl-1-hydroxypropane-2-nitrosothiol (4a). A solution of **3a** (4.40 g, 41.5 mmol) in CH_2Cl_2 (50 mL) was added to *t*-BuONO (90% tech, 5.50 mL, 41.5 mmol). The resulting green reaction mixture was stirred for 10 min at room temperature and then evaporated under reduced pressure at 40 °C to furnish **4a** (4.60 g, 82%) as a dark green oil: ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 6 H), 1.95 (brs, 1H), 4.17 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 25.1, 57.7, 70.5; HRMS *m*/*z* calcd for $C_4H_{10}NO_2S$ (M + 1) 136.0432, found 136.0435.

2-Ethyl-2-(nitrosothio)butan-1-ol (4b). Compound **4b** was synthesized in a manner similar to the synthesis of **4a** from **3b**.[34] **4b** was obtained as a green oil: ^{1}H NMR ($CDCl_3$) δ 1.90 (t, $J = 7.4$ Hz, 6 H), 2.29 (m, 4 H), 2.16 (brs, 1H), 4.34 (s, 4 H); ^{13}C NMR δ 7.9, 26.8, 66.5, 67.0.

[(Nitrosothio)cyclohexyl]methan-1-ol (4c). Compound **4c** was synthesized in a manner similar to the synthesis of **4a** from **3c**.[35] **4c** was obtained as a green oil: ^{1}H NMR ($CDCl_3$) δ 1.45−1.85 (m, 5 H), 2.05−2.13 (m, 3 H), 2.45−2.55 (m, 2 H), 4.26 (s, 2 H); ^{13}C NMR δ 21.9 (2C), 25.6, 33.2 (2 C), 63.6, 70.8.

2-{([({[(2-Hydroxyethyl)amino]methyl}cyclohexyl)disulfanyl]methyl)amino}ethan-1-ol (5a). A mixture of **2c** (10 g, 35 mmol), ethanolamine (4.26 g, 69.8 mmol) and $MgSO_4$ (10 g) in dry $CHCl_3$ (100 mL) was heated under reflux for 8 h. The solid was removed by filtration, and the solvent was evaporated under reduced pressure to obtain a viscous yellow liquid. The crude product was dissolved in MeOH (125 mL), and $NaBH_4$ (3.3 g, 87.3 mmol) was added in small portions over 10 min. The resulting solution was stirred at room temperature for 1 h. The solvent was evaporated, and the crude material was dissolved in a mixture of water (200 mL) and EtOAc (100 mL). The organic layer was recovered, and the aqueous layer was extracted with EtOAc (100 mL). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to give a colorless viscous liquid. This product was further purified as follows: The liquid **5a** was dissolved in Et_2O (50 mL), and HCl in Et_2O was added dropwise to form a white salt. The salt was washed with Et_2O (2 × 50 mL), and the solid was dissolved in water (100 mL). The aqueous solution was washed with Et_2O (100 mL), and the Et_2O layer was discarded. The aqueous layer was made basic with 15% NH_4OH (10 mL) to form a white suspension which was extracted with EtOAc (2 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give **5a** (12.2 g, 93%) as a viscous liquid: ^{1}H NMR ($CDCl_3$) δ 1.20−1.95 (m, 20 H), 2.66 (s, 4 H), 2.78 (t, $J = 5.2$ Hz, 4 H), 3.61 (t, $J = 5.2$ Hz, 4 H); ^{13}C NMR δ 22.7, 25.8, 34.3, 51.5, 54.7, 60.6, 68.2. Anal. (HCl salt) ($C_{18}H_{38}\ N_2O_2S_2Cl_2$) C, H, N, S, Cl.

3-{([({[(3-Hydroxypropyl)amino]methyl}cyclohexyl)disulfanyl]methyl)amino}propan-1-ol (5b). Compound **5b** was synthesized in a manner similar to the synthesis of **5a**, using propanolamine (10.5 g, 140 mmol), **2c** (20 g, 70 mmol) and $NaBH_4$ (5.3 g, 139.6 mmol) to give **5b** (27.5 g, 97%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.25−1.80 (m, 24 H), 2.67 (s, 4 H), 2.91 (t, $J = 5.5$ Hz, 4 H), 3.84 (t, $J = 5.3$ Hz, 4 H); ^{13}C NMR δ 22.2, 25.8, 30.4, 34.3, 50.6, 54.4, 57.1, 64.7; MS *m*/*z* 405 (M + 1).

2-{([({[(2-Hydroxyethyl)methylamino]methyl}cyclohexyl)disulfanyl]methyl)amino}ethan-1-ol (6a). A mixture of **5a** (12.2 g, 32.4 mmol), 38% formaldehyde (35 mL) and methanol (70 mL) was stirred at room temperature under N_2 for 12 h. The solution was diluted with water (100 mL) and extracted with EtOAC (3 × 100 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give an oil. The crude product was dissolved in MeOH (120 mL), and $NaBH_4$ (3.05 g, 80.6 mmol) was added in small portions over 10 min. The resulting solution was stirred at room temperature for 1 h. Solvent was evaporated, and the crude material was dissolved in a mixture of water (200 mL) and EtOAc (100 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 100 mL).

The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to give **6a** (11.6 g, 88.5%) as a colorless viscous liquid. The product solidified on standing: mp 65−70 °C; ^1H NMR ($CDCl_3$) δ 1.23−1.65 (m, 20 H), 2.34 (s, 6 H), 2.59 (s, 4 H), 2.65 (t, $J = 5.3$ Hz, 4 H), 3.60 (t, $J = 5.3$ Hz, 4 H); ^{13}C NMR δ 22.3, 25.6, 33.4, 44.2, 55.9, 59.2, 61.7, 67.4. Anal. ($C_{20}H_{40}N_2O_2S_2$) C, H, N, S.

2-{Methyl[(sulfanylcyclohexyl)methyl]amino}ethan-1-ol (7a). To a stirred solution of **6a** (11.6 g, 28.7 mmol) in dry THF (100 mL) was added 1 M $LiAlH_4$ in THF (43 mL, 43 mmol) dropwise at room temperature under N_2. The resulting clear solution was stirred at room temperature for 1 h. The excess $LiAlH_4$ was destroyed by careful addition of solid $Na_2SO_4{\cdot}10H_2O$ (2 g). EtOAc (100 mL) was added, and the precipitate was removed by filtration. The precipitate was washed with 10% MeOH in CH_2Cl_2 (2 × 50 mL), and the combined filtrate was evaporated under reduced pressure to give **7a** (9.2 g, 79%) as a viscous liquid: ^1H NMR ($CDCl_3$) δ 1.05−1.25 (m, 2 H), 1.45−1.85 (m, 8 H), 2.34 (s, 3 H), 2.50 (s, 2 H), 2.64 (t, $J = 5.4$ Hz, 2 H), 3.56 (t, $J = 5.4$ Hz, 2 H); ^{13}C NMR δ 22.2, 25.9, 38.2, 44.8, 52.2, 59.4, 62.1, 72.2; HRMS *m/z* calcd for $C_{10}H_{20}NOS$ ($M^+ - 1$) 202.1265, found 202.1261.

5-{Methyl[(sulfanylcyclohexyl)methyl]amino}pentan-1-ol (7d). Compound **7d** was synthesized in a manner similar to that of **7a** using disulfide **6d** (7.4 g, 15.14 mmol), 1 M $LiAlH_4$ (30 mL, 30 mmol) and THF (50 mL). Workup similar to that of **7a** gave **7d** (6.1 g, 82%) as a viscous liquid: ^1H NMR ($CDCl_3$) δ 0.95−1.60 (m, 16 H), 2.16 (s, 3 H), 2.26 (s, 2 H), 2.32 (t, $J = 7.4$ Hz, 2 H), 3.45 (t, $J = 6.6$ Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 22.4, 23.4, 25.9, 27.4, 32.7, 37.9, 44.9, 52.3, 60.6, 62.7, 72.1; MS *m/z* 246 (M + 1).

Di(1,3-oxazaperhydroin-3-ylmethyl)cyclohexyl Disulfide (9). Propanolamine (15.7 g, 209.5 mmol) in MeOH (50 mL) was added to a stirred suspension of disulfide **2c** (30 g, 104.7 mmol) in MeOH (150 mL) at room temperature. The disulfide was gradually dissolved, forming a light brown solution which was stirred at room temperature for 3 h. $NaBH_4$ (4 g, 104.73 mmol) was added in small portions over 10 min, and the reaction mixture was stirred at room temperature for 1 h. Formaldehyde solution (38%, 120 mL) was added, and the resulting cloudy solution was stirred at room temperature for 1 h. The flask was placed in a freezer (−20 °C) for 12 h after which time the clear supernatant was decanted. The gummy mass remaining in the flask was shaken well with MeOH (50 mL) to produce a white solid which was filtered and dried in vacuo (6 h) to give **9** (34 g, 75.7%) as a white powder. Compound **9** (24 g, 56 mmol) was dissolved in boiling MeOH (150 mL), and the resulting clear solution was left in a freezer for 12 h. The solid was filtered and dried in vacuo to give **9** (21 g) as a white crystalline solid: mp 65−66 °C; ^1H NMR ($CDCl_3$) δ 1.20−1.80 (m, 24 H), 2.92 (s, 4 H), 3.06 (t, $J = 5.3$ Hz, 4 H), 3.93 (t, $J = 5.3$ Hz, 4 H), 4.39 (s, 4 H); ^{13}C δ 22.1, 22.7, 25.6, 32.3, 52.6, 55.8, 60.9, 67.7, 86. Anal. ($C_{22}H_{40}N_2O_2S_2$) C, H, N, S.

3-{([({[(3-Hydroxypropyl)methylamino]methyl}cyclohexyl)disulfanyl]methyl)amino}ethan-1-ol (6b). To a stirred solution of **9** (4.6 g, 10.7 mmol) in glacial HOAc (30 mL) was added $NaBH_4$ (1 g, 26.3 mmol) in small portions over 5 min at room temperature, and the resulting solution was stirred for 4 h. The solution was poured onto crushed ice (10 g), made basic with concentrated NH_4OH, and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to give **6b** (4.6 g, 99%) as a pale yellow oil: ^1H NMR ($CDCl_3$) δ 1.20−1.65 (m, 24 H), 2.29 (s, 6 H), 2.48 (s, 4 H), 2.66 (t, $J = 5.8$ Hz, 4 H), 3.76 (t, $J = 5.2$ Hz, 4 H); ^{13}C NMR δ 22.4, 25.6, 28.7, 33.4, 44.4, 55.7, 60.8, 64.0, 68.2; MS *m/z* 433 (M + 1), 216; HRMS *m/z* calcd for $C_{22}H_{44}N_2O_2S_2$ (M + 1) 433.2922, found 433.2910.

3-{Methyl[(sulfanylcyclohexyl)methyl]amino}propan-1-ol (7b). (a) To a stirred solution of 1 M $LiAlH_4$ in THF (18 mL, 18 mmol) was added **9** (5.00 g, 11.66 mmol) in THF (25 mL) dropwise at room temperature under N_2. The resulting clear solution was stirred at room temperature for 3 h, and the excess $LiAlH_4$ was destroyed by careful addition of $Na_2SO_4{\cdot}10H_2O$ (5 g). A solution of 10% MeOH in CH_2Cl_2 (50 mL) was added, and the precipitated solid was removed by filtration. The solid was washed with 10% MeOH in CH_2Cl_2 (2 × 50 mL). The combined filtrate was concentrated under reduced pressure and dried in vacuo for 3 h to give **7b** (4.9 g, 97%) as a viscous liquid: ^1H NMR ($CDCl_3$) δ 1.11−1.78 (m, 12 H), 2.33 (s, 3 H), 2.46 (s, 2 H), 2.69 (t, $J = 6.3$ Hz, 2 H), 3.75 (t, $J = 5.1$ Hz, 2 H); ^{13}C NMR δ 22.1, 25.8, 37.8, 44.5, 51.5, 60.4, 63.5, 73.34; MS *m/z* 218 (M + 1).

(b) Powdered zinc (10 g, 153 mmol) was suspended in a solution of **6b** (5.3 g, 12.3 mmol) in glacial HOAc (40 mL). The resulting slurry was stirred at room temperature under N_2 for 80 min. The inorganic solid was removed by filtration and washed with HOAc (30 mL). The filtrate was poured onto crushed ice (∼200 g) and made basic with concentrated NH_4OH (90 mL). The white precipitate was extracted with EtOAc (3 × 150 mL) and washed with brine (2 × 100 mL), then water (2 × 100 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give **7b** (4.5 g, 84.3%) as an oil.

7b·HCl. A saturated solution of HCl in dry Et_2O was added dropwise to a solution of **7b** (4.9 g, free base) in dry Et_2O (50 mL) to form an insoluble gummy precipitate. The solvent was decanted, and the residue was washed with Et_2O (2 × 50 mL) and dried in vacuo for 12 h to give **7b·HCl** (4.8 g) as a gummy semisolid.

2-{Methyl[((nitrosothio)cyclohexyl)methyl]amino}ethan-1-ol (8a). Nitrosylated tether **8a** was synthesized as similar to **8b** using **7a** (1.05 g, 5.2 mmol) MeOH (7 mL), concentrated HCl (0.5 mL) and *t*-BuONO (0.76 g, 6.5 mL). The crude product was flash chromatographed on silica gel eluting with EtOAc/hexane (5:1) afforded **8a** (0.2 g, 17%): ^1H NMR ($CDCl_3$) δ 1.42−1.75 (m, 6 H), 2.02−2.11 (m, 2 H), 2.33 (s, 3 H), 2.48−2.56 (m, 2 H), 2.7 (t, $J = 6$ Hz, 2 H), 3.23 (s, 2 H), 3.53 (t, $J = 6$ Hz, 2 H); ^{13}C NMR δ 22.14, 25.6, 34.7, 44.1, 58.9, 61.7, 64.2, 69.3; MS *m/z* 233 (M + 1), 202 (M − 30, −NO).

3-{Methyl[((nitrosothio)cyclohexyl)methyl]amino}propan-1-ol (8b). *t*-BuONO (3.03 mL, 25.9 mmol) was added to a stirred solution of **7b** (4.5 g, 20.7 mmol) and concentrated HCl (1.5 mL) in MeOH (30 mL) under N_2 at room temperature. The green solution was stirred for 18 min and poured onto crushed ice (∼200 g). The mixture was made basic with 10% Na_2CO_3 (35 mL) and extracted with EtOAc (2 × 120 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give **8b** as a green viscous oil (4.8 g, 94%): TLC $R_f = 0.23$ (hexane/EtOAc, 1:1; green); ^1H NMR ($CDCl_3$) δ 1.45−1.80 (m, 8 H), 2.09 (m, 2 H), 2.38 (s, 3 H), 2.53 (d, $J = 14$ Hz, 2 H), 2.74 (t, $J = 5.8$ Hz, 2 H), 3.24 (s, 2 H), 3.77 (t, $J = 5.2$ Hz, 2 H); ^{13}C NMR δ 21.9, 25.3, 28.7, 34.3, 44.2, 60.1, 63.1, 63.7, 69.7.

4-{Methyl[((nitrosothio)cyclohexyl)methyl]amino}butan-1-ol (8c). *t*-BuONO (3 mL, 25.97 mmol) was added to a stirred solution of **7c** (5.0 g, 21.6 mmol) and concentrated HCl (2 mL) in EtOH (25 mL) under N_2 at room temperature. The resulting green solution was stirred for 15 min and poured onto crushed ice (∼100 g). The mixture was made basic with 10% Na_2CO_3 (20 mL) and extracted with EtOAc (2 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated. (During the workup, some **8c** decomposed.) Flash chromatography of the crude material on silica gel eluting with EtOAc/hexane (1:1) afforded **8c** (0.89 g, 16%) as a green viscous oil: ^1H NMR ($CDCl_3$) δ 1.33−1.70 (m, 10 H), 2.01−2.12 (m, 2 H), 2.26 (s, 3 H), 2.26−2.50 (m, 4 H), 3.12 (s, 2 H), 3.53 (t, $J = 5.7$, 2 H); ^{13}C NMR δ 22.2, 25.1, 25.5, 31.3, 34.5, 44.7, 60.9, 62.9, 64.1, 69.5.

5-{Methyl[((nitrosothio)cyclohexyl)methyl]amino}pentan-1-ol (8d). *t*-BuONO (3 mL, 25.97 mmol) was added to a stirred solution of **7d** (4.00 g, 14.93 mmol) and concentrated HCl (2 mL) in CH_2Cl_2 (20 mL) under N_2 at room temperature. The resulting green solution was stirred for 15 min and poured onto crushed ice (∼100 g). The mixture was made basic with 10% Na_2CO_3 (20 mL) and extracted with EtOAC (2 × 50 mL). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. (During the workup, some of the desired product decomposed.) Flash chromatography of the crude material on silica gel eluting with EtOAc/hexane (1:1) afforded **8d** (0.8 g, 14%) as a green oil: ^{1}H NMR (CDCl$_3$) δ 1.1−1.8 (m, 12 H), 2.01−2.19 (m, 2 H), 2.25 (s, 3 H), 2.25−2.45 (m, 4 H), 3.05 (s, 2 H), 3.54 (t, $J = 6.6$, 2 H); ^{13}C NMR δ 22.3, 23.3, 25.5, 27.2, 32.6, 34.3, 44.9, 60.4, 62.8, 64.5, 68.7; MS m/z 275 (M + 1).

3-{Benzyl[(sulfanylcyclohexyl)methyl]amino}propan-1-ol (11). Solid K$_2$CO$_3$ (10 g) was added to a stirred solution of disulfide **5b** (6.5 g, 16.06 mmol) and benzyl bromide (3.8 mL, 32.12 mmol) in acetone (75 mL). The solution was heated at 60 °C for 12 h. The solid was filtered, and the filtrate was concentrated. The crude product was flash chromatographed on silica gel eluting with EtOAc/Hex (1:1) to afford disulfide **10** (8.6 g, 91.6%) as a viscous oil. Compound **10** (8.00 g, 13.67 mmol) was dissolved in THF (50 mL), and 1 M LiAlH$_4$ in THF (26 mL, 26 mmol) was added dropwise at 0 °C. The mixture was then heated at 60 °C for 18 h and then the reaction mixture was cooled to room temperature. Solid Na$_2$SO$_4$·10H$_2$O (~10 g) was added in small portions over 5 min to form a white precipitate. A solution of MeOH/CH$_2$Cl$_2$ (1:10, 100 mL) was added, and the solid was removed by filtration. The solid was washed with MeOH/CH$_2$Cl$_2$ (1:10, 100 mL). The filtrate was concentrated and dried in vacuo to give **11** (5.6 g, 70%) as an oil: ^{1}H NMR (CDCl$_3$) δ 1.00−1.80 (m, 8 H), 2.57 (s, 2 H), 2. 70 (t, $J = 6.5$ Hz, 2H), 3.68 (s, 2 H), 3.41−3.71 (m, 2 H), 7.15−7.36 (m, 5 H); ^{13}C NMR δ 22.2, 25.8, 29.1, 36.0, 38.2, 54.9, 60.1, 62.5, 68.9, 127.2, 128.3, 129.3, 138.6; MS m/z 294 (M + 1); HRMS m/z calcd for C$_{17}$H$_{26}$NOS (M − 1) 292.1734, found 292.1730.

3-({[(Nitrosothio)cyclohexyl]methyl}benzylamino)-propan-1-ol (12). *t*-BuONO (2.8 mL, 21.3 mmol) was added to a stirred solution of **11** (5.0 g, 20.7 mmol) and concentrated HCl (2 mL) in MeOH (30 mL) under N$_2$ at room temperature. The workup similar to **8b** gave crude material which was chromatographed on silica gel eluting with EtOAc/hexane (1:2) to afford **12** (4.24 g, 76%): TLC $R_f = 0.53$ (hexane/EtOAc, 2:1, green); ^{1}H NMR (CDCl$_3$) δ 1.40−1.80 (m, 6 H), 1.76 (t, $J = 6.1$ Hz, 2 H), 2.01−2.09 (m, 2 H), 2.43−2.48 (m, 2 H), 2.74 (t, $J = 6.5$ Hz, 2 H), 3.34 (s, 2 H), 3.69 (t, $J = 5.8$ Hz, 2 H), 3.74 (s, 2 H), 7.15−7.40 (m, 5 H); ^{13}C NMR 22.1, 25.5, 28.9, 34.6,, 55.1, 59.9, 62.6, 63.8, 65.8, 127.3, 128.3, 129.3, 138.2; MS m/z 323 (M$^+$ + 1).

Acetate 13. Acetic anhydride (7.8 g, 77.1 mmol) was added to a stirred solution of disulfide **5b** (5.2 g, 12.8 mmol), DMAP (0.1 g) and Et$_3$N (2 mL) in CH$_2$Cl$_2$ (50 mL). The solution was heated at 60 °C for 4 h, stirred at room temperature for 12 h and concentrated. The crude product was flash chromatographed on silica gel eluting with MeOH/CH$_2$Cl$_2$ (1:19) to afford disulfide **13** (5.3 g, 72%) as a viscous oil: ^{1}H NMR (CDCl$_3$) δ 1.00−1.95 (m, 24 H), 2.30 (s, 6 H), 2.38 (s, 6 H), 3.42−3.52 (m, 8 H), 4.03 (t, $J = 5.9$ Hz, 4 H); ^{13}C NMR δ 20.8, 21.6, 22.1, 25.5, 26.4, 27.9, 33.8, 47.8, 52.7, 56.3, 61.7, 170.8, 171.8; MS m/z 573 (M + 1).

3-{Ethyl[(sulfanylcyclohexyl)methyl]amino}propan-1-ol (14). Compound **13** (5.2 g, 9.1 mmol) was dissolved in THF (50 mL), and 1 M LiAlH$_4$ in THF (18 mL, 18 mmol) was added dropwise at 0 °C. The mixture was then heated at 60 °C for 18 h and then the reaction mixture was cooled to room temperature. Solid Na$_2$SO$_4$·10H$_2$O (~10 g) was added in small portions over 5 min to form a white precipitate. A solution of MeOH/CH$_2$Cl$_2$ (1:10, 100 mL) was added, and the solid was removed by filtration. The solid was washed with MeOH/CH$_2$Cl$_2$ (1:10, 100 mL). The filtrate was concentrated and dried in vacuo to give **11** (3.3 g, 78%) as an oil: ^{1}H NMR (CDCl$_3$) δ 0.96 (t, $J = 5.2$ Hz, 2H), 1.19−1.80 (m, 13 H), 2.52 (s, 2 H), 2.72 (t, $J = 5.8$ Hz, 2 H), 2.62 (q, $J = 7.1$ Hz, 2 H), 3.76 (t, $J = 5.2$ Hz, 2 H); ^{13}C NMR δ 10.3, 22.4, 25.5, 28.4, 33.3, 48.2, 55.6, 55.7, 63.4, 64.9; MS m/z 232 (M + 1).

3-(Ethyl{[(nitrosothio)cyclohexyl]methyl}amino)-propan-1-ol (15). *t*-BuONO (1.9 mL, 18.7 mmol) was added to a stirred solution of the HCl salt of crude **14** (4.0 g, 14.9 mmol) in CH$_2$Cl$_2$ (25 mL) under N$_2$ at room temperature. The resulting green solution was stirred for 15 min and poured onto crushed ice (100 g). The mixture was made basic with 10% Na$_2$CO$_3$ (20 mL) and extracted with EtOAc (2 × 50 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated. Flash chromatography of the crude material on silica gel eluting with EtOAc/hexane (1:2) afforded **15** (1.3 g, 32.6%) as a green viscous oil: ^{1}H NMR (CDCl$_3$) δ 0.91 (t, $J = 7.1$, 3 H), 1.30−1.66 (m, 8 H), 1.98−2.07 (m, 2 H), 2.42−2.53 (m, 2 H), 2.61 (q, $J = 7.1$ Hz, 2 H), 2.71 (t, $J = 6.1$ Hz, 2 H), 3.13 (s, 2 H), 3.68 (t, $J = 5.5$ Hz, 2 H); ^{13}C NMR δ 10.4, 22.2, 25.4, 28.4, 34.5, 48.2, 55.5, 63.4, 63.8, 66.3.

2-Methyl-2-(nitrosothio)propyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (20a). A solution of diclofenac (8.30 g, 28.0 mmol), DCC (5.80 g, 28.0 mmol), and **4a** (3.80 g, 28.0 mmol) in CH$_2$Cl$_2$ (100 mL) was stirred at room temperature for 40 min. The precipitate was removed by filtration, and the filtrate was concentrated. The crude material was purified by flash chromatography on silica gel eluting with EtOAc/hexane (1:9) to give a green oil which was dissolved in EtOAc/pentane (1:5) and left in a freezer (−20 °C) for 12 h to produce **20a** (8.2 g, 71%) as red rods: TLC $R_f = 0.62$ (EtOAc/hexane, 1:9); mp 48 °C; ^{1}H NMR (CDCl$_3$) δ 1.89 (s, 6 H), 3.84 (s, 2 H), 4.72 (s, 2 H), 6.54−7.36 (m, 8 H); ^{13}C NMR δ 25.7, 38.2, 54.7, 71.4, 118.3, 122.1, 123.9, 124.0, 128.0, 129.3, 137.7, 142.6, 171.9. Anal. (C$_{18}$H$_{18}$N$_2$O$_3$SCl$_2$) C, H, N, S, Cl.

2-Ethyl-2-(nitrosothio)butyl 2-{2-[(2,6-Dichlorophenyl)-amino]phenyl}acetate (20b). DCC (2.43 g, 21.46 mmol) in CH$_2$Cl$_2$ (25 mL) was added dropwise to a stirred solution of **4b** (1.75 g, 10.72 mmol), diclofenac (3.17 g, 10.72 mmol), and DMAP (0.10 g) in dry CH$_2$Cl$_2$ (25 mL) at 0 °C over 15 min. The suspension was then stirred at 0 °C for 30 min. The solid was removed by filtration and washed with CH$_2$Cl$_2$ (25 mL). The filtrate was concentrated under reduced pressure at 40 °C and chromatographed on silica gel eluting with EtOAc/hexane (1:10) to afford **20 b** (4.3 g, 91%) as a green oil which crystallized on standing in a freezer (−20 °C) for 12 h: mp 50−51 °C; TLC $R_f = 0.80$ (hexane/EtOAc, 9:1); ^{1}H NMR (CDCl$_3$) δ 0.88 (t, $J = 7.5$, 6 H), 2.04−2.24 (m, 4 H), 3.68 (s, 2 H), 4.72 (m, 2 H), 6.41 (d, $J = 7.9$ Hz, 1 H), 6.66 (s, 1 H), 6.79−7.12 (d, $J = 7.4$ Hz, 1 H), 7.20 (d, $J = 8.1$ Hz, 2 H); ^{13}C NMR δ 8.1, 27.7, 38.3, 63.0, 68.1, 118.4, 122.1, 124.0, 124.1, 128.1, 128.8, 129.4, 130.8, 137.7, 142.6,172.1; MS m/z 441 (M + 1). Anal. (C$_{20}$H$_{22}$N$_2$O$_3$SCl$_2$) C, H, N, S, Cl.

[(Nitrosothio)cyclohexyl]methyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (20c). Compound **20c** was synthesized in a manner similar to the synthesis of **20b** using **4c** (2 g, 11.4 mmol), DCC (2.8 g, 13.5 mmol), diclofenac (4.0 g, 13.5 mmol) and DMAP (0.3 g, 2.4 mmol) in CH$_2$Cl$_2$ (100 mL). Chromatography of the crude product on silica gel eluting with EtOAc/hexane (1:40) and followed by recrystallization from EtOAc/MeOH (1:4) afforded **20c** (4.0 g, 77%) as a green solid: mp 61−63 °C; ^{1}H NMR (CDCl$_3$) δ 1.33−1.78 (m, 6 H), 2.02−2.12 (m, 2 H), 2.40−2.51 (m, 2 H), 3.82 (s, 2 H), 4.80 (s, 2 H), 6.55 (d, $J = 8$ Hz, 1 H), 6.79 (s, 1 H), 6.92−7.00 (m, 2 H), 7.10−7.20 (m, 2 H), 7.34 (d, $J = 8$ Hz, 2 H); ^{13}C NMR δ 21.7, 25.3, 33.2, 38.4, 60.2, 71.3, 118.4, 122.1, 124.0, 124.1, 128.1, 128.8, 129.4, 130.8, 137.7, 142.7, 172.2. Anal. (C$_{21}$H$_{22}$N$_2$O$_3$SCl$_2$) C, H, N, S, Cl.

2-(Methyl[(nitrosothiocyclohexyl)methyl]amino)-ethyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (21a). (a) *t*-BuONO (0.46 g, 4.42 mmol) was added to a stirred solution of **16.HCl** (2.28 g, 4.42 mmol) in CH$_2$Cl$_2$ (50 mL) at −78 °C. The cooling bath was removed, and the green solution was stirred for 10 min and then concentrated under reduced pressure to give a green foam. The green foam was dissolved in EtOAc (25 mL), ice (~5 g) was added, and the mixture was first washed with 10% Na$_2$CO$_3$ (10 mL) and then with water (25 mL). The organic layer was dried over Na$_2$SO$_4$ and concentrated under reduced pressure to give an oil. Flash chromatography of the crude product on silica gel eluting with EtOAc/hexane (1:19) afforded **21a** (1.92 g, 85.2%) as a green viscous oil.

(b) Alternatively, compound **21a** was synthesized in a manner similar to the synthesis of **21b** using DCC (0.49 g, 2.38 mmol), **8a** (0.46 g, 1.98 mmol), diclofenac (0.59 g, 2.00

mmol), DMAP (0.025 g) and CH_2Cl_2 (15 mL). Chromatography of the crude coupling product on silica gel eluting with EtOAc/hexane (1:9) afforded **21a** (1.0 g, 98%) as a green oil: TLC R_f = 0.47 (Hex/EtOAc, 9:1, green); ^{1}H NMR ($CDCl_3$) δ 1.25−1.70 (m, 6 H), 2.05−2.14 (m, 2 H), 2.35−2.44 (m, 2 H), 2.37 (s, 3 H), 2.81 (t, *J* = 5.9 Hz, 2 H), 3.19 (s, 2 H), 3.80 (s, 2 H), 4.21 (t, *J* = 5.9 Hz, 2 H), 6.54 (d, *J* = 8.0 Hz, 1 H), 6.90−6.99 (m, 3 H), 7.08−7.13 (m, 1 H), 7.20 (d, *J* = 7.4 Hz, 1 H), 7.32 (d, *J* = 8.0 Hz, 2 H); ^{13}C NMR δ 22.2, 25.6, 34.0, 38.6, 45.0, 58.4, 63.0, 64.4, 69.0, 118.2, 122.0, 124.0, 124.2, 128.0, 128.8, 129.4, 130.9, 137.8, 142.7, 172.2; HPLC t_R = 4.6 min (system A, 98%).

21a·HCl. A saturated solution of HCl gas in Et_2O was added dropwise to a solution of **21a** (1.8 g) in dry Et_2O (30 mL) to form an insoluble green gum. The solvent was evaporated under reduced pressure to give a green foam, which was triturated with hexane (25 mL) to afford a green suspension. The solvent was evaporated under reduced pressure, and the product was dried in vacuo for 12 h to give **21a·HCl** (1.86 g) as a green powder: mp 105−107 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.50−1.90 (m, 6 H), 2.52 (bs, 2 H), 2.88 (s, 3 H), 3.35−3.52 (m, 2 H), 3.80−4.20 (m, 2 H), 3.82 (s, 2 H), 4.72 (brs, 2 H), 6.52 (d, *J* = 7.6 Hz, 2 H), 6.92 (t, *J* = 7.3 Hz, 1 H), 7.01 (t, *J* = 7.7 Hz, 1 H), 7.09−7.22 (m, 2 H), 7.34 (d, *J* = 8.0 Hz, 2 H), 12.61 (brs, 1 H).

3-(Methyl[(nitrosothiocyclohexyl)methyl]amino)propyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (21b). DCC (4.4 g, 21.46 mmol) in CH_2Cl_2 (30 mL) was added dropwise to a stirred solution of **8b** (4.2 g, 17 mmol), diclofenac (5.3 g, 17.9 mmol), and DMAP (0.15 g) in dry CH_2Cl_2 (30 mL) at 0 °C over 15 min. The suspension was then stirred at 0 °C for 30 min. The precipitate was removed by filtration and washed with CH_2Cl_2 (25 mL). The filtrate was concentrated at 40 °C. Hexane (100 mL) was added, and the precipitated solid was removed by filtration. The filtrate was concentrated under reduced pressure to give an oil which was dissolved in EtOAc (10 mL) and MeOH (40 mL). The solution was filtered, and the filtrate was heated gently at 40 °C for 2 min and then left in the freezer (- 20 °C) overnight (12 h). The green crystals which formed were collected by filtration and dried in vacuo 6 h to give **21b** (8.4 g, 94%): mp 58−60 °C; TLC R_f = 0.46 (hexane/EtOAc, 9:1); ^{1}H NMR ($CDCl_3$) δ 1.40−1.77 (m, 6 H), 1.84 (p, *J* = 6.8 Hz, 2 H), 2.08−2.18 (m, 2 H), 2.35 (s, 3 H), 2.47 (d, *J* = 13.9 Hz, 2 H), 2.58 (t, *J* = 7.1 Hz, 2 H), 3.16 (s, 2 H), 3.85 (s, 2 H), 4.22 (t, *J* = 6.4 Hz, 2 H), 6.61 (d, *J* = 7.9 Hz, 1 H), 6.97−7.05 (m, 3H), 7.16 (t, *J* = 5 Hz, 1H), 7.28 (d, *J* = 7.4 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2 H); ^{13}C NMR δ 22.2, 25.5, 26.7, 34.2, 38.6, 44.5, 56.5, 63.2, 64.4, 68.9, 118.2, 121.9, 123.9, 124.3, 127.8, 128.8, 129.4, 130.8, 137.8, 142.7, 172.3. Anal. ($C_{25}H_{31}N_3O_3SCl_2$) C, H, N, S, Cl..

4-(Methyl[(nitrosothiocyclohexyl)methyl]amino)butyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (21c). Compound **21c** was synthesized in a manner similar to the synthesis of **21b** using DCC (0.77 g, 3.7 mmol), **8c** (0.65 g, 2.49 mmol), diclofenac (0.927 g, 3.13 mmol), DMAP (0.025 g) and CH_2Cl_2 (40 mL). Chromatography of the crude product on silica gel eluting with EtOAc/hexane (1:9) afforded **21c** (1.1 g, 82%) as a green oil: ^{1}H NMR ($CDCl_3$) δ 1.20−1.70 (m, 10 H), 2.00−2.15 (m, 2 H), 2.21 (s, 3 H), 2.28−2.42 (m, 4 H), 3.04 (s, 2 H), 3.75 (s, 2 H), 4.08 (t, *J* = 6.6 Hz, 2 H), 6.48 (d, *J* = 7.9 Hz, 1 H), 6.86−6.94 (m, 3 H), 7.03−7.08 (m, 1 H),7 0.16−7.19 (m, 1H), 7.27 (d, *J* = 8.1 Hz, 2 H); ^{13}C NMR δ 22.3, 23.9, 25.6, 26.3, 34.3, 38.7, 44.7, 59.9, 64.6, 65.3, 68.8, 118.2, 122.0, 124.0, 124.4, 127.9, 128.8, 129.5, 130.8, 137.8, 142.7, 172.4; MS *m/z* 538 (M + 1); HPLC t_R = 4.9 min (system A, 100%).

5-(Methyl[(nitrosothiocyclohexyl)methyl]amino)pentyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (21d). Compound **21d** was synthesized in a manner similar to the synthesis of **21b** using DCC (0.75 g, 3.66 mmol), **8d** (0.80 g, 2.91 mmol), diclofenac (0.93 g, 3.05 mmol), DMAP (0.1 g) and CH_2Cl_2 (40 mL). Chromatography of the crude product on silica gel eluting with EtOAc/Hex (1:9) afforded **21d** (1.1 g, 69%) as a green oil: TLC R_f = 0.40 (hexane/EtOAc, 9:1); ^{1}H NMR ($CDCl_3$) δ 1.18−1.66 (m, 12 H), 2.03−2.12 (m, 2 H), 2.25 (s, 3 H), 2.31−2.47 (m, 4 H), 3.05 (s, 2 H), 3.76 (s, 2 H), 4.08 (t, *J* = 6.7 Hz, 2 H), 6.49 (d, *J* = 7.9 Hz, 1 H), 6.87−6.95 (m, 3 H), 7.04−7.09 (m, 1H), 7.18 (dd, *J* = 1.1 and 7.4, 1 H), 7.28 (d, *J* = 8.0 Hz, 2 H); ^{13}C NMR δ 22.3, 23.4, 25.6, 27.1, 28.5, 34.3, 38.7, 44.9, 60.3, 64.5, 65.3, 68.8, 118.2, 121.9, 123.9, 124.4, 127.9, 128.8, 129.4, 130.8, 137.8, 142.7, 172.4; MS *m/z* 552 (M + 1); HPLC t_R = 5.4 min (system A, 98.4%).

3-([(Nitrosothiocyclohexyl)methyl]benzylamino)propyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (22a). Compound **22a** was synthesized in a manner similar to the synthesis of **21b** using DCC (0.87 g, 4.23 mmol), **12** (0.82 g, 2.54 mmol), diclofenac (1.04 g, 3.53 mmol), DMAP (0.025 g) and CH_2Cl_2 (50 mL). Chromatography of the crude product on silica gel eluting with EtOAc/hexane (1:19) afforded **22a** (1.20 g, 80%) as a green oil: TLC R_f = 0.58 (hexane/EtOAc 9:1); ^{1}H NMR ($CDCl_3$) δ 1.30−1.80 (m, 8 H), 2.01−2.12 (m, 2 H), 2.30−2.68 (m, 4 H), 3.26 (s, 2 H), 3.67 (s, 2 H), 3.69 (s, 2 H), 4.03 (t, *J* = 6.3 Hz, 2 H), 6.50 (d, *J* = 7.9 Hz, 1 H), 6.78−6.96 (m, 3 H), 7.01−7.14 (m, 2 H), 7.16−731(m, 7H); ^{13}C NMR δ 22.3, 25.6, 25.9, 34.5, 38.6, 52.1, 60.7, 63.4, 64.5, 65.9, 118.2, 122.0, 124.1, 124.4, 127.2, 127.9, 128.3, 128.7, 128.9, 129.5, 130.9, 137.8, 139.2, 142.7, 172.4; MS *m/z* 601 (M + 1); HPLC t_R = 6.5 min (system A, 99%).

3-(Ethyl[(nitrosothiocyclohexyl)methyl]amino)propyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (22b). Compound **22b** was synthesized in a manner similar to the synthesis of **21b** using DCC (4.4 g, 21.46 mmol), **15** (1.1 g, 4.22 mmol), diclofenac (1.37 g, 4.64 mmol), DMAP (0.025 g) and CH_2Cl_2 (50 mL). Chromatography of the crude product on silica gel eluting with EtOAc/hexane (1:9) afforded **22b** (1.65 g, 73%) as a green oil: TLC R_f = 0.33 (hexane/EtOAc, 9:1); ^{1}H NMR ($CDCl_3$) δ 0.84 (t, *J* = 7.0, 3 H), 1.22−1.70 (m, 8 H), 1.96−2.04 (m, 2 H), 2.33−2.55 (m, 6 H), 3.06 (s, 2 H), 3.72 (s, 2 H), 4.08 (t, *J* = 6.4 Hz, 2 H), 6.46 (d, *J* = 7.9 Hz, 1 H), 6.85−6.92 (m, 3 H), 7.01−7.06 (m, 1H), 7.14 (d, *J* = 7.4 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 2 H); ^{13}C NMR δ 11.1, 22.3, 25.6, 26.4, 34.2, 38.7, 48.9, 51.5, 63.5, 64.6, 66.0, 118.2, 122.0, 124.0, 124.4, 127.9, 128.8, 129.5, 130.8, 137.8, 142.7, 172.4; MS *m/z* 538 (M + 1); HPLC t_R = 8.4 min (system A, 95%). Anal. ($C_{26}H_{33}N_3O_3SCl_2$) C, H, N, S, Cl.

2-{[({[([{2-(2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetyloxy)ethyl}methylamino]methyl)cyclohexyl]disulfanyl}cyclohexyl)methyl]amino}ethyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (19). DCC (6.1 g, 29.6 mmol) in CH_2Cl_2 (160 mL) was added dropwise to a stirred solution of **6a** (12.0 g, 29.8 mmol) and diclofenac (24.2 g, 81.7 mmol) in CH_2Cl_2 (160 mL) over 1 h at room temperature. The mixture was then stirred for an additional 24 h at room temperature. The solid was removed by filtration and washed with CH_2Cl_2 (100 mL). The filtrate was concentrated under reduced pressure, and the residue was dissolved in Et_2O. A saturated solution of HCl gas in Et_2O was added to the solution until it became acidic. The precipitated yellow powder was filtered onto a Buchner funnel and washed with Et_2O (600 mL). The resulting white powder was dried in vacuo to afford **19·HCl** (26.0 g, 84%): mp 162−165 °C; ^{1}H NMR ($CDCl_3$) δ 1.20−1.90 (m, 10 H), 2.36 (s, 3 H), 2.55 (s, 2 H), 2.78 (t, *J* = 6 Hz, 2 H), 3.82 (s, 2 H), 4.23 (t, *J* = 6 Hz, 2 H), 6.54 (d, *J* = 8 Hz, 1 H), 6.92−7.0 (m, 3 H), 7.09−7.25 (m, 2 H), 7.33 (d, *J* = 8 Hz, 2 H); ^{13}C NMR δ 22.3, 32.6, 38.7, 45.3, 55.9, 58.5, 63.2, 118.2, 121.9, 123.9, 124.3, 127.9, 128.8, 129.4, 130.8, 137.8, 142.7, 172.3.

2-{Methyl[(sulfanylcyclohexyl)methyl]amino}ethyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (16). (a) DCC (1.21 g, 5.89 mmol) in CH_2Cl_2 (40 mL) was added dropwise to a stirred solution of **7a** (1.0 g, 4.91 mmol) and diclofenac (1.45 g, 4.91 mmol) in dry CH_2Cl_2 (50 mL) over 1 h. The suspension was then stirred at room temperature for 2 h. The solid was removed by filtration, and then was washed with CH_2Cl_2 (2 × 20 mL). The filtrate was concentrated under reduced pressure, and the crude material was triturated with hexane (2 × 25 mL) and filtered to remove DCU and the lactam byproduct (**18**). The solvent was evaporated to give a viscous oil which was chromatographed on silica gel eluting with EtOAc/hexane (1:19) afforded **16** (1.35 g, 57%): TLC R_f = 0.35 (hexane/EtOAc, 9:1; $KMNO_4$ brown); ^{1}H NMR ($CDCl_3$) δ 1.15−1.18 (m, 1 H), 1.29−1.80 (m, 9 H), 2.12 (bs, 1 H), 2.42 (s, 3 H), 2.52 (s, 2 H), 2.86 (t, J = 5.9 Hz, 2 H), 3.84 (s, 2 H), 4.28 (t, J = 5.9 Hz, 2 H), 6.56 (d, J = 7.9 Hz, 1 H), 6.93−7.00 (m, 3 H), 7.14 (t, J = 6.7 Hz, 1 H), 7.23 (d, J = 6.7 Hz, 1 H), 7.34 (d, J = 8.0 Hz, 2 H), ^{13}C NMR δ 22.3, 25.9, 37.6, 38.6, 45.2, 52.2, 58.5, 60.2, 63.1, 72.1, 118.1, 121.9, 123.9, 124.1, 127.8, 128.7, 129.3, 130.8, 137.7, 142.6, 172.2; MS m/z 510 (M + 1). Anal. ($C_{24}H_{30}N_2O_2Cl_2S_2$) C, H, N, S, Cl.

(b) 19 (18 g, 18.1 mmol) was dissolved in glacial HOAc (300 mL), and powdered zinc (36 g, 550 mmol) was added to this solution. The resulting slurry was stirred under N_2 for 6 h at room temperature, and the inorganic solid was removed by filtration. The solid was washed with HOAc (50 mL), and the residue was made basic with aqueous 15% NH_4OH. The product was then extracted with EtOAc (2 × 150 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give an oil, which was dissolved in Et_2O (100 mL). A saturated solution of HCl gas in Et_2O was added to acidify the Et_2O solution. The precipitate was filtered and washed with Et_2O (2 × 100 mL). The resulting white solid was dried in vacuo for 12 h to afford **16·HCl** (16 g, 86%): mp 113 °C; ^{1}H NMR ($CDCl_3$) δ 1.14−1.30 (m, 1 H), 1.34−2.00 (m, 9 H), 2.95−3.01 (m, 1 H), 2.96 (d, J = 4.2 Hz, 3 H), 3.30 (d, J = 13.5 Hz, 1 H), 3.43−3.60 (m, 3 H), 3.86 (s, 2 H), 4.66−4.82 (m, 2 H), 6.51−6.54 (m, 2 H), 6.94 (t, J = 7.4 Hz, 1 H), 7.02 (t, J = 8.0 Hz, 1 H), 7.06−7.16 (m, 1 H), 7.20 (d, J = 7.4 Hz, 1 H), 7.35 (d, J = 8.0 Hz, 2 H), 11.66 (bs, 1 H).

(Sulfanylcyclohexyl)methyl 2-{2-[(2,6-Dichlorophenyl)-amino]phenyl}acetate (23). DCC (2.25 g, 10.94 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a stirred solution of 3c (1.60 g, 10.95 mmol) and diclofenac (2.72 g, 9.12 mmol) in dry CH_2Cl_2 (25 mL) over 30 min at 0 °C. The suspension was then stirred at 0 °C for 1 h. Workup and purification (EtOAc/hexane, 1:19) similar to **16** afforded **23** (2.45 g, 63%): ^{1}H NMR ($CDCl_3$) δ 1.17−1.80 (m, 10 H), 3.88 (s, 2 H), 4.18 (s, 2 H), 6.57 (d, J = 7.9 Hz, 1 H), 6.84 (bs, 1 H), 6.96−7.02 (m, 2 H), 7.12−7.17 (m, 1 H), 7.26 (d, J = 6.7 Hz, 1 H), 7.35 (d, J = 8.0 Hz, 2 H); ^{13}C NMR δ 21.8, 25.7, 36.0, 38.5, 48.4, 74.2, 118.4, 122.1, 124.0, 124.4, 128.0, 128.8, 129.5, 130.8, 137.8, 142.7, 172.1; MS m/z 425 (M + 1); HPLC t_R = 9.0 min (system A, 100%). Anal. ($C_{21}H_{23}NO_2Cl_2S$) C, H, N, S, Cl.

3-{Methyl[(sulfanylcyclohexyl)methyl]amino}propyl 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetate (24). DCC (1.45 g, 7.03 mmol) in CH_2Cl_2 (25 mL) was added dropwise to a stirred solution of **7b** (1.53 g, 7.03 mmol) and diclofenac (2.08 g, 4.91 mmol) in dry CH_2Cl_2 (25 mL) over 1 h at room temperature. The suspension was then stirred at room temperature for 1 h. Workup and purification (EtOAc/hexane, 1:9) similar to **16** afforded **24** (0.65 g, 20%): TLC R_f = 0.58 (hexane/EtOAc, 4:1; $KMNO_4$ brown); ^{1}H NMR ($CDCl_3$) δ 0.99−1.80 (m, 12 H), 1.94 (bs, 1 H), 2.23 (s, 3 H), 2.34 (s, 2 H), 2.47 (t, J = 7.2 Hz, 2 H), 3.70 (s, 2 H), 4.13 (t, J = 6.5 Hz, 2 H), 6.45 (d, J = 7.9 Hz, 1 H), 6.82−6.90 (m, 3 H), 6.99−7.04 (m, 1 H), 7.12 (d, J = 7.4 Hz, 1 H), 7.23 (d, J = 8.0 Hz, 2 H); ^{13}C NMR δ 22.4, 25.9, 26.9, 37.8, 38.6, 44.7, 52.1, 56.7, 63.4, 72.2, 118.2, 121.9, 123.9, 124.3, 127.8, 128.8, 129.4, 130.7, 137.7, 142.6, 172.2; MS m/z 495 (M + 1); HPLC t_R = 4.3 min (system A, 100%). Anal. ($C_{25}H_{32}N_2O_2Cl_2S_1$) C, H, N, S, Cl.

Biological Assays Compound Formulation. All test compounds, including diclofenac sodium (Sigma Chemical Co., St. Louis, MO), were formulated just prior to administration in either 5% (by volume) or 50% (by volume) aqueous propylene glycol (PEG) containing 0.5% methocel in order to obtain a homogeneous suspension for oral animal dosing. The more lipophilic S-NO-diclofenac derivatives (i.e., **21a**−**21d**, **22a**, **22b**) required the greater PEG concentration. Procedurally, the desired amount of test compound was weighed and dissolved in aqueous PEG and then diluted with 0.5% methocel until the 5% or 50% concentration was attained. In all experiments, comparison between diclofenac sodium and a given S-NO-diclofenac derivative was made with the two compounds administered in the same vehicle.

Relative Bioavailability Determination. Relative compound bioavailability was indexed as the amount of circulating diclofenac present in mouse blood after oral dosing. Male CD-1 mice (Charles River Laboratories, Wilmington, MA) were fasted overnight (≈17 h) with water ad libitum. Mice were administered by oral gavage either a fixed dose (20 μmol/kg) of diclofenac sodium or an equimolar dose of a S-NO-diclofenac derivative as the appropriate suspension. The mice were anesthetized by ketamine (90 mg/kg)/xylazine (10 mg/kg) at 30, 60 and 120 min postdosing. A different group of mice was used for each time point, and blood samples were collected through cardiac puncture into a heparinized syringe. The heparinized blood was then centrifuged at 4 °C for 7 min at 850*g*, and the plasma was collected and stored at −20 °C until analysis. Plasma diclofenac was quantified in organic extracts by HPLC based upon the response of the spectrophotometric detector to known amounts of authentic diclofenac with meclofenamic acid as the internal standard.[43] Relative bioavailability over the 2-h period was then calculated and expressed relative to that of an equimolar dose (20 μmol/kg) of diclofenac in the same vehicle as the integrated area under the pharmacokinetic plot of blood diclofenac level vs time after dosing. The peak diclofenac plasma concentration within the 2-h sampling period was also quantified. Mice administered vehicle only evidenced no circulating diclofenac (data not shown). Data are expressed as mean plasma levels of diclofenac ± SEM.

Analgesic Activity (Writhing Test). The phenylbenzoquinone (PBQ)-induced writhing test was used to determine analgesic activity.[36] Male CD-1 mice weighing 20−25 g were fasted overnight (≈17 h) with water ad libitum. Vehicle or test compound (100 μmol/kg) was administered by oral gavage as a suspension with a constant dose volume of 2.5 mL/kg body weight at least 9 mice/group. One hour after dosing, each mouse received an intraperitoneal injection (2 mg/kg body weight) of PBQ solution. Five minutes after the PBQ injection, the number of writhes by each mouse over the next 5-min period was scored. Analgesic activity is expressed as the inhibition of the PBQ-induced writhing due to test compound.

Antiinflammatory Activity (Paw Edema Test). Antiinflammatory activity was assessed in the carrageenan-induced paw edema assay.[37] Male Sprague−Dawley rats (200−250 g) (Charles River Laboratories, Wilmington, MA) were fasted overnight (≈17 h) with water ad libitum. A test compound (100 μmol/kg) or appropriate vehicle was administered by oral gavage at a constant dose volume of 5 mL/kg body weight, with at least 5 rats/group. One hour after dosing, 50 μL of 1% carrageenan solution was injected into the subplantar region of the right front paw. Paw volume was measured immediately and 3 h after carrageenan injection by a water-displacement method. Antiinflammatory activity was quantified as the incremental reduction of the carrageenan-induced increase in paw volume due to test compound.

Gastric Lesion Assessment. Male Sprague−Dawley rats (180−200 g) were fasted for 24 h with water ad libitum and then dosed by oral gavage with diclofenac sodium or a S-NO-diclofenac derivative administered in a constant volume of 5 mL/kg at a final dose of 100 μmol/kg. Fasting was continued for an additional 18 h, at which time the animals were euthanized with CO_2. The stomachs were dissected along the greater curvature, washed with a directed stream of 0.9% saline, pinned open on a sylgard-lined Petri dish, and examined by light microscopy to assess the presence of gastric lesions.[39] Gastric lesion score is expressed in mm of lesion and calculated by summing the length of all lesions in a given stomach.

Statistics. All data are expressed as the mean ± SEM and were analyzed by performing analysis of variance followed by a Student−Neuman−Keuls post hoc test to determine the significance level with a Super Anova computer program.

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