Structure-Based Design, Synthesis, and Biological Evaluation of Indomethacin Derivatives as Cyclooxygenase-2 Inhibiting Nitric Oxide Donors§


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Received October 11, 2006

Indomethacin, a nonselective cyclooxygenase (COX) inhibitor, was modified in three distinct regions in an attempt both to increase cyclooxygenase-2 (COX-2) selectivity and to enhance drug safety by covalent attachment of an organic nitrate moiety as a nitric oxide donor. A human whole-blood COX assay shows the modifications on the 3-acetic acid part of the indomethacin yielding an amide-nitrate derivative 61 conferred COX-2 selectivity. Along with their respective des-nitrate analogs, for example, 31 and 62, the nitrates 32 and 61 were effective antiinflammatory agents in the rat air-pouch model. After oral dosing, though, only 32 increased nitrate and nitrite levels in rat plasma, indicating that its nitrate tether served as a nitric oxide donor in vivo. In a rat gastric injury model, examples 31 and 32 both show a 98% reduction in gastric lesion score compared to that of indomethacin. In addition, the nitrated derivative 32 inducing 85% fewer gastric lesions when coadministered with aspirin as compared to the combination of aspirin and valdecoxib.

Introduction

Common adverse effects of nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g., aspirin, indomethacin, naproxen, and ibuprofen) are gastrointestinal (GI) bleeding, ulceration, and more severely, perforation. Prostaglandins (PGs) synthesized by the “housekeeping” cyclooxygenase-1 (COX-1) enzyme mediate many physiological functions, including GI cytoprotection. The immediate hypothesis after the discovery of the “inducible” COX-2 enzyme and its increased expression at the sites of inflammation was that the proinflammatory PGs produced by COX-2 caused pain and fever.1-3 Therefore, a selective COX-2 inhibitor was expected to exert the anti-inflammatory, analgesic and antipretic effects of conventional NSAIDs without the undesired GI side effects associated with COX-1 inhibition. The subsequent discovery that COX-2 is constitutively expressed in the brain, the spinal cord, and the kidneys4-5 suggested that the COX-2 derived PGs have beneficial effects in, for example, ulcer healing6 and regulation of renal function.7 The physiological importance of COX-2 was subsequently manifested in the clinic as increased cardiovascular and renal risks associated with COX-2 inhibitors,8,9 particularly, when administered concurrently with aspirin.10-11 The cardio-renal liability of COX-2 inhibitor drugs has severely restricted their use as anti-inflammatory agents, especially for those with a very high selectivity for the COX-2 isomem that may obviate physiological effects of COX-2 derived PGs and induced a prothrombotic state.

Nitric oxide (NO) also plays many important physiological roles, including, vasoregulation, GI tissue protection,12 wound healing,13 and inhibition of platelet activity.14 Because of its cytoprotective effects and its potent antiplatelet and vasorelaxant properties, adjunctive NO has been considered a plausible means for improving the GI safety of traditional NSAIDs. COX-inhibiting nitric oxide donors (CINODs) are currently under laboratory and clinical investigation for using NO to improve and enhance traditional NSAIDs,13,15-28 as well as for COX-2 selective inhibitors.29,30 Especially in predisposed cardiovascular patients, the increased cardiovascular risk of COX-2 selective inhibitors and their gastric toxicity when administered with aspirin prophylaxis might be ameliorated by supplementary NO.

The crystal structures of the inhibitor-bound COXs show that the catalytic binding site is a long hydrophobic channel. From a combination of the information from the 3-D crystal structures, site-directed mutation studies, and kinetic experiments, the rationale for inhibitor binding and COX-2 selectivity are summarized as follows: Arg12031 at the entrance of the catalytic active site forms a salt bridge with the carboxylic acid end of the traditional NSAIDs, an interaction important for COX-1 inhibition by traditional NSAIDs, but not crucial for COX-2 inhibition.32,33 Therefore, carboxylic acids are not a structural requirement for COX-2 selective inhibitors. Other amino acids involved in the hydrogen-bonding network at the entrance for binding inhibitors are Tyr355 and Glu524.34 In COX-1, Ile523 blocks the diaryl-heterocycle-type selective inhibitors from entering the catalytic site.35 Because of the shorter side chain of Val523 in COX-2, a side-pocket is created (17% increased size) next to the main catalytic binding site. This side pocket is referred to as the “selective binding site” because traditional NSAIDs do not use this space. In the crystal structures of 1CX2 and 6COX, the aryl-sulfonamide group of SC-558, a bromo-analog of the COX-2 selective inhibitor celecoxib, penetrates deep into this side pocket and interacts with polar groups His90, Glu192, and Arg513.36 There is a possibility that the hydrogen-bonding network extends to nearby Glu524, which is part of the hydrogen-bonding network at the entrance. In COX-1, His513’s side chain is shorter, so even if the inhibitor can bypass Ile523, it likely cannot interact with His513. The use of this side-pocket and established hydrogen bonding to form a tight-binding slowly reversible complex was suggested to be the
resulted a tight-binding, slowly reversible enzyme (E) complex.44,45

The induced conformational changes after binding with inhibitors caused by the flexibility and sequence differences of the peptide chain have also been suggested to play a role in isoform selectivity. For example, one of the sequence differences between COX-1 and COX-2 is located at the junction of α-helices C and D, which is the framework in front of the catalytic binding site.38 As a result of an extra amino acid inserted between α-helices C and D in COX-2, the α-helix D that contains Arg120 has more flexibility at the entrance of the binding site. The combination of these observations with the kinetic data leads to a possible inhibition profile being proposed: E + I ⇌ [EI] ⇌ EI*.39–43 The induced conformational change resulted a tight-binding, slowly reversible enzyme (E)–inhibitor (I) complex, EI*. COX-1 inhibition is usually time independent, whereas COX-2 inhibition is time dependent. Therefore, the equilibrium between weak complex [EI] and tight complex EI* can influence selectivity, as may multiple stages of binding complexes.44,45

There have been several reports of using traditional NSAID as pharmacophores for the design and synthesis of new COX-2 selective inhibitors, including aspirin,46 meloxicam,47 nimesulide,48 meclofenamic acid,37 ketoprofen,49 flurbiprofen,50 and indomethacin.51–56 Herein, we report the design and synthesis of a nitric oxide-donating COX-2 selective inhibitor modified from indomethacin that showed modest COX-2 selectivity in a human whole-blood assay with improved GI safety, especially when coadministered with aspirin.  

**Molecule Design.** As shown in Figure 1, three regions of modifications were preformed with the goal of enhancing the COX-2 selectivity based on the information just summarized, and improving its safety profile as a COX-2 selective inhibitor by attaching a NO donor. From a comparison of the crystal structures of inhibitor-bound COX-2 enzymes, 4COX (with indomethacin) and 1CX2 (with SC-558), the 5-methoxy group and the 6-position of the indomethacin are very close to Val523 and the methoxy group lines up with the sulfonamide group of SC-558. Thus, the modifications on the 5- or 6-positions of the indole ring were intended to increase the steric interaction with Ile523 in COX-1, the probability of establishing a hydrogen-bond with Arg513 in the selective binding site of COX-2, or both. The second region of modification was at the N-1 position of the indole ring. The 4-chlorobenzoyl group of the indomethacin, which binds in the apical pocket of the binding site, will be replaced with cycloalkylalkyl and arylalkyl groups to explore other hydrophobic residues that can bind in this cavity. Although the specific sequence differences outside the binding site that are responsible for the selectivity were not identified by Kalgutkar et al.,37 there are two uncharged polar amino acids just outside the entrance on α-helix D, Tyr115 and Ser119 in COX-2 instead of Leu115 and Val119 in COX-1,55 that may influence the inhibitor binding by establishing hydrogen bonds with a polar-end tether. Finally, since a carboxylic acid-end is not a structural requirement for COX-2 inhibition, the 3-acetic acid group of indomethacin was converted to alternative functional groups and attached with either an alcohol or a nitrate as a NO donor. These polar-end tethers may also serve to establish possible hydrogen-bonding with Tyr115, Ser119, or both outside the binding channel and, hence, may increase the COX-2 selectivity.

**Syntheses.** The nitrate tethers for use as potential NO donors were synthesized either according to or modified from literature procedures. As shown in Scheme 1, the nitric acid salts of 3-(nitrooxy)propylamine 2 and 2,2-dimethyl-3-(nitrooxy)propylamine 4 were prepared from the nitration of the corresponding amino alcohols with acetyl nitrate, prepared in situ from the acid group of indomethacin was converted to alternative functional groups and attached with either an alcohol or a nitrate as a NO donor. The free base of Tris−trinitrate 6 was prepared according to a literature procedure and was treated with dry hydrochloric acid in ethyl ether to afford the salt for storage.57 All amine-nitrate derivatives were not stable at room temperature for a long period of time and therefore were kept in the freezer as ammonium salts. 3-(Nitrooxy)propan-1-ol 58 was prepared as shown in Scheme 1, the nitric acid salts of 3-(nitrooxy)propylamine 2 and 2,2-dimethyl-3-(nitrooxy)propylamine 4 were prepared from the nitration of the corresponding amino alcohols with acetyl nitrate, prepared in situ from 90% fuming nitric acid and acetic anhydride. The free base of Tris−trinitrate 6 was prepared according to a literature procedure and was treated with dry hydrochloric acid in ethyl ether to afford the salt for storage.57

The 5-O-sulfamate compound 14 was prepared as shown in Scheme 2. Demethylation of indomethacin with boron tribromide in methylene chloride afforded the phenolic compound 12,59 a possible indomethacin metabolite. The amine-nitrate tether 2 and 3-aminopropional 1 were coupled to the 3-acetic acid in the presence of N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDAC) to give examples 13 and 15, respectively. The phenolic group was then transformed to O-sulfamate 14 by reaction with sulfamoyl chloride in N-methyl-2-pyrrolidinone.60

The 4- and 6-chloro derivatives of indomethacin were prepared starting from a Fisher indole synthesis as shown in Scheme 3. Condensation between 3-chloro-4-methoxyphenylhydrazine 16, which was prepared from 3-chloro-p-anisidine following a literature procedure,61 and benzyl levulinate in
refluxing acetic acid gave compounds 17 and 18 in a ratio of 1:2. These two regioisomers were separated by column chromatography and were reacted with 4-chlorobenzoyl chloride in the presence of 4-dimethylaminopyridine (DMAP) to obtain 19 and 20, respectively. 6-Chloro indomethacin 21 was obtained by hydrogenation of benzyl ester 20 in the presence of 10% Pd on carbon. The amine-nitrate tether 2 was then coupled to the carboxylic acid to afford compound 22.

Syntheses to replace the 4-chlorobenzoyl group of indomethacin are shown in Scheme 4. Fisher indole synthesis using 4-methoxyphenylhydrazine and ethyl levulinate in acetic acid, followed by N-1 alkylation with 4-(bromomethyl)-1-(methylsulfonyl)benzene using potassium tert-butoxide as base, gave compound 24a. After hydrolysis with aqueous sodium hydroxide in THF, the acid 25a was coupled with amine-nitrate tether 2 to give example 26a. The N-cyclohexylmethyl derivatives 26b was synthesized using the same procedure as 26a except (bromomethyl)cyclohexane was used for N-1 alkylation of compound 23. The nitrate 29b was prepared from the reduction of ethyl ester 24b with lithium aluminum hydride, followed by the bromination with hydrobromic acid and then reaction with silver nitrate.

As shown in Scheme 5, the amide derivatives of indomethacin, compounds 30–33, were all prepared in the same manner by coupling with an amine-alcohol or an amine-nitrate in the presence of EDAC. The amide derivative 34 and hydroxamic acid 35 were prepared from the reaction of the acyl chloride of indomethacin with Tris-trinitrate or hydroxylamine. Acetyl-derivatives of hydroxamic acid, examples 36 and 37, were obtained by controlling the amount of acetic anhydride used in the reaction. The O-alkyl hydroxamic acid derivatives, examples 38–40, were prepared from the coupling reaction with the corresponding O-alkyl hydroxylamines. The O-alkyl-hydroxylamines 43 and 44 were prepared from O-alkylation of N-hydroxyphthalimide with bromoalcohols in the presence of sodium acetate followed by deprotection with hydrazine.

Other modifications on the 3-acetic acid of indomethacin started from borane reduction of indomethacin to give ethanol derivative 45, as shown in Scheme 6. The ethanol derivative 45 was then transformed to nitrate 47, bromide 48, and azide 54 through a common intermediate, mesylate 46. The phosphonic acid 50 were synthesized via an Abuzov reaction by reacting bromide 48 with trimethyl phosphite, followed by hydrolysis with bromotrimethylsilane. The carboxylsulfamide 51 and carboxylsulfamate 52 were prepared from the reaction of ethanol derivative 45 and chlorosulfonyl isocyanate followed by the addition of nitrate tethers 2 or 8. Phosgene was used in the preparation of carbamate 53 through the formation of the chloroformate of 45. The amine

Scheme 2. Synthesis of 5-Position Modified Indomethacin and its Derivatives

Scheme 3. Synthesis of 4- and 6-Chloro-indomethacin Derivatives
derivative 55 was prepared from the hydrogenation of azide 54 in the presence of acetic acid and was converted to the hydrochloride salt to prevent possible N-1 benzoyl group transposition to the primary amine. The benzyl nitrate tether 10 was coupled to the amine 55 in the presence of triethylamine and EDAC to afford the inverse amide 56. The urea derivative 57 was prepared through the formation of the carbamoyl imidazole intermediate of amine 55, followed by the addition of amine-nitrate tether 2. The sulfonamide derivatives 58 and 60 were obtained from the reaction of the corresponding sulfonyl chloride with amine 55 and were converted to their nitrate derivatives, examples 59 and 61, with silver nitrate in refluxing acetonitrile. Although 4-(bromomethyl) benzenesulfonyl chloride was used in the preparation of sulfonamide derivative 58, the chloromethyl derivative was isolated because of the halogen exchange in the reaction mixture. Initial attempts to prepare alcohol 62, not shown in the scheme, through the conversion of chloride 60 to acetate, followed by hydrolysis, proved unsuccessful. Under the basic conditions for hydrolysis of the acetate, a mixture of a cyclic sulfonamide, removal of 4-chlorobenzoyl group product, or both were obtained. The alcohol derivative 62 was prepared from the hydrogenation of the nitrate 61 using 10% Pd/C as catalyst. Hydrogenation usually will reduce chlorobenzene, but in our preparation of examples 55 and 62, the N-1 chlorobenzoyl group remains intact.

Results and Discussion

COX Inhibitory Activity. Compounds were assayed for COX inhibition using a human whole-blood assay. After
initial screening at three concentrations, COX-1 (100 µM) and COX-2 (1 and 10 µM), the active compounds were selected for IC$_{50}$ determination. The complete screening data of all compounds is available as Supporting Information. The most promising compounds were further examined using in vivo experiments for antiinflammatory activity, gastric tolerability, and NO donor activity.

In our studies, as shown in Table 1, indomethacin (11) slightly favors COX-1 inhibition and celecoxib showed 11.7-fold COX-2 selectivity. The demethylated indomethacin, example 12, favors COX-2 inhibition by 3-fold, and incorporation of an amide tether as in examples 13 and 15 slightly decreases the selectivity. The 5-O-sulfamate group in example 14 does restore COX-2 selectivity by greater than 4-fold. This suggests the 5-O-sulfamate group might be entering the selective binding site and interacting with Arg513 as we anticipated, but simultaneously lowering the COX inhibitory potency. Both benzyl esters of 4- and 6-chloro-indomethacin, examples 19 and 20, showed weak inhibitory activity (data not shown in Table 1). 6-Chloro-indomethacin 21 appears to more favor inhibition of COX-1 but is not as potent as indomethacin. A chlorine atom at the 6-position is either not large enough to cause unfavorable interaction with Ile523 or, perhaps, is too big and causes indomethacin to adopt another binding mode. As suggested by Loll et al., another possible conformation for 4′-iodo-indomethacin binding in COX-1 is a trans form, as depicted in the crystal structure of 1PGG. Example 22, attaching an amide linker to 6-chloro indomethacin favors COX-2 inhibition by almost 3-fold.

Replacement of the 4-chlorobenzoyl group of indomethacin with a 4-bromobenzyl group was reported to give excellent COX-2 selectivity. In our series, replacement of the 4-chlorobenzoyl group with a (methylsulfonyl)benzyl, example 25a, or a cyclohexylmethyl group, example 25b, severely compromised the inhibition of both COX isozymes. The attachment of an amide-nitrate linker did not improve the inhibitory activity for the (methylsulfonyl)benzyl derivative, example 26a, but did cause a minor improvement for the cyclohexylmethyl derivative 26b. The ethanol derivative of cyclohexylmethyl series, example 27b, and its nitrate derivative 29b also show weak inhibitory activity.

The indomethacin alkyl-amide derivatives 30–33 increased COX-2 inhibition from 2- to 5-fold. Decreased selectivity of compound 33 is most likely caused by the branched side chain still fitting well in the lobby region of COX-1 and thus exhibiting better affinity for this subtype. However, this size effect did not impart a selectivity advantage for COX-2 because the lobby region of COX-2 has more flexibility. An alkyl group with
increased steric bulk, example 34, diminishes the COX-2 selectivity in this series. These results contrast with data from a purified enzyme assay in which a two-carbon alkyl amide analogue of compound 31 was reported to have a 290-fold selectivity for COX-2.\textsuperscript{54} The derivatives of hydroxamic acid 35–37 showed no COX-2 selectivity. Because the immediate binding site for the acid group is conserved in both COX enzymes, a short aliphatic chain most likely will not have a strong influence on COX selectivity. However, hydroxamic acid derivatives 38–40 favor COX-1 inhibition as opposed to the amide derivatives 30–33. Perhaps the preferred binding conformation of hydroxamic acid directs the alkyl chain to the surface of the enzyme instead of in-line with binding channel to the lobby region.

The nitrate 47, phosphoric acid 50, carboxylsulfamide 51, carboxylsulfamate 52, carbamate 53, and amine 55 derived from alcohol 45 lost their ability to inhibit COX isozymes. The inverse amide 56, urea 57, and aryl-sulfonamide 59 derived from amine 55 also showed no COX-2 selectivity. However, the nitrate of alkyl-sulfonamides, example 61, showed 9-fold selectivity, and its alcohol, example 62, showed 4-fold COX-2 selectivity. Both nitrate derivatives 32 and 61 showed better COX-2 selectivity than the corresponding alcohols, examples 31 and 62. The improved selectivity of nitrate derivatives was also reported previously in pyrazoles derivatives.\textsuperscript{39}

**In Vivo Pharmacological Profiling.** In the rat air pouch model, amide derivatives 31 and 32 and alkyl-sulfonamide derivatives 61 and 62 significantly reduced the PG\textsubscript{E\%} level when administered intrapouch by 97%, 99%, 59% and 98%, respectively, as shown in Figure 2. The oral dosing air pouch experiment, as shown in Figure 3, also showed these four compounds significantly reduced the PG\textsubscript{E\%} level as evidence of their antiinflammatory activity. These two sets of experiments suggested example 62 has the best bioavailability and least protein binding affinity followed by 31, 32 and 61. However, only example 32 increased rat plasma nitrite plus nitrate (NO\textsubscript{x}) concentration, as shown in Figure 4, indicating that the nitrate tether acted as a NO donor. Since the attachment of a NO donor to improve the safety profiles of COX-2 inhibitor drugs is the primary goal of this research, the pair of propoxy-amide derivatives, examples 31 and 32, were further examined for GI tolerance.

The amide derivatives, 31 and 32, both show a 98% reduction in gastric lesion score when compared to equimolar indomethacin as shown in Figure 5. Although low-dose aspirin is routinely used for cardiovascular prophylaxis, concomitant use of aspirin and a COX-2 inhibitor increases gastric damage when compared to using either aspirin or the COX-2 inhibitor alone.\textsuperscript{10,11,60} This adverse effect was not significant for example 32 when administered with background aspirin treatment compared to the valdecoxib and aspirin treatment group as shown in Figure 6. The gastric-sparing effect (85% reduction) is especially noteworthy given the 4.7-fold greater dose of 32 versus valdecoxib when coadministered with aspirin.

**Conclusion.** We have identified several modifications of indomethacin yielding derivatives that increase this NSAID’s COX-2 selectivity and safety: the 5-phenol 12 and 5-O-sulfamate 14 derivatives, the alkyl-amide derivatives 30–33, and the inverted alkyl-sulfonamide derivatives 61 and 62. The 5-O-sulfamate derivative 14 was COX-2 selective but at higher concentration. The nitrated sulfonamide derivative 61 showed the highest COX-2 selectivity, but the nitrate tether did not increase plasma NO\textsubscript{x} concentration in vivo. With the assumption that the binding mode did not change because of these modifications, a straight-chain, polar-end tether might have some influence on COX binding and selectivity. Potential interactions between the straight-chain polar group and Tyr115, Ser119, or both in the lobby region would need to be confirmed through mutation studies. Nevertheless,
we have successfully designed and synthesized a NO-enhanced COX-2 selective inhibitor, example 32 (IC$_{50}$ = 1.2 µM with 5-fold selectivity), from indomethacin which is an effective antiinflammatory agent in vivo, while being exceptionally well tolerated without apparent GI liability by itself and, most notably, when coadministered with aspirin. On the basis of a prior detailed analysis of indomethacin phenethylamide metabolism in the rat, the amide linker of example 32 should be virtually immune to amidase hydrolysis such that negligible indomethacin would be generated. Consequently, our approach toward an NO-enhanced anti-inflammatory agent differs from the “pro-drug” approach commonly used in the synthesis of nitrated NSAID esters.

Experimental Section

Reagents and solvents were used as obtained from commercial suppliers. $^1$H and $^{13}$C NMR spectra were recorded on a 300 (Bruker AMX) or 400 MHz (Bruker AVANCE) spectrometer, and the chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane. Mass spectra were recorded on a PE SCIEX, API 150EX instrument using the turbo ion spray atmospheric pressure ionization method. Melting points were determined on a MEL-TEMP apparatus and are uncorrected. Elemental analyses were performed by Robertson Microlit Laboratories (Madison, NJ). Flash chromatography was performed using EMD silica gel 60H. Thin-layer chromatography was carried out on EMD silica gel 60 F$_{254}$ TLC plates and visualized under UV or by staining with phosphomolybdate or KMnO$_4$ solution.

Human Whole-Blood COX Inhibition Assay. Whole human blood from consenting donors of either sex who had not taken any NSAIDs for two weeks were collected in sodium heparin (20 units/mL). Test compounds at various concentrations, dissolved and diluted in DMSO at 1000 times the final concentrations, were added in duplicate to 1 mL per well aliquots of blood in a 24-well plate. After 15 min of incubation at 37 °C in a CO$_2$ incubator, LPS at 10 µg/mL was added to the appropriate wells to induce COX-2. At 4.5 h after test compound addition, A23187 was added at 25 µM to other wells to activate COX-1. Vehicle control wells received equal volumes of DMSO. At 30 min after A23187 activation, that is, 5 h after LPS addition, reactions were terminated by placing the 24-well plates on ice and adding 2 mM EGTA. Plasma was collected and extracted with methanol overnight at -20 °C. After evaporation, the thromboxane B$_2$ (TXB$_2$) in each sample was measured in duplicate with an enzyme-linked immunosassay kit (Cayman Chemical, Ann Arbor, MI). The results were normalized against vehicle (control) values and expressed as % control of COX activity, and an IC$_{50}$ was determined over the concentration range tested.

Rat Carrageenan Air Pouch Studies. Male Sprague–Dawley rats (180–200 g) were purchased from Charles River Laboratories (Kingston or Raleigh). Animal care and use were in accord with NitroMed’s IACUC guidelines. Rats were randomly housed 5 per cage and allowed to acclimate on a 12/12 reverse light/dark cycle with standard chow and water available ad libitum for at least 48 h before the experiment. Air pouches were produced by subcutaneous injection of 20 mL of sterile air on day (-6) into the intrascapular area on the back of the anesthetized rats. An additional 10 mL of sterile air was injected into the pouch on day (-3) to keep the pouch open and allow the interior membrane to develop. On day (-1), rats were fasted 18 h before the experiment (water ad libitum) in cages equipped with metal floor racks. On day 0, the pulverized test compounds in 0.5% methocel or vehicle were either injected into the pouch or dosed orally in blinded fashion, 1 h prior to carrageenan injection (1 mL of 1.0% carrageenan in saline). After 4 h, PBS/heparin (Invitrogen Corporation, Carlsbad, CA) was injected into the pouch, which was then massaged gently for 15–20 s. The rat was then quickly sacrificed using carbon dioxide, and the inflammatory exudate was collected from the pouch. The exudates were assayed for PGE$_2$ with an enzyme-linked immunoassay kit (Cayman).

NO$_3$ in Rat Plasma. Rat plasma was collected by heart puncture at the termination of the air-pouch (4 h after oral dosing) described above. Plasma NO$_3$ (the sum of inorganic nitrite and nitrate) was quantified with a fluorometric kit (Cayman).

Rat Gastric Injury Model. Rats were acclimated as described for the air-pouch model (above) and were fasted 18 h before the experiment (water ad libitum) in cages equipped with metal floor racks. Water was removed 1 h before the experiment and returned after dosing and removed again 1 h prior to examination of the stomach. The pulverized test compounds were homogenized with a glass/Teflon pestle homogenizer in 0.5% methocel and were prepared immediately before dosing. The test compounds were administered intragastrically (p.o.) at a dose volume of 1 mL/kg using an 18-gauge gavage needle. The rats were sacrificed 3 h after dosing using carbon dioxide. The stomach was removed, opened along the greater curvature, rinsed, mounted on a Petri dish, and digitally photographed. The images were analyzed by staff blinded to drug treatment using Image J Analysis software for visible hemorrhagic lesions. The length of each lesion was recorded, and all lesion lengths were summed as the total lesion score. Values are given as the means ± SEM for 4–12 animals. The significance of differences between means was evaluated using a one-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test. P < 0.05 was considered significant.

Aspirin-Induced Rat Gastric Damage Model. A minimum of 72 h was allowed for acclimation of rats, as described above. The experiment was performed in six groups with 3–11 animals per group. All test compounds including aspirin were pulverized with a mortar and pestle and homogenized by vortexing with 2–3 layers of glass beads in sufficient amount of vehicle. All compounds were prepared immediately before dosing and were administered intragastrically using gavage needles at a dose volume of 1.0 mL/kg. Aspirin was suspended in 1.0% methocel, whereas test compounds were suspended in 0.5% methocel. Four groups of animals were dosed intragastrically with aspirin (25 mg/kg); then the test compounds were dosed 2 min later. After 3 h, the rats were sacrificed and subjected to gastric lesion scoring as described above.

3-(Nitrooxy)propylamine Nitric Acid Salt (2). A solution of 3-amino-1-propanol (6.17 g, 82.2 mmol) was added, dropwise, to an ice-cooled solution of fuming nitric acid (90%, 12 mL in acetic anhydride (50 mL)). The reaction was stirred in an ice-bath for 10 min and then at room temperature for 10 min. The solvent was evaporated under vacuum at 40 °C. The residue was stirred in Et$_2$O (200 mL) until the product precipitated. The mixture was filtered, and the white crystalline solid was dried in vacuo to give the title compound (12.1 g, 80% yield). $^1$H NMR (DMSO-$d_6$, 300 MHz): δ 4.57 (br. t, 2 H), 2.8–3.0 (m, 2H), 1.98–1.93 (m, 2H). $^{13}$C NMR (DMSO-$d_6$, 75 MHz): δ 70.9, 36.1, 24.5. MS (API-TIS): m/z 121 (M – NO$_3$)$^+$. 

Figure 6. Aspirin-induced rat gastric lesions. Values are means ± SEM (n = 3–11). Statistical analysis by ANOVA, followed by Newman–Keuls multiple comparison test ((**p < 0.001 vs all other groups). V$_1$: vehicle (1.0% methylcellulose). V$_2$: vehicle (0.5% methylcellulose). asp: aspirin (139 µmol/kg). val: valdecoxib (9.5 µmol/kg).
2,2-Dimethyl-3-(nitrooxy)propylamine Nitric Acid Salt (4). A solution of 3-amino-2,2-dimethylpropionol (6.0 g, 82.2 mmol) in EtOAc (40 mL) was added, dropwise, to an ice-cooled solution of fuming HNO3 (90%, 8 mL) in acetic anhydride (50 mL). The reaction was stirred in an ice-bath for 10 min and an additional 10 min at room temperature. The solvent was evaporated under vacuum at 40 °C. The residue was dried in Et2O (200 mL) until the product precipitated. The mixture was filtered, and the white solid was dried in vacuo to give the title compound (35% yield). 1H NMR (300 MHz, CD3OD): δ 4.78 (br, 2H), 6.88 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.8 Hz, 1H), 6.84 (d, J = 2.1 Hz, 1H), 5.64 (dd, J = 8.9, 2.4 Hz, 1H), 4.47 (t, J = 6.4 Hz, 2H), 3.43 (s, 2H), 3.13 (br. q, 2H), 2.19 (s, 3H), 1.79 (m, 2H). 13C NMR (75 MHz, DMSO-d6): δ 169.8, 167.9, 153.4, 137.5, 134.9, 134.5, 131.4, 129.7, 129.7, 114.6, 114.0, 112.0, 103.7, 71.7, 35.3, 31.3, 26.5, 13.4. MS (API-TIS): m/z 446 (MH+)4.

1-[4-(Chlorophenyl)carbonyl]-2-methyl-3-(N-[3-(nitrooxy)-propyl]carbaamoyl) methylindol-5-yl Aminosulfonate (14). A solution of 3-amino-2,2-dimethylpropionol (0.19 g, 1.7 mmol) and 13 (0.19 g, 0.09 mmol) in N-methylpyrrolidinone (NMP, 3 mL) was stirred at room temperature for 4 h. The reaction was quenched with brine and dried over Na2SO4, filtered, and concentrated. The crude material was dissolved in CH2Cl2 (50 mL) and stirred with water (30 mL) overnight to remove trace amounts of NMP. The white solid suspended in the CH2Cl2 layer was collected and dried under vacuum (0.105 g, 48% yield). mp: 146–148 °C. 1H NMR (300 MHz, DMSO-d6): δ 8.11 (br. t, 1H), 7.90 (br. s, 2H), 7.71 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 2.1 Hz, 1H), 7.11 (d, J = 8.9 Hz, 1H), 7.01 (dd, J = 8.9, 2.1 Hz, 1H), 4.47 (t, J = 6.2 Hz, 2H), 3.52 (s, 2H), 3.13 (br. q, 2H), 2.22 (s, 3H), 1.79 (m, 2H). 13C NMR (75 MHz, DMSO-d6): δ 169.8, 167.9, 153.4, 137.5, 134.9, 134.5, 131.1, 129.6, 129.1, 114.6, 114.0, 112.0, 103.7, 71.7, 35.3, 31.3, 26.5, 13.4. MS(API-TIS): m/z 525 (MH+)5.

2-[1-[4-(Chlorophenyl)carbonyl]-5-hydroxy-2-methylindol-3-yl]-N-(3-hydroxypropyl)acetamide (15). A solution of 12 (0.84 g, 2.4 mmol), 3-amino-1-propanol (0.2 mL, 2.6 mmol), DMAP (35 mg, 0.29 mmol), EDAC (0.57 g, 2.97 mmol), and NEt3 (0.67 mL, 2.75 mmol) in CH2Cl2 (100 mL) was stirred at room temperature overnight. The reaction mixture was partitioned between 3 N HCl (30 mL) and CH2Cl2 (50 mL × 2). The combined organic extracts were washed with water and brine, dried over Na2SO4, filtered, and concentrated. The product was separated by silica gel column chromatography eluted with MeOH/CHCl3 (gradient from 1:2 to 1:5) to obtain the title compound (0.56 g, 57% yield). 1H NMR (300 MHz, 10% CD3OD/CDCl3): δ 7.65 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.5 Hz, 2H), 6.81–6.8 (m, 2H), 6.68 (br. t, 1H), 6.61 (dd, J = 8.9, 2.4 Hz, 1H), 3.57 (s, 2H), 3.54 (t, J = 5.8 Hz, 2H), 3.32 (q, J = 5.8 Hz, 2H), 2.36 (s, 3H), 1.61 (m, 2H). 13C NMR (75 MHz, 10% CD3OD/CDCl3): δ 171.8, 168.6, 153.1, 139.4, 136.0, 133.6, 130.1, 130.4, 129.1, 115.0, 112.5, 102.8, 59.5, 36.9, 31.9, 31.4, 13.0. MS(API-TIS): m/z 401 (MH+)6.

3-Chloro-4-methoxyphenylhydrazine Hydrochloride (16). A solution of NaNO2 (4.77 g, 69.1 mmol) in water (15 mL) was added to an ice-cold solution of 3-chloro-4-anisidine in 6 N HCl (100 mL) and stirred for 20 min. The resulting solution was washed with water and brine, dried over Na2SO4, filtered, and concentrated. The resulting solid was washed with methanol and DCM (50 mL) and stirred at room temperature overnight. The reaction mixture was cooled down to room temperature. The crude material was filtered, dissolved in MeOH (500 mL), and dried over Na2SO4, filtered, and concentrated. The resulting yellowish solid was washed with EtOAc and dried under vacuum to obtain the title compound (13.62 g, 99% yield). 1H NMR (300 MHz, DMSO-d6): δ 10.4 (br. H), 7.24 (d, J = 2.1 Hz, 1H), 7.2–7.0 (m, 2H), 3.8 (s, 3H). 13C NMR (75 MHz, DMSO-d6): δ 149.8, 139.6, 121.2, 117.4, 115.4, 113.5, 56.4.

Phenethyl 2-[4-(chloro-5-methoxy-2-methylindol-3-yl)-N-[3-(nitrooxy)propyl]acetamide (17) and Phenethyl 2-(6-chloro-5-methoxy-2-methylindol-3-yl)acetate (18). Benzyl levulinate (7.23 g, 35.1 mmol) and 16 (7.53 g, 36.0 mmol) was heated to reflux in acetic acid (100 mL) for 3 h. After the mixture was cooled down to room temperature, acetic acid was removed under vacuum. The residue was partitioned between water (100 mL) and EtOAc (100 mL × 2). The combined organic extracts were washed with saturated NaHCO3, 3 N HCl, water, and brine, dried over Na2SO4, filtered, and concentrated. The product was separated by silica gel column chromatography eluted with EtOAc/hexane (gradient from 1:3 to 1:2, Rf = 0.2, 0.1). The data for the more-polar compound 17 (1.96 g, 16% yield) follow: mp: 124–125 °C. 1H NMR (300 MHz, CDCl3): δ 7.93 (br, 1H), 7.4–7.3 (m, 5H), 6.98 (d, J = 8.7 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.8 Hz, 1H), 6.84 (d, J = 2.1 Hz, 1H), 5.64 (dd, J = 8.9, 2.4 Hz, 1H), 4.47 (t, J = 6.4 Hz, 2H), 3.43 (s, 2H), 3.13 (br. q, 2H), 2.19 (s, 3H), 1.79 (m, 2H). 13C NMR (75 MHz, DMSO-d6): δ 169.8, 167.9, 153.4, 137.5, 134.9, 134.5, 131.1, 129.6, 129.1, 114.6, 114.0, 112.0, 103.7, 71.7, 35.3, 31.3, 26.5, 13.4. MS (API-TIS): m/z 446 (MH+)4.
with 5% MeOH/EtO₂ and, dried under vacuum to obtain the title compound as a yellowish solid (0.61 g, 59% yield), mp: 154–155 °C. 

1H NMR (300 MHz, CDCl₃): δ 7.66 (d, J = 8.5 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H), 7.15 (s, 1H), 6.92 (s, 1H), 5.73 (br. t, 1H), 4.42 (t, J = 6.2 Hz, 2H), 3.92 (s, 3H), 3.64 (2H, 3H), 3.34 (br. q, 2H), 2.33 (s, 3H), 1.89 (m, 2H). 

13C NMR (75 MHz, CDCl₃): δ 171.8, 168.6, 168.0, 153.7, 135.7, 130.3, 128.4, 128.2, 128.0, 117.1, 111.7, 104.1, 100.8, 66.5, 56.5, 30.4, 11.6. MS (API-TIS): m/z 494 (MH⁺). 

Ethyl 2-(5-methoxy-2-methylindol-3-yl)acetate (23). A mixture of 4-methoxyphenylhydrazine hydrochloride (26 g, 0.149 mol), ethyl levulinate (21.5 g, 0.149 mol), and sodium acetate (12.2 g, 0.149 mol) in glacial acetic acid (200 mL) was heated at reflux for 3 h. The reaction mixture was concentrated to dryness. The residue was dissolved in ethanol (50 mL), treated with water, and neutralized with aqueous K₂CO₃ and brine. The organic phase was dried over Na₂SO₄, filtered, and concentrated under vacuum. The crude material was chromatographed on silica gel (70–230 mesh), eluted with CH₂Cl₂/Et₂O (1:1, 120 mL) and CH₂Cl₂/Et₂O (1:1, 120 mL) to give the title compound (28 g, 76% yield) as a viscous oil. 

1H NMR (300 MHz, CDCl₃): δ 7.88 (s, 2H), 7.51 to 7.49 (m, 4H), 6.78 (m, 2H), 3.84 (s, 3H), 3.64 (2H, 3H), 2.40 (s, 3H), 1.23 (t, J = 7.1 Hz, 3H). 

(MS (API-TIS): m/z 416 (MH⁺)). 

2-[(5-Methoxy-2-methyl-1,3-benzodioxolyl)(phenyl)methyl]-2-methylindol-3-yl)acetic acid (25a). To a stirred solution of 24a (2.01 g, 4.84 mmol) in THF (50 mL) was added 2 N NaOH (50 mL). The mixture was stirred at room temperature for 4 h and concentrated to remove the volatiles. After acidification to pH 2 with 2 N HCl, the mixture was extracted with EtOAc twice. The organic phase was dried over Na₂SO₄, filtered, and concentrated under vacuum. The solid was collected, washed with 5% MeOH/EtO₂ and, dried under vacuum to obtain the title compound as a yellowish solid (0.61 g, 59% yield), mp: 154–155 °C. 

1H NMR (300 MHz, CDCl₃): δ 7.66 (d, J = 8.5 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H), 7.15 (s, 1H), 6.92 (s, 1H), 5.73 (br. t, 1H), 4.42 (t, J = 6.2 Hz, 2H), 3.92 (s, 3H), 3.64 (2H, 3H), 3.34 (br. q, 2H), 2.33 (s, 3H), 1.89 (m, 2H). 

13C NMR (75 MHz, CDCl₃): δ 171.8, 168.6, 168.0, 153.7, 135.7, 130.3, 128.4, 128.2, 128.0, 117.1, 111.7, 104.1, 100.8, 66.5, 56.5, 30.4, 11.6. MS (API-TIS): m/z 494 (MH⁺). 

Anal. (C₂₆H₂₁Cl₂NO₄) C, H, N.

Ethyl 2-[(5-methoxy-2-methylindol-3-yl)acetate (23). To a stirred solution of 23 (5.40 g, 21.9 mmol) in THF (50 mL) was added t-BuOK (1.0 M in THF, 5.87 mL, 5.87 mmol) via syringe at room temperature under N₂. The mixture was heated at a gentle reflux for 16 h. After it was cooled, the mixture was poured onto ice, neutralized with 1 N HCl, and extracted with EtOAc twice. The organics were dried over Na₂SO₄, filtered, and concentrated. Chromatography of the residue (1:1 EtOAc/hexane, silica gel) afforded the title compound (28 g, 76% yield) as a viscous oil. 

1H NMR (300 MHz, CDCl₃): δ 7.83 (br. s, 1H), 7.06 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 6.77–6.73 (m, 1H), 4.12 (q, J = 7.1 Hz, 2H), 3.84 (s, 3H), 3.64 (m, 2H, 1H), 1.23 (t, J = 7.1 Hz, 3H). (MS (API-TIS): m/z 428 (MH⁺)).

2-(5-Methoxy-2-methyl-1,3-benzodioxolyl)(phenyl)methyl)-2-methylindol-3-yl)acetic acid (25a). To a stirred solution of 24a (2.01 g, 4.84 mmol) in THF (50 mL) was added 2 N NaOH (50 mL). The mixture was stirred at room temperature for 4 h and concentrated to remove the volatiles. After acidification to pH 2 with 2 N HCl, the mixture was extracted with EtOAc twice. The organic phase was dried over Na₂SO₄, filtered, and concentrated under vacuum. The solid was collected, washed with 5% MeOH/EtO₂ and, dried under vacuum to obtain the title compound as a yellowish solid (0.61 g, 59% yield), mp: 154–155 °C. 

1H NMR (300 MHz, CDCl₃): δ 7.66 (d, J = 8.5 Hz, 2H), 7.51 (d, J = 8.5 Hz, 2H), 7.15 (s, 1H), 6.92 (s, 1H), 5.73 (br. t, 1H), 4.42 (t, J = 6.2 Hz, 2H), 3.92 (s, 3H), 3.64 (2H, 3H), 3.34 (br. q, 2H), 2.33 (s, 3H), 1.89 (m, 2H). 

13C NMR (75 MHz, CDCl₃): δ 171.8, 168.6, 168.0, 153.7, 135.7, 130.3, 128.4, 128.2, 128.0, 117.1, 111.7, 104.1, 100.8, 66.5, 56.5, 30.4, 11.6. MS (API-TIS): m/z 494 (MH⁺).
was cooled, the mixture was poured onto ice, acidified to pH 2 with 2 N HCl, and extracted with EtOAc twice. The organics were dried over Na2SO4, filtered, and concentrated. Chromatography (2–10% EtOAc in hexane gradient, silica gel) afforded the title compound (3.88 g, 52% yield) as an oil.

1H NMR (300 MHz, CDCl3): δ 7.15 (d, J = 8.8 Hz, 1H), 7.04 (d, J = 2.4 Hz, 1H), 6.83–6.79 (m, 1H), 4.14 (q, J = 7.1 Hz, 2H), 3.88 (s, 3H), 3.85 (m, 2H), 3.69 (s, 2H), 2.40 (s, 3H), 1.73–1.67 (m, 6H), 1.26 (t, J = 7.1 Hz, 3H). (C8H15NO2) C, H, N. MS (API-TIS): m/z 347 (MH+) +.

2-[1-(Cyclohexylmethyl)-5-methoxy-2-methylindol-3-yl]acetamide (25b). To a stirred solution of 24b (740 mg, 2.16 mmol) in 1:1 THF:MeOH (20 mL) was added 2 N NaOH (5 mL). After the mixture was stirred at room temperature overnight, it was acidified to pH 1 with 1 N HCl and extracted with EtOAc twice. The organics were washed with brine, dried over Na2SO4, filtered, and concentrated to give a tan solid. Recrystallization from EtOAc (10 mL) afforded the title compound (560 mg, 82% yield) as a white solid.

1H NMR (300 MHz, CDCl3): δ 11.6–9.5 (br, 1H), 7.13 (d, J = 8.8 Hz, 1H), 6.97 (d, J = 2.3 Hz, 1H), 6.78 (dd, J = 6.4, 2.4 Hz, 1H), 3.84 (s, 3H), 3.82 (m, 2H), 3.69 (s, 2H), 2.36 (s, 3H), 1.7–1.5 (m, 6H), 1.2–0.9 (m, 9H). MS (API-TIS): m/z 316.2 (MH+) +.

2-[1-(Cyclohexylmethyl)-5-methoxy-2-methylindol-3-yl]-N-[3-(nitroxy)propyl]acetamide (26b). To a stirred solution of 24b (230 mg, 0.73 mmol), EDAC (280 mg, 1.46 mmol), and 1 (161 mg, 0.88 mmol) in acetonitrile (10 mL) was added AgNO3 (0.419 g, 2.46 mmol). The mixture was poured onto solid Na2SO4. The combined filtrate and EtOAc washings were concentrated before filtration. The filter cake was washed several times with acetonitrile (10 mL) and the precipitate was air-dried.

The organics were washed with brine, dried over Na2SO4, filtered, treated with brine, and concentrated. Chromatography (1.9 EtOAc/hexane, silica gel) afforded the title compound (1.23 g, 68% yield) as a white solid.

1H NMR (300 MHz, CDCl3): δ 7.17 (d, J = 8.8 Hz, 1H), 6.9–6.8 (m, 2H), 6.84 (s, 1H), 5.83 (br. t, 1H), 4.35 (t, J = 6.3 Hz, 2H), 3.85 (d, J = 7.4 Hz, 2H), 3.82 (s, 3H), 3.65 (m, 2H), 3.25 (m, 2H), 2.33 (m, 3H), 1.84 (t, J = 6.5 Hz, 2H), 1.8–1.5 (m, 6H), 1.2–1.0 (m, 6H). MS (API-TIS): m/z 418 (MH+) +.

2-[1-(Cyclohexylmethyl)-5-methoxy-2-methylindol-3-yl]ethan-1-ol (27b). To a stirred solution of 24b (0.520 g, 1.52 mmol) in THF (15 mL) was added 1 M LiAlH4 in THF (3 mL, 3 mmol), and the mixture was stirred at room temperature for 45 min. The mixture was poured onto solid Na2SO4·10H2O and aged for 10 min before filtration. The filter cake was washed several times with EtOAc. The combined filtrate and EtOAc washings were concentrated, and the residue was purified by chromatography (1:2 EtOAc/hexane, silica gel) to give the title compound (0.450 g, 98% yield) as an oil.

1H NMR (300 MHz, CDCl3): δ 7.34 (d, J = 8.8 Hz, 1H), 7.12 (d, J = 7.2 Hz, 1H), 6.52 (d, J = 2.3 Hz, 1H), 3.82 (s, 3H), 3.73 (s, 3H), 3.90–3.81 (m, 4H), 2.97 (t, J = 6.5 Hz, 2H), 2.05 (s, 3H), 1.8–0.99 (m, 12 H). 13C NMR (75 MHz, CDCl3): δ 154.6, 134.7, 131.7, 128.0, 111.0, 110.0, 106.2, 100.1, 62.7, 55.8, 49.7, 38.9, 31.1, 28.0, 26.2, 25.8, 10.5. MS (API-TIS): m/z 302 (MH+) +.

3-(2-Bromoethyl)-1-(cyclohexylmethyl)-5-methoxy-2-methylindol-28b). To a stirred solution of 27b (0.430 g, 1.43 mmol) in toluene (15 mL) was added 48% aqueous HBr (1.59 mL, 14.3 mmol), and the mixture was heated at reflux for 2 h. After it was cooled, the mixture was diluted with EtOAc, washed with saturated aqueous NaHCO3, and water. The organic layer was dried over Na2SO4, filtered, and concentrated. Chromatography (1:19 EtOAc/hexane, silica gel) of the residue afforded the title compound (0.45 g, 86% yield) as a foam.

1H NMR (300 MHz, CDCl3): δ 7.16 (d, J = 8.8 Hz, 1H), 6.95 (d, J = 2.4 Hz, 1H), 6.81 (dd, J = 8.8, 2.4 Hz, 1H), 3.87 (s, 3H), 3.86–3.83 (m, 2H), 3.52 (m, 2H), 2.36 (t, J = 8.3 Hz, 2H), 2.36 (s, 3H), 1.8–0.99 (m, 11H). MS (API-TIS): m/z 364, 366 (MH+) + for 18Br and 19Br, respectively.

2-[1-(Cyclohexylmethyl)-5-methoxy-2-methylindol-3-yl]nitrobenzene (28b). To a stirred solution of 28a (0.450 g, 1.23 mmol) in acetonitrile (10 mL) was added AgNO3 (0.419 g, 2.46 mmol). The mixture was stirred at 15 h, at which point the bromide had been completely consumed, as indicated by TLC. Upon concentration to dryness, the residue was taken up with EtOAc, treated with brine, and agitated for 10 min. The reaction mixture was filtered, and the organic layer was separated from the filtrate, dried over Na2SO4, filtered, and concentrated. Chromatography (1.9 EtOAc/hexane, silica gel) of the residue afforded the title compound (0.345 g, 81% yield) as a yellow oil.

1H NMR (300 MHz, CDCl3): δ 7.16 (d, J = 8.8 Hz, 1H), 6.97 (d, J = 2.0 Hz, 1H), 6.82 (dd, J = 8.8, 2.0 Hz, 1H), 4.58 (t, J = 7.5 Hz, 2H), 3.90 (s, 3H), 3.88–3.84 (m, 2H), 3.12 (t, J = 7.3 Hz, 2H), 2.36 (s, 3H), 1.74–1.57 (m, 6H), 1.01 (s, 10H). MS (API-TIS): m/z 347 (MH+) +. Anal. (C18H14NO2) C, H, N.
N-[2-(2-Dimethyl-3-(nitroxy)propyl]-2-[1-[4-(chlorophenyl)-carbonyl]-5-methoxy-2-methylindol-3-yl]acetamide (33). A solution of indomethacin (1.15 g, 3.2 mmol), 4 (0.70 g, 3.3 mmol), EDAC (0.74 g, 3.9 mmol), and NEt₃ (0.95 mL, 6.8 mmol) in CH₂Cl₂ (40 mL) was stirred at room temperature for 2 h. The reaction was partitioned between 3 N HCl (50 mL) and CH₂Cl₂ (100 mL). The organic layer was washed with 3 N HCl, water, and brine, dried over Na₂SO₄, filtered, and concentrated. The residue was triturated with Et₂O (60 mL). The solid was collected, washed with MeOH, and dried under vacuum to obtain the title compound as a white solid (0.57 g, 37% yield). mp: 153–156 °C. ¹H NMR (300 MHz, CDCl₃): dę 9.1 (br, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.46 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 2.3 Hz, 1H), 6.90 (d, J = 9.0 Hz, 1H), 6.69 (dd, J = 9.0, 2.3 Hz, 1H), 3.84 (s, 3H), 3.73 (s, 2H), 2.38 (s, 2H), 1.26 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): ował 168.4, 168.3, 156.2, 139.4, 136.4, 133.4, 131.1, 130.8, 129.1, 115.0, 112.3, 111.0, 100.9, 55.5, 29.4, 18.5. MS (API-TIS): m/z 415 (MH)⁺. Anal. (C₁₂H₁₀ClIN₂O₂) C, H, N.

(N-Acetyl-2-[1-[4-(chlorophenyl)carbonyl]-5-methoxy-2-methylindol-3-yl)acetamido) Acetate (37). A solution of 35 (0.42 g, 1.13 mmol) in a mixture of THF (10 mL), DMSO (2 mL), and acetic anhydride (5 mL) was stirred at room temperature overnight. The solvents were evaporated under vacuum. The residue was dissolved in EtOAc (100 mL), washed with saturated NaHCO₃, water, and brine, dried over Na₂SO₄, filtered, and concentrated. The residue was triturated with Et₂O, and the solid was collected and dried under vacuum to obtain the title compound (0.32 g, 61% yield). mp: 156–158 °C. ¹H NMR (300 MHz, CDCl₃): dę 7.67 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 6.67 (d, J = 9.0 Hz, 1H), 6.64 (d, J = 2.3 Hz, 1H), 6.66 (dd, J = 9.0, 2.3 Hz, 1H), 4.06 (br, s, 2H), 3.82 (s, 2H), 2.42 (s, 2H), 2.33 (s, 2H), 2.27 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): oğal 168.2, 167.9, 167.4, 155.1, 139.2, 136.4, 133.8, 131.2, 130.8, 130.5, 129.1, 115.0, 111.8, 111.2, 100.2, 55.7, 32.2, 24.3, 17.9, 13.4. MS (API-TIS): m/z 457 (MH)⁺. Anal. (C₁₉H₁₅ClIN₂O₂) C, H, N.
The combined organic extracts were washed with 3 N HCl, water, and brine, dried over Na2SO4, filtered, and concentrated. The resulting crude material was dissolved in ethyl acetate and triturated with hexane. The solid was collected and dried under vacuum to obtain the title compound as a yellowish solid (2.40 g, 81% yield). mp: 112–114 °C. 1H NMR (300 MHz, CDCl3): δ 9.15 (br. s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.47 (d, J = 8.5 Hz, 2H), 6.92 (d, J = 2.4 Hz, 1H), 6.84 (d, J = 9.0 Hz, 1H), 6.67 (dd, J = 9.0, 2.4 Hz, 1H), 3.99 (t, J = 5.7 Hz, 2H), 3.81 (s, 3H), 3.74 (t, J = 5.7 Hz, 2H), 3.59 (s, 2H), 2.37 (s, 3H), 1.80–1.73 (m, 2H). 13C NMR (75 MHz, CDCl3): δ 168.3, 168.2, 153.0, 139.3, 133.3, 134.4, 131.1, 130.7, 130.2, 129.0, 114.9, 111.7, 101.1, 74.5, 59.4, 55.6, 30.4, 29.2, 13.2, MS (API-TIS): m/z 431 (MH+)1.

2-(2-Hydroxyethoxy)benzof[1]azolidine-3,1-dione (41). A solution of sodium acetate (33.21 g, 0.4 mol), N,N-dihydroxyphthalimide (22.0 g, 0.135 mol), and 2-bromothanol (50.6 g, 0.4 mol) in DMSO (400 mL) was heated to 70 °C for 6 h. After it was cooled down to room temperature, water (400 mL) was added to the reaction mixture, and it was extracted with CH2Cl2 (250 mL × 3). The combined organic extracts were washed with water (250 mL × 2), 3 N HCl (150 mL), and brine, dried over Na2SO4, filtered, and concentrated. The resulting crude material was washed with 50% ethanol in water (120 mL), filtered, and dried under vacuum to obtain the title compound as a white solid (70% yield) from 3-bromoprop-1-ol by following the procedure for 41. mp: 78–80 °C. 1H NMR (300 MHz, CDCl3): δ 7.9–7.75 (m, 4H), 4.39 (t, J = 6.0 Hz, 2H), 3.93 (t, J = 6.0 Hz, 2H), 3.04 (br. s, 1H), 2.02 (m, 2H). 13C NMR (75 MHz, CDCl3): δ 165.2, 135.8, 130.2, 124.3, 80.5, 60.8. MS (API-TIS): m/z 222 (MH+)1.

2-(2-Hydroxypropoxy)benzo[c]azolidine-3,1-dione (42). The title compound was prepared as a white solid (70% yield) from 3-bromoprop-1-ol by following the procedure for 41. mp: 78–80 °C. 1H NMR (300 MHz, CDCl3): δ 7.9–7.75 (m, 4H), 4.39 (t, J = 6.0 Hz, 2H), 3.93 (t, J = 6.0 Hz, 2H), 3.04 (br. s, 1H), 2.02 (m, 2H). 13C NMR (75 MHz, CDCl3): δ 163.7, 134.5, 128.6, 123.5, 75.8, 58.8, 30.7. MS (API-TIS): m/z 222 (MH+)1.

2-(Aminoxy)ethan-1-ol (43). A solution of 41 (15.57 g, 75.2 mmol) and hydrazine hydrate (5.4 mL, 0.11 mol) in MeOH (150 mL) was heated to 70 °C for 1.5 h. After the mixture was cooled down to room temperature, CHCl3 (100 mL) was added to the reaction mixture. The resulting slurry was filtered and washed with CHCl3 (100 mL × 2). The filtrate was concentrated, and the residue was distilled under vacuum (0.025 mmHg) at 75–80 °C to obtain a colorless oil (4.54 g, 78% yield). 1H NMR (300 MHz, CDCl3): δ 3.78 (s, 4H). 13C NMR (75 MHz, CDCl3): δ 76.3, 60.7. 2-[2-(2-Hydroxyethoxy)propoxy]ethan-1-ol (44). The title compound was prepared as a colorless oil (87% yield), by following the procedure for 43, and was distilled under vacuum (0.05 mmHg) at 90–95 °C. 1H NMR (300 MHz, CDCl3): δ 6.0–5.0 (br. 3H), 3.79 (t, J = 6.1 Hz, 2H), 3.67 (t, J = 6.1 Hz, 2H), 1.81 (m, 2H). 13C NMR (75 MHz, CDCl3): δ 73.0, 59.2, 31.2.

4-Chlorophenyl 3-(2-hydroxyethyl)-5-methoxy-2-methylindol-3-yethyl dimethoxyphosphino-1-one (49). A solution of BH3·SMe2 in THF (2 M, 15 mL) was heated to 70 °C in acetone (100 mL) for 24 h. After the mixture was cooled to room temperature, the acetone was evaporated under vacuum. The residue was dissolved in CH2Cl2 (100 mL), washed with water and brine, dried over Na2SO4, filtered, and concentrated. The product was separated by silica gel column chromatography eluted with EtOAc/hexane (1:10, Rf = 0.25) to obtain the title compound (0.96 g, 79% yield). The product can be recrystallized from EtO and MeOH, mp: 92–93 °C. 1H NMR (300 MHz, CDCl3): δ 7.63 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 6.90 (d, J = 2.5 Hz, 1H), 6.90 (d, J = 8.9 Hz, 1H), 6.66 (d, J = 8.9, 2.5 Hz, 1H), 3.82 (s, 3H), 3.56 (t, J = 7.6 Hz, 2H), 3.21 (t, J = 7.6 Hz, 2H), 2.34 (s, 3H). 13C NMR (75 MHz, CDCl3): δ 168.1, 155.9, 139.1, 135.4, 133.9, 131.0, 130.9, 129.0, 116.6, 111.9, 111.3, 109.0, 55.7, 31.4, 27.6. MS (API-TIS): m/z 406 (MH+)1.

2-[1-(4-Chlorophenyl)carbonyl]-5-methoxy-2-methylindol-3-yl]ethyl Methylsulfonate (46). Triethyamine (0.22 mL, 1.6 mmol) and methanesulfonyl chloride (0.15 mL, 1.9 mmol) were added to a solution of 45 (0.44 g, 1.3 mmol) in CH2Cl2 (15 mL), and the mixture was stirred at room temperature for 2 h. The reaction was partitioned between 3 N HCl (10 mL) and CH2Cl2 (30 mL). The organic extract was washed with water and brine, dried over Na2SO4, filtered, concentrated, and dried under vacuum. The product, >95% purity from NMR analysis, was used in the next step without purification. 1H NMR (300 MHz, CDCl3): δ 7.64 (d, J = 8.5 Hz, 2H), 7.47 (d, J = 8.5 Hz, 2H), 6.95 (d, J = 2.4 Hz, 1H), 6.89 (d, J = 9.0 Hz, 1H), 6.67 (dd, J = 9.0, 2.4 Hz, 1H), 4.39 (t, J = 7.0 Hz, 2H), 3.83 (s, 3H), 3.13 (t, J = 7.0 Hz, 2H), 2.92 (s, 3H), 2.35 (s, 3H). 13C NMR (75 MHz, CDCl3): δ 168.1, 155.9, 139.1, 135.6, 133.7, 131.0, 130.7, 130.3, 129.0, 114.9, 113.7, 111.4, 100.8, 68.5, 55.6, 37.2, 24.4, 13.1. MS (API-TIS): m/z 422 (MH+)1.

4-Chlorophenyl 5-methoxy-2-methyl-3-[2-(nitr oxy)ethyl]indol-3 yethyl Ketone (47). The crude product of 46 and tetrabutylammonium nitrate (0.47 g, 1.54 mmol) was heated to 100 °C overnight in toluene (15 mL). After it was cooled to room temperature, the reaction mixture was dissolved in EtOAc (100 mL), washed with water and brine, dried over Na2SO4, filtered, and concentrated. The product was separated by silica gel column chromatography eluted with EtOAc/hexane (1:10, Rf = 0.25) to obtain the title compound (0.96 g, 79% yield).
triturated with EtO (100 mL). The off-white solid was collected, washed with EtOAc, and then dried under vacuum (0.51 g, 57% yield). mp: 165–167 °C. \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.62 (d, \(J = 8.4\) Hz, 2H), 7.52 (d, \(J = 8.4\) Hz, 2H), 7.01 (d, \(J = 2.1\) Hz, 1H), 6.92 (d, \(J = 9.0\) Hz, 1H), 6.64 (dd, \(J = 9.0, 2.1\) Hz, 1H), 3.81 (s, 3H), 2.95 (m, 2H), 2.27 (s, 3H), 1.99 (m, 2H). \(^{13}C\) NMR (75 MHz, CDCl\(_3\)): \(\delta\) 169.9, 157.7, 140.0, 135.9, 134.9, 132.4, 132.2, 131.8, 130.2, 120.5 (d, \(J_{CP} = 18\) Hz), 116.1, 112.5, 102.1, 55.1, 28.4, 21.6, 13.0, 7.4. MS (APPI-TIS): \(m/z\) 408 (M+H\(^{+}\))

(2-[1-(4-Chlorophenyl]carbonyl]-5-methoxy-2-methylindol-3-yl)ethoxy-
N-[3-(nitroxy)propyl]aminolufonio)sulfuric acid (51). Chlorosulfonyl isocyanate (115 µL, 1.3 mmol) and NEt\(_3\) (250 µL, 1.8 mmol) were added to an ice-cold solution of 45 (0.40 g, 1.2 mmol) in CHCl\(_3\) (50 mL) and stirred in the ice-bath for 30 min. Compound 2 (0.26 g, 1.4 mmol) and NEt\(_3\) (250 µL, 1.8 mmol) were added to the reaction, and the temperature allowed to warm slowly in the ice-bath to room temperature. After 3 h, the reaction mixture was diluted with CH\(_2\)Cl\(_2\) (80 mL), washed with 3 N HCl, water, and brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated.

The product was recrystallized from CHCl\(_3\)hexane (1:1, \(R_f = 0.32\)) to obtain the title compound (0.14 g, 17% yield). mp: 44–46 °C. \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.64 (d, \(J = 8.5\) Hz, 2H), 7.43 (d, \(J = 8.5\) Hz, 2H), 6.91 (d, \(J = 2.5\) Hz, 1H), 6.89 (d, \(J = 9.0\) Hz, 1H), 6.67 (dd, \(J = 9.0, 2.5\) Hz, 1H), 3.83 (s, 3H), 3.49 (t, \(J = 7.0\) Hz, 2H), 2.94 (t, \(J = 7.0\) Hz, 2H), 2.37 (s, 3H). \(^{13}C\) NMR (75 MHz, CDCl\(_3\)): \(\delta\) 168.1, 155.9, 139.1, 135.4, 133.9, 131.1, 130.9, 129.0, 115.5, 115.0, 111.2, 101.0, 55.7, 50.7, 24.1, 13.1.

(3-2-Azidoethyl]-5-methoxy-2-methylindol-4-yl)chlorophenyl
Ketone (54). To the crude product 46 (1.51 g, 4.4 mmol), was added Na\(_2\)CO\(_3\) (0.58 g, 8.9 mmol), and the mixture was heated to 70 °C in DMSO (50 mL) for 2 h. After the mixture was cooled to room temperature, the DMSO was evaporated under vacuum. The residue was dissolved in CH\(_2\)Cl\(_2\) (150 mL), washed with water and brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated under vacuum to obtain the title compound. The product, >95% purity from NMR analysis, was used in the next step without purification. An analytical sample was obtained by silica gel column chromatography eluted with EtOAc/hexane (1:1, \(R_f = 0.45\)). \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.64 (d, \(J = 8.5\) Hz, 2H), 7.45 (d, \(J = 8.5\) Hz, 2H), 6.91 (d, \(J = 2.5\) Hz, 1H), 6.89 (d, \(J = 9.0\) Hz, 1H), 6.67 (dd, \(J = 9.0, 2.5\) Hz, 1H), 3.83 (s, 3H), 3.49 (t, \(J = 7.0\) Hz, 2H), 2.94 (t, \(J = 7.0\) Hz, 2H), 2.37 (s, 3H). \(^{13}C\) NMR (75 MHz, CDCl\(_3\)): \(\delta\) 168.2, 155.9, 139.1, 135.4, 133.9, 131.1, 130.9, 129.0, 115.5, 115.0, 111.2, 101.0, 55.7, 50.7, 24.1, 13.1.

N-2-([4-Chlorophenyl]carbonyl]-5-methoxy-2-methylindol-3-yl)ethyl]
N-[4-(nitroxy)methyl]phenyl)carboxamide (56). A mixture of 55 (1.01 g, 2.7 mmol), 10 (0.59 g, 3.0 mmol), DMAP (0.4 g, 3.3 mmol), EDAC (0.67 g, 3.5 mmol), and NEt\(_3\) (1.6 mL, 11.5 mmol) in CH\(_2\)Cl\(_2\) (30 mL) and THF (20 mL) was stirred at room temperature for 2 days. The reaction mixture was partitioned between 3 N HCl (30 mL) and EtOAc (50 mL × 2). The combined organic extracts were washed with water and brine, dried over Na\(_2\)SO\(_4\), filtered, and concentrated. The product was separated by silica gel column chromatography eluted with EtOAc/hexane (2:3, \(R_f = 0.25\)) to obtain the title compound (0.07 g, 28% yield).

The product can be recrystallized from CHCl\(_3\)/hexane. mp: 51–52 °C. \(^{1}H\) NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.61 (d, \(J = 8.5\) Hz, 2H), 7.43 (d, \(J = 8.5\) Hz, 2H), 6.96 (d, \(J = 2.1\) Hz, 1H), 6.87 (d, \(J = 9.0\) Hz, 1H), 6.64 (dd, \(J = 9.0, 2.1\) Hz, 1H), 5.15 (br, 1H, 4.44 (t, \(J = 5.9\) Hz, 2H), 4.23 (t, \(J = 6.7\) Hz, 2H), 3.81 (s, 3H), 3.32 (br, q, 2H), 2.96 (t, \(J = 6.7\) Hz, 2H), 2.32 (s, 3H), 1.88 (m, 2H). \(^{13}C\) NMR (75 MHz, CDCl\(_3\)): \(\delta\) 168.1, 156.4, 155.8, 138.9, 135.1, 133.9, 130.9, 130.7, 128.9, 115.3, 114.8, 111.0, 101.2, 70.6, 55.5, 37.3, 27.2, 23.9, 13.0. MS (APPI-TIS): \(m/z\) 490 (M+H\(^{+}\))

N,N'-Carbonyldimidazole (79 mg, 0.49 mmol) and 55 (0.16 g, 0.46 mmol) in CH\(_2\)Cl\(_2\) (10 mL) were stirred at room temperature for 45 min. Compound 2 (85 mg, 0.46 mmol) and NEt\(_3\) (0.07 mL, 0.5 mmol) in THF (5 mL) was added to the resulting solution, and
the mixture was stirred for an additional 5 h. The reaction mixture was partitioned between 3 N HCl (30 mL) and CH₂Cl₂ (50 mL). The organic extract was washed with water and brine, dried over Na₂SO₄, filtered, and concentrated. The product was separated by silica gel column chromatography eluted with EtOAc/hexane (gradient from 2:1 to 4:1, Rf = 0.33 in 2:1) to obtain the title compound (0.081 g, 40% yield). The product can be further purified by silica gel column chromatography eluted with EtOAc/hexane (2:1, Rf = 0.25) to obtain the title compound (0.83 g, 53% yield). mp: 58–60 °C. Anal. (C₂₂H₂₄ClN₃O₇S) C, H, N.

References

Supporting Information Available: COX inhibition screening data of all compounds and combustion analyses results. This material is available free of charge via the Internet at http://pubs.acs.org.
selective and nonselective inhibitors of cyclooxygenase-2 (COX-2) inhibiting NO donors.


