Full Paper

Aminocarbonyloxymethyl Ester Prodrugs of Flufenamic Acid and Diclofenac: Suppressing the Rearrangement Pathway in Aqueous Media

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Aminocarbonyloxymethyl ester prodrugs are known to undergo rearrangement in aqueous solutions to form the corresponding *N*-acylamine side product via an O → N intramolecular acyl transfer from the carbamate conjugate base. Novel aminocarbonyloxymethyl esters of diclofenac and flufenamic acid containing amino acid amide carriers were synthesized and evaluated as potential prodrugs displaying less ability to undergo rearrangement. These compounds were prepared in reasonable yield by a four-step synthetic method that uses the appropriate *N*-Boc-protected amino acid *N*-hydroxysuccinimide ester and secondary amine and chloromethyl chloroformate as key reactants. Their reactivity in pH 7.4 buffer and 80% human plasma at 37°C was assessed by RP-HPLC. The aminocarbonyloxymethyl esters containing a secondary carbamate group derived from amino acids such as glycine or phenylalanine were hydrolyzed quantitatively to the parent drug both in non-enzymatic and enzymatic conditions, with no rearrangement product being detected. The oral bioavailability in rats was determined for selected diclofenac derivatives. These derivatives displayed a bioavailability of 25 to 68% relative to that of diclofenac, probably due to their poor aqueous solubility and lipophilicity. These results suggest that further optimization of aminocarbonyloxymethyl esters as potential prodrugs for non-steroidal anti-inflammatory drugs require the use of amino acid carriers with ionizable groups to improve aqueous solubility.

Keywords: Aminocarbonyloxymethyl Esters / Bioavailability / NSAIDs / Prodrugs

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of rheumatic and inflammatory conditions [1]. The pharmacological activity of NSAIDs is related to the blocking of prostaglandin H_2 (PGH_2) biosynthesis from arachidonic acid by inhibiting the activity of cyclooxygenases (COXs) [2]. The COX enzyme exists in two isoforms: a constitutive isoform, COX-1, found in most tissues including stomach, kidney, and platelets, and an inducible isoform, COX-2, expressed at the site of inflammation [3, 4]. Classical NSAIDs, such as ibuprofen, flufenamic acid, diclofenac, and aspirin, preferentially inhibit COX-1 [5, 6], thus suppressing the biosynthesis of prostaglandins that maintain gastric mucosal integrity and leading to gastrointestinal (GI) side effects [6], including ulceration and hemorrhage. Moreover, the carboxylic acid function present in most classical NSAIDs can also impart local GI irritation, called direct contact effect [7 – 9]. Since the introduction of spe-

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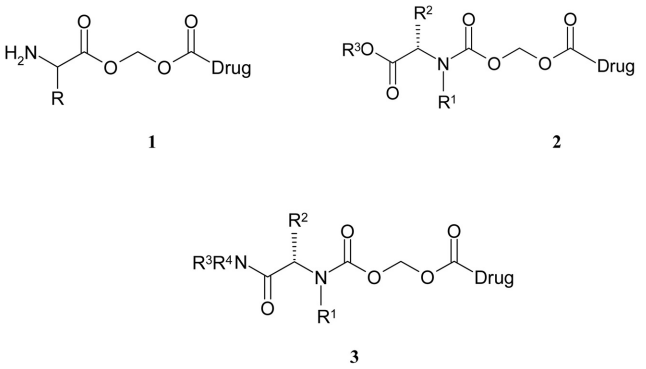
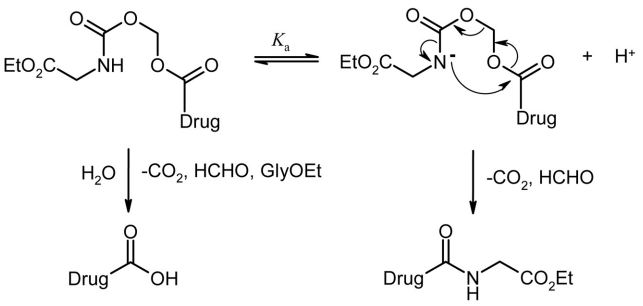


Figure 1. Molecular structures of acyloxymethyl-type derivatives **1** and aminocarbonyloxymethyl derivatives **2** and **3**.

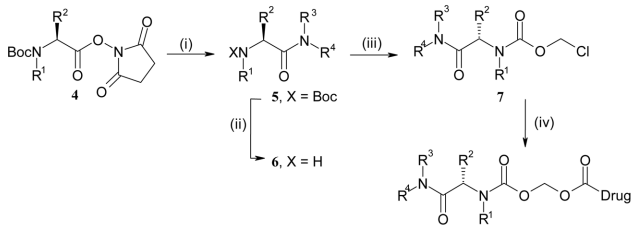
cific COX-2 inhibitors, which are less harmful to the GI tract [6 – 10], the use of conventional NSAIDs has declined. However, the safety profile of COX-2 inhibitors has been questioned due to the risk of ulcer complications in high-risk individuals [11, 12] and to cardiovascular adverse effects that, ultimately, lead to high drop-out rates [13 – 15]. Thus, the need for NSAIDs with improved GI tolerability still exists.

One approach that has been used to decrease NSAID-induced GI toxicity without adversely affecting their anti-inflammatory activity is to mask the carboxylic acid group by synthesizing the corresponding ester prodrugs [7 – 9]. A major requisite for these prodrugs is that they must be readily hydrolyzed, enzymatically or chemically, after oral absorption to quantitatively release the parent drug [16]. In addition, the pro-moiety should be non-toxic and readily excreted. Since they offer enormous potential in terms of low toxicity, amino acids have been considered the ideal carriers for the development of prodrugs. An obvious approach for carboxylic acids prodrugs that contain an α-amino acid carrier is to prepare acyloxymethyl-type derivatives, *e.g.* **1** (Fig. 1) [17]. The presence of the basic α-amino group has been reported to improve the water-solubility of prodrugs **1**, but unfortunately, usually leads to chemically unstable compounds [17, 18]. This is due to the strong electron-withdrawing effect of the protonated amino group at physiological pH and to the ability of the unprotonated amino group to enhance hydrolysis via nucleophilic or general-base catalysis [16] and to undergo intramolecular rearrangement to form an amide [17].

Recently, we synthesized a series of aminocarbonyloxymethyl esters, **2**, (Fig. 1) designed as carboxylic acid prodrugs [19]. Conceptually, this approach is based on the low chemical reactivity of carbamates in neutral aqueous media [20] and corresponds to an inversion of the amino acid position in the potentially unstable counterparts **1**.



Scheme 1. Rearrangements of several prodrugs **2** in pH, 7.4 buffer to form the corresponding *N*-acylamines.



Reagents: (i) NHR^3R^4, DCM; (ii) CF_3CO_2H/DCM (1 : 1); (iii) **6**, $ClCO_2CH_2Cl$, TEA, DCM; (iv) flufenamic acid (sodium salt) or diclofenac (sodium salt), TEA, TBAB, DCM.

	R^1	R^2	R^3	R^4
(a)	H	$PhCH_2$	$-(CH_2)_5-$	
(b)	H	$PhCH_2$	Et	Et
(c)	$-(CH_2)_3-$		Et	Et
(d)	Me	H	Et	Et
(e)	H	H	Et	Et
(f)	H	$PhCH_2$	$-(CH_2)_2O(CH_2)_2-$	
(g)	H	H	Et	Et
(h)	H	$PhCH_2$	Et	Et

Scheme 2. Preparation of aminocarbonyloxymethyl derivatives **3**.

Prodrugs **2** are readily hydrolyzed to the parent compound in human plasma while displaying reasonable stability in aqueous solutions. However, several of the prodrugs **2** that contain a secondary carbamate group, particularly those containing glycine (**2**, $R^1 = R^2 = H$), undergo rearrangement in pH 7.4 buffer, to form the corresponding *N*-acylamine most probably via an O → N intramolecular acyl transfer from the carbamate conjugate base (Scheme 1) [19]. This rearrangement pathway is significantly reduced or suppressed when sterically-hindered aminoacids (*e.g.* Phe or Val and *N*-methylglycine (sarcosine)) are used as carriers. To further evaluate the potential of aminocarbonyloxymethyl esters as prodrugs for NSAIDs, we have prepared a series of aminocarbonyloxymethyl derivatives **3** of flufenamic acid and diclofenac, in which amino acid amides are used as carriers (Scheme 2). The replacement of the amino acid ester by

an amino acid amide is expected to decrease the carbamate pK_a by ca. 1.2 units [21] and thus suppress the O → N rearrangements compared with **2**. We have also carried out a kinetic study that evaluates the influence of the amino acid amide pro-moiety on the chemical reactivity and stability of aminocarbonyloxymethyl prodrugs of flufenamic acid and diclofenac **3** in human plasma. Since the oral bioavailability of diclofenac is only 50% [22], we have compared the bioavailability of several diclofenac derivatives **2** and **3** with diclofenac itself.

Results and discussion

Synthesis

The target prodrugs **3** were synthesised via the corresponding amino acid amide derivatives **6** (Scheme 2). The *N*-Boc protected *N*-hydroxysuccinimidyl amino acid ester **4** reacted with the appropriate amine to afford the corresponding *N*-Boc protected derivatives **5** in yields of 70–90%. These latter derivatives, **5a–e**, upon treatment with trifluoroacetic acid (TFA) to remove the Boc-protecting group, gave as oils the deprotected amino acid derivatives **6** isolated in 90–95% yields. Compounds **6** reacted with chloromethyl chloroformate in dichloromethane (DCM), in the presence of the phase-transfer catalyst tetrabutylammonium (Bu_4NBr, TBAB) and triethylamine, to form the corresponding chloromethyl intermediates **7** in about 50% yield (Scheme 2). Finally, reaction of **7** with the appropriate sodium salt of diclofenac or flufenamic acid in DCM formed the target prodrug **3** in variable yield ranging from 20% to 50%. The structure of compounds **3a–h** follows from their spectroscopic and analytical data. In particular, the ^{1}H-NMR spectra of the derivatives **3a, 3b, 3f**, and **3h** revealed by the diastereotopic nature of the CH_2Ph protons which resonate as doublet of doublets at approx. 3.3 ppm. The diastereotopic nature of the OCH_2O was also evident for compounds **3b** and **3h** as revealed by the presence of a pair of doublets at approx. 6.0 ppm. In the proline derivative **3c** the OCH_2O signal appears as two pairs of doublets, reflecting the diastereotopic nature of the methylene protons as well as the presence of *syn*- and *anti*-rotamers. The spectroscopic detection of rotamers has been reported for other prolyl carbamate frameworks, similar to the barrier of acyclic tertiary amides [23, 24].

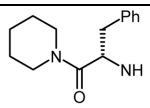
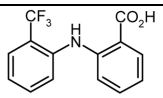
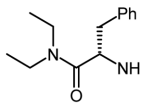
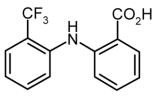
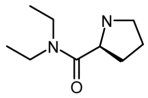
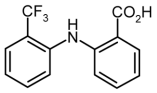
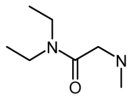
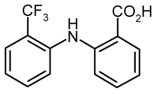
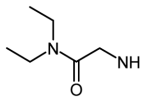
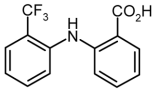
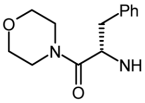
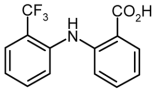
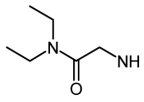
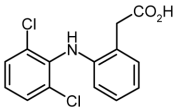
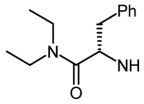
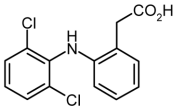
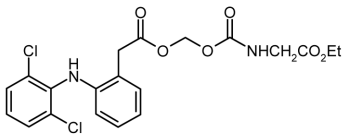
Products and kinetics of chemical hydrolysis

The hydrolysis of *N*-aminocarbonyloxymethyl ester prodrugs **3** proceeds with quantitative formation of the parent carboxylic acid at pH 7.4. As we had anticipated, and in contrast to their ester counterparts **2**, no *N*-acyl amino acid derivatives were detected during the hydrolysis of secondary carbamates **3a**, **b**, **d–h**. Thus, compounds **3** do not undergo the rearrangement involving an O → N intramolecular acyl transfer from the carbamate conjugate base (Scheme 1) that can be observed for compounds **2**. Not surprisingly, the hydrolysis of tertiary carbamates **3c**, **d**, which lack an ionisable carbamate NH, also proceeded with quantitative formation of the parent carboxylic acid.

The hydrolysis of compounds **3a–h** was studied in isotonic pH 7.4 phosphate buffer at 37°C, and the corresponding half-lives are given in Table 1. Inspection of Table 1 shows that compounds **3** are very stable in pH 7.4 buffer, hydrolyzing with half-lives ranging between 10 h and 16 d. This represents a major increase in chemical stability over their ester counterparts **2**. For example, the diclofenac *N*,*N*-diethylamide derivative **3g** is hydrolyzed about 20 times more slowly than its ethyl ester counterpart **2a**. A similar effect is observed for the flufenamic acid derivative **3e**, which is hydrolyzed approx. nine times more slowly than the corresponding ethyl ester prodrug [19]. The large differences in chemical reactivity between the amino acid ester **2** and amide **3** derivatives, can be ascribed to differences both in the electronic (*i.e.* pK_a) and steric properties of the terminal amino acid amide and ester moieties, which are likely to affect both the rearrangement and hydrolysis pathways. For example, the carbamate group of compound **3g** is expected to be about 2 pK_a units less acidic than its counterpart **2a**, based on the inductive effects of the substituent on the carbamate NH group [21; see section 3.8]. The rearrangement pathway finds some parallel to the E1cb elimination-addition mechanism operating in the hydrolysis of secondary carbamates in that it also involves the formation of the carbamate conjugate base [20]. However, the effect exerted by the *N*-substituents of secondary carbamates, *e.g.* **8** (Fig. 2), on the rate of alkaline hydrolysis is not large, as indicated by the Hammett ρ value of 0.64 [20], thus suggesting that the estimated drop in the pK_a of **3** relatively to that of **2** should reduce the rate of rearrangement; however, it is unlikely this effect could be enough to suppress completely this pathway.

Another major difference between amino acid amides **3** and their amino acid ester counterparts **2** is the size of the amino acid C-terminal substituent. In the absence of volume descriptors for the $CONR^3R^4$ amide in **3**, we have determined the molecular refractivity (MR) and molar volume (MV) for $CH_3CONR^3R^4$ using the ACDlabs ChemSketch 8.0 freeware [25], and compared it with that of $CH_3CO_2CH_2CH_3$. The calculated MR and MV values are 35.83 and 128.7 cm^3 for the piperidine amide, 33.58 and 131.9 cm^3 for the *N*,*N*-diethylamide, and 22.35 and

Table 1. Kinetic data for the hydrolysis of compounds **3a – 3h** and the corresponding ester **2a** in pH 7.4 isotonic phosphate buffer and in 80% human plasma at 37°C, together with their apparent partition coefficients at pH 7.4 (Log P_{app}; mean ± SD, n = 3).

Compound	NR^3R^4	R^3CO_2H	pH 7.4 phosphate buffer	80% Human plasma	Log P_{app}
			$t_{1/2}$ (h)	$t_{1/2}$ (min)	
3a			164	434	4.22 ± 0.01
3b			160	301	4.06± 0.34
3c			385	462	4.00 ± 0.29
3d			124	26	3.65 ± 0.07
3e			160	16.6	[a]
3f			157	126	3.79 ± 0.16
3g			13.3	3.77	3.84 ± 0.05
3h			22.6	26.9	[a]
2a			0.98	4.17	4.16 ± 0.15

[a] Not determined.

98.0 cm^3 for the ethyl ester. These results imply that the amide groups in **3** are significantly bulkier than the ethyl ester moiety in **2**, thus suggesting that this effect might significantly contribute to the suppression of the rearrangement pathway for prodrugs **3**.

Quite interestingly, the glycine derivative **3e**, which is a secondary carbamate, hydrolyzes essentially at the same rate as its tertiary carbamate (sarcosine) counterpart **3d**. This contrasts with the observed differences between the rates of alkaline hydrolysis of secondary

(E1cb mechanism) and tertiary carbamates (classical addition-elimination mechanism). For example, blocking the carbamate acidic NH proton, *e.g.* by methyl substitution, usually leads to dramatic reductions in the rate of alkaline hydrolysis, typically by a factor of 10^3 to 10^8 [26]. Therefore, the absence of any reduction in reactivity of the sarcosine derivative **3d** when compared to its glycine counterpart **3e** is also consistent with the absence of the rearrangement pathway.

The rates of hydrolysis for the flufenamic acid derivatives at pH 7.4 are seven to twelve times smaller than those of the diclofenac counterparts (*e.g.* **3e** versus **3g**), reflecting the steric hindrance exerted by the *ortho*-anilino substituent of flufenamic acid. The nature of the amino acid pro-moiety also affects the rate of hydrolysis at pH 7.4, but to a lesser extent than the ester moiety. For example, the phenylalanine piperidine **3a** and diethylamide **3b** hydrolyze at the same rate as their glycine counterpart **3e**. In contrast, the proline derivative **3c** is hydrolyzed ca. three times more slowly than the sarcosine derivative **3d**, a result that can be accounted in terms of the steric crowding exerted by the bulky pyrrolidine ring.

Hydrolysis in human plasma

Compounds **3** are quantitatively hydrolyzed to the corresponding parent carboxylic acid in human plasma, with first-order kinetics for at least four half-lives. As with the hydrolysis in pH 7.4 buffer, no traceable amounts of the rearrangement product were detected for any compound, which contrasts with the formation of ca. 20% of rearrangement product for the human plasma hydrolysis of the closely related secondary *N*-acyloxyalkoxycarbonyl derivatives of aniline **9** (R = Ph) but compares with that of the benzylamine counterpart **9** (R = CH_2Ph) (Fig. 2), which hydrolyzed in human plasma almost exclusively to the parent amine [27].

Human plasma enzymes accelerated the rate of hydrolysis of aminocarbonyloxymethyl esters **3**, as revealed by the half-lives for hydrolysis in 80% human plasma ranging from 4 min to 7 h (Table 1). Reactivity in human plasma appears to be dependent on the nature of substituents in both the amino acid carrier and the parent carboxylic acid. For example, the glycine derivative of flufenamic acid **3e** is hydrolyzed about 18 times faster than the more sterically hindered phenylalanine counterpart **3b**. Similarly, compound **3g**, the glycine derivative of diclofenac, is hydrolyzed approximately seven times faster than the phenylalanine counterpart **3h**. The diclofenac derivatives hydrolyze four to ten times faster than their flufenamic acid analogues, reflecting the sterical hindrance exerted by the *ortho*-anilino substituent on the latter compounds.

8 9

Figure 2. Molecular structure of secondary carbamates **8** and *N*-acyloxyalkoxycarbonyl derivatives of aniline (R = Ph) and benzylamine (R = CH_2Ph) **9.**

Table 2. Pharmacokinetic parameters of diclofenac after oral administration of diclofenac sodium and prodrugs **2a**, **3g**, and **3h** in rats. (Mean ± SE, n = 5).

Compound	C_{max} of diclofenac (μg/mL)	AUC_{0-4h} (μg.h/mL)
Diclofenac	3.374 ± 0.63	9.069
2a	2.330 ± 0.27	6.143
3g	1.012 ± 0.22	2.219
3h	2.257 ± 0.30	5.552

Relative bioavailability studies

The plasma concentration-time curves of diclofenac after oral administration of the free drug and of the prodrugs **3g**, **3h**, and **2a** are shown in Fig. 3. Detectable concentrations of diclofenac were observed for 4 h both when free drug and prodrugs were given orally to rats. This result indicates that compounds **3** are hydrolyzed *in vivo* to the parent diclofenac and thus confirm the prodrug nature of these compounds. The peak plasma concentration C_{max} for the free drug was 3.374 μg/mL within 1 h (T_{max}), whereas for the prodrug **2a** it was 2.330 μg/mL after a period of 30 min. There was a reduction in the extent of drug absorption between the free drug and the prodrugs **2a**, **3g**, and **3h** as revealed by the area under the plasma concentration curve (AUC) (Table 2). Equimolar doses of **2a** and **3h** resulted in 68% and 61% level of plasma diclo-

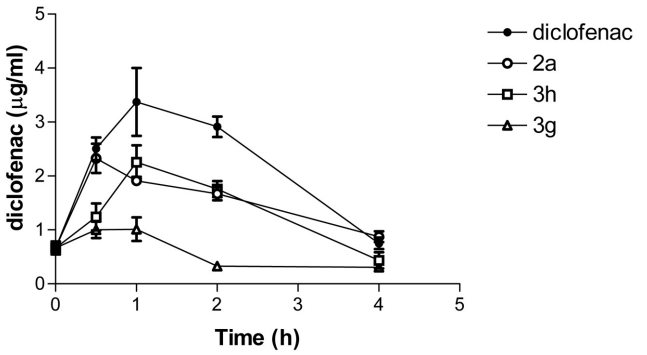


Figure 3. Mean plasma concentration-time profiles of diclofenac after oral administration of diclofenac sodium and prodrugs **2a**, **3g**, and **3h** in rats.

fenac of that from an equimolar dose of diclofenac sodium (100%). These results are comparable with previous studies with nitrosothiolesters of diclofenac, which also display a reduced oral bioavailability compared to the parent drug [28]. For prodrugs **3**, this effect might be ascribed to the high lipophilicity, as indicated by the log P_{app} values around 4 (Table 1). In conclusion, the novel aminocarbonyloxymethyl prodrugs **3** of flufenamic acid and diclofenac, containing amino acid amide pro-moieties, display an unusually high stability in pH 7.4 buffers combined with a good susceptibility to plasma-mediated regeneration to the parent drug. Moreover, the previously reported O → N intramolecular acyl transfer from the carbamate conjugate base observed with the ester counterparts **2** was completely suppressed in prodrugs **3**. The quantitative delivery of the parent drug in buffers and in human plasma suggests that the oral bioavailability can be further improved by increasing aqueous solubility using amino acid carriers with ionizable groups.

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Experimental

Materials and chemicals

Melting points were determined using a Kofler camera Bock Monoscop M (C. Reichert, Vienna, Austria) and are uncorrected. Elemental analyses were performed either by Instituto Tecnológico Nuclear, Sacavém, Portugal or by Perkin-Elmer Analyzer 2400, Department of Pharmaceutical Chemistry, University of Kuopio. FTIR spectra were recorded using a Nicolet Impact 400 spectrophotometer (Nicolet, Madison, WI, USA). The high-performance liquid chromatography (HPLC) system consisted of a Merck Hitachi L-7110 pump with a L-7400 UV detector, a manual sample injection module equipped with a 20 µL loop, a Merck LiChrospher® 100 RP_8 125 cm × 4.6 mm (5 µm) column equipped with a Merck LiChrocart pre-column (Merck, Germany). ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solutions and were performed by Bruker AM 400 WB (Bruker). Coupling constants *J* are expressed in Hz. The carboxylic acids were purchased from Sigma. All other chemicals and solvents were of reagent grade, except buffers substances and HPLC solvents which were analytical grade and LiChrosolv® grade, respectively. Column chromatography was performed using silica gel 60 mesh 70 – 230 (Merck).

General procedures for the synthesis of prodrugs 3a – 3h

Typically, the amine (2.5 mmol) was added dropwise to a stirred solution of the *N*-*t*-Boc-amino acid-*N*-hydroxysuccinimidyl ester **4** (2.5 mmol) in dichloromethane (5 mL) (Scheme 2). After 48 h at room temperature the solvent was evaporated and the residue purified by column chromatography (eluant: diethyl ether). To the protected compound **5**, a solution of trifluoroacetic acid in dichloromethane (50% w/v) was added at room temperature (4.5 mL for 1 mmol of the protected compound). After stirring for 30 min the solvent was evaporated and the residue dissolved in a solution of NaOH. The mixture was extracted with ethyl acetate, and the organic phase dried (anhydrous Na_2SO_4) and evaporated to afford the deprotected compound **6**. To a dichloromethane solution of the deprotected compound **6** was added chloromethyl chloroformate (1.1 mol eq., in dichloromethane). After stirring at −10°C during 25 min and 75 min at room temperature, the reaction mixture was filtered, the filtrate was washed with water, dried, and evaporated to obtain the corresponding chloromethylcarbamate **7**. A solution of the sodium salt of the appropriate carboxylic acid (1.1 mol eq.) and tetrabutylammonium bromide (1.1 mol equivalent) in tetrahydrofuran (5 mL) was added to a solution of the intermediate **7** in tetrahydrofuran (5 mL) and triethylamine (1.1 mol equivalent). After stirring for 24 h at room temperature, the solvent was evaporated and the resultant compound purified by column chromatography using a mixture of diethyl ether and light petroleum (40 : 60) as eluent.

*Synthesis of **3a***

*N-[N-(tert-Butoxycarbonyl)phenylalanyl]piperidine **5a***

Prepared from BocPheOSuc, **4a** (905.9 mg, 2.5 mmol) and piperidine (212.9 mg, 2.5 mmol). Yield: 86% (714.7 mg, 2.1 mmol). ^{1}H-NMR: 1.01 (1H, brs, C*H* pip), 1.41 – 1.79 (14H, m, (CH_3)$_3$ and C*H* pip), 2.95 – 3.05 (3H, m, NC*H* pip and CH_2Ph), 3.25 (1H, ddd, *J* = 18.0, 7.7, 3.4, NC*H* pip), 3.44-3.50 (2H, m, NC*H* pip), 4.85 (1H, q, *J* = 7.3, α-C*H*), 5.45 (1H, brs, *J* = 7.9, N*H*), 7.18 – 7.28 (5H, m, Ar*H*).

*N-(Phenylalanyl)piperidineamide **6a***

Yield: 95% (473.9 mg, 2.0 mmol). ^{1}H-NMR: 1.16 – 1.28 (1H, brs, C*H* pip), 1.41 – 1.61 (5H, m, CH_2 pip), 2.73 (2H, brs, NH_2), 2.86 (1H, dd, *J* = 13.2, 8.5, C*H*Ph), 2.96 (1H, 2 × dd, *J* = 13.2, 6.1, C*H*Ph), 3.11 – 3.14 (1H, m, NC*H* pip), 3.31 – 3.36 (1H, m, NC*H* pip), 3.48 – 3.60 (2H, m, NCH_2 pip), 4.10 (1H, dd, *J* = 8.0, 7.0, α-C*H*), 7.20 – 7.32 (5H, m, Ar*H*).

*N-[2-(Chloromethyloxycarbonylamino)-3-phenylpropanoyl]piperidine **7a***

To a solution of **6a** (2.0 mmol, in 5 mL CH_2Cl_2) was added chloromethyl chloroformate (2.0 mmol, 263.0 mg in 5 mL CH_2Cl_2) yielding an oil (292.1 mg, 0.9 mmol). Yield: 46%. ^{1}H-NMR: 1.05 – 1.90 (6H, m, CH_2 pip), 3.00 – 3.04 (2H, m, NCH_2 pip), 3.12 – 3.27 (2H, m, CH_2Ph), 3.46-3.52 (2H, m, CH_2 pip), 4.90 (1H, q, *J* = 7.5, α-C*H*), 5.69 (1H, d, *J* = 6.2, OC*H*Cl), 5.73 (1H, d, *J* = 6.2, OC*H*Cl), 6.13 (1H, broad d, *J* = 8.0, N*H*), 7.16 – 7.30 (5H, m, ArH).

*Compound **3a***

Prepared from **7a** (292.1 mg, 0.9 mmol) and flufenamic acid (264.3 mg, 0.9 mmol). Yield: 39% (210.8 mg, 0.4 mmol). M. p. 58 – 60°C. ν_{max}/cm^{-1}: 3315, 1732, 1710, 1629. ^{1}H-NMR: 1.36 – 1.56 (6H, m, CH_2CH_2CH_2), 2.95 – 3.04 (3H, m, NCH_2 and C*H*Ph), 3.21 – 3.26 (1H, m, C*H*Ph), 3.44 – 3.51 (2H, m, NCH_2), 4.89 and 4.90 (1H, ddd, *J* = 8.8, 8.7, 5.4, a-C*H*), 5.93 (1H, d, *J* = 8.4, N*H*), 5.96 (2H, s, OCH_2O), 6.80 – 6.82 (1H, m, Ar*H*), 7.14 – 7.50 (11H, m, Ar*H*), 8.04 – 8.06 (1H, m, Ar*H*), 9.46 (1H, s, N*H*). EIMS (%): 570.1 (100) [M$^+$]. Calculated for $C_{30}H_{30}N_3O_5F_3$: C, 63.3; H, 5.3; N, 7.4%. Found: C, 63.2; H, 5.4; N, 7.4%.

*Synthesis of **3b***

*N-[N-(tert-Butoxycarbonyl)phenylalanyl]-N,N-diethylamine **5b***

Prepared from BocPheOSuc **4a** (906.0, mg, 2.5 mmol) and diethylamine (182.8 mg, 2.5 mmol). Yield: 78% (624.9 mg, 1.9 mmol). ^{1}H-NMR: 0.99 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.10 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.40 (9H, s, (CH_3)$_3$), 2.92–3.14 (5H, m, NCH_2 and CH_2Ph), 3.5 (1H, dq, *J* = 14.0, 6.3, NC*H*) 4.73 (1H, apparent q, *J* = 7.5, α-CH), 5.37 (1H, d, *J* = 7.5, N*H*), 7.16–7.24 (5H, m, Ar*H*).

*N-(Phenylalanyl)-N,N-diethylamine **6b***

Yield: 95% (407.6 mg,1.9 mmol). ^{1}H-NMR: 1.10 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.15 (3H, t, *J* = 7.0, CH_3CH$_2$), 2.60 (2H, s, NH_2), 2.97 (2H, m, CH$_3$CH_2), 3.12 (1H, m, C*H*HPh), 3.26 (2H, m, CH$_3$CH_2), 3.52 (1H, m, CH*H*Ph), 4.10 (1H, dd, *J* = 8.7, 7.0, α-C*H*), 7.20–7.40 (5H, m, Ar*H*).

*N-[2-(Chloromethyloxycarbonylamino)-3-phenylpropanoyl]-N,N-diethylamine **7b***

To a solution of **6b** (407.6 mg in 5 mL CH_2Cl_2) was added chloromethyl chloroformate (1.9 mmol, 238.5 mg in 5 mL CH_2Cl_2) yielding an oil (309.7 mg, 1.0 mmol). Yield: 54%. ^{1}H-NMR: 1.01 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.05 (3H, t, *J* = 7.0, CH_3CH$_2$), 2.90–3.15 (5H, m, NCH*H* and CH_2Ph), 3.50–3.58 (1H, dq, *J* = 12.5, 8.0, NC*H*H), 4.78–4.81 (1H, m, α-C*H*), 5.67 (1H, d, *J* = 5.8, OC*H*Cl), 5.75 (1H, d, *J* = 5.8, OC*H*Cl), 6.09 (1H, d broad, *J* = 8.0, N*H*), 7.17-7.29 (5H, m, Ar*H*).

*Compound **3b***

Prepared from **7b** (309.67 mg, 0.99 mmol) and flufenamic acid (sodium salt) (278.4 mg, 1.0 mmol). Yield: 39% (217.5 mg, 0.4 mmol). Oil. ν_{max}/cm^{-1}: 3323, 3275, 1736, 1699, 1633. ^{1}H-NMR: 0.99 (3H, t, *J* = 7.0, CH_3CH$_2$) and 1.04 (3H, t, *J* = 7.0, CH_3CH$_2$), 2.90–3.11 (5H, m, NCH_2 and CH_2Ph), 3.49–3.57 (1H, m, NC*H*), 4.78 and 4.80 (1H, ddd, *J* = 8.53, 8.35, 6.18, a-C*H*), 5.83 (1H, d, *J* = 8.7, N*H*), 5.94 (1H, d, *J* = 5.81, OC*H*O), 5.97 (1H, d, *J* = 5.81, OC*H*O), 6.80–6.83 (1H, m, Ar*H*), 7.17–7.50 (11H, m, Ar*H*), 8.01–8.04 (1H, m, Ar*H*), 9.46 (1H, s, N*H*). EIMS (%): 558 (100) [M$^+$]. Calculated for $C_{29}H_{30}N_3O_5F_3$: C, 62.5, H, 5.4, N, 7.5%. Found: C, 62.4; H, 5.4; N, 7.5%.

*Synthesis of **3c***

*N-(tert-Butoxycarbonyl)proline diethylamide **5c***

Prepared from BocProOSuc **4b** (780.8 mg, 2.5 mmol) and diethylamine (182.8 mg, 2.5 mmol). Yield: 68% (459.6 mg, 1.7 mmol). ^{1}H-NMR: 1.10 and 1.14 (3H, 2 × t, *J* = 7.0, CH_3CH$_2$), 1.23 and 1.25 (3H, 2 × t, *J* = 7.0, CH_3CH$_2$), 1.41 and 1.45 (9H, 2 × s, (CH_3)$_3$), 1.64–2.22 (4H, m, 2 × CH_2 pro), 3.20–3.66 (6H, m, NCH_2 and CH_2 Pro), 4.44 and 4.60 (1H, 2 × dd, *J* = 4.0, 8.3, α-C*H*).

*Proline diethylamide **6c***

Yield: 95% (275.8 mg, 1.6 mmol). ^{1}H-NMR: 1.12 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.23 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.71–1.78 (1H, m, C*H* Pro), 1.85–2.05 (2H, m, CH_2 Pro), 2.25–2.32 (1H, m, C*H* Pro), 3.15 (1H, brs, N*H*), 3.27–3.44 (6H, m, CH$_3$CH_2 and NCH_2 Pro), 4.32 (1H, brs, α-C*H*).

*N-(Chloromethyloxycarbonyl)proline diethylamide **7c***

To a solution of **6c** (275.8 mg in 5 mL CH_2Cl_2) was added chloromethyl chloroformate (208.9 mg in 5 mL CH_2Cl_2) yielding an oil (170.8 mg, 0.7 mmol). Yield: 40%. ^{1}H-NMR: 1.12 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.23 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.71–1.78 (1H, m, C*H* Pro), 1.85–2.05 (2H, m, CH_2 Pro), 2.25–2.32 (1H, m, C*H* Pro), 3.27–3.44 (6H, m, CH$_3$CH_2 and NCH_2 Pro), 4.32 (1H, brs, α-C*H*), 5.95, 5.98 and 6.06 (2H, 3 × overlapping doublets, *J* = 5.6, OCH_2Cl).

*Compound **3c***

Prepared from **7c** (170.8 mg, 0.7 mmol) and flufenamic acid (sodium salt) (182.8 mg, 0.7 mmol). Yield: 37% (122.0 mg, 0.2 mmol). Oil. ν_{max}/cm^{-1}: 3325, 1717, 1687, 1644. ^{1}H-NMR: 1.01, 1.13, 1.21 and 1.28 (6H, 4 × t, *J* = 7.0, CH_3CH$_2$), 1.85–1.92 (2H, m, CH_2 Pro), 2.05–2.24 (2H, m, CH_2 Pro), 3.11–3.74 (6H, m, CH$_3$CH_2 and NCH_2 Pro), 4.55 and 4.66 (1H, 2 × dd, *J* = 8.2, 3.6, α-C*H*), 5.92, 5.93, 5.98, and 6.08 (2H, 4 × d, *J* = 5.8, OCH_2O), 6.79–6.84 (1H, m, Ar*H*), 7.25–7.49 (6H, m, Ar*H*), 8.03-8.06 (1H, m, Ar*H*), 9.42, 9.46 (1H, 2 × s, N*H*). EIMS (%): 508.1 (91) [M$^+$]. Calculated for $C_{25}H_{28}N_3O_5F_3$: C, 59.2, H, 5.7, N, 8.3%. Found: C, 59.0, H, 5.7, N, 8.3%.

*Synthesis of **3d***

*N-(tert-Butoxycarbonyl)sarcosine diethylamide **5d***

Prepared from BocSarOSuc **4c** (715.7 mg, 2.5 mmol) and diethylamine (182.8 mg, 2.5 mmol). Yield: 79% (482.6 mg, 2.0 mmol). ^{1}H-NMR: 1.13 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.19 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.43 and 1.47 (9H, 2 × s, (CH_3)$_3$), 2.93 and 2.95 (3H, 2 × s, NCH_3), 3.28-3.32 (2H, m, NCH_2CH$_3$), 3.35–3.42 (2H, m, NCH_2CH$_3$), 3.94 and 4.05 (2H, 2 × s, NCH_2CO).

*Sarcosine diethylamide **6d***

Yield: 90% (257.0 mg, 1.8 mmol). Oil ^{1}H-NMR: 1.10 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.20 (3H, t, *J* = 7.0, CH_3CH$_2$), 2.54 (3H, s, NCH_3), 3.26 (2H, q, *J* = 7.0, CH$_3$CH_2), 3.39 (2H, q, *J* = 7.0, CH$_3$CH_2), 3.58 (2H, s, NCH_2), 3.86 (1H, brs, N*H*).

*N-(Chloromethyloxycarbonylsarcosine diethylamide **7d***

To a solution of **6d** (257.0 mg in 5 mL CH_2Cl_2) was added chloromethyl chloroformate (1.8 mmol, 229.5 mg in 5 mL CH_2Cl_2) yielding an oil (421.3 mg, 1.8 mmol). Yield: 100% (syn/anti ratio = 0.5) [29, 30]. ^{1}H-NMR: 1.13 (3H, t, *J* = 7.0, CH_3CH$_2$), 1.22 (3H, t, *J* = 7.0, CH_3CH$_2$), 3.04 (3H, s, NCH_3), 3.29 (2H, q, *J* = 7.2, NCH_2), 3.39 (2H, q, *J* = 7.0, NCH_2), 4.05 and 4.11 (2H, 2 × s, COCH_2N), 5.76 and 5.79 (2H, 2 × s, OCH_2Cl).

*Compound **3d***

Prepared from **7d** (421.3 mg) and flufenamic acid (sodium salt) (500.5 mg, 0.7 mmol). Yield: 37% (317.8 mg, 0.7 mmol). Oil. ν_{max}/cm^{-1}: 3335, 1727, 1697, 1663. ^{1}H-NMR: 1.13, 1.17, 1.21, 1.26 (6H, 4 × t, *J* = 7.1, CH_3CH$_2$), 3.03 and 3.04 (3H, 2 × s, NCH_3), 3.21, 3.28, 3.29, 3.38 (4H, 4 × q, *J* = 7.0, CH$_3$CH_2), 4.02 and 4.10 (2H, 2 × s, NCH_2), 5.98 and 6.03 (2H, 2 × s, OCH_2O), 6.79–6.84 (1H, m, Ar*H*), 7.25–7.49 (6H, m, ArH), 8.04–8.06 (1H, m, Ar*H*), 9.41 and 9.45 (1H, 2 × s, N*H*). EIMS (%): 482.1 (100) [M$^+$]. Calculated for $C_{23}H_{26}N_3O_5F_3$: C, 57.4, H, 5.4, N, 8.7%. Found: C, 56.7; H, 5.6; N, 8.7%.

*Synthesis of **3e***

*N-(tert-Butoxycarbonyl)glycine diethylamide **6e***

Prepared from BocGlyOSuc **4d** (2.4 g, 8.9 mmol) and diethylamine (650.9 mg, 8.9 mmol). Yield: 90% (1.9 g, 8.0 mmol). ^{1}H-NMR: 1.10 (3H, t, *J* = 7.0, C*H*$_3$CH$_2$), 1.30 (3H, t, *J* = 7.0, CH$_3$C*H*$_2$), 1.47 (9H, s, (C*H*$_3$)$_3$), 3.17 – 3.67 (4H, m, 2 × CH$_3$C*H*$_2$), 4.00 (2H, d, *J* = 4.0, NHC*H*$_2$CO), 5.57 (1H, brs, N*H*CH$_2$CO).

*Glycine diethylamide **6e***

Yield: 66% (689.9 mg, 5.3 mmol). ^{1}H-NMR: 1.07 (3H, t, *J* = 7.0, C*H*$_3$CH$_2$), 1.23 (3H, t, *J* = 7.0, CH$_3$C*H*$_2$), 3.10 – 3.93 (8H, m, CH$_3$C*H*$_2$, C*H*$_2$CO and N*H*$_2$).

*N-(Chloromethyloxycarbonylglycine diethylamide **7e***

To a solution of **6e** (5.3 mmol, 689.9 mg in 10 mL CH_2Cl_2) was added chloromethyl chloroformate (5.3 mmol, 683.4 mg in 10 mL CH_2Cl_2) yielding an oil (532.2 mg, 2.4 mmol). Yield: 45%. ^{1}H-NMR: 1.13 (3H, t, *J* = 7.0, C*H*$_3$CH$_2$), 1.30 (3H, t, *J* = 7.0, C*H*$_3$CH$_2$), 3.30 (2H, q, *J* = 7.0, CH$_3$C*H*$_2$), 3.51 (2H, q, *J* = 7.0, CH$_3$C*H*$_2$), 4.13 (2H, d, *J* = 4.0, NHC*H*$_2$CO), 5.87 (2H, s, OC*H*$_2$Cl), 6.23 (1H, brs, CON*H*CH$_2$).

*Compound **3e***

Prepared from **7e** (243 mg, 1.1 mmol) and flufenamic acid (sodium salt) (309.3 mg, 1.1 mmol). Yield: 65% (332.1 mg, 0.7 mmol). M. p. 119 – 1200C. ν_{max}/cm^{-1}: 3333, 3279, 1739, 1677, 1631. ^{1}H-NMR: 1.14 (3H, t, *J* = 7.2, C*H*$_3$CH$_2$), 1.21 (3H, t, *J* = 7.2, C*H*$_3$CH$_2$), 3.26 (2H, q, *J* = 7.2, CH$_3$C*H*$_2$), 3.41 (2H, q, *J* = 7.2, CH$_3$C*H*$_2$), 4.04 (2H, d, *J* = 4.0, NC*H*$_2$CO), 6.02 (2H, s, OC*H*$_2$O), 6.08 (1H, broad t, CON*H*CH$_2$), 6.80 – 6.84 (1H, m, Ar*H*), 7.27 – 7.55 (6H, m, Ar*H*), 8.06 (1H, dd, *J* = 8.2, 1.4, Ar*H*), 9.45 (1H, s, N*H*). EIMS: 468.9 (45) [MH$^+$], 935.9 (35) [M$_2$H$^+$], 957.9 (100) [M$_2$Na$^+$]. Calculated for $C_{22}H_{24}N_3O_5F_3$: C, 56.5; H, 5.2; N, 9.0%. Found: C, 56.4, H, 5.4, N, 8.92%.

*Synthesis of **3f***

*N-[(tert-Butoxycarbonyl)phenylalanyl]morpholine **5f***

Prepared from BocPheOSuc **4a** (905.9 mg, 2.5 mmol) and morpholine (217.8 mg, 2.5 mmol). Yield: 87% (729.0 mg, 2.2 mmol). ^{1}H-NMR: 1.38 (9H, s, (C*H*$_3$)$_3$), 2.87 – 2.99 (3H, m, NC*H*$_2$ and C*H*Ph), 3.02 (1H, dd, *J* = 12.2, 5.0, C*H*Ph), 3.25 – 3.31 (1H, m, NC*H*H), 3.39-3.61 (5H, m, NC*H*H and 2 × C*H*$_2$O), 4.80 (1H, q, *J* = 8.0, α-C*H*), 5.45 (1H, d, *J* = 8.0, N*H*), 7.10 – 7.30 (5H, m, Ar*H*).

*N-(Phenylalanyl)morpholine **6f***

Yield: 90% (459.2 mg, 1.9 mmol). ^{1}H-NMR: 2.91 – 3.10 (7H, m, C*H*$_2$Ph and CH*H*NC*H*$_2$ and N*H*$_2$), 3.35 (1H, brs, NC*H*H), 3.50 (2H, brs, OC*H*$_2$), 3.60 (2H, brs, OC*H*$_2$), 4.10 (1H, t, *J* = 6.8, α-C*H*), 7.10 – 7.30 (5H, m, Ar*H*).

*N-[(2-Chloromethyloxycarbonylamino)-3-phenylpropanoyl]morpholine **7f***

To a solution of **6f** (459.2 mg in 10 mL CH_2Cl_2) was added chloromethyl chloroformate (1.9 mmol, 252.7 mg in 5 mL CH_2Cl_2) yielding an oil (300.6 mg, 0.9 mmol). Yield: 47%. ^{1}H-NMR: 2.62 (4H, m, C*H*$_2$NC*H*$_2$), 2.87 (2H, m, C*H*$_2$Ph), 3.60 (4H, m, C*H*$_2$OC*H*$_2$), 4.83 – 4.88 (1H, m, α-C*H*), 5.71 (1H, d, *J* = 6.1, OC*H*Cl), 5.74 (1H, d, *J* = 6.1, OC*H*Cl), 6.01 (1H, d broad, *J* = 7.8, N*H*), 7.17-7.32 (5H, m, Ar*H*).

*Compound **3f***

Prepared from **7f** (300.6 mg, 0.9 mmol) and flufenamic acid (sodium salt) (258.7 mg, 0.9 mmol). Yield: 27% (142.9 mg, 0.3 mmol). Oil ν_{max}/cm^{-1}: 3325, 1737, 1698, 1639. ^{1}H-NMR: 2.89 – 2.93 (3H, m, C*H*$_2$NC*H* morphol), 2.97 (1H, dd, *J* = 13.3, 9.2, C*H*Ph), 3.07 (1H, dd, *J* = 13.3, 5.3, C*H*Ph), 3.23 – 3.29 (1H, m, NCH), 3.40-3.60 (4H, m, C*H*$_2$OC*H*$_2$), 4.85 (1H, ddd, *J* = 8.8, 8.7, 5.4, a-C*H*), 5.90 (1H, d, *J* = 8.5, N*H*), 5.97 (2H, s, OC*H*$_2$O), 6.80 – 6.83 (1H, m, Ar*H*), 7.17 – 7.50 (11H, m, Ar*H*), 8.03 (1H, d, *J* = 7.9, Ar*H*), 9.45 (1H, s, NH). EIMS (%): 572.0 (100) [MH$^+$]. Calculated for $C_{29}H_{28}N_3O_6F_3$: C, 60.9; H, 4.9; N, 7.4%. Found: C, 60.8; H, 5.0; N, 7.4%.

*Synthesis of compound **3g***

Prepared from **7e** (302.3 mg, 1.4 mmol) and diclofenac (sodium salt) (432.6 mg, 1.4 mmol). Yield: 13% (83.3 mg, 0.2 mmol). M. p. 105 – 1060C. ν_{max}/cm^{-1}. 3393, 3335, 3090, 2982, 1746, 1644; ^{1}H-NMR: 1.07 (3H, t, *J* = 7.2, C*H*$_3$CH$_2$), 1.13 (3H, t, *J* = 7.2, C*H*$_3$CH$_2$), 3.18 (2H, q, *J* = 7.2, CH$_3$C*H*$_2$), 3.34 (2H, q, *J* = 7.2, CH$_3$C*H*$_2$), 3.80 (2H, s, ArC*H*$_2$CO), 3.94 (2H, d, *J* = 4.0, NHC*H*$_2$CO), 5.74 (2H, s, OC*H*$_2$O), 5.93 (1H, broad t, CON*H*CH$_2$), 6.49 (1H, d, *J* = 8.0, Ar*H*), 6.69 (1H, brs, ArN*H*Ar), 7.16 – 7.28 (6H, m, Ar*H*). EIMS: 482 (69) [MH$^+$], 505 (80) [MNa$^+$], 988 (78) [M$_2$Na$^+$]. Calculated for $C_{22}H_{25}N_3O_5Cl_2$, C, 54.78; H, 4.22; N, 8.71%. Found: C, 54.62; H, 4.43; N, 8.07%.

*Synthesis of compound **3h***

Prepared from **7a** (824.8 mg, 2.6 mmol) and diclofenac (sodium salt) (839.8 mg, 2.6 mmol). Yield: 23% (343.0 mg, 0.6 mmol). M. p. 33 – 340C. ν_{max}/cm^{-1}: 3325, 3266, 3060, 2973, 1737, 1629. ^{1}H-NMR: 0.91 (3H, t, *J* = 7.2, C*H*$_3$CH$_2$), 0.97 (3H, t, *J* = 7.2, C*H*$_3$CH$_2$), 2.80 – 3.01 (6H, m, CH$_3$C*H*$_2$, CH$_3$C*H*$_2$, PhC*H*$_2$), 3.79 (2H, s, ArC*H*$_2$CO), 4.70 (1H, ddd, *J* = 8.8, 8.4, 6.4, NHC*H*), 5.68 (1H, d, *J* = 6.0, OC*H*$_2$O), 5.71 (1H, d, *J* = 6.0, OC*H*$_2$O), 5.80 (1H, d, *J* = 6.0, CON*H*CH), 6.49 (1H, broad d, *J* = 8.8, Ar*H*), 6.68 (1H, s, ArN*H*Ar), 6.87 – 7.28 (11H, m, Ar*H*); ^{13}C-NMR: 12.75 (*C*H$_3$CH$_2$), 14.12 (*C*H$_3$CH$_2$), 38.29 (CH$_{3C}$H$_2$), 40.25 (CH$_{3C}$H$_2$), 40.55 (*C*H$_2$Ph), 41.61 (NH*C*H$_2$CO), 51.97 (HN*C*H(CH$_2$Ph)CO, 80.52 (O*C*H$_2$O), 118.54 – 153.71 (*C*HAr), 170.03 (CH$_{2C}$O), 171.25 (NH*C*O). Calculated for $C_{29}H_{31}N_3O_5Cl_2$: C, 60.84; H, 5.46; N, 7.34%. Found: C, 60.18; H, 5.64; N, 6.99%.

Apparent partition coefficients

The apparent partition coefficients (log P_{app}) of prodrugs were determined at room temperature using octanol-pH 7.4 phosphate buffer. Each phase was mutually saturated before the experiment. The volumes of each phase were chosen so that solute concentrations in the aqueous phase after distribution were readily measurable. The compounds were dissolved in 1-octanol and the octanol-phosphate buffer mixtures were shaken for 30 min to reach an equilibrium distribution. Each phase was analyzed separately by HPLC, but the organic phase was diluted in acetonitrile (1 : 10) before HPLC analysis. Partition coefficients P_{app} were calculated from the ratio of the peak area in octanol to the peak area in buffer.

Hydrolysis in buffer solution

Reaction mixtures were analysed using HPLC. Usually, a 10 μL aliquot of a 10^{-2} M stock solution of substrate in acetonitrile was added to 10 mL of the appropriate thermostatted buffer solu-

tion. At regular intervals, samples of the reaction mixture were analysed by HPLC using the following conditions: mobile phase, acetonitrile/water (the composition of which depended on the compound); flow rate 1.0 mL/min; detector wavelength, 230 nm.

Hydrolysis in human plasma

Human plasma was obtained from the pooled, heparinized blood of healthy donors, and was frozen and stored at −70°C prior to use. For the hydrolysis experiments, the substrates were incubated at 37°C in human plasma that had been diluted to 80% (v/v) with pH 7.4 isotonic phosphate buffer. At appropriate intervals, aliquots of 200 μL were added to 400 μL of acetonitrile to both quench the reaction and precipitate plasma proteins. These samples were centrifuged and the supernatant analyzed by HPLC for the presence of substrate and products.

Animal model

Male Wistar rats (Harlan Iberica, Barcelona, Spain) weighing 150 to 200 g were randomized into four groups and each of these groups subdivided into five groups containing five animals each. Animals were administered, by oral gavage, either a fixed dose (10 mg/kg) of diclofenac sodium or an equimolar dose of a diclofenac derivative (**2a**, **3g**, and **3h**) in a suspension of propylenglycol-400 (mg/mL). Blood samples were collected at 0 (predose), 30 min, 1, 2, and 4 h. (five rats per time point; five time points per rat). Blood samples were collected through cardiac puncture into heparinized syringe. The heparinized blood was then centrifuged at 4°C for 7 min at 850*g*, and the plasma was collected and stored at −20°C until analysis.

Relative bioavailability determination

Relative compound bioavailability was indexed as the amount of circulating diclofenac present in the blood of rats after oral dosing. Plasma concentrations of diclofenac sodium were quantified using a reverse phase HPLC method at room temperature. Briefly, 475 μL of rat plasma was mixed with 25 μL of internal standard (flufenamic acid, 30 μg/mL), and with 300 μL of acetonitrile to precipitate plasma proteins. The contents of the tube were vortexed for 2 min, and then centrifuged. The supernatant was analyzed by HPLC. The mobile phase consisted of acetonitrile-acetate buffer (pH 3.3; 50 : 50 v/v). The flow rate was 1 mL/min and detection was performed at 280 nm. Under the chromatographic conditions employed, the diclofenac and internal standard eluted at 4.1 and 5.8 min, respectively. A linear concentration-response relationship was found within 0.025–2.5 μg/mL ($r^2 > 0.998$) range.

Estimation of the pK_a differences between the carbamate group of prodrugs 2 and 3

To estimate the pK_a differences between the secondary carbamate group of prodrugs **2** and **3**, we have assumed that the equation established for secondary amides, RCONHR′, [21] applies:

$$pK_a = 22 - 3.1\,\Sigma^*$$

The difference between compounds **2** and **3** lies in the R′ substituent, which corresponds to the amino acid pro-moiety. For the glycine derivatives, the Taft σ^* values for the methyl ester, $CH_2CO_2CH_3$, ethyl ester, $CH_2CO_2CH_2CH_3$, and amide, CH_2CONH_2 groups, are 1.06, 0.82 and 0.31, respectively; thus, the difference in pK_a ranges from $3.1(1.06 - 0.31) \approx 2.3$ to $3.1\,(0.82 - 0.31) \approx 1.6$.

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