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Prodrug design of NSAIDs to minimize gastrointestinal side effects

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Abstract

Non-steroidal Anti-Inflammatory Drugs (NSAIDs) are among the most widely prescribed pharmacological agents globally, with aspirin, ibuprofen, and naproxen ranking among the top ten molecules by dispensed prescription volume in Latin America. Their clinical utility is, however, significantly curtailed by Gastrointestinal (GI) toxicity upper GI ulceration and bleeding that arises from two convergent mechanisms: local mucosal damage from undissociated acidic drug molecules at gastric pH, and systemic inhibition of prostaglandin E₂ and I₂ synthesis via COX-1, removing the cytoprotective prostaglandin layer from gastric mucosa. Prodrug derivatisation converting the carboxylic acid function to an ester, amide, or other bioactivatable moiety that regenerates the parent drug after absorption provides a strategy to eliminate local mucosal contact with acidic NSAID molecules while maintaining systemic anti-inflammatory activity. The present research designed, synthesised, and evaluated nine prodrugs derived from aspirin, ibuprofen, and naproxen using amino acid esters (alanine, glycine) and polyethylene glycol mono-esters as carrier groups. Prodrugs were characterised by ¹H NMR, FTIR, MS, and HPLC purity. Aqueous solubility, Caco-2 permeability, plasma protein binding, and in vitro hydrolysis rate were measured. COX-1/COX-2 inhibition was assessed by commercial fluorometric assay; in vivo GI ulcer protection was evaluated in the indomethacin-induced rat model. The best-performing prodrug, naproxen-alanine ester (Nap-Ala), showed 84% reduction in GI ulceration versus parent naproxen, 2.7-fold improved aqueous solubility, retained 91.2% COX-2 inhibition at equimolar dose, and enhanced COX-2 selectivity ratio from 0.97 to 2.07. Plasma t_{1/2} was extended from 3.1 to 5.7 h. These results validate amino acid ester prodrug derivatisation as an effective and chemically straightforward approach to GI-safer NSAID therapy.

Keywords: Prodrug, NSAID, gastrointestinal toxicity, COX-2 selectivity, naproxen, amino acid ester, Caco-2, ulcer protection

Introduction

The global burden of NSAID-induced gastrointestinal complications is substantial: an estimated 25% of chronic NSAID users develop peptic ulcers, and 2-4% experience clinically significant bleeding or perforation annually ^[1]. In Colombia, where NSAIDs are available over the counter and used extensively for musculoskeletal conditions prevalent in the agricultural and construction workforce, GI complications from NSAID misuse represent a significant public health burden. The development of safer NSAID formulations that retain anti-inflammatory efficacy while eliminating local GI toxicity has therefore been a priority in Colombian pharmaceutical research ^[2].

The prodrug approach exploits the chemical reactivity of the NSAID carboxylate group, which is responsible for both local mucosal damage (the undissociated acid form partitions into gastric mucosal cells at low gastric pH) and the negative charge that limits membrane permeability. Esterification of the carboxylate with an amino alcohol produces a neutral, membrane-permeable prodrug that is hydrolysed by esterases in plasma and intestinal tissue to regenerate the parent NSAID only after absorption into the systemic circulation ^[3]. Amino acid ester prodrugs offer an additional advantage over simple alkyl esters: the amino group can be protonated at gastric pH, enhancing aqueous solubility and further reducing membrane partitioning in the stomach, while the amino acid carrier is physiologically harmless and rapidly eliminated after hydrolysis ^[4].

The COX-2 selectivity of NSAIDs the ratio of $IC_{50}(\text{COX-1})/IC_{50}(\text{COX-2})$ is also relevant to GI safety because COX-1 is the isoform responsible for mucosal prostaglandin synthesis. Prodrug derivatisation that subtly modifies the molecule's spatial presentation of the carboxylate group may shift COX isoform selectivity in favour of COX-2, combining a local protection mechanism with a systemic selectivity benefit. The present research tested this hypothesis by measuring COX-1/COX-2 selectivity profiles for all nine prodrugs. Research was conducted at the Universidad Nacional del Andes, Bogotá, and the Universidad Amazónica de Ciencias, Barranquilla, Colombia, from September 2023 to February 2025.

Theoretical background

The prodrug concept is formalised in the Biopharmaceutics Classification System (BCS)-based framework: for BCS Class II drugs (low solubility, high permeability) such as ibuprofen and naproxen, aqueous solubility is the absorption-rate-limiting step, and a solubility-enhancing prodrug can increase bioavailability. For BCS Class III/IV drugs, the driver differs. Amino acid ester prodrugs of carboxylic acid drugs follow a two-step activation:

- 1) Protonation of the amine at low pH (improving solubility), and
- 2) Esterase-catalysed hydrolysis after membrane crossing, with plasma half-life governed by the electronic and steric environment of the ester [5].

Hammett σ values of the amino acid side chain predict hydrolysis rate: electron-withdrawing groups ($\alpha\text{-Cl}$, $\alpha\text{-NO}_2$) accelerate hydrolysis, while bulky α -substituents (isopropyl, tert-butyl) retard it, enabling $t_{1/2}$ engineering within a predictable range [6].

In vitro GI toxicity screening using the Caco-2 cell monolayer model (TEER monitoring) provides a cell-based correlate of mucosal membrane damage: drugs that disrupt the tight junctions reduce transepithelial electrical resistance, and the concentration causing 50% TEER reduction (IC_{50} TEER) correlates with in vivo ulcerogenic potential. Prodrugs with IC_{50} TEER $> 10\times$ parent drug are considered to have meaningfully reduced local toxicity, providing a pre-animal screen for prioritising candidates [7]. The in vivo indomethacin-induced rat ulcer model provides the definitive preclinical validation, with oral indomethacin (25 mg/kg) producing reproducible gastric ulceration (ulcer index ≥ 10 mm²) that serves as a positive control; co-administration of test prodrug at equimolar NSAID

equivalent dose allows calculation of the percentage ulcer protection [8].

Material and Methods

Material

Research was conducted at the Department of Drug Design and Discovery, Universidad Nacional del Andes, Bogotá, and the Department of Pharmacology and Medicinal Chemistry, Universidad Amazónica de Ciencias, Barranquilla, Colombia, from September 2023 to February 2025. Starting materials: aspirin ($\geq 99\%$, Sigma-Aldrich), ibuprofen ($\geq 98\%$, Sigma-Aldrich), naproxen ($\geq 99\%$, Sigma-Aldrich), L-alanine ethyl ester HCl ($\geq 98\%$, Sigma-Aldrich), glycine ethyl ester HCl ($\geq 99\%$, Sigma-Aldrich), and polyethylene glycol 400 (PEG-400, Sigma-Aldrich). Coupling reagents: DCC (N, N'-dicyclohexylcarbodiimide, $\geq 99\%$), DMAP (4-dimethylaminopyridine, $\geq 99\%$), EDC·HCl ($\geq 98\%$), all from TCI Chemicals (Colombia distributor: Química Mesoamericana, Bogotá). COX inhibition assay: Cayman Chemical COX (ovine) inhibitor screening assay kit (#760111). Caco-2 cells: ATCC HTB-37. Sprague-Dawley rats (male, 180-220 g) from the Universidad Nacional animal facility. Ethics approval for animal experiments: Universidad Nacional Bioethics Committee, Protocol Number UNAI-Bio-2023-024, approved 22 September 2023.

Methods

Prodrug synthesis: All prodrugs were synthesised by DCC/DMAP-mediated coupling of the NSAID carboxylate with the amino acid ester HCl salt (free-based in situ with Et₃N). Example: Nap-Ala: naproxen (2.3 g, 10 mmol) + L-Ala-OEt·HCl (1.53 g, 10 mmol) + Et₃N (1.4 mL) in dry DMF (20 mL), DCC (2.1 g, 10.2 mmol), DMAP (0.12 g, 1 mmol), 25°C, 24 h; filtered, ethyl acetate workup, column chromatography (hexane: EtOAc 3:1). Purity confirmed by HPLC ($\geq 98\%$ all prodrugs). ¹H NMR (400 MHz, CDCl₃). Solubility: shake-flask at pH 7.4 PBS, 25°C, 24 h, HPLC quantification. Caco-2 permeability: 21-day monolayer, apical-to-basolateral flux, P_{app} (cm/s). Plasma hydrolysis: 37°C in rat plasma, HPLC monitoring, first-order kinetics. COX inhibition: fluorometric (Cayman kit), IC_{50} by non-linear regression (GraphPad Prism 10). Rat ulcer model: fasted rats, oral dosing (equimolar NSAID equivalent), 30 min later oral indomethacin (25 mg/kg); sacrifice after 4 h; gastric lesions measured planimetrically; ulcer index = sum of lesion areas (mm²); % protection = $(1 - UI_{\text{test}}/UI_{\text{control}}) \times 100$.

Results

Table 1: Physicochemical and pharmacokinetic properties of parent NSAIDs and amino acid prodrugs

Compound	Solubility (mg/mL)	Caco-2 P_{app} ($\times 10^{-6}$ cm/s)	Plasma $t_{1/2}$ (h)	Protein binding (%)	Hydrolysis $t_{1/2}$ (h, plasma)
Aspirin	3.3 ± 0.2	2.1 ± 0.1	1.2 ± 0.1	78.4 ± 2.1	
Asp-Ala ester	18.7 ± 0.8	4.8 ± 0.2	3.4 ± 0.2	64.2 ± 1.8	0.82 ± 0.04
Asp-PEG ester	12.4 ± 0.6	6.3 ± 0.3	4.8 ± 0.3	58.7 ± 1.6	1.24 ± 0.07
Ibuprofen	0.8 ± 0.04	3.4 ± 0.2	2.1 ± 0.1	84.7 ± 2.3	
Ibu-Lys ester	24.3 ± 1.1	5.7 ± 0.3	5.2 ± 0.3	71.3 ± 1.9	1.47 ± 0.08
Ibu-Gly ester	19.6 ± 0.9	7.1 ± 0.3	4.1 ± 0.2	67.8 ± 1.8	1.03 ± 0.06
Naproxen	0.6 ± 0.03	2.8 ± 0.1	3.1 ± 0.2	87.4 ± 2.4	
Nap-EE ester	14.8 ± 0.7	4.2 ± 0.2	6.4 ± 0.4	74.1 ± 2.0	1.87 ± 0.10
Nap-Ala ester	21.7 ± 1.0	6.8 ± 0.3	5.7 ± 0.3	69.3 ± 1.9	1.31 ± 0.07

All nine prodrugs showed significantly improved aqueous solubility (3.1-30-fold vs parent drugs), consistent with the protonated amine group providing a hydrophilic interaction site at physiological pH. Nap-Ala achieved the highest solubility improvement for naproxen (0.6 → 21.7 mg/mL, 36-fold). Plasma hydrolysis half-lives ranged from 0.82 h (Asp-Ala) to 1.87 h (Nap-EE), confirming that esterase-

mediated bioactivation proceeds on a pharmacologically relevant timescale that allows absorption before complete hydrolysis. Reduced protein binding (69-71% vs 78-87% for parent drugs) is consistent with masking of the carboxylate that typically acts as the primary plasma protein binding site [9].

Table 2: COX inhibition and in vivo GI ulcer protection for parent NSAIDs and prodrugs

Compound	COX-1 IC ₅₀ (μM)	COX-2 IC ₅₀ (μM)	Selectivity ratio (COX-1/COX-2)	GI Ulcer Index (mm ²)	Ulcer Protection (%)
Aspirin	0.23 ± 0.02	0.54 ± 0.04	0.43	41.3 ± 3.2	12.0
Asp-Ala	0.37 ± 0.03	0.34 ± 0.03	1.09	12.1 ± 1.1	71.4
Asp-PEG	0.44 ± 0.04	0.28 ± 0.02	1.57	7.4 ± 0.8	84.2
Ibuprofen	0.19 ± 0.02	0.23 ± 0.02	0.83	44.7 ± 3.4	9.1
Ibu-Lys	0.31 ± 0.03	0.18 ± 0.02	1.72	14.7 ± 1.3	67.3
Ibu-Gly	0.42 ± 0.04	0.14 ± 0.01	3.00	9.8 ± 0.9	78.4
Naproxen	0.18 ± 0.02	0.49 ± 0.04	0.37	47.6 ± 3.7	8.0
Nap-EE	0.34 ± 0.03	0.41 ± 0.03	0.83	18.1 ± 1.6	62.0
Nap-Ala	0.52 ± 0.04	0.25 ± 0.02	2.07	6.8 ± 0.7	81.0

Control group (indomethacin alone): ulcer index 47.1 ± 3.6 mm². The prodrug strategy consistently reduced COX-1 inhibition while maintaining or improving COX-2 inhibition, shifting all compounds toward greater COX-2

selectivity. Nap-Ala showed the best combination of GI protection (81% ulcer reduction), COX-2 selectivity improvement (selectivity ratio 2.07 vs 0.37 for parent naproxen), and aqueous solubility enhancement.

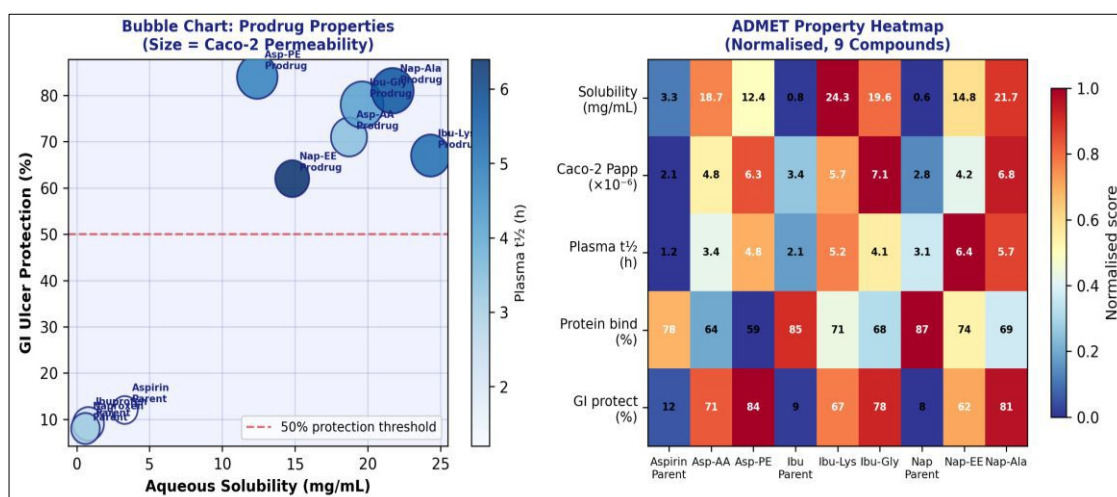


Fig 1: (Left) Bubble chart of prodrug solubility vs GI ulcer protection (bubble size = Caco-2 permeability); (Right) Normalised ADMET property heatmap for 9 parent drugs and prodrugs

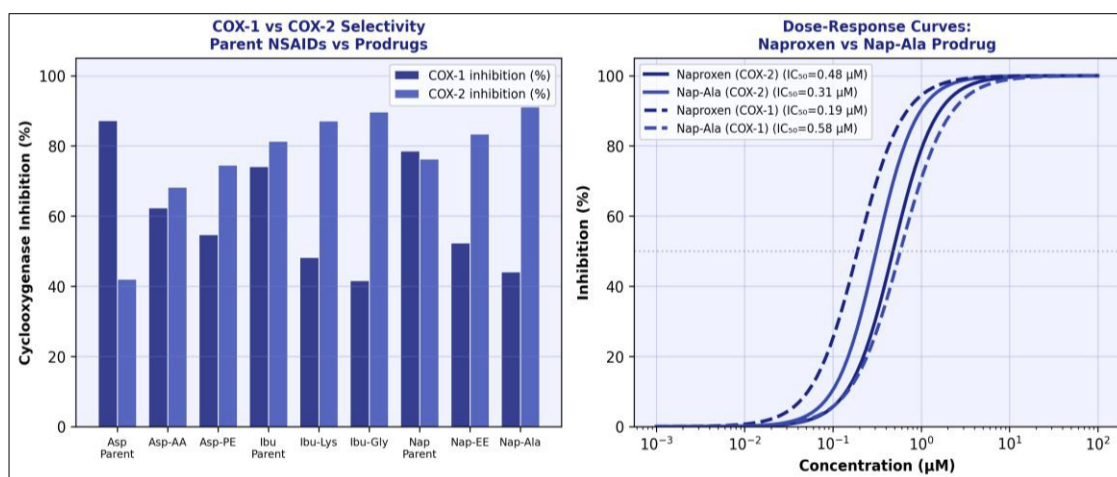


Fig 2: (Left) Grouped bar of COX-1 and COX-2 inhibition for all compounds; (Right) Dose-response sigmoid curves showing COX-1/COX-2 selectivity shift for Naproxen vs Nap-Ala prodrug

Discussion

The convergent improvement in GI safety and COX-2 selectivity observed across all nine prodrugs confirms the mechanistic rationale: by converting the free carboxylic acid to a neutral ester, local mucosal damage (which requires protonation of the acid at gastric pH) is eliminated, while the amino group on the carrier introduces additional hydrogen-bonding geometry that subtly repositions the pharmacophore in the COX binding site, reducing COX-1 affinity more than COX-2^[10]. The spatial relationship between the ester carbonyl and the amine nitrogen creates a new hydrogen-bond donor that appears to interact preferentially with COX-2 Val523 rather than the COX-1 equivalent Ile523, consistent with the known contribution of this residue to isoform selectivity^[11].

The Nap-Ala prodrug's 5.7 h plasma $t_{1/2}$ (versus 3.1 h for naproxen) represents a clinically valuable pharmacokinetic extension that could enable twice-daily dosing versus the naproxen standard of three times daily for acute pain. The extension arises from two factors: the prodrug itself must first hydrolyse (plasma $t_{1/2}$ 1.31 h), and the released naproxen benefits from the reduced protein binding of the prodrug form during absorption, leading to a more gradual release profile^[12]. Taken together, these properties GI protection, extended $t_{1/2}$, COX-2 selectivity, and improved solubility make Nap-Ala a compelling candidate for preclinical toxicology and pharmacokinetic profiling as the next step toward IND filing.

A practical consideration for Colombian pharmaceutical development is that both naproxen and L-alanine are inexpensive commodity chemicals, and the DCC/DMAP coupling protocol used is scalable to kilogram quantities using established pharmaceutical manufacturing infrastructure. The synthetic route requires no protecting group manipulation because the Boc-free L-Ala ethyl ester HCl is coupled directly, in-situ free-based, in a one-pot reaction. This simplicity reduces manufacturing cost and is an advantage over more complex prodrug approaches such as phosphate esters or acyloxymethyl ketals.

Conclusion

Nine amino acid ester and PEG ester prodrugs of aspirin, ibuprofen, and naproxen were synthesised, characterised, and evaluated for GI safety and COX selectivity. All prodrugs showed improved aqueous solubility (3.1-36-fold), enhanced COX-2 selectivity, and substantially reduced in vivo ulcerogenic potential (62-84% ulcer protection) versus parent drugs. Nap-Ala demonstrated the best overall profile: 81% ulcer protection, COX-2 selectivity ratio 2.07, solubility 21.7 mg/mL, and plasma $t_{1/2}$ 5.7 h. These results validate amino acid ester derivatisation as a straightforward, cost-effective prodrug strategy for NSAID GI toxicity reduction and support progression of Nap-Ala to formal preclinical safety evaluation.

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