NO-NSAIDs. Part 3: Nitric Oxide-Releasing Prodrugs of Non-steroidal Anti-inflammatory Drugs

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In continuation of our efforts to discover novel nitric oxide-releasing non-steroidal anti-inflammatory drugs (NO-NSAIDs) as potentially "Safe NSAIDs," we report herein the design, synthesis and evaluation of 21 new NO-NSAIDs of commonly used NSAIDs such as aspirin, diclofenac, naproxen, flurbiprofen, ketoprofen, sulindac, ibuprofen and indomethacin. These prodrugs have NO-releasing disulfide linker attached to a parent NSAID *via* linkages such as an ester (compounds 9–16), a double ester (compounds 17–24), an imide (compounds 25–30) or an amide (compounds 31–33). Among these NO-NSAIDs, the ester-containing NO-aspirin (9), NO-diclofenac (10), NO-naproxen (11), and the imide-containing NO-aspirin (25), NO-flurbiprofen (27) and NO-ketoprofen (28) have shown promising oral absorption, anti-inflammatory activity and NO-releasing property, and also protected rats from NSAID-induced gastric damage. NO-aspirin compound 25, on further co-evaluation with aspirin at equimolar doses, exhibited comparable dose-dependent pharmacokinetics, inhibition of gastric mucosal prostaglandin E_2 (PGE_2) synthesis and analgesic properties to those of aspirin, but retained its gastric-sparing properties even after doubling its oral dose. These promising NO-NSAIDs could therefore represent a new class of potentially "Safe NSAIDs" for the treatment of arthritic pain and inflammation.

Key words nitric oxide-aspirin; nitric oxide-diclofenac; nitric oxide-naproxen; nitric oxide-flurbiprofen; nitric oxide-ketoprofen

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of osteoarthritis, rheumatoid arthritis and musculoskeletal pain.[1,2] However, chronic use of NSAIDs has been associated with an increased risk of gastrointestinal (GI) complications.[3,4] Lately, a new class of nitric oxide (NO)-releasable prodrugs of NSAIDs (NO-NSAIDs) has been developed with an objective to overcome the risks associated with NSAIDs.[5] Naproxcinod (Fig. 1) is one such compound at late stage development.[6]

In this perspective, during the past six years, we have been engaged in the design of novel NO-NSAIDs as potentially gastric-sparing "Safe NSAIDs" by the application of our disulfide linker and prodrug technology.[7] In a recent publication of preliminary results from this study, we reported NO-aspirin and NO-diclofenac as potentially "safe" prodrugs of aspirin (**1**) and diclofenac (**2**), respectively.[8] Among the reported NO-diclofenac prodrugs (Fig. 2), the mono ester prodrug **10** had shown excellent oral bioavailability and anti-inflammatory activity comparable to those of diclofenac (**2**) in rat models. However, in case of NO-aspirin prodrugs (Fig. 2), the bioavailability and anti-inflammatory activity of the mono ester compound **9**, although better than those of double ester compound **17**, were not comparable to those of aspirin (**1**). We had also witnessed zero bioavailability for the amide-containing aspirin compound **31**. The promising compounds **9** and **10** had also shown NO-releasing properties and protected rats from NSAID-induced gastric damage which could be attributable to the beneficial effects of nitric oxide released from these prodrugs.

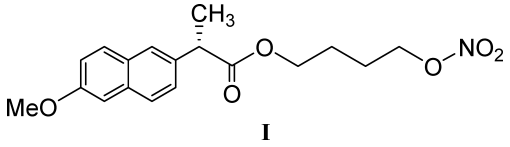

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Fig. 1. Structure of Naproxcinod (**I**)

We have further evaluated the promising NO-diclofenac prodrug **10** for dose-dependent pharmacokinetics and anti-inflammatory activity in three different models of inflammation (*i.e.*, acute, chronic and systemic), and published those results elsewhere.[9] In those studies, the NO-diclofenac prodrug **10**, rather uniquely, inhibited the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α (38.1% inhibition), interleukin (IL)-1β (42.8% inhibition) and IL-6 (59.3% inhibition). In contrast, diclofenac (**2**) did not inhibit the lipopolysaccharide (LPS)-induced production of IL-1β and IL-6 and also, rather surprisingly, it increased the levels of TNF-α (79.6% enhancement). The interaction between NO and cytokines is still ambiguous within the complex interplay among inflammatory mediators.[10] Depending on the type and phase of inflammatory condition, NO can exert both

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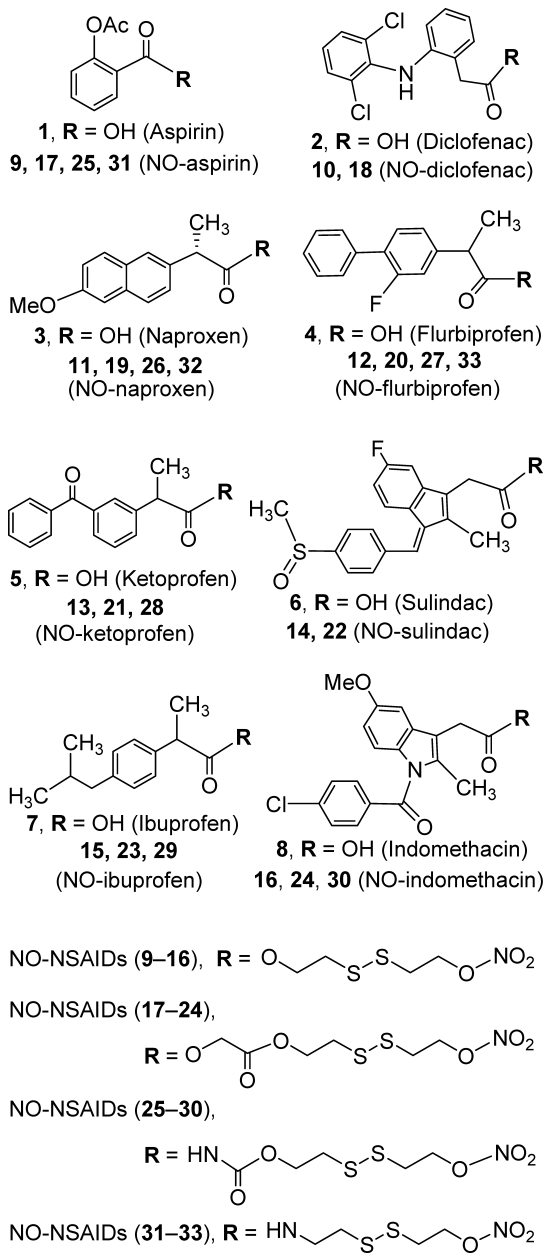


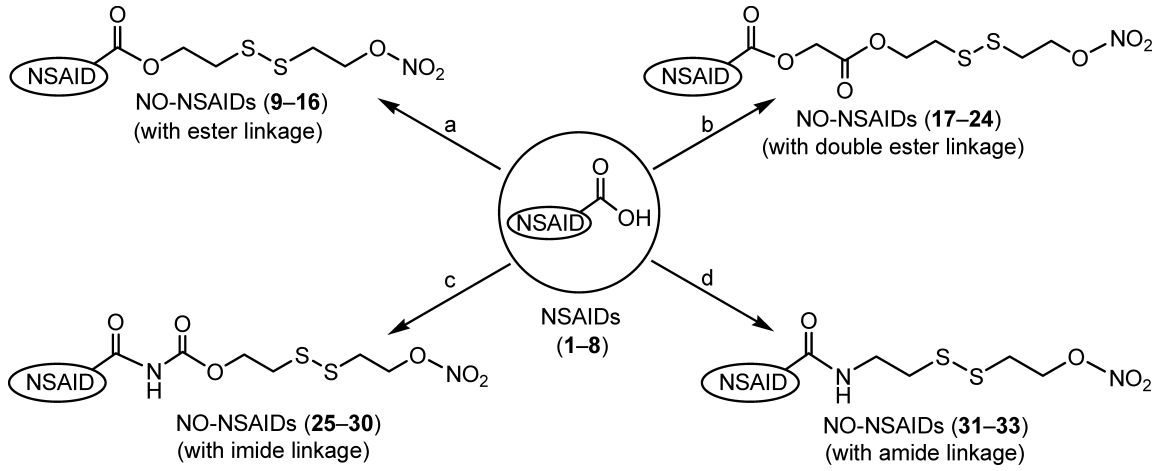
Fig. 2. Structures of NSAIDs (**1**–**8**) and Synthesized NO-NSAIDs (**9**–**33**)

pro-inflammatory[10–13] and anti-inflammatory effects.[14–17] The small amount of NO generated from constitutive nitric oxide synthase (cNOS), which includes endothelial (eNOS) and neuronal (nNOS), is important for regulation of physiological homeostasis, whereas the large amount of NO produced from inducible nitric oxide synthase (iNOS), which can remain active for several hours or even days after expression, has been implicated in pathophysiology of several diseases and chronic inflammatory conditions.[18] However, NO-releasing derivatives of cyclooxygenase inhibitors are generally known to exhibit enhanced anti-inflammatory activity and the improved efficacy of these derivatives are most likely caused by its ability to suppress leukocyte infiltration and scavenge peroxynitrite.[19] We therefore believe that the NO released from NO-diclofenac prodrug **10** could be responsible for the inhibition of pro-inflammatory cytokines.

Encouraged by the successful identification of a potentially "safe NO-diclofenac" prodrug **10**, we have further extended the application of the "SS-nitrate" strategy to explore the possibility of discovering potentially "safe NO-NSAID" prodrugs of other widely used NSAIDs such as naproxen (**3**), flurbiprofen (**4**), ketoprofen (**5**), sulindac (**6**), ibuprofen (**7**) and indomethacin (**8**) (Fig. 2) and the results from those studies are reported in this article.

NO-NSAID Design The most important design feature in our NO-NSAIDs is the presence of a disulfide linkage at *β*-position to the nitrate ester group. Organic nitrates containing a sulfur atom at *β*-position to a nitrate group were originally termed as "SS-nitrates,"[20] and they were designed to prove the hypothesized sulfhydryl-dependent mechanism of NO release from organic nitrates such as glyceryl trinitrate (GTN).[21,22] Such 'SS-nitrates' exhibited good stability in neutral aqueous solution but released significant amounts of NO in aqueous medium at biologically relevant pH on addition of sulfhydryl-containing compounds.[20] By partly adopting this "SS-nitrate" feature into our disulfide-linker containing prodrug strategy,[7] we have designed, synthesized and evaluated NO-NSAID prodrugs that contain a NO-releasing nitrate moiety at *β*-position to the disulfide group, which is, in turn, covalently bonded through "CH_2CH_2" chain to the carboxyl group of parent NSAIDs *via* linkages such as an ester (compounds **9**–**16**), a double ester (compounds **17**–**24**), an imide (compounds **25**–**30**) or an amide (compounds **31**–**33**) as shown in Fig. 2. As proposed earlier, the disulfide group plays a prominent role in the mechanism of release of NO from these compounds.[8,23]

NO-NSAID Synthesis General methods used for the synthesis of NO-NSAIDs **9**–**33** are outlined in Chart 1. In general, the desired NO-NSAIDs were synthesized from the parent NSAIDs by activation of their carboxylic acid function followed by appendage of appropriate linker intermediates **34**–**38a** and **38b** (Fig. 3) in the presence of a suitable base and a suitable solvent. Thus, the ester-linked NO-NSAIDs **9**–**16** were synthesized by employing one of the four standard methods of carboxylic acid activation followed by their reaction with linker intermediate **36** (**35** in the case of NO-diclofenac **10**) as depicted in Chart 2. The nitro linker intermediate **36** was prepared from the mono-bromide intermediate **34** as reported earlier.[8] NO-aspirin **9** and NO-ibuprofen **15** were synthesized in 45−54% yields by first converting the parent drugs aspirin (**1**) and ibuprofen (**7**) into their respective acid chlorides using oxalyl chloride and *N*,*N*-dimethylformamide (DMF) method, followed by their reaction with intermediate **36** in the presence of triethylamine. In case of naproxen (**3**), flurbiprofen (**4**), ketoprofen (**5**) and indomethacin (**8**), they were first converted to their respective imidazolide species using *N*,*N*′-carbonyldiimidazole (CDI), followed by their *in situ* reaction with intermediate **36** to afford 28–67% of the corresponding NO-NSAIDs **11**–**13** and **16**. NO-sulindac **14** was prepared in 53% yield by coupling sulindac (**6**) directly with intermediate **36** in presence of *N*,*N*′-dicyclohexyl carbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP). Previously, we synthesized NO-diclofenac **10** by reacting diclofenac (**2**) with intermediate **36** in presence of DCC and DMAP. However, we noticed formation of significant amounts (*ca.* 30%) of diclofenac lactam **39** (Fig. 3). This lactam formation was more pronounced when we attempted to synthesize



Reagents and conditions: a) see Chart 2 for details; b) 1. Cs_2CO_3, MeOH/THF or K_2CO_3, DMF, 2. **37**, THF/DMF, 15−80%; c) see Chart 3 for details; d) 1. **38a**, TFA/CH_2Cl_2 (1 : 1), 30 min; 2. oxalyl chloride, CH_2Cl_2 or *N*,*N*′-carbonyldiimidazole, THF; 3. **38b**, Et_3N, CH_2Cl_2, 17–34%.

Chart 1. Synthesis of NO-NSAIDs (**9**–**33**)

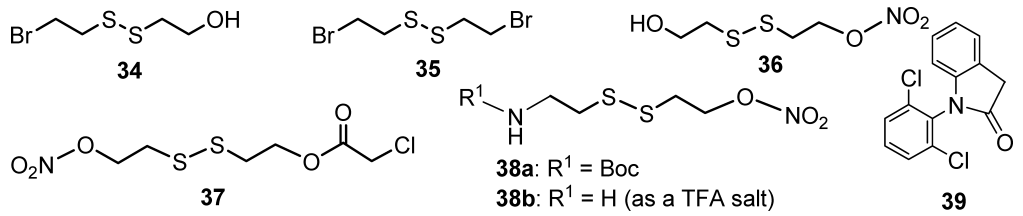
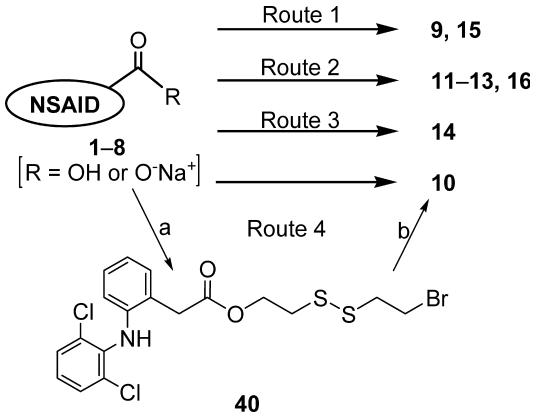
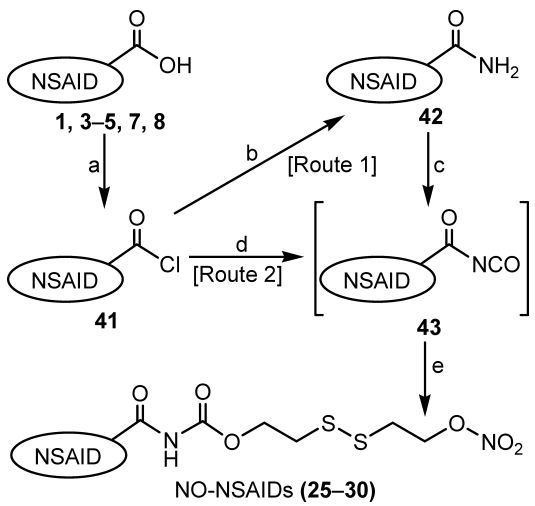


Fig. 3. Structures of Linker Intermediates (**34**–**38a**, **38b** and **39**)



Reagents and conditions: Route 1: a) aspirin (**1**) or ibuprofen (**7**), oxalyl chloride, DMF (cat. amt.), benzene; b) **36**, Et_3N, CH_2Cl_2, 45–54%; Route 2: naproxen (**3**) or flurbiprofen (**4**) or ketoprofen (**5**) or indomethacin (**8**), *N*,*N*′-carbonyldiimidazole, CH_2Cl_2 or THF or $CHCl_3$, followed by **36**, 28–67%; Route 3: sulindac (**6**), **36**, DCC, DMAP, CH_2Cl_2, 53%; Route 4: a) diclofenac sodium (**2a**), **35**, DMF, 50%; b) $AgNO_3$, acetonitrile, 49%.

Chart 2. Synthesis of NO-NSAIDs (**9**–**16**) with Ester Linkage



Reagents and conditions: (a) oxalyl chloride, DMF (cat. amt.)/thionyl chloride, benzene; (b) ammonia gas, CH_2Cl_2, 0°C, 0.5 h, 90–100%; (c) oxalyl chloride, CH_2Cl_2, reflux, 16 h[24]; (d) silver cyanate, benzene, reflux, 0.5 h[25]; (e) **36**, benzene, 16 h, 10–36%.

Chart 3. Synthesis of NO-NSAIDs (**25**–**30**) with Imide Linkage

NO-diclofenac **10** by the reaction of intermediate **36** with diclofenac acid chloride in presence of a base such as triethylamine. We have therefore used an alternative two step procedure for the synthesis of NO-diclofenac **10** as shown in route 4 of Chart 2. Thus, diclofenac was first converted to its sodium salt, which was then coupled with the dibromo linker intermediate **35** to give the bromide intermediate **40** in 50% yield, which was further treated with silver nitrate to afford the target compound **10** in 49% yield.

The double ester linkage-containing NO-NSAIDs **17**–**24** were prepared in 15–80% yields by first converting the parent NSAIDs into their respective metal carboxylate salts and then treating those salts with linker intermediate **37** (Chart 1, Fig. 3). The intermediate **37** was prepared from intermediate **36** as reported earlier.[8] Thus, NSAIDs **1**–**7** were converted to their respective cesium carboxylate salts by treatment with cesium carbonate, which on reaction with linker intermediate **37** yielded NO-NSAIDs **17**–**23**. The NO-indomethacin prodrug

24 could not be made by the above method. However, it was made (15% yield) by the reaction of potassium carboxylate salt of indomethacin with intermediate **37**. Synthesis of imide linkage-containing NO-NSAIDs **25**–**30** was achieved by first converting the NSAIDs into their respective acid chlorides **41** by oxalyl chloride and DMF method (Chart 3). Bubbling of ammonia gas into the solution containing acid chlorides **41** at 0°C afforded the corresponding NSAID-amides **42** (Chart 3) in 90–100% yields. The said amides **42** were then refluxed with oxalyl chloride in dichloromethane to yield their respective acyl isocyanates **43**. Addition of linker intermediate **36** to these highly reactive acyl isocyanates **43** afforded NO-NSAIDs **26**–**30** (*via* Route 1)[24] in 10–36% yields, but the NO-aspirin **25** could not be made *via* Route 1. However, the NO-aspirin **25** could be made *via* Route 2[25] in 35% yield by the reaction of aspirin acid chloride with silver cyanate to yield the desired aspirin acyl isocyanate intermediate **43** followed by its reaction with linker intermediate **36**.

NO-NSAIDs **31**–**33** containing amide linkages were prepared in three steps as shown in Chart 1 by converting the parent NSAID into either its acid chloride or imidazolide, followed by reaction with linker intermediate **38b** in presence of triethylamine. Thus, in the first step, linker intermediate **38b** was prepared by the deprotection of the Boc group of linker intermediate **38a**[8] using trifluoroacetic acid. In the second step, the NSAIDs were converted to their respective acid chlorides by treatment with thionyl chloride or oxalyl chloride/DMF (or imidazolide by the reaction with CDI). In the last step, reaction of the amine intermediate (trifluoroacetic acid (TFA) salt) **38b** with the respective NSAID acid chlorides in the presence of a suitable base and solvent yielded the amide-containing NO-NSAIDs **31**–**33** in 17–34% yields.

Results and Discussion

Biological Evaluation The NO-NSAIDs **9**–**33** were evaluated *in vivo* to establish their pharmacokinetics and/or anti-inflammatory activity using rat as animal model. Compounds with more promising bioavailability and/or anti-inflammatory activity were selected and evaluated further for their NO-releasing capabilities as well as their propensity for sparing/inducing gastric lesions *vis-à-vis* their parent NSAIDs.

The bioavailability (area under curve (*AUC*)) data presented for NO-NSAIDs correspond to the released respective parent NSAID, except in the case of aspirin and NO-aspirins, where the *AUC* data corresponds to plasma salicylate concentrations, since aspirin and NO-aspirin get converted rapidly to salicylic acid by enzymatic hydrolysis *in vivo*. All NSAIDs showed faster absorption with T_{max} between 15 and 30 min, whereas NO-NSAIDs showed delayed T_{max} with significant release of parent NSAID over a longer period of time.

As mentioned earlier, the previously reported NO-aspirin compounds **9** and **17** did not show comparable bioavailability and anti-inflammatory activity to those of aspirin.[8] We now report a new "imide-containing" NO-aspirin prodrug **25** (**P1539**) that exhibited nearly comparable bioavailability and anti-inflammatory activity to those of aspirin[26] (Table 1). Thus, the imide group is found to be the best linkage for creating a promising prodrug in aspirin series using the specified linkers.

Since the data obtained on NO-aspirin and NO-diclofenac prodrugs exhibited a strong correlation between plasma drug concentration and anti-inflammatory activity, some of the reported NO-NSAIDs were screened for either pharmacokinetics or anti-inflammatory activity, depending on availability of resources.

Among naproxen series[26] (Table 1), the ester prodrug **11** (**P1853**) showed maximum anti-inflammatory activity, followed by the double ester prodrug **19**. The imide prodrug **26** and the amide prodrug **32** were inactive. In pharmacokinetic (PK) studies, as anticipated, the ester prodrug **11** showed significant oral bioavailability (*AUC* value), which is only slightly less than the *AUC* value of naproxen. However, the ester prodrug **11** exhibited nearly comparable oral bioavailability, anti-inflammatory activity and NO release profile to those of naproxcinod (I)[27] (Table 1), which is an investigational drug in advanced clinical development.[6] Thus, the ester group is found to be the best linkage for generation of a promising prodrug in naproxen series using the specified linkers.

While none of the listed NO-NSAID prodrugs of flurbiprofen, ketoprofen, sulindac, ibuprofen and indomethacin showed comparable bioavailability to that of their respective parent NSAID, the ester prodrugs of all the reported NSAIDs, with the lone exception of indomethacin series, showed superior bioavailability compared to the corresponding double ester, imide and amide prodrug counterparts in their respective series. In the indomethacin series, the double ester prodrug exhibited better bioavailability than the ester and the imide prodrugs. Thus, the following is the order of bioavailability in each of the series of NO-NSAIDs (Table 1)[26]: NO-flurbiprofen: ester prodrug **12**>double ester prodrug **20**>imide prodrug **27**>amide prodrug **33**; NO-ketoprofen: ester prodrug **13**>double ester prodrug **21**>imide prodrug **28**; NO-sulindac: ester prodrug **14**>double ester prodrug **22**; NO-ibuprofen: ester prodrug **15**>double ester prodrug **23**>imide prodrug **29**; NO-indomethacin: double ester prodrug **24**>ester prodrug **16**>imide prodrug **30**.

We have also evaluated anti-inflammatory activities of all the reported prodrugs of NO-flurbiprofen and only the ester and imide prodrugs of NO-ketoprofen. In contrast to the trend that was seen in other series of NO-NSAIDs, the imide prodrug **27** exhibited better anti-inflammatory activity than that of ester prodrug **12** and double ester prodrug **20**, although its bioavailability is less than those of ester prodrug **12** and double ester prodrug **20**. In the case of NO-ketoprofen series too, the imide prodrug **28** showed better anti-inflammatory activity than that of ester prodrug **13**, although its bioavailability is less than that of **13**[26] (Table 1). It is rather surprising to see the enhanced anti-inflammatory activities for the imide-containing flurbiprofen prodrug **27** and ketoprofen prodrug **28**, in spite of their low bioavailability. However, as mentioned earlier, NO-releasing compounds generally exhibit enhanced anti-inflammatory activity[19] which supports our belief that the enhanced anti-inflammatory effects shown by our compounds **10**, **25**, **27** and **28** could also be partly due to the NO released from these NO-NSAIDs. We have measured *in vivo* NO-release of the promising NO-NSAIDs **10**, **11**, **25**, **27** and **28** in rats. As anticipated, all tested NO-NSAIDs released significant amounts of NO. However, the NO release profile (rate, extent and fate) of these NO-NSAIDS is varied from one compound to another which may be due to differences in their physicochemical properties. The corresponding plasma nitrate/nitrite *AUC* values are shown in Table 1.[26]

Table 1. Oral Bioavailability (*AUC*), Anti-inflammatory Activity and NO Release Data of NO-NSAIDs[a)]

Compd.[a)]	*AUC*[b)] (μg/mL·h)	Activity (% inhibition)[c,d)]		*AUC* of NO_x (nitrite/nitrate) [μM·h][k)]	
		4 h	6 h	Compd. (*Z* mg/kg eq)	Vehicle[l)] (1 mL/kg)
Aspirin (**1**)	4051±312	42±9	31±8	—	—
9 (P1538)[e)]	1234±93**	20±12	17±7	—	—
17 (P1911)[e)]	892±140**	Inactive	Inactive	—	—
25 (P1539)	3836±164[f)]	48±15	28±16	3681 (*Z*=150)	330.4
31 (P1537)[e)]	0	—	—	—	—
Diclofenac (**2**)[e)]	65±2	55±9	21±12		
10 (P2026)[e)]	60±5[f)]	53±7	20±13	612 (*Z*=5)	305
18 (P1912)[e)]	49±2*	34±10	Inactive	—	—
Naproxen (**3**)	238±8	61±6	50±6	—	—
11 (P1853)	187±12*	45±4	35±5	1082 (*Z*=10)	433
19 (P1945)	—	44±9	14±6	—	—
26 (P1882)	—	Inactive	Inactive	—	—
32 (P1854)	—	Inactive	Inactive	—	—
Naproxcinod (**I**)	181±5*	53±10[g)]	49±8[h)]	842.3 (*Z*=10)	449
Flurbiprofen (**4**)	180±15	68±8	47±5	—	—
12 (P1887)	88±11**	46±13[i)]	34±10[i)]	—	—
20 (P1873)	53±5**	57±9[i)]	46±6[i)]	—	—
27 (P1888)	27±7**	61±17[i)]	48±2[i)]	1122 (*Z*=10)	418.7
33 (P1889)	4±0.2**	23±5[i)]	16±5[i)]	—	—
Ketoprofen (**5**)	90±11	58±6	40±10	—	—
13 (P1897)	58±8[f)]	47±7[j)]	20±12[j)]	—	—
21 (P1973)	38±8**	—	—	—	—
28 (P1898)	23±6**	68±1[j)]	46±7[j)]	707 (*Z*=30)	363.5
Sulindac (**6**)	2498±139	—	—	—	—
14 (P1995)	850±108**	—	—	—	—
22 (P2025)	329±44**	—	—	—	—
Ibuprofen (**7**)	82±3	—	—	—	—
15 (P1970)	36±2**	—	—	—	—
23 (P1972)	28±7**	—	—	—	—
29 (P1971)	26±4**	—	—	—	—
Indomethacin (**8**)	323±7	—	—	—	—
16 (P1975)	57±13**	—	—	—	—
24 (P5058)	96±14**	—	—	—	—
30 (P5057)	22±6**	—	—	—	—

a) All prodrugs were administered orally to rats at equimolar dose to their respective parent drugs (10 mg/kg for diclofenac, naproxen, flurbiprofen, ibuprofen and indomethacin; 150 mg/kg for aspirin, 15 mg/kg for ketoprofen and 100 mg/kg for sulindac).[26,27)] *b*) For aspirin and its prodrugs, plasma salicylate levels were estimated to calculate bioavailability. *c*) Anti-inflammatory activity is expressed as % inhibition of paw edema volume over control rats. *d*) All values are expressed as mean±S.E.M. (n=3–4 for pharmacokinetic study; n=5–8 for anti-inflammatory activity). *e*) Reported earlier[8)]; included here for comparison. *f*) Statistically not significant (*i.e.*, $p>0.05$ *versus* parent drug). *g*) Anti-inflammatory activity at 3 h for naproxcinod. *h*) Anti-inflammatory activity at 5 h for naproxcinod. *i*) 20 mg/kg equivalent dose. *j*) 30 mg/kg equivalent dose. *k*) Pooled data (n=3–5). *l*) 100% polyethylene glycol 400 (PEG400) was used as vehicle for NO_x estimation study. *$p<0.05$, **$p<0.01$ *versus* parent drug; —=Not evaluated.

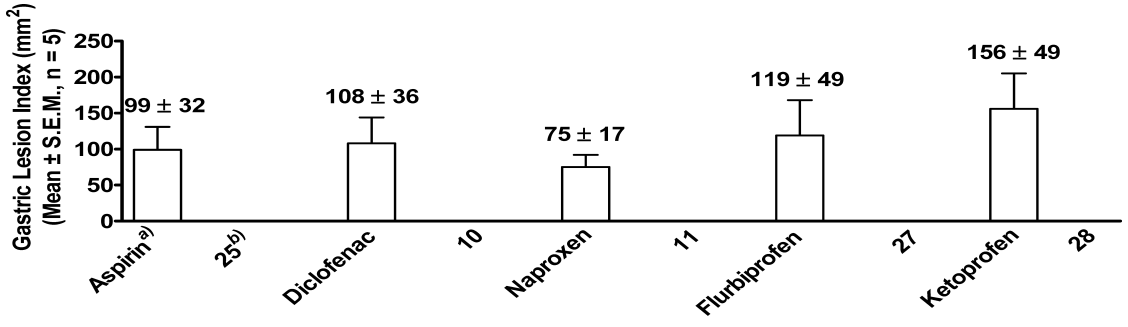


Fig. 4.[28)] Gastric Lesion Index of Selected NSAIDs *versus* Corresponding NO-NSAIDs (*i.e.*, **25**, **10**, **11**, **27** and **28**) Measured as Gastric Lesion Area (mm^2)

All NSAIDS (except aspirin) were dosed at 100 mg/kg and all NO-NSAIDs (except NO-aspirin) were dosed at 100 mg/kg equimolar to their respective parent NSAIDs; 0.5% (w/v) carboxymethyl-cellulose (CMC) containing 1% (v/v) Tween80 was used as vehicle. *a*) Used aspirin oral dose of 150 mg/kg. *b*) Used NO-Aspirin oral dose of 150 mg/kg equimolar to aspirin.

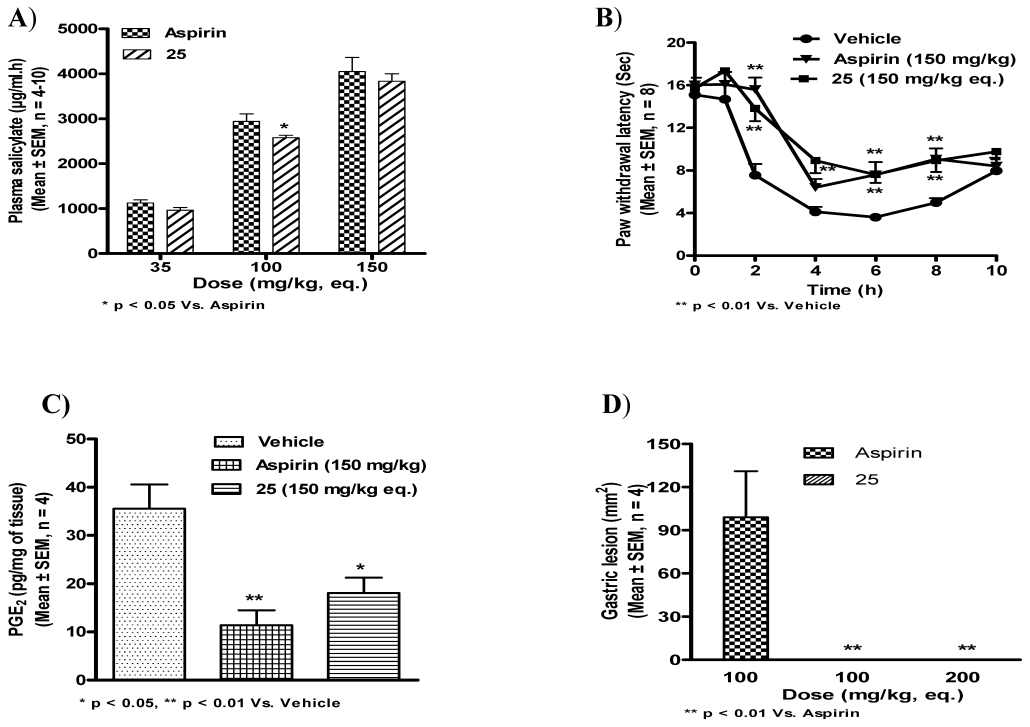


Fig. 5. Pharmacokinetic and Pharmcodynamic Effects of Oral Aspirin and NO-Aspirin Compound **25** (**P1539**) in Rat

(A) Dose dependent pharmacokinetics of aspirin and **25**; (B) analgesic activity of aspirin and **25**; (C) inhibition of gastric mucosal PGE_2 production by aspirin and **25**; (D) effect of aspirin and **25** (at equal and double doses) on gastric mucosa (gastric lesion index in mm^2).

Since all the prodrugs in NO-sulindac, NO-ibuprofen and NO-indomethacin series exhibited poor bioavailability when compared to those of their respective parent NSAIDs (Table 1),[26] we did not carry out further evaluation of these compounds.

We also selected the promising NO-NSAIDs **10**, **11**, **25**, **27** and **28** and studied their gastric tolerance compared to their respective parent NSAIDs in rats and the results of those experiments are presented graphically in Fig. 4.[28]

While all parent NSAIDs caused significant gastric damage, none of the tested NO-NSAIDs at equimolar doses caused any gastric lesions. We believe that the observed gastric-sparing effect could be attributable to the physiological actions of NO released from these NO-NSAIDs. However, additional factors such as masking of free carboxylic acid group of NSAID as an ester also might be partly contributing to the gastric-sparing effect of these compounds.[29–31]

Based on its promising oral bioavailability, anti-inflammatory and gastric-sparing properties, we have selected the NO-aspirin compound **25** for further evaluation and determined its: A) dose-dependent pharmacokinetics; B) analgesic activity in carrageenan-induced hind paw model of inflammatory pain; C) effect on reducing mucosal prostaglandin E_2 (PGE_2) levels *via* inhibition of cyclooxygenase-1 (COX-1); and D) gastric-sparing effects at equal as well as at double the doses and compared the results with those of aspirin at equimolar doses (Fig. 5).

Thus, as shown in Fig. 5A, compound **25** exhibited dose-dependent oral absorption and its overall bioavailability was nearly comparable to that of aspirin at equimolar doses. As anticipated, compound **25** as well as aspirin at equimolar doses exhibited comparable anti-hyperalgesic effects in carrageenan-induced hind paw model of hyperalgesia in rat (Fig. 5B). Similar to aspirin, NO-aspirin **25** also caused significant reduction in PGE_2 synthesis (Fig. 5C), which is an indirect measure of COX-1 inhibition. By inhibiting COX-1, which led to reduction in gastroprotective PGE_2 levels, aspirin caused significant gastric lesions. Although compound **25** also caused significant reduction in PGE_2 levels in gastric mucosa, it did not lead to any gastric damage even after doubling its dose (Fig. 5D). This result further confirms our belief that the unique gastric-sparing property exhibited by compound **25** could be attributable to NO released from this promising molecule.

Table 2. Stability of Aspirin (**1**), Flurbiprofen (**4**), Ester Prodrugs **9** and **12**, and the Imide Prodrugs **25** and **27** in SGF[a,b]

Incubation time	Prodrug **25**		
	% **25**	% **SA**[c]	Half-life ($t_{1/2}$)
0 min	96	0	
5 min	59	34	
10 min	33	57	7 min
15 min	15	72	
30 min	0	90	
60 min	0	90	

a) Values given are average of triplicate experiments. *b*) Aspirin (**1**), flurbiprofen (**4**), ester prodrugs **9** and **12**, and the imide prodrug **27** remained stable for 60 min (study time) in SGF at 37°C. *c*) **SA**=salicylic acid; other expected metabolites such as aspirin, aspirin amide and salicylamide are not formed (for structures of metabolites, see Fig. 6).

Metabolic Stability of NO-NSAIDs in Biological Fluids We have checked metabolic stability of a few representative NO-NSAIDs such as ester prodrugs **9** and **12** and imide pro-

drugs **25** and **27** along with their respective parent drugs aspirin (**1**) and flurbiprofen (**4**), in biological fluids such as simulated gastric fluid (SGF) (Table 2) and human plasma (Table 3) and identified their metabolite(s) (Fig. 6). Additionally, the stability of imide prodrug **27** was also tested in rat plasma (Table 3).

While aspirin (**1**), the ester prodrugs **9** and **12**, and the imide prodrug **27** remained stable on incubation in SGF at 37°C up to 60 min (study period), the imide prodrug **25** degraded completely within 30 min [half-life ($t_{1/2}$)=7 min] under similar conditions to give salicylic acid (**SA**) as the sole metabolite (Table 2). However, as expected, all the tested NO-NSAIDs degraded at varied rates [*i.e.*, $t_{1/2}$=between 9, 150 min] on incubation in human plasma at 37°C for up to 2 h (up to 4 h in case of flurbiprofen prodrugs **12**, **27**) (Table 3). Here also, only the imide prodrug **25** degraded completely within 30 min ($t_{1/2}$=9 min) to give **SA** as the sole metabolite.

Degradation of the remaining prodrugs (including aspirin) was not complete even after 2 h (4 h in case of flurbiprofen prodrugs **12**, **27**) of incubation in human plasma. Thus, at 2 h incubation time, there remained about 18% of intact ester prodrug **9** ($t_{1/2}$=55 min) and 82% of released **SA** metabolite. Surprisingly, degradation of flurbiprofen ester prodrug **12** was very slow ($t_{1/2}$=200 min) and even after 4 h incubation time, there still remained 40% of intact prodrug and 60% of released flurbiprofen (**4**). The flurbiprofen imide prodrug **27** also degraded very slowly ($t_{1/2}$=150 min), but rather interestingly, this imide prodrug released the expected metabolites, flurbiprofen (**4**) and flurbiprofen amide (**44**) (Fig. 6). Thus, at 4 h incubation, there still remained 32% of intact imide prodrug **27** along with metabolites **4** (21%) and **44** (23%). Interestingly, this flurbiprofen imide prodrug **27** degraded faster ($t_{1/2}$=85 min) and released more of flurbiprofen amide (**44**) in rat plasma. Thus, when prodrug **27** was incubated in rat plasma at 37°C for 4 h, there remained only, as shown in parentheses in Table 3, 8% of intact imide prodrug **27** along with the metabolites **4** (6%) and **44** (78%). Thus, although both the aspirin prodrug **25** and the flurbiprofen prodrug **27** contain the imide linkages in their structures, they exhibit different metabolic stability, and also metabolize differently in biological fluids. However, in PK study in rats, the flurbiprofen imide prodrug **27** released flurbiprofen (**4**) as the sole metabolite. We could not detect flurbiprofen amide (**44**) even at early time points of PK study. It seems that the metabolite **44** is either not formed or might be getting hydrolyzed to **4** as soon as it is formed under *in vivo* conditions.

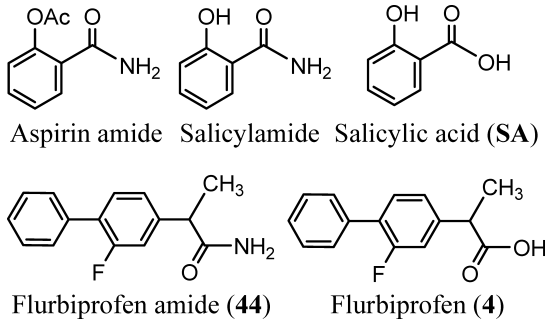


Fig. 6. Structures of Some of the Expected Metabolites from Prodrugs **9**, **12**, **25** and **27**

Since NO-aspirin **25** is expected to be hydrolyzed immediately after oral administration, the anti-inflammatory effects shown by the compound **25** could be caused by the released salicylate. It is also possible that both salicylic acid and NO could be contributing to the anti-inflammatory effect of compound **25**. In order to understand the possible role of NO in this process, we have evaluated anti-inflammatory effects of the nitro linker **36** *in vivo* in rats.[32] For comparison, we have also simultaneously evaluated anti-inflammatory effects of the non-nitro linker ($HOCH_2CH_2SSCH_2CH_2OH$), salicylic acid (**SA**) and 1 : 1 mixture of nitro linker **36** and **SA** and the results are presented in Table 4.

Surprisingly, both non-nitro and nitro linkers exhibited significant anti-inflammatory activity over control. The slightly enhanced activity of nitro linker **36** over non-nitro linker, although not significant, could be attributable to NO released from the linker. As anticipated, an equimolar mixture of **SA** and nitro linker **36** exhibited a slightly better anti-inflammatory activity than either of constituents individually. However, the difference in activity is not significant when compared with salicylate, but it reached significance when compared with nitro linker **36**. We therefore can not attribute this enhanced activity solely to NO as the nitro linker **36** and/or the non-nitro linker (after de-nitration) could as well contribute to

Table 3. Stability of Aspirin (**1**), Ester Prodrugs **9** and **12**, and Imide Prodrugs **25** and **27** in Human Plasma[a)]

Incubation time	Aspirin (**1**)		Prodrug **9**		Prodrug **25**		Prodrug **12**		Prodrug **27**		
	% **1**	% **SA**	% **9**	% **SA**	% **25**	% **SA**[b)]	% **12**	% **4**	% **27**	% **4**	% **44**
0 min	100	0	99	0	96	0.5	98	0	97	0	0
5 min	89	11	95	5	58	42	—	—	—	—	—
10 min	78	20	87	10	40	60	96	3	94	0	3
15 min	69	31	76	17	8	92	—	—	— (95)[c)]	— (1)[c)]	— (3)[c)]
30 min	68	32	67	33	0	98	83	12	86 (90)[c)]	0 (1)[c)]	6 (8)[c)]
60 min	58	42	46	54	0	98	82	17	76 (82)[c)]	4 (1)[c)]	8 (15)[c)]
120 min	38	62	18	82	0	98	57	43	56 (32)[c)]	9 (6)[c)]	14 (56)[c)]
180 min	—	—	—	—	—	—	55	45	48	14	17
240 min	—	—	—	—	—	—	40	60	32 (8)[c)]	21 (6)[c)]	23 (78)[c)]
Half-lives ($t_{1/2}$)	70 min		55 min		9 min		200 min		150 min (85 min)[c)]		

a) Values given are average of triplicate experiments. *b*) Other expected metabolites such as aspirin, aspirin amide and salicylamide are not formed (for structures, see Fig. 6). *c*) Values in parentheses are for % of indicated prodrugs or metabolites in rat plasma at the specified incubation times; **SA**=salicylic acid; **44**=flurbiprofen amide (for structure, see Fig. 6); —=not done.

the observed enhancement of activity of the mixture of salicylate and nitro linker **36**.

Mechanism of Drug Release A plausible mechanism for the release of NO and NSAID (native drug) from NO-NSAIDs containing ester linkage has been proposed in our earlier paper.[8,23] We now propose a plausible mechanism for the metabolic degradation of NO-NSAIDs containing imide linkages by taking aspirin imide prodrug **25** and flurbiprofen imide prodrug **27** as representative examples as depicted in Chart 4. We believe that these imide prodrugs **25** and **27**, because of their distinct differences in metabolic stability ($t_{1/2}$=9 min *versus* 150 min) and degradation pattern in rat and/or human plasma, qualify themselves as ideal candidates for such mechanistic studies. Thus, in case of aspirin imide prodrug **25**, hydrolysis by plasma esterases would lead to a transient intermediate *o*-phenoxybenzoyl isocyanate (**TI1**), which can break down *via o*-phenoxy-assisted cleavage to yield hydrogen cyanate (which in turn can hydrolyze to ammonia and carbon dioxide as shown) and *o*-oxoketene (**TI2A**)[33] *via* pathway a and/or the lactone form **TI2B**[33,34] *via* pathway b. The geometrically restricted *o*-oxoketene intermediate **TI2A**, being a highly reactive species, would rapidly undergo hydration to give a six-membered cyclic transition state **TS1**, which could quickly achieve aromatization *via* rearrangement to yield **SA** as the sole metabolite.[34] The transient intermediate **TI2B** [*i.e.*, benzodioxetane] could either equilibrate with *o*-oxoketene **TI2A** or directly undergo hydration to yield **SA**.[33,34] The stability of such lactone form (*i.e.*, **TI2B**) was reported to be nearly equal to *o*-oxoketene (**TI2A**) and it could be attributed to aromatic stabilization, which might be partially offset by its ring strain. The photochemical inter-conversion of **TI2A** and **TI2B** has also been reported earlier.[35]

Table 4. Anti-inflammatory Activity of Non-nitro Linker, Nitro Linker **36**, Salicylic Acid (**SA**) and an Equimolar Mixture of **SA** and Nitro Linker **36** in Rats[a]

Compd.[b]	Anti-inflammatory activity (% inhibition)[c]
(Non-nitro) linker	41.99±25.85*
Nitro linker **36**	59.26±12.00*
SA	74.10±19.01*
SA+**36** (1 : 1)	87.64±9.34*,**

a) Notably, the current batch of rats exhibited better sensitivity to all the test compounds. *b*) All the compounds were dosed at 832.60 μM/kg, which is equivalent to 150 mg/kg dose of aspirin. *c*) Anti-inflammatory activity is expressed as % inhibition of paw edema volume over control rats at 6 h after dosing. All values are expressed as mean±S.E.M. (n=5–6). *Statistically significant (*i.e.*, $p<0.05$ *versus* control). ** Statistically significant (*i.e.*, $p<0.05$ *versus* nitro linker **36**).

Metabolic degradation of flurbiprofen imide prodrug **27** is expected to proceed *via* two different pathways to yield the metabolites flurbiprofen (**4**) and flurbiprofen amide (**44**), respectively (Chart 4). Thus, the prodrug **27** is expected to be hydrolyzed by plasma esterases to yield a transient acyl isocyanate intermediate **TI3**. This reactive intermediate **TI3** can break down into hydrogen cyanate (which can further hydrolyze to ammonia and carbon dioxide as shown) and highly unstable transient intermediates **TI4A** and **TI4B** (pathway a), which can rapidly undergo hydration to yield flurbiprofen (**4**).

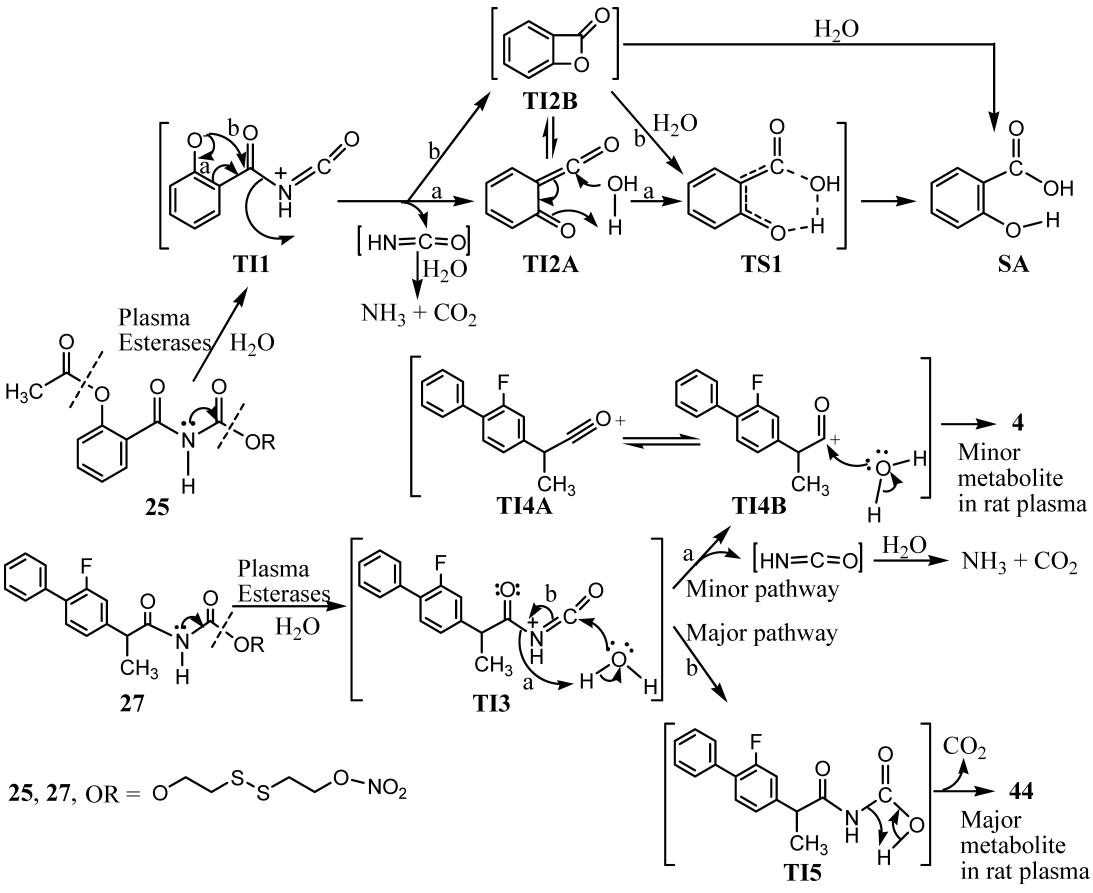


Chart 4. Plausible Mechanisms for the Metabolic Degradation of Imide Prodrugs **25** and **27** in Rat and/or Human Plasma

Table 5. Determination of Sulfhydryl-Assisted Nitric Oxide (NO) Release Profiles of a Disulfide-Containing Compound **11** (NO-Naproxen) and a Non-disulfide-Containing Compound **I** (Naproxcinod)

Compd.	Nitrate/nitrite concentration (μM)[a]			
	100 μM	50 μM	20 μM	10 μM
Standard ($NaNO_3$)	99.88±4.12[b] (<16.00)[c]	48.90±0.30[b] (<16.00)[c]	21.87±0.16[b] (<16.00)[c]	<16.00[b] (<16.00)[c]
I (Naproxcinod)	20.67±0.44[b] (<16.00)[c]	<16.00[b] (<16.00)[c]	<16.00[b] (<16.00)[c]	<16.00[b] (<16.00)[c]
11 (P1853)	100.0±4.00[b] (<16.00)[c]	50.9±3.60[b] (<16.00)[c]	22.80±2.80[b] (<16.00)[c]	16.50±0.60[b] (<16.00)[c]
Blank	<16.00[b] (<16.00)[c]	<16.00[b] (<16.00)[c]	<16.00[b] (<16.00)[c]	<16.00[b] (<16.00)[c]

a) Used Griess Nitrate/Nitrite Assay Kit from Sigma-Aldrich; Values are expressed as mean±S.E.M. (n=3 experiments) and a value of <16.00 represents a minimum detection level. *b*) Values are obtained after treatment with nitrate reductase. *c*) Values in parentheses are obtained before treatment with nitrate reductase.

Alternatively, the highly reactive acyl isocyanate intermediate **TI3** can rapidly undergo hydrolysis to yield yet another unstable transient intermediate **TI5**, which can rapidly lose carbon dioxide to yield flurbiprofen amide (**44**).

Thus, we have discovered a novel and unique aspirin imide prodrug **25**, that: (1) metabolized quickly in biological fluids such as SGF or human plasma and released **SA** as the sole metabolite; in contrast, the imide prodrugs of other NSAIDs, such as flurbiprofen imide prodrug **27**, exhibited good stability in SGF and metabolized slowly and differently in human plasma and rat plasma by releasing the expected metabolites, flurbiprofen (**4**) and flurbiprofen amide (**44**); (2) exhibited comparable and dose-dependent pharmacokinetics, anti-inflammatory and analgesic properties to those of aspirin; (3) exhibited comparable inhibition of mucosal PGE_2 levels to that of aspirin; and (4) exhibited gastric-sparing properties.

Mechanistic Studies on NO Release By design, our NO-NSAIDs carry salient features of both our disulfide linker[7] and the known SS-nitrates.[20] We have proposed a plausible sulfhydryl-dependent mechanism of NO release from our NO-NSAIDs in our earlier paper.[8,23] In light of FDA's recent negative decision on Nicox's naproxcinod (**I**),[6] we wanted to determine the similarities as well as differences in NO release profiles of naproxcinod (**I**) and our NO-NSAIDs. We have selected an appropriate ester containing NO-naproxen prodrug **11** as it is the closest structural analog of naproxcinod (**I**) but differs only by having a disulfide group in its linker. As discussed above, both these compounds exhibited nearly similar anti-inflammatory activity and oral bioavailability (Table 1) (*i.e.*, *AUC* values are comparable although they showed different T_{max} as well as C_{max}).[26,27] In principle, prodrug **11** is a simple organic nitrate like naproxcinod (**I**), but by having a disulfide group at β-position to the nitrate group in its structure, it also falls into the category of 'SS-nitrates.' When these two compounds were evaluated *in vivo* in rats at equimolar oral doses, we observed significantly different NO release profiles [*i.e.*, *AUC* of NO_x (nitrate/nitrite): 1082 μM·h *versus* 842 μM·h; T_{max}: 2 h *versus* 4 h; C_{max}: *ca.* 200 μM *versus ca.* 125 μM] for prodrug **11** and naproxcinod (**I**), respectively[26,27] (Table 1). Since prodrug **11** possesses SS-nitrate features, we expected it to release NO (*i.e.*, nitrate/nitrite) on treatment with a sulfhydryl-containing compound.[20] Based on known hypothesis of sulfhydryl-dependent mechanism of NO release from glyceryl nitrate (GTN),[36,37] we also wanted to determine whether a simple organic nitrate such as naproxcinod (**I**) can release NO on treatment with a sulfhydryl-containing compound. Accordingly, when prodrug **11** and naproxcinod (**I**) were individually treated with sulfhydryl-containing compound such as dithiothreitol (DTT) at biological relevant temperature (*ca.* 37°C) and pH (*ca.* 7.4), only prodrug **11** exhibited nearly quantitative release of NO (Table 5). This result confirms that our disulfide-containing NO-NSAIDs (**9**–**33**) indeed show characteristic properties of SS-nitrates. We also suspect that our NO-NSAIDs (**9**–**33**) and other organic nitrates such as naproxcinod (**I**) metabolize and release NO *in vivo* by different mechanisms and may therefore show different pharmacological outcome.

Conclusion

In summary, we have designed, synthesized, and evaluated a few novel NO-NSAID prodrugs of commonly used NSAIDs such as aspirin, diclofenac, naproxen, flurbiprofen, ketoprofen, sulindac, ibuprofen and indomethacin. These prodrugs have NO-releasing disulfide linker attached to the parent NSAID *via* linkages such as an ester (compounds **9**–**16**), a double ester (compounds **17**–**24**), an imide (compounds **25**–**30**), or an amide (compounds **31**–**33**). These NO-NSAIDs were evaluated for their oral bioavailability, anti-inflammatory activity, NO-releasing capability and gastric-sparing effects in rats. Among the tested NO-NSAIDs, the ester containing NO-aspirin (**9**), NO-diclofenac (**10**), NO-naproxen (**11**), and the imide-containing NO-aspirin (**25**), NO-flurbiprofen (**27**) and NO-ketoprofen (**28**) have shown promising pharmacokinetic and/or anti-inflammatory activities. However, only the ester prodrugs of diclofenac (**10**) and naproxen (**11**), and the imide prodrug of aspirin (**25**) have shown significant oral absorption and proportional anti-inflammatory activities that are nearly comparable to those of their respective parent NSAIDs. These promising NO-NSAIDs have also shown NO-releasing properties and protected rats from NSAID-induced gastric damage which could be attributable to the physiological actions of NO released from these prodrugs. NO-aspirin compound **25**, on further co-evaluation with aspirin at equimolar doses, exhibited comparable dose-dependent pharmacokinetics, inhibition of gastric mucosal PGE_2 synthesis and analgesic properties to those of aspirin, but retained its gastric-sparing properties even after doubling its oral dose. This prodrug **25** also showed less stability and metabolized differently (when compared with imide prodrugs of other NSAIDs such as flurbiprofen imide prodrug **27**) on incubation in biological fluids such as SGF and human plasma. However, in pharmacokinetics studies, all the reported NO-NSAIDs (except aspirin or NO-aspirin compounds, which release only salicylate) released their respective NSAIDs as sole metabolites. Based on the above data, we anticipate that these promising NO-NSAIDs could represent a new class of potentially "Safe NSAIDs" for the treatment of

arthritic pain and inflammation. However, further studies are required to establish cardiovascular safety of these novel NO-NSAIDs over conventional NSAIDs.

Experimental

Chemistry. General Methods Melting points were determined using a capillary melting point apparatus and were uncorrected. All solvents and reagents were of laboratory reagent grade and were used without purification. Dry solvents were prepared by standard methods. Analytical TLC was performed on 0.25 mm silica gel 60 F_{254} plates and visualized under UV light (254 nm). IR spectra were recorded on Perkin Elmer Spectrum One FTIR spectrometer; samples were prepared using either the KBR pellet method (for solids) or were run neat using NaCl disc (for oils). ^{1}H- and ^{13}C-NMR spectra were acquired using Bruker AVANCE 300 and Bruker AVANCE 500 spectrometers. Chemical shifts are reported as δ values in ppm using tetramethylsilane as an internal standard, and coupling constants (J) are given in Hertz. Signal multiplicities are represented by: s=singlet, d=doublet, dd=doublet of doublet, t=triplet, q=quartet, m=multiplet, br s=broad singlet. MS and high resolution (HR)-MS data were obtained using a Bruker-Micro-Q-time-of-flight (TOF) instrument. Reverse phase HPLC was performed on Waters HPLC using Waters X-terra RP-18 analytical column (Dimension: 150 mm×3.9 mm, 5 micron). Silica gel column chromatography was carried out using (150–300 mesh) silica gel. Acetylsalicylic acid (aspirin) was purchased from Dr. Reddy's Laboratories, Hyderabad; Diclofenac sodium was purchased from Aarti Drugs Ltd., Mumbai. Naproxen was purchased from Divi's Labs, Hyderabad. Flurbiprofen was purchased from Sun Pharmaceutical Industries Ltd., Ahmednagar; Ketoprofen was purchased from BEC Chemicals, Roha; Ibuprofen, Sulindac and Indomethacin were purchased from Sigma-Aldrich Co., U.S.A.

General Procedure for the Synthesis of NO-NSAIDs (9 and 15) with Ester Linkage Oxalyl chloride (45 mmol) was added to a solution of the appropriate NSAID (30 mmol) in benzene (40 mL). The reaction mixture was heated at 50°C for 1 h, concentrated, re-dissolved in benzene (40 mL) and treated with a solution of **36** (30 mmol) and triethylamine (45 mmol) in dichloromethane (20 mL) at 0°C. After stirring for 16 h, the reaction mixture was concentrated and partitioned between ethyl acetate and 0.5 N HCl. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The crude compound was purified by column chromatography (silica gel, 5–10% ethyl acetate in petroleum ether) to afford the target compounds **9** and **15**.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-2-acetoxybenzoate (9) The synthesis of this compound, along with spectral and analytical data, has been described in our earlier publication.[8)]

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-2-(4-isobutylphenyl)propanoate (15) The title compound was obtained as a light yellow oil (45%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89 (d, J=6.6 Hz, 6H, –CH($C\underline{H}_3$)$_2$), 1.49 (d, J=7.2 Hz, 3H, –CHC$\underline{H}_3$), 1.79–1.88 (m, 1H, –C$\underline{H}$(CH$_3$)$_2$), 2.44 (d, J=7.2 Hz, 2H, –ArC$\underline{H}_2$–), 2.81–2.91 (m, 4H, –$CH_2S_2CH_2$–), 3.70 (q, J=7.2 Hz, 1H, –C$\underline{H}$CH$_3$), 4.33 (t, J=6.9 Hz, 2H, –$CO_2C\underline{H}_2$–), 4.63 (t, J=6.9 Hz, 2H, –CH_2ONO_2), 7.10 (d, J=8.1 Hz, 2H, Ar-H), 7.20 (d, J=7.8 Hz, 2H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.51 (–CH$\underline{C}$H$_3$), 22.38 (–CH($\underline{C}$H$_3$)$_2$), 30.19 (–$\underline{C}$H(CH$_3$)$_2$), 34.77 (–SCH_2–), 37.16 (–SCH_2–), 45.01 (–$\underline{C}$HCH$_3$), 45.61 (–Ar$\underline{C}H_2$–), 62.22 (–$CO_2\underline{C}H_2$–), 70.57 (–CH_2ONO_2), 127.17 (Ar-C), 129.39 (Ar-C), 137.40 (Ar-C), 140.71 (Ar-C), 174.13 (–CO–); IR (NaCl) cm^{-1}: 2955 (C–H), 1737 (C=O), 1634 (Ar-C=C), 1279 (–NO_2), 1160 (C–O); HR-MS-electrospray ionization (ESI) (m/z) [M+Na]$^+$ Calcd for $C_{17}H_{25}NNaO_5S_2$: 410.1066; Found: 410.1055 (mass accuracy: 2.68 ppm); *Anal.* Calcd for $C_{17}H_{25}NO_5S_2$: C 52.69, H 6.50, N 3.61; Found: C 53.20, H, 6.85, N, 3.56.

General Procedure for the Synthesis of NO-NSAIDs (11–13, 16) with Ester Linkage To a solution of the appropriate NSAID (5.0 mmol) in dichloromethane (20 mL) was added *N,N′*-carbonyldiimidazole (7.5 mmol). After stirring for 2 h, a solution of **36** (5.0 mmol) in dichloromethane (15 mL) was added, and the mixture was stirred at room temperature for 16 h. Reaction mixture was washed successively with sat. $NaHCO_3$ solution and brine, dried (Na_2SO_4) and concentrated. The crude product was purified by silica gel column chromatography (50–70% petroleum ether in dichloromethane) to afford the target compounds **11**–**13** and **16**.

(*S*)-2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-2-(6-methoxynaphthalen-2-yl)propanoate (11) The title compound was obtained as a light yellow oil (46%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.60 (d, J=6.9 Hz, 3H, –CHC$\underline{H}_3$), 2.84 (t, J=6.9 Hz, 2H, –SCH_2–), 2.90 (t, J=6.3 Hz, 2H, –SCH_2–), 3.86–3.90 (m, 1H, –C$\underline{H}$CH$_3$), 3.93 (s, 3H, –OCH_3), 4.34 (dt, J=1.2, 6.3 Hz, 2H, –CO_2CH_2–), 4.56 (t, J=6.9 Hz, 2H, –CH_2ONO_2), 7.10–7.18 (m, 2H, Ar-H), 7.42 (dd, J=1.1, 8.4 Hz, 1H, Ar-H), 7.71 (m, 3H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.49 (–CH$\underline{C}$H$_3$), 34.79 (–SCH_2–), 37.15 (–SCH_2–), 45.39 (–$\underline{C}$HCH$_3$), 55.33 (–OCH_3), 62.30 (–$CO_2\underline{C}H_2$–), 70.53 (–CH_2ONO_2), 105.56 (Ar-C), 119.14 (Ar-C), 126.01 (Ar-C), 126.19 (Ar-C), 127.22 (Ar-C), 128.89 (Ar-C), 129.26 (Ar-C), 133.73 (Ar-C), 135.33 (Ar-C), 157.71 (Ar-C), 174.44 (–CO–). IR (NaCl) cm^{-1}: 2934 (C–H), 1735 (C=O), 1635 (Ar-C=C), 1277 (–NO_2); HR-MS-ESI (m/z) [M+Na]$^+$ Calcd for $C_{18}H_{21}NNaO_6S_2$: 434.0702, Found: 434.0688 (mass accuracy: 5.4 ppm); *Anal.* Calcd for $C_{18}H_{21}NO_6S_2$: C 52.54, H 5.14, N 3.40; Found: C 53.36, H 5.21, N 3.50.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanoate (12) The title compound was obtained as a light yellow oil (43%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.55 (d, J=7.20 Hz, 3H, –CH_3), 2.86–2.95 (m, 4H, –$CH_2S_2CH_2$–), 3.78 (q, J=7.0 Hz, 1H, –CH–), 4.38 (t, J=4.9, 7.2 Hz, 2H, –CO_2CH_2–), 4.66 (t, J=6.7 Hz, 2H, –CH_2ONO_2), 7.11–7.66 (m, 2H, Ar-H), 7.30–7.50 (m, 6H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 17.84 (–CH_3), 34.39 (–SCH_2–), 36.65 (–SCH_2–), 44.45 (–CH–), 62.03 (–$CO_2\underline{C}H_2$–), 70.08 (–CH_2ONO_2), 114.63 (Ar-C), 114.94 (Ar-C), 123.08 (Ar-C), 127.22 (Ar-C), 127.54 (Ar-C), 128.43 (Ar-C), 130.35 (Ar-C), 134.92 (Ar-C), 140.93 (Ar-C), 141.03 (Ar-C), 157.55 (Ar-C), 160.84 (Ar-C), 173.23 (–CO–); IR (NaCl) cm^{-1}: 2936 (C–H), 1737 (C=O), 1634 (Ar-C=C), 1278 (–NO_2), 1171 (C–O); HR-MS-ESI (m/z) [M+Na]$^+$ Calcd for $C_{19}H_{20}FNNaO_5S_2$: 448.0659; Found 448.0655 (mass accuracy: 0.89 ppm); *Anal.* Calcd for $C_{19}H_{20}FNO_5S_2$: C 53.63, H 4.74, N 3.29, S 15.07; Found: C 53.98, H 4.81, N 3.63, S 15.46.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-2-(3-benzoylphenyl)propanoate (13) The title compound was obtained as a light yellow oil (28%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.54 (d, J=7.2 Hz, 3H, –CH_3), 2.91–2.93 (m, 4H, –$CH_2S_2CH_2$–), 3.82

(q, J=6.6 Hz, 1H, –CH–), 4.34 (t, J=6.1 Hz, 2H, $-CO_2CH_2-$), 4.64 (t, J=6.5 Hz, 2H, $-CH_2ONO_2$), 7.42–8.0 (m, 9H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 17.89 ($-CH_3$), 34.38 ($-SCH_2-$), 36.63 ($-SCH_2-$), 44.82 (–CH–), 61.97 ($-CO_2\underline{C}H_2-$), 70.06 ($-CH_2ONO_2$), 127.82 (Ar-C), 128.07 (Ar-C), 128.65 (Ar-C), 129.56 (Ar-C), 130.99 (Ar-C), 132.04 (Ar-C), 136.96 (Ar-C), 137.50 (Ar-C), 140.05 (Ar-C), 173.30 ($-CO_2-$), 195.94 (Ar$\underline{C}$O–); IR (NaCl) cm^{-1}: 2978 (C–H), 1736 (C=O), 1634 (Ar-C=C), 1280 ($-NO_2$), 1164 (C–O); HR-MS-ESI (m/z) $[M+Na]^+$ Calcd for $C_{20}H_{21}NNaO_6S_2$: 458.0702; Found 458.0698 (mass accuracy: 0.87 ppm); *Anal.* Calcd for $C_{20}H_{21}NO_6S_2$: C 55.16, H 4.86, N 3.22, S 14.73; Found: C 55.14, H 4.81, N 3.48, S 15.09.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetate (16) The title compound was obtained as an off-white solid (67%). mp 91–93°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.40 (s, 3H, Ar-C$\underline{H}_3$), 2.92–2.94 (m, 4H, $-CH_2S_2CH_2-$), 3.71 (s, 2H, Ar-C$\underline{H}_2-$), 3.86 (s, 3H, $-OCH_3$), 4.39 (t, J=6.4 Hz, 2H, $-CO_2CH_2-$), 4.64 (t, J=4.7 Hz, 2H, $-CH_2ONO_2$), 6.71 (d, J=2.7 Hz, 1H, Ar-H), 6.89 (d, J=9.0 Hz, 1H, Ar-H), 6.98 (d, J=2.7 Hz, 1H, Ar-H), 7.49 (d, J=8.4 Hz, 2H, Ar-H), 7.68 (d, J=8.4 Hz, 2H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 13.86 (Ar-$\underline{C}H_3$), 30.72 (Ar-$\underline{C}H_2-$), 35.44 ($-SCH_2-$), 37.75 ($-SCH_2-$), 56.24 ($-OCH_3$), 63.00 ($-CO_2\underline{C}H_2-$), 71.04 ($-CH_2ONO_2$), 101.85 (Ar-C), 112.11 (Ar-C), 112.69 (Ar-C), 115.45 (Ar-C), 129.62 (Ar-C), 131.04 (Ar-C), 131.31 (Ar-C), 131.68 (Ar-C), 134.34 (Ar-C), 136.54 (Ar-C), 139.80 (Ar-C), 156.56 (Ar-C), 168.78 (–N–CO–), 172.07 (Ar$CH_2\underline{C}$O–). IR (KBr) cm^{-1}: 2930 (C–H), 1722 (C=O), 1641 (Ar-C=C), 1278 ($-NO_2$), 1133 (C–O); HR-MS-ESI (m/z) $[M+Na]^+$ Calcd for $C_{23}H_{23}ClN_2NaO_7S_2$: 561.0527; Found: 561.0511 (mass accuracy: 2.85 ppm); *Anal.* Calcd for $C_{23}H_{23}ClN_2O_7S_2$: C 51.25, H 4.30, N 5.20; Found: C 51.41, H 4.36, N 5.36.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (10) We have reported the synthesis of this compound by a different method in our earlier publication.[8] Here, we report an alternative method: To a solution of diclofenac sodium (2.80 g, 8.80 mmol) in DMF (20 mL) at 0°C, was added a solution of dibromide **35** (2.46 g, 8.80 mmol) in DMF (10 mL) over a period of 15 min. The resulting mixture was stirred overnight at room temperature. The reaction mixture was quenched with water, extracted with dichloromethane, washed with water and brine, dried (Na_2SO_4) and concentrated. The crude material was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to give the intermediate **40** as a pale yellow viscous liquid (2.18 g, 50%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.95 (t, J=6.6 Hz, 2H, $-SCH_2-$), 3.05 (t, J=8.4 Hz, 2H, $-SCH_2-$), 3.58 (t, J=8.4 Hz, 2H, $-CH_2Br$), 3.84 (s, 2H, –Ar$\underline{C}H_2-$), 4.40 (t, J=6.6 Hz, 2H, $-CO_2CH_2-$), 6.55 (d, J=8.1 Hz, 1H, Ar-H), 6.82 (br s, 1H, –NH–), 6.94–7.01 (m, 2H, Ar-H), 7.10–7.16 (m, 1H, Ar-H), 7.22–7.28 (m, 1H, Ar-H), 7.34 (d, J=8.1 Hz, 2H, Ar-H); MS m/z 494 $[M+H]^+$. To a solution of intermediate **40** (2.00 g, 4.05 mmol) in acetonitrile (20 mL) was added a solution of silver nitrate (0.86 g, 5.07 mmol) in acetonitrile (10 mL). The resulting mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with ethyl acetate (50 mL) and filtered through celite. The filtrate was concentrated, dried (Na_2SO_4) and purified by silica gel column chromatography (5–10% ethyl acetate in petroleum ether) to afford the compound **10** as a pale yellow viscous liquid (0.95 g, 49%). The characterization data (spectral and analytical) obtained for the compound **10** is identical to that reported earlier.[8]

(*Z*)-2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)-acetate (14) To a solution of sulindac (1.00 g, 2.80 mmol) and linker **36** (0.78 g, 3.91 mmol) in dichloromethane (20 mL) at RT were added DCC (0.69 g, 3.36 mmol) and DMAP (0.41 g, 3.36 mmol), and the mixture was stirred for 10 h. The reaction mixture was filtered and concentrated, and the residue purified by silica gel column chromatography (5% acetone in dichloromethane) to afford **14** as a yellow solid (0.80 g, 53%). mp 171–172°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.21 (s, 3H, Ar-C$\underline{H}_3$), 2.81 (s, 3H, $-S(O)CH_3$), 2.90–2.96 (m, 4H, $-CH_2S_2CH_2-$), 3.59 (s, 2H, ArC$\underline{H}_2-$), 4.38 (t, J=6.6 Hz, 2H, $-CO_2CH_2-$), 4.64 (t, J=6.6 Hz, 2H, $-CH_2ONO_2$), 6.53–6.60 (m, 1H, Ar-H), 6.88 (dd, J=2.4, 9.0 Hz, 1H, –C=CH–), 7.13–7.17 (m, 1H, Ar-H), 7.16 (s, 1H, Ar-H), 7.65–7.73 (m, 4H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 10.0 (Ar-$\underline{C}H_3$), 31.1 (Ar-$\underline{C}H_2-$), 34.4 ($-SCH_2-$), 36.7 ($-SCH_2-$), 43.3 ($-S(O)CH_3$), 62.1 ($-CO_2\underline{C}H_2-$), 70.0 ($-CH_2ONO_2$), 105.4 (Ar-C), 105.7 (Ar-C), 110.1 (Ar-C), 110.4 (Ar-C), 123.1 (Ar-C), 123.3 (Ar-C), 127.8 (Ar-C), 128.9 (Ar-C), 129.7 (Ar-C), 130.9 (Ar=$\underline{C}$–), 137.9 (Ar-C), 139.1 (Ar-C), 141.0 (Ar-C), 145.0 (Ar-C), 146.0 (Ar-C), 146.1 (Ar-C), 169.4 (–CO–); IR (KBr) cm^{-1}: 2922 (C–H), 1727 (C=O), 1625 (Ar-C=C), 1278 ($-NO_2$), 1169 (C–O), 1049 (S=O); HR-MS-ESI (m/z) $[M+H]^+$ Calcd for $C_{24}H_{25}FNO_6S_3$: 538.0823; Found: 538.0806 (mass accuracy: 3.16 ppm); *Anal.* Calcd for $C_{24}H_{24}FNO_6S_3$: C 53.61, H 4.50, N 2.61; Found: C 54.70, H 4.84, N 2.56.

General Procedure for the Synthesis of NO-NSAIDs (17, 19–23) with Double Ester Linkage A solution of the desired NSAID (13 mmol) and cesium carbonate (13 mmol) in methanol (40 mL) was stirred for 24 h at room temperature. Methanol was removed under vacuum. The residual cesium salt of the NSAID was suspended in a 1 : 1 mixture of tetrahydrofuran (THF) : DMF (40 mL) and treated with a solution of linker intermediate **37** (13 mmol) in THF (10 mL). After 2 h, the reaction mixture was concentrated, re-dissolved in ethyl acetate, washed with water, dried (Na_2SO_4) and concentrated. The crude material was purified by silica gel column chromatography (5–10% ethyl acetate in petroleum ether) to afford the target compounds **17** and **19**–**23**.

2-(2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl-2-acetoxybenzoate (17) The synthesis of this compound, along with spectral and analytical data, has been described in our earlier publication.[8]

(*S*)-2-(2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl-2-(6-methoxy naphthalen-2-yl)propanoate (19) The title compound was obtained as a pale yellow gum (44%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.62 (d, J=7.2 Hz, 3H, –CHC$\underline{H}_3$), 2.80 (t, J=6.3 Hz, 2H, $-SCH_2-$), 2.90 (t, J=6.6 Hz, 2H, $-SCH_2-$), 3.91 (s, 3H, $-OCH_3$), 3.96 (q, J=7.2 Hz, 1H), 4.35 (t, J=6.6 Hz, 2H, $-CO_2CH_2-$), 4.61–4.68 (m, 4H, $-CO_2CH_2CO_2-$, $-CH_2ONO_2$), 7.12–7.16 (m, 2H, Ar-H), 7.42 (dd, J=1.5, 8.4 Hz, 1H, Ar-H), 7.71 (d, J=8.4 Hz, 3H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 17.97 (–CH$\underline{C}$H3), 34.32 ($-SCH_2-$), 36.19 ($-SCH_2-$), 44.56 (–$\underline{C}$HCH$_3$), 54.81 ($-OCH_3$), 60.34 ($-CO_2\underline{C}H_2CH_2S-$), 62.28 ($-CO_2\underline{C}$H2CO$_2-$), 70.06 ($-CH_2ONO_2$), 105.12 (Ar-C), 118.54 (Ar-C), 125.68 (Ar-C), 125.77 (Ar-C), 126.67 (Ar-C), 128.40 (Ar-C), 128.78 (Ar-C), 133.29 (Ar-C), 134.46 (Ar-C), 157.20 (Ar-C), 167.01 ($OCH_2\underline{C}O-$), 173.49 ($CH_3CH\underline{C}O-$); IR (NaCl)

cm^{-1}: 2950 (C–H), 1747 (C=O), 1633 (Ar-C=C), 1278 (–NO_2), 1166 (C–O); HR-MS-ESI (*m/z*) $[M+K]^+$ Calcd for $C_{20}H_{23}KNO_8S_2$: 508.0497; Found: 508.0504 (mass accuracy: −1.38 ppm); *Anal.* Calcd for $C_{20}H_{23}NO_8S_2$: C 51.16, H 4.94, N 2.98; Found: C 51.28, H 5.05, N 3.17.

2-(2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl-2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanoate (20) The title compound was obtained as a pale yellow gum (80%). ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: 1.58 (d, *J*=7.5 Hz, 3H, –CH_3), 2.80–2.94 (m, 4H, –$CH_2S_2CH_2$–), 3.88 (q, *J*=7.0 Hz, 1H, –CH–), 4.40 (t, *J*=6.5 Hz, 2H, –CO_2CH_2–), 4.64–4.68 (m, 4H, –$CO_2CH_2CO_2$–, –CH_2ONO_2), 7.14–7.19 (m, 2H, Ar-H), 7.34–7.45 (m, 4H, Ar-H), 7.52–7.54 (m, 2H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$) *δ*: 18.4 (–CH_3), 34.8 (–SCH_2–), 36.7 (–SCH_2–), 44.6 (–CH–), 60.9 (–$CO_2\underline{C}H_2$–), 62.8 (–$CO_2\underline{C}H_2CO_2$–), 70.5 (–CH_2ONO_2), 115.3 (Ar-C), 115.5 (Ar-C), 123.7 (Ar-C), 127.7 (Ar-C), 128.4 (Ar-C), 128.9 (Ar-C), 130.8 (Ar-C), 135.4 (Ar-C), 140.1 (Ar-C), 159.0 (Ar-C), 167.4 (–$OCH_2\underline{C}O$–), 173.3 ($CH_3CH\underline{C}O$–); IR (NaCl) cm^{-1}: 2950 (C–H), 1746 (C=O), 1634 (Ar-C=C), 1279 (–NO_2), 1164 (C–O); HR-MS-ESI (*m/z*) $[M+Na]^+$ Calcd for $C_{21}H_{22}FNNaO_7S_2$: 506.0719; Found: 506.0707 (mass accuracy: −2.5 ppm); *Anal.* Calcd for $C_{21}H_{22}FNO_7S_2$: C 52.16, H 4.59, N 2.90; Found: C 52.83, H 4.45, N 2.46.

2-(2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl-2-(3-benzoylphenyl)propanoate (21) The title compound was obtained as a pale yellow oil (40%). ^{1}H-NMR (300 MHz, $CDCl_3$) *δ*: 1.59 (d, *J*=7.2 Hz, 3H, –CH_3), 2.89 (t, *J*=6.6 Hz, 2H, –SCH_2–), 2.95 (t, *J*=6.6 Hz, 2H, –SCH_2–), 3.92 (q, *J*=6.9 Hz, 1H, –CH–), 4.39 (t, *J*=6.6 Hz, 2H, –CO_2CH_2), 4.62–4.68 (m, 4H, –$CO_2CH_2CO_2$–, –CH_2ONO_2), 7.48 (t, *J*=7.8 Hz, 3H, Ar-H), 7.59 (d, *J*=7.5 Hz, 2H, Ar-H), 7.69 (d, *J*=7.8 Hz, 1H, Ar-H), 7.80 (d, *J*=8.7 Hz, 3H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) *δ*: 16.40 (–CH_3), 34.82 (–SCH_2–), 36.72 (–SCH_2–), 45.00 (–CH–), 60.91 (–$CO_2\underline{C}H_2$–), 62.84 (–$CO_2\underline{C}H_2CO_2$–), 70.58 (–CH_2ONO_2), 128.33 (Ar-C), 128.58 (Ar-C), 129.23 (Ar-C), 129.30 (Ar-C), 130.10 (Ar-C), 131.69 (Ar-C), 132.55 (Ar-C), 137.43 (Ar-C), 137.94 (Ar-C), 140.18 (Ar-C), 167.38 (–$OCH_2\underline{C}O$–), 173.44 ($CH_3CH\underline{C}O$–), 196.44 (Ar$\underline{C}O$–); IR (NaCl) cm^{-1}: 2950 (C–H), 1746 (C=O), 1634 (Ar-C=C), 1280 (–NO_2), 1156 (C–O); HR-MS-ESI (*m/z*) $[M+Na]^+$ Calcd for $C_{22}H_{23}NNaO_8S_2$: 516.0757; Found: 516.0767 (mass accuracy: −1.94 ppm); *Anal.* Calcd for $C_{22}H_{23}NO_8S_2$: C 53.54, H 4.70, N 2.84; Found: C 52.75, H 4.80, N 2.99.

(*Z*)-2-(2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl-2-(5-fluoro-2-methyl-1-(4-(methylsulfinyl)benzylidene)-1*H*-inden-3-yl)acetate (22) The title compound was obtained as a light yellow solid (56%). mp 74–75°C; ^{1}H-NMR (300 MHz, $CDCl_3$) *δ*: 2.22 (s, 3H, Ar-C$\underline{H}_3$), 2.81 (s, 3H, –S(O)CH_3), 2.86–2.97 (m, 4H, –$CH_2S_2CH_2$–), 3.68 (s, 2H, Ar-C$\underline{H}_2$–), 4.41 (t, *J*=6.6 Hz, 2H, –CO_2CH_2–), 4.66 (t, *J*=6.6 Hz, 2H, –CH_2ONO_2), 4.67 (s, 2H, –$CO_2CH_2CO_2$–), 6.54–6.60 (m, 1H, Ar-H), 6.90 (dd, *J*=2.4, 9.0 Hz, 1H, –C=CH–), 7.13–7.16 (m, 1H, Ar-H), 7.17 (s, 1H, Ar-H), 7.65–7.73 (m, 4H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) *δ*: 10.0 (Ar-$\underline{C}H_3$), 30.6 (Ar-$\underline{C}H_2$–), 34.3 (–SCH_2–), 36.2 (–SCH_2–), 43.4 (–S(O)CH_3), 60.5 (–$CO_2\underline{C}H_2CO_2$–), 62.3 (–CO_2CH_2–), 70.0 (–CH_2ONO_2), 105.5 (Ar-C), 105.8 (Ar-C), 110.1 (Ar-C), 110.4 (Ar-C), 123.1 (Ar-C), 123.3 (Ar-C), 127.9 (Ar-C), 128.9 (Ar-C), 129.7 (–C=$\underline{C}$H–), 130.6 (Ar-C), 138.1 (Ar-C), 139.0 (Ar-C), 141.0 (Ar-C), 145.1 (Ar-C), 145.9 (Ar-C), 146.0 (Ar-C), 166.8 (Ar$CH_2\underline{C}$O–), 169.0 (–$OCH_2\underline{C}$O–); IR (KBr) cm^{-1}: 2958 (C–H), 1741 (C=O), 1630 (Ar-C=C), 1278 (–NO_2), 1166 (C–O), 1049 (S=O); HR-MS-ESI (*m/z*) $[M+H]^+$ Calcd for $C_{26}H_{27}FNO_8S_3$: 596.0871; Found: 596.0877 (mass accuracy: 1.01 ppm); *Anal.* Calcd for $C_{26}H_{26}FNO_8S_3$: C 52.42, H 4.40, N 2.35; Found: C 52.17, H 4.31, N, 2.39.

2-(2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl-2-(4-isobutylphenyl)propanoate (23) The title compound was obtained as a pale yellow oil (36%). ^{1}H-NMR (300 MHz, $CDCl_3$) *δ*: 0.89 (d, *J*=6.6 Hz, 6H, –CH(C$\underline{H}_3$)$_2$), 1.54 (d, *J*=7.2 Hz, 3H, –CHC$\underline{H}_3$), 1.80–1.87 (m, 1H, –C$\underline{H}$(CH$_3$)$_2$), 2.44 (d, *J*=7.2 Hz, 2H, –ArC$\underline{H}_2$–), 2.88 (t, *J*=6.6 Hz, 2H, –SCH_2–), 2.95 (t, *J*=6.9 Hz, 2H, –SCH_2–), 3.82 (q, *J*=7.2 Hz, 1H, –C$\underline{H}CH_3$), 4.38 (t, *J*=6.6 Hz, 2H, –CO_2CH_2–), 4.62–4.69 (m, 4H, –$CO_2CH_2CO_2$–, –CH_2ONO_2), 7.11 (d, *J*=7.8 Hz, 2H), 7.23 (d, *J*=7.8 Hz, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) *δ*: 18.56 (–CH$\underline{C}H_3$), 22.41 (–CH($\underline{C}H_3$)$_2$), 30.18 (–$\underline{C}$H(CH$_3$)$_2$), 34.85 (–SCH_2–), 36.76 (–SCH_2–), 44.74 (–$\underline{C}HCH_3$), 45.00 (–Ar$\underline{C}$H2–), 60.82 (–$CO_2\underline{C}H_2$–), 62.78 (–$CO_2\underline{C}H_2CO_2$–), 70.60 (–CH_2ONO_2), 127.31 (Ar-C), 129.39 (Ar-C), 137.06 (Ar-C), 140.79 (Ar-C), 167.61 (–$OCH_2\underline{C}O_2$–), 174.13 ($CH_3CH\underline{C}O$–); IR (NaCl) cm^{-1}: 2955 (C–H), 1746 (C=O), 1634 (Ar-C=C), 1278 (–NO_2), 1151 (C–O); HR-MS-ESI (*m/z*) $[M+K]^+$ Calcd for $C_{19}H_{27}KNO_7S_2$: 484.0861; Found: 484.0864 (mass accuracy: −0.62 ppm); *Anal.* Calcd for $C_{19}H_{27}NO_7S_2$: C 51.22, H 6.11, N 3.14; Found: C 51.94, H 6.08, N 3.34.

2-(2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl-2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (18) The synthesis of this compound, along with spectral and analytical data, has been described in our earlier publication.[8]

2-(2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)-2-oxoethyl-2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)-acetate (24) To a solution of indomethacin (2.5 g, 7.0 mmol) in DMF (40 mL) were added potassium carbonate (2.4 g, 17.5 mmol) and the intermediate **37** (1.9 g, 7.0 mmol) and the mixture was stirred for 16 h. DMF was removed under vacuum. The residue was partitioned between water and ethyl acetate. The organic layer was washed with water and brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (10% ethyl acetate in petroleum ether) to afford **24** as a light green gum (0.60 g, 15%). ^{1}H-NMR (300 MHz, $CDCl_3$) *δ*: 2.41 (s, 3H, Ar-C$\underline{H}_3$), 2.94 (m, 4H, –$CH_2S_2CH_2$–), 3.81 (s, 2H, Ar-C$\underline{H}_2$–), 3.86 (s, 3H, –OCH_3), 4.42 (t, *J*=5.7 Hz, 2H, –CO_2CH_2–), 4.62–4.67 (m, 4H, –$CO_2CH_2CO_2$–, –CH_2ONO_2), 6.69 (d, *J*=8.4 Hz, 1H, Ar-H), 6.90 (d, *J*=8.7 Hz, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 7.49 (d, *J*=7.8 Hz, 2H, Ar-H), 7.68 (d, *J*=7.8 Hz, 2H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) *δ*: 13.85 (Ar-$\underline{C}H_3$), 30.24 (Ar$\underline{C}H_2$–), 35.36 (–SCH_2–), 37.25 (–SCH_2–), 56.23 (–OCH_3), 61.46 (–$CO_2\underline{C}H_2CO_2$–), 63.34 (–$CO_2\underline{C}H_2$–), 71.06 (–CH_2ONO_2), 101.82 (Ar-C), 112.22 (Ar-C), 112.37 (Ar-C), 115.44 (Ar-C), 129.63 (Ar-C), 131.01 (Ar-C), 131.30 (Ar-C), 131.70 (Ar-C), 134.33 (Ar-C), 136.67 (Ar-C), 139.81 (Ar-C), 156.58 (Ar-C), 167.87 (–N–CO–), 168.79 (Ar$CH_2\underline{C}$O–), 170.71 (–$OCH_2\underline{C}$O–); IR (NaCl) cm^{-1}: 2927 (C–H), 1735 (C=O), 1636 (Ar-C=C), 1278 (–NO_2), 1149 (C–O); MS *m/z*: 597.1 $[M+H]^+$; HR-MS-ESI (*m/z*) $[M+H]^+$ Calcd for $C_{25}H_{26}ClN_2O_9S_2$: 597.0763; Found: 597.0739 (mass accuracy: 4.02 ppm); *Anal.* Calcd for $C_{25}H_{25}ClN_2O_9S_2$: C 50.29, H 4.22, N 4.69; Found: C 51.19, H 4.31, N 4.38.

Synthesis of NO-NSAID (25) with Imide Linkage 2-(((2-((2-(Nitrooxy)ethyl)disulfanyl)ethoxy)carbonyl)car-

bamoyl)phenylacetate (25) Thionyl chloride (0.81 mL, 11.10 mmol) was added to a solution of aspirin (**1**, 1.00 g, 5.55 mmol) in benzene (8 mL) at 0°C. The reaction mixture was heated at 70°C for 1.5 h. Silver cyanate (0.99 g, 6.66 mmol) was added to the mixture containing aspirin acid chloride and the mixture was refluxed for 0.5 h to get the corresponding acyl isocyanate **43**.[25] The mixture was allowed to cool to room temperature. A solution of **36** (0.70 g, 3.51 mmol) in benzene (4 mL) was added to the mixture containing the intermediate aspirin acyl isocyanate **43** and the mixture was stirred for 8 h to generate the imide prodrug **25**. The reaction mixture was concentrated and the residue was partitioned between ethyl acetate (40 mL) and water (40 mL). The organic layer was washed with brine (40 mL), dried (Na_2SO_4) and concentrated. The crude material was purified by silica gel column chromatography (5–10% ethyl acetate in hexane) to afford **25** as a colourless oil (0.50 g, 35%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.22 (s, 3H, $-COCH_3$), 2.87–2.95 (m, 4H, $-CH_2S_2CH_2-$), 4.31 (t, J=6.0 Hz, 2H, $-CO_2CH_2-$), 4.67 (t, J=7.5 Hz, 2H, $-CH_2ONO_2$), 5.73 (br s, 1H, –NH–), 7.05 (d, J=9.0 Hz, 1H, Ar-H), 7.19 (t, J=7.5 Hz, 1H, Ar-H), 7.61 (t, J=6.0 Hz, 1H, Ar-H), 7.97 (d, J=6.0 Hz, 1H, Ar-H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 25.0 ($-CH_3$), 34.7 ($-SCH_2-$), 37.2 ($-SCH_2-$), 62.9 ($-CO_2\underline{C}H_2CH_2-$), 70.6 ($-CH_2ONO_2$), 107.0 (Ar-C), 113.1 (Ar-C), 117.4 (Ar-C), 123.6 (Ar-C), 129.6 (Ar-C), 136.9 (Ar-C), 152.6 (–CONH–), 154.4 (Ar-$\underline{C}O_2-$), 159.6 ($CH_3\underline{C}O_2-$); IR (NaCl) cm^{-1}: 3305 (N–H), 2954 (C–H), 1731 (C=O), 1633 (Ar-C=C), 1278 ($-NO_2$), 1156 (C–O); HR-MS-ESI (*m/z*) $[M+Na]^+$ Calcd for $C_{14}H_{16}N_2NaO_8S_2$: 427.0246; Found: 427.0229 (mass accuracy: −3.8 ppm); *Anal.* Calcd for $C_{14}H_{16}N_2O_8S_2$: C 41.58, H 3.99, N 6.93, S 15.86; Found: C 41.85, H 3.97, N 6.65, S 15.78.

General Procedure for the Synthesis of NO-NSAIDs (26–30) with Imide Linkage To a solution of the appropriate NSAID (**3**–**5**, **7**, **8**) (30 mmol) in benzene (70 mL) was added DMF (2 drops) followed by oxalyl chloride (36 mmol) and stirred at room temperature for 3 h. The solvent was removed under vacuum. The residue (containing acid chlorides of respective NSAIDs) was dissolved in dichloromethane (100 mL) and cooled to 0°C. Ammonia gas was passed slowly through the solution for 30 min. The reaction mixture was washed successively with sat. $NaHCO_3$ solution and brine. The organic layer was dried (Na_2SO_4) and concentrated to afford the respective NSAID amides. To a stirred suspension of the NSAID amide (30 mmol) in 1,2-dichloroethane (100 mL) was added oxalyl chloride (36 mmol) and refluxed for 16 h to get the corresponding acyl isocyanate **43**.[24] The reaction mixture was cooled to room temperature. A solution of **36** (30 mmol) in 1,2-dichloroethane (10 mL) was added to the above mixture and the mixture was stirred for 16 h at room temperature to generate the corresponding imide containing prodrugs **26**–**30**. The reaction mixture was concentrated; the residue was taken up in ethyl acetate, washed with sat. $NaHCO_3$ solution, brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (1% methanol in dichloromethane) to afford the target compounds **26**–**30**.

(*S*)-2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-(2-(6-methoxynaphthalen-2-yl)propanoyl)carbamate (26) The intermediate naproxen amide was obtained as a white solid (100%). ^{1}H-NMR (300 MHz, CD_3OD) δ: 1.53 (d, J=6.9 Hz, 3H, $-CHC\underline{H}_3$), 3.68 (q, J=7.2 Hz, 1H, $-C\underline{H}CH_3$), 7.0–7.16 (m, 2H, Ar-H), 7.37–7.47 (m, 1H, Ar-H), 7.68–7.63 (m, 3H, Ar-H); MS *m/z* 429.27 $[M+H]^+$. Using this amide, the title compound was obtained as a white solid (36%). mp 145–147°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.56 (d, J=6.9 Hz, 3H, $-CHC\underline{H}_3$), 2.86 (m, 4H, $-CH_2S_2CH_2-$), 3.91 (s, 3H, $-OCH_3$), 4.31–4.33 (m, 3H, $-CO_2CH_2-$, $-C\underline{H}CH_3$), 4.59 (t, J=6.6 Hz, 2H, $-CH_2ONO_2$), 7.11–7.16 (m, 2H, Ar-H), 7.37–7.47 (m, 1H, Ar-H), 7.68–7.63 (m, 3H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.26 ($-CH\underline{C}H_3$), 34.05 ($-SCH_2-$), 36.15 ($-SCH_2-$), 45.14 ($-\underline{C}HCH_3$), 55.12 ($-OCH_3$), 62.44 ($-CO_2\underline{C}H_2-$), 71.07 ($-CH_2ONO_2$), 105.66 (Ar-C), 118.69 (Ar-C), 125.72 (Ar-C), 126.27 (Ar-C), 126.85 (Ar-C), 128.30 (Ar-C), 129.10 (Ar-C), 133.27 (Ar-C), 135.78 (Ar-C), 150.87 (Ar-C), 157.14 (–OCONH–), 173.17 ($-CO_2NH\underline{C}O-$); IR (KBr) cm^{-1}: 3248 (N–H), 2960 (C–H), 1749 (C=O), 1635 (Ar-C=C), 1278 ($-NO_2$), 1180 (C–O); HR-MS-ESI (*m/z*) $[M+Na]^+$ Calcd for $C_{19}H_{22}N_2NaO_7S_2$: 477.0761; Found: 477.0766 (mass accuracy: −1.05 ppm); *Anal.* Calcd for $C_{19}H_{22}N_2O_7S_2$: C 50.22, H 4.88, N 6.16; Found: C 50.92, H 4.92, N 6.28.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-(2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanoyl)carbamate (27) The intermediate flurbiprofen amide was obtained as a white solid (90%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.55 (d, J=6.9 Hz, 3H, $-CH_3$), 3.66 (q, J=7.2 Hz, 1H, –CH–), 5.63 (s, 1H, $-NH_2$), 5.95 (s, 1H, $-NH_2$), 7.1–7.20 (m, 2H, Ar-H), 7.38–7.56 (m, 6H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.32 ($-CH_3$), 46.00 (–CH–), 115.15 (Ar-C), 115.46 (Ar-C), 123.59 (Ar-C), 123.63 (Ar-C), 127.79 (Ar-C), 128.50 (Ar-C), 128.91 (Ar-C), 128.94 (Ar-C), 131.14 (Ar-C), 131.20 (Ar-C), 135.29 (Ar-C), 142.32 (Ar-C), 142.42 (–CO–); MS *m/z* 244.14 $[M+H]^+$. Using this amide, the title compound was obtained as a white solid (10%). mp: 58–60°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.54 (d, J=6.9 Hz, 3H, $-CH_3$), 2.95 (m, 4H, $-CH_2S_2CH_2-$), 4.40–4.43 (m, 3H, $-CO_2CH_2-$, $-C\underline{H}CH_3$), 4.68 (t, J=6.6 Hz, 2H, $-CH_2ONO_2$), 7.13–7.19 (m, 2H, Ar-H), 7.35–7.55 (m, 6H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.28 ($-CH_3$), 34.36 ($-SCH_2-$), 36.35 ($-SCH_2-$), 44.59 (–CH–), 63.17 ($-CO_2\underline{C}H_2-$), 70.01 ($-CH_2ONO_2$), 115.03 (Ar-C), 115.33 (Ar-C), 123.40 (Ar-C), 127.27 (Ar-C), 127.98 (Ar-C), 128.42 (Ar-C), 130.54 (Ar-C), 134.82 (Ar-C), 140.65 (Ar-C), 140.79 (Ar-C), 150.38 (Ar-C), 157.60 (Ar-C), 160.89 ($-NHCO_2-$), 173.32 ($CH_3CH\underline{C}O-$); IR (KBr) cm^{-1}: 3259 (N–H), 2978 (C–H), 1752 (C=O), 1646 (Ar-C=C), 1272 ($-NO_2$), 1175 (C–O); HR-MS-ESI (*m/z*) $[M+Na]^+$ Calcd for $C_{20}H_{21}FN_2NaO_6S_2$: 491.0717; Found: 491.0718 (mass accuracy: −0.2 ppm); *Anal.* Calcd for $C_{20}H_{21}FN_2O_6S_2$: C 51.27, H 4.52, N 5.98, S 13.69; Found: C 51.24, H 4.48, N 5.97, S 13.71.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl(2-(3-benzoylphenyl)propanoyl)carbamate (28) The intermediate ketoprofen amide was obtained as a white solid (100%). ^{1}H-NMR (300 MHz, CD_3OD) δ: 1.55 (d, J=7.2 Hz, 3H, $-CH_3$), 3.68 (q, J=6.9 Hz, 1H, –CH–), 5.54–5.69 (m, 2H, $-NH_2$), 7.43–7.80 (m, 9H, Ar-H); MS *m/z* 453.97 $[M+H]^+$. Using this amide, the title compound was obtained as a pale yellow oil (18%) ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.46 (d, J=7.1 Hz, 3H, $-CH_3$), 2.96–3.0 (m, 4H, $-CH_2S_2CH_2-$), 4.00 (q, J=6.8 Hz, 1H, –CH–), 4.37 (t, J=6.2 Hz, 2H, $-CO_2CH_2-$), 4.68 (t, J=6.4 Hz, 2H, $-CH_2ONO_2$), 7.47–4.77 (m, 9H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.40 ($-CH_3$), 34.35 ($-SCH_2-$), 36.30 ($-SCH_2-$), 45.05 (–CH–), 63.13 ($-CO_2CH_2-$), 70.09 ($-CH_2ONO_2$), 127.84 (Ar-C), 128.28 (Ar-C), 128.77 (Ar-C), 129.05 (Ar-

C), 129.60 (Ar-C), 131.29 (Ar-C), 132.12 (Ar-C), 136.84 (Ar-C), 137.62 (Ar-C), 139.84 (Ar-C), 150.26 (–$NHCO_2$–), 173.27 ($CH_3CH\underline{C}O$–), 195.90 (Ar$\underline{C}O$–); IR (NaCl) cm^{-1}: 3281 (N–H), 2977 (C–H), 1770 (C=O), 1634 (Ar-C=C), 1279 (–NO_2), 1199 (C–O); HR-MS-ESI (*m/z*) $[M+Na]^+$ Calcd for $C_{21}H_{22}N_2NaO_7S_2$: 501.0761; Found: 501.0770 (mass accuracy: −1.8 ppm); *Anal.* Calcd for $C_{21}H_{22}N_2O_7S_2$: C 52.71, H 4.63, N 5.85, S 13.40; Found: C 52.62, H 4.63, N 6.15, S 13.73.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl(2-(4-isobutylphenyl)propanoyl)carbamate (29) The intermediate ibuprofen amide was obtained as a white solid (100%). ^{1}H-NMR (300 MHz, CD_3OD) δ: 0.88 (d, J=6.3 Hz, 6H, –CH($C\underline{H}_3$)$_2$), 1.41 (d, J=7.2 Hz, 3H, –CHC$\underline{H}_3$), 1.78–1.87 (m, 1H, –C$\underline{H}$(CH_3)$_2$), 2.41 (d, J=7.2 Hz, 2H, –ArC$\underline{H}_2$–), 3.62 (q, J=7.2 Hz, 1H, –C$\underline{H}CH_3$), 7.00 (d, J=8.1 Hz, 2H, Ar-H), 7.24 (d, J=7.8 Hz, 2H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 17.45 (–CH$\underline{C}H_3$), 21.30 (–CH($\underline{C}H_3$)$_2$), 30.06 (–$\underline{C}$H(CH_3)$_2$), 44.64 (–$\underline{C}HCH_3$), 45.34 (–Ar$\underline{C}H_2$–), 126.77 (Ar-C), 128.87 (Ar-C), 129.46 (Ar-C), 138.98 (Ar-C), 140.11 (Ar-C), 178.82 (–CO–). Using this amide, the title compound was obtained as a yellow solid (32%) mp 62-64°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.89 (d, J=6.6 Hz, 6H, –CH($C\underline{H}_3$)$_2$), 1.49 (d, J=7.2 Hz, 3H, –CHC$\underline{H}_3$), 1.80–1.86 (m, 1H, –C$\underline{H}$(CH_3)$_2$), 2.45 (d, J=6.9 Hz, 2H, –ArC$\underline{H}_2$–), 2.92 (q, J=6.9 Hz, 4H, –$CH_2S_2CH_2$–), 4.18 (q, J=6.9 Hz, 1H, –C$\underline{H}CH_3$), 4.36 (t, J=6.3 Hz, 2H, –CO_2CH_2–), 4.67 (t, J=6.9 Hz, 2H, –CH_2ONO_2), 7.11 (d, J=8.1 Hz, 2H, Ar-H), 7.20 (d, J=8.1 Hz, 2H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.65 (–CH$\underline{C}H_3$), 22.37 (–CH($\underline{C}H_3$)$_2$), 30.15 (–$\underline{C}$H(CH_3)$_2$), 34.79 (–SCH_2–), 36.83 (–SCH_2–), 45.01 (–$\underline{C}HCH_3$), 45.93 (–Ar$\underline{C}H_2$–), 63.52 (–$CO_2\underline{C}H_2$–), 70.58 (–CH_2ONO_2), 127.52 (Ar-C), 129.73 (Ar-C), 136.99 (Ar-C), 141.15 (Ar-C), 150.66 (–$NHCO_2$–), 173.00 ($CH_3CH\underline{C}O$–); IR (KBr) cm^{-1}: 3248 (N–H), 2951 (C–H), 1752 (C=O), 1635 (Ar-C=C), 1279 (–NO_2), 1170 (C–O); HR-MS-ESI (*m/z*) $[M+Na]^+$ Calcd for $C_{18}H_{26}N_2NaO_6S_2$: 453.1124; Found: 453.1110 (mass accuracy: 2.43 ppm); *Anal.* Calcd for $C_{18}H_{26}N_2O_6S_2$: C 50.21, H 6.09, N 6.51; Found: C 50.54, H 6.14, N 6.47.

2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl)-carbamate (30) The intermediate indomethacin amide was obtained as a white solid (100%), from which the title compound was obtained as a pale yellow solid (10%) mp 122–124°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.39 (s, 3H, Ar-C$\underline{H}_3$), 2.98 (d, J=6.5 Hz, 2H, –SCH_2–), 3.00 (d, J=6.5 Hz, 2H, –SCH_2–), 3.84 (s, 3H, –OCH_3), 4.00 (s, 2H, Ar-C$\underline{H}_2$–), 4.47 (t, J=6.5 Hz, 2H, –CO_2CH_2–), 4.71 (t, J=6.5 Hz, 2H, –CH_2ONO_2), 6.70 (dd, J=2.0, 9.0 Hz, 1H, Ar-H), 6.89 (m, 2H, Ar-H), 7.49 (d, J=8.5 Hz, 2H, Ar-H), 7.69 (d, J=8.0 Hz, 2H, Ar-H), 7.73 (br s, 1H, –NH–); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 13.94 (Ar-$\underline{C}H_3$), 32.71 (Ar-$\underline{C}H_2$–), 35.39 (–SCH_2–), 37.33 (–SCH_2–), 56.25 (–OCH_3), 64.31 (–$CO_2\underline{C}H_2$–), 71.11 (–CH_2ONO_2), 101.64 (Ar-C), 112.04 (Ar-C), 112.33 (Ar-C), 115.55 (Ar-C), 129.66 (Ar-C), 131.01 (Ar-C), 131.38 (Ar-C), 131.71 (Ar-C), 134.23 (Ar-C), 137.14 (Ar-C), 139.91 (Ar-C), 151.73 (Ar-C), 156.65 (–N–CO–), 168.79 (–$NHCO_2$–), 170.83 (Ar$CH_2\underline{C}O$–); IR (KBr) cm^{-1}: 3241 (N–H), 2959 (C–H), 1756 (C=O), 1690 (Ar-C=C), 1281 (–NO_2), 1217 (C–O); MS *m/z* 582.1 $[M+H]^+$; HR-MS-ESI (*m/z*) $[M+Na]^+$ Calcd for $C_{24}H_{24}ClN_3NaO_8S_2$: 604.0586; Found: 604.0599 (mass accuracy: 2.15 ppm); *Anal.* Calcd for $C_{24}H_{24}ClN_3NaO_8S_2$: C 47.64, H 4.00, N 6.95; Found: C 47.00, H 3.88, N 7.07.

General Procedure for the Synthesis of NO-NSAIDs (31–32) with Amide Linkage To an ice-cold solution of **38a** (7 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (TFA, 28 mmol) and stirred for 1 h. Excess TFA and solvent was distilled off and the residue was dried in vacuum to afford the TFA salt **38b**. Separately, to an ice-cold solution of the appropriate NSAID (7 mmol) in dichloromethane (10 mL) was added oxalyl chloride (9 mmol), followed by 2 drops of DMF and stirred for 1.5 h. The intermediate NSAID acid chloride was concentrated, dried and dissolved in dichloromethane (10 mL). A solution of the above TFA salt (**38b**) in dichloromethane (10 mL) was added at 0°C to a solution of the NSAID acid chloride, followed by drop-wise addition of triethylamine (21 mmol) and stirred for 2 h. The reaction mixture was washed with water, brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (10–30% ethyl acetate in petroleum ether) to afford the target compounds **31** and **32**.

2-((2-((2-(Nitrooxy)ethyl)disulfanyl)ethyl)carbamoyl)-phenylacetate (31) The synthesis of this compound, along with spectral and analytical data, has been described in our earlier publication.[8)]

(*S*)-2-((2-(2-(6-Methoxynaphthalen-2-yl)propanamido)-ethyl)disulfanyl)ethylnitrate (32) The title compound was obtained as a colorless gum (17%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.63 (d, J=2.4 Hz, 3H, –CHC$\underline{H}_3$), 2.76–2.83 (m, 4H, –$CH_2S_2CH_2$–), 3.53 (q, J=3.6 Hz, 2H), 3.93 (q, J=4.5 Hz, 1H, –C$\underline{H}CH_3$), 3.94 (s, 3H, –OCH_3), 4.58 (t, J=3.9 Hz, 2H, –CH_2ONO_2), 5.7 (br s, 1H), 7.14–7.19 (m, 2H, Ar-H), 7.39–7.39 (m, 1H, Ar-H), 7.69–7.76 (m, 3H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 18.71 (–CH$\underline{C}H_3$), 34.89 (–SCH_2–), 38.14 (–SCH_2–), 38.91 (–$NHCH_2$–), 47.41 (–$\underline{C}HCH_3$), 55.71 (–OCH_3), 70.87 (–CH_2ONO_2), 105.98 (Ar-C), 119.65 (Ar-C), 126.62 (Ar-C), 128.01 (Ar-C), 129.34 (Ar-C), 129.55 (Ar-C), 134.15 (Ar-C), 136.51 (Ar-C), 158.18 (Ar-C), 174.93 (–CO–); IR (NaCl) cm^{-1}: 3299 (N–H), 2936 (C–H), 1645 (C=O), 1546 (Ar-C=C), 1278 (–NO_2), 1216 (C–O); MS *m/z* 410.99 $[M+H]^+$; HR-MS-ESI (*m/z*) $[M+H]^+$ Calcd for $C_{18}H_{23}N_2O_5S_2$: 411.1043; Found: 411.1031 (mass accuracy: 2.79 ppm); *Anal.* Calcd for $C_{18}H_{22}N_2O_5S_2$: C 52.66, H 5.40, N 6.82; Found: C 52.20, H 5.98, N 6.43.

2-((2-(2-(2-Fluoro-[1,1′-biphenyl]-4-yl)propanamido)ethyl)disulfanyl)ethylnitrate (33) To an ice-cold solution of **38a** (3.00 g, 10.24 mmol) in dichloromethane (50 mL) was added trifluoroacetic acid (3.00 mL, 40.96 mmol) and stirred for 1 h. Excess TFA and solvent was distilled off and the residue was dried in vacuum to afford the TFA salt **38b**. Separately, a solution of flurbiprofen (2.50 g, 10.24 mmol) and *N*,*N*′-carbonyldiimidazole (3.31 g, 20.48 mmol) in THF (35 mL) was stirred for 4 h, then treated with the above TFA salt **38b** and triethylamine (4.30 mL, 30.72 mmol). This mixture was stirred for 16 h and concentrated under vacuum. The residue was dissolved in ethyl acetate, washed with sat. $NaHCO_3$ solution and brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (25% dichloromethane in petroleum ether) to afford **33** as a white solid (1.50 g, 34%). mp 48–50°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.55 (d, J=6.9 Hz, 3H, –CH_3), 2.82 (t, J=6.3 Hz, 2H, –SCH_2–), 2.92 (t, J=6.9 Hz, 2H, –SCH_2–), 3.58 (m, 2H, –$NHC\underline{H}_2$–), 4.67 (t, J=6.6 Hz, 2H, –CH_2ONO_2), 5.8 (br s, 1H, –NH–), 7.14 (t, J=6.9 Hz, 2H, Ar-H), 7.35–7.55 (m, 6H, Ar-H); ^{13}C-NMR (75 MHz, $CDCl_3$)

δ: 17.94 ($-CH_3$), 34.17 ($-SCH_2-$), 37.31 ($-SCH_2-$), 37.98 ($-NHCH_2-$), 46.13 (–CH–), 70.06 ($-CH_2ONO_2$), 114.66 (Ar-C), 114.97 (Ar-C), 123.14 (Ar-C), 127.28 (Ar-C), 128.00 (Ar-C), 128.41 (Ar-C), 130.64 (Ar-C), 134.80 (Ar-C), 141.90 (Ar-C), 142.01 (Ar-C), 159.22 (Ar-C), 157.12 (Ar-C), 173.17 (–CO–); IR (KBr) cm^{-1}: 3267 (N–H), 2969 (C–H), 1752 (C=O), 1627 (Ar-C=C), 1281 ($-NO_2$), 1231 (C–O); HR-MS-ESI (*m/z*) $[M+H]^+$ Calcd for $C_{19}H_{22}FN_2O_4S_2$: 425.1000; Found: 425.0994 (mass accuracy: 1.41 ppm); *Anal.* Calcd for $C_{19}H_{21}FN_2O_4S_2$: C 53.76, H 4.99, N 6.60, S 15.11; Found: C 53.89, H 5.18, N 6.03, S 15.35.

Biological Methods. Animals For all our animal studies, male Sprague-Dawley (SD) and Wistar rats (175–325 g) were procured from the animal facility, Piramal Life Sciences Limited, Mumbai, India. The animals were fed with normal pellet diet and water *ad libitum*, and maintained in standard environmental conditions (temperature 22±2°C, humidity 60±5%, and 12 h light/12 h dark cycle). Rats were housed in a group of three per cage and acclimatized to the laboratory condition one week prior to experiments. All the study protocols were approved by Institutional Animal Ethics Committee (IAEC), and performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Government of India.

Materials Carrageenan (λ-Carrageenan, Type IV) was purchased from Sigma Chemicals, St. Louis, MO, U.S.A.

Dose Preparation NSAIDs and equivalent doses of NO-NSAIDs were weighed and solution formulations were prepared in 100% polyethylene glycol (PEG400). These solution formulations were used for the pharmacokinetics and pharmcodynamic studies. Since PEG400 is reported to possess gastro-protective effect,[38] suspension formulations of drugs were prepared in 0.5% (w/v) carboxymethylcellulose (CMC) containing 1% (v/v) Tween80 for gastric ulcerogenic studies and for estimation of gastric mucosal PGE_2 levels. The drug formulations were prepared freshly just before dosing and were vortexed thoroughly before administration to each animal. Carrageenan (1%) was suspended in physiological saline and a uniform solution was achieved by warming (58°C) with constant agitation for 1 h.

Biological Studies. Oral Bioavailability Study The pharmacokinetics studies of NO-NSAIDs were carried out in SD rats. Aspirin (150 mg/kg), sulindac (100 mg/kg), diclofenac, naproxen, flurbiprofen, ketoprofen and indomethacin (each at 10 mg/kg) or equimolar doses of respective NO-NSAIDs were administered orally to overnight fasted rats. Blood was collected in heparinized micro-centrifuge tubes from retro-orbital plexus under isoflurane anesthesia (Animal Anesthesia Unit, Surgivet Inc. Waukesha, WI, U.S.A.) at different time points and plasma was obtained by centrifugation at 10000 rpm for 10 min at 4°C. The plasma samples were processed with acetonitrile and were further centrifuged at 10000 rpm for 10 min at 4°C to remove plasma proteins. The supernatant samples obtained were analyzed by HPLC (Waters 2695 Separations Module with 2996 Photodiode Array Detector; Waters Corp., Milford, MA, U.S.A.).

Anti-inflammatory Assay To assess the anti-inflammatory activity of compounds, the rat paw edema model was used, as previously reported.[39] Briefly, right hind paw of SD rats was marked with an indelible ink marker at the lateral malleolus of the ankle and dipped up to the mark in water displacement cylinder of plethysmometer (Letika, Model No. LE7500) and basal paw volume was recorded. Aspirin (150 mg/kg), diclofenac, naproxen, flurbiprofen (each at 10 mg/kg) and ketoprofen (15 mg/kg) or equimolar doses of respective NO-NSAIDs (exception: NO-flurbiprofen at 20 mg/kg equivalent (eq.) and NO-ketoprofen at 30 mg/kg eq.) were administered orally to overnight fasted rats. One hour after compound administration, carrageenan (100 μL, 1% w/v) suspension was injected intra-plantarly in the right hind paw. Paw volume were measured at 4 and 6 h post injection. Change in paw volume was obtained by subtracting basal paw volume from the post-injection paw volumes at specified time points and the percent inhibition of paw edema was calculated.

Analgesic Assay Analgesic activity was evaluated in carrageenan-induced hind paw model of inflammatory pain where threshold response to a thermal noxious stimulus was determined using Hargreaves's test.[40] Briefly, SD rats were tested for paw withdrawal latency in response to noxious thermal stimuli using a Plantar Analgesia Meter (IITC Life Sciences Inc., CA, U.S.A.). Animals were placed in Plexiglas cages with a glass floor. After 5 min of habituation, a radiant heat light source was focused onto the plantar surface of right hind paw until the animal lifted the paw away from the heat source. The basal paw withdrawal latency (in seconds) was recorded for each animal. A cut-off latency of 20 s was used to avoid tissue damage. Aspirin (150 mg/kg) or NO-aspirin compound **25** (150 mg/kg eq.) was administered orally. One hour after compound administration, carrageenan (100 μL, 1% w/v) suspension was injected intra-plantarly in the right hind paw. The paw withdrawal latency of the ipsilateral paw was measured at various time points (*i.e.*, 1, 2, 4, 6, 8, 10 h) after carrageenan injection.

Acute Gastric Ulcerogenesis Study The acute ulcerogenic potential of compounds was evaluated in SD rats according to the reported procedure.[41] Vehicle (1 mL/kg), aspirin (150 mg/kg), diclofenac, naproxen, flurbiprofen and ketoprofen (each at 100 mg/kg) or equimolar doses of respective NO-NSAIDs were administered orally to overnight fasted rats. Three hours (aspirin groups) or five hours (for other NSAID groups) later, the rats were euthanized and their stomachs were removed and opened along the greater curvature. The stomach were rinsed with physiological saline and then fixed with 2% formalin saline for 5 min, spread and pinned on paper. The images were captured using a stereomicroscope (at 0.5× magnification) attached to a digital camera (Stemi 2000, Zeiss, Germany). An observer unaware of the treatment regimen quantified the hemorrhagic ulcer lesions from the images using Image Pro Plus 5.1 software (Media Cybernetics, Bethesda, MD, U.S.A.). The total area of ulcer lesions for each stomach were measured and expressed as mm^2 and the average total lesion area for each treatment group was calculated.

Estimation of Gastric Mucosal PGE_2 Levels Vehicle, aspirin (150 mg/kg) or compound **25** (150 mg/kg eq.) was administered orally to the overnight fasted SD rats. Three hours later, rats were euthanized, stomachs were removed and mucosa (*ca.* 100 mg) was obtained by scraping the gastric tissue with the help of ice-cold glass slide. The mucosa was weighed and transferred to a tube containing 100% ethanol and 0.1 M indomethacin. The samples were then homogenized and centrifuged for 10 min at 12000 rpm at 4°C. The supernatant was collected and used for estimation of PGE_2 by EIA kit

(Cayman Chemical Co., Ann Arbor, MI, U.S.A.).[42)]

Estimation of Plasma NO_x Plasma NO_x (nitrate/nitrite) was estimated as per the Griess method described previously,[42)] with minor modifications. Briefly, aspirin (150 mg/kg), diclofenac, naproxen, flurbiprofen (each drug at 10 mg/kg) and ketoprofen (15 mg/kg) or equimolar doses of respective NO-NSAIDs (exceptions: NO-diclofenac compound **10** at 5 mg/kg eq and NO-ketoprofen compound **28** at 30 mg/kg eq) were administered orally to overnight fasted Wister rats and blood was collected in heparinized micro-centrifuge tubes from the retro-orbital plexus at various time points under light isoflurane anesthesia (Animal Anesthesia Unit, Surgivet Inc. Waukesha, WI, U.S.A.). Samples were centrifuged at 10000 rpm for 10 min at 4°C to obtain plasma that was passed through Multi-Screen filter plate with Ultracel-10 membrane (Millipore Corp. Billerica, MA, U.S.A.) for further purification. The filtrate was collected and stored at −20°C. Nitrate/nitrite, an indirect measure for plasma NO release, was estimated in aliquots of the samples by the Griess method using commercially available nitrate/nitrite assay kit (Sigma Chemicals, St. Louis, MO, U.S.A.).

Statistical Analysis Statistical analyses of data consisting of three or more groups were performed using one-way analysis of variance (one-way ANOVA) followed by *post-hoc* Dunnett's multiple comparison test, and values of $p<0.05$ were considered as significant. For data consisting of two groups, analyses were performed using student's *t*-test and values of $p<0.05$ were considered as significant. All analyses were carried out using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, U.S.A.). For data consisting of only pooled/mean values, the statistical analysis could not be performed.

***In Vitro* Metabolic Stability Studies in Biological Fluids. Preparation of Biological Fluids** Simulated Gastric Fluid (SGF): SGF was prepared according to a procedure described in Test Solution-USP. Thus, 0.20 g of sodium chloride and 0.32 g of purified pepsin (Sigma), which is derived from porcine stomach mucosa with an activity of 800 to 2500 units per mg of protein, were dissolved in 0.70 mL of hydrochloric acid and sufficient water to make 100 mL. This test solution has a pH of about 1.2 and it was utilized in further *in vitro* studies.

Rat Plasma: Rat plasma was harvested from in-house rats. Fresh blood was collected from the male rat using a retro-orbital bleeding method into a tube containing heparin (50 IU/mL blood). After collection of blood, plasma was separated from the blood by centrifugation at 9000 rpm for 5 min at 4°C. The supernatant plasma was separated and utilized for the *in vitro* experiments.

Human Plasma: Human plasma was similarly obtained by processing the blood taken from healthy human male volunteers (age group 25–35 years) who have not consumed any NSAIDS one week prior to the collection of blood. This plasma was utilized for the *in vitro* experiments.

In Vitro Metabolic Stability of Aspirin (**1**), Ester Prodrugs **9** and **12**, and Imide Prodrugs **25** and **27** in SGF, Human Plasma and Rat Plasma: The test compound solution in acetonitrile (10 μL of 100 μM solution) was dissolved in SGF or Human Plasma or Rat Plasma (990 μL). The resulting reaction mixtures were incubated at 37°C. At specified time intervals, aliquots (60 μL) were withdrawn and added to acetonitrile (200 μL) and mixed well by vortexing for 2 min. The mixture was centrifuged at 13000 rpm for 15 min at 4°C, and the supernatant analyzed by HPLC. The amounts (area percentages) of the remaining intact prodrug (if any) and the released metabolite(s) were estimated by HPLC.

***In Vitro* Studies on DTT-Mediated NO Release from NO-NSAIDs 11 and Naproxcinod (I). Preparation of Solutions** Dithiothreitol: Five milligrams of DTT was dissolved in 2 mL of acetonitrile/water (4 : 1).

Samples: One hundred micromolars solutions of test compounds were prepared in acetonitrile/water (4 : 1).

Procedure: One hundred microliters of DTT solution was added to 1 mL of 100 μM test compound solution and the pH of the mixture was adjusted to *ca.* 7.5 by adding 0.1 N $NaHCO_3$. The mixture was stirred at RT under nitrogen and the DTT mediated decomposition of test compound was monitored by HPLC. After 2 h, about 2/3 of compound **11** decomposed (Note: Naproxcinod (**I**) remained stable). At this stage, additional 50 mL of DTT solution was added followed by 0.1 N $NaHCO_3$ to adjust pH to *ca.* 7.5 and the mixture was stirred at RT under nitrogen for additional 1 h. HPLC analysis of the mixture at this stage indicated >95% decomposition of compound **11** (Note: Naproxcinod (**I**) remained stable). During this reaction period (*ca.* 3 h), while naproxcinod (**I**) remained stable, compound **11** (t_R=9.37 min) was converted into another unidentified compound (t_R=10.38 min). The resulting mixture was freeze dried. HPLC analysis of the freeze dried residue indicated mainly two unidentified peaks at t_R 9.85 min (52%) and t_R 10.33 min (48%) with no trace of compound **11** peak (t_R 9.37 min). Freeze dried naproxcinod containing mixture showed only the naproxcinod peak with no signs of DTT-mediated decomposition. The residue obtained after freeze drying was dissolved in 1 mL of PBS buffer and the liberated NO (*i.e.*, nitrate/nitrite levels) was estimated by Griess method as described above. Blank experiments (*i.e.*, without adding the test compounds) were simultaneously performed as control.

Analysis of Metabolites: This was performed by using HPLC instrument (Waters alliance), pump 2695, and PDA detector 2996 with the following chromatographic parameters: wavelength-210 nm; column, Waters X-Terra RP-18, 150×3.9 mm, 5 μm; injection volume, 25 μL; run time, 13 min. Mode of operation was linear gradient with mobile phase A: Acetonitrile and B: 0.1% TFA in water (filtered and degassed). Flow rate was 1.0 mL/min at 25°C.

Analysis of Naproxen: Compounds were analyzed at room temperature on a Waters X-Terra RP 18 column (150×3.5 mm, particle size 5 μm; Waters Corp., Milford, MA, U.S.A.) and eluted with a gradient of acetonitrile (A) and 0.1% v/v TFA in water (B) at a flow rate of 1 mL/min as per the following schedule: 0–10 min, 20% A, 10–14 min, 100% A, 14–18 min, 20% A. UV absorbance was monitored at PDA 3D Max plot wavelength.

Analysis of Flurbiprofen: Compounds were analyzed at room temperature on a Waters X-Terra RP 18 column (150×3.5 mm, particle size 5 μm; Waters Corp., Milford, MA, U.S.A.) and eluted with a gradient of acetonitrile (A) and 0.1% v/v TFA (B) in water at a flow rate of 1 mL/min as per the following schedule: 0–10 min, 30% A, 10–13 min, 100% A, 13–18 min, 30% A. UV absorbance was monitored at PDA 3D Max plot wavelength.

Analysis of Ketoprofen, Ibuprofen and Indomethacin:

Compounds were analyzed at room temperature on a Waters X-Terra RP 18 column (150×3.5 mm, particle size 5 μm; Waters Corp., Milford, MA, U.S.A.) and eluted with a gradient of acetonitrile (A) and 0.1% v/v TFA (B) in water at a flow rate of 1 mL/min as per the following schedule: 0–4 min, 25% A, 4–12 min, 85% A, 12–18 min, 25% A. UV absorbance was monitored at PDA 3D Max plot wavelength.

Analysis of Sulindac: Compounds were separated at room temperature on a Waters X-Terra RP 18 column (150×3.5 mm, particle size 5 μm; Waters Corp., Milford, MA, U.S.A.) and eluted with a gradient of acetonitrile (A) and 0.1% v/v TFA (B) in water at a flow rate of 1 mL/min as per the following schedule: 0–4 min, 15% A, 4–14 min, 80% A, 14–18 min, 15% A. UV absorbance was monitored at PDA 3D Max plot wavelength.

Analysis of Aspirin and Diclofenac: Analytical methods of aspirin and diclofenac were carried out as reported earlier.[8)]

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27) Graphical representation of oral bioavailability, anti-inflammatory activity and NO release data for naproxcinod (I) and their corresponding NSAIDs or control are presented as Fig. S1 in the supporting information.
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