NSAID Conjugates with Carnosine and Amino Acids

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Abstract: Benzotriazole-mediated syntheses of novel bioconjugates of nonsteroidal anti-inflammatory drugs with carnosine and with amino acids were prepared in yields of 50–97% as potential drug candidates.

Key words: NSAIDs, carnosine, amino acid, prodrug, conjugates, coupling, benzotriazole methodology

Nonsteroidal anti-inflammatory drugs (NSAIDs) including ibuprofen (**1** + **1′**), mefenamic acid (**2**), diclofenac (**3**), naproxen (**4**), indomethacin (**5**) (Figure 1), and many others are highly effective analgesic, antipyretic, and anti-inflammatory agents. It is generally believed that they act by inhibiting prostaglandin synthesis, particularly in inflamed tissue.[1,2] They do not interfere with the actual disease process, but rather offer symptom relief, even when administered chronically. However, drugs of this type are responsible for substantial morbidity and mortality as a result of complications associated with gastroduodenal disorders, such as perforation and bleeding,[3–5] The NSAIDs often cause dyspepsia and adverse upper gastrointestinal (GI) effects, and frequently require GI co-medications and diagnostic procedures.

NSAIDs affect several biological processes such as inflammation (COX inhibition), angiogenesis, cell differentiation, and apoptosis (induction independent of COX inhibition), which in turn potentially influence carcinogenesis.[6] However, NSAIDs are not currently recommended for routine use as cancer preventive agents due to their chronic safety issues.

To improve the GI tolerance of NSAIDs by temporarily masking the free carboxylic group, numerous ester and amide prodrugs of NSAIDs have been designed and evaluated.[7–9] but it has not yet been possible to avoid the systemic side effects of the parent drugs by this approach.

Carnosine (**6**) and its derivatives such as polaprezinc (PZ, **7**) (Figure 2), a chelate of zinc and L-carnosine, are widely used as an anti-ulcer drugs.[10] Depending on dose, PZ can prevent gastric mucosal lesions and mucosal cell damages induced by ethanol,[11] monochloramine,[12] histamine,[13] HCl-aspirin,[13] indomethacin,[13] water-immersion stress,[13] burn shock,[14] ischemia reperfusion,[14,15] *Helicobacter pylori* associated gastritis,[16] duodenal ulcers induced by mepirizole,[13] and colitis induced by 2,4,6-trinitrobenzenesulfonic acid.[17]

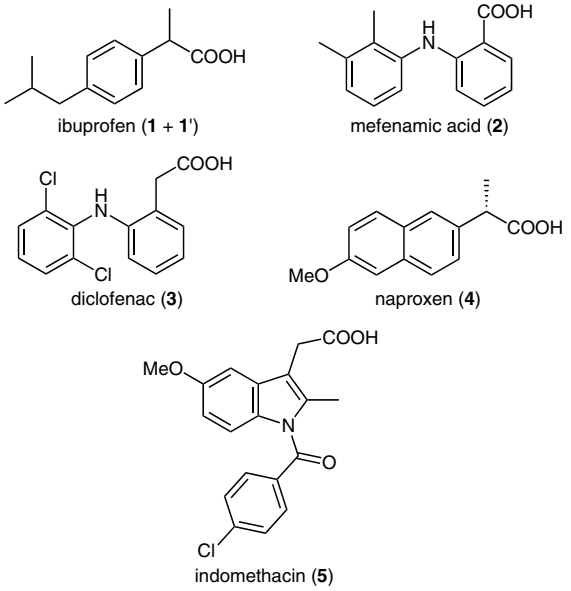

Figure 1 Nonsteroidal anti-inflammatory drugs **1**–**5**

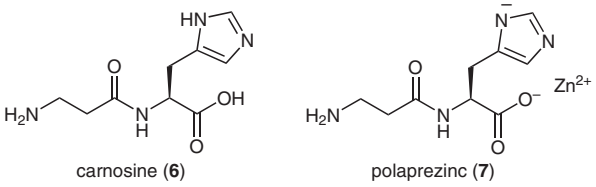

Figure 2 Carnosine (**6**) and polaprezinc (**7**)

Facino et al. reported recently that L-carnosine, an endogenous dipeptide present at millimolar concentrations in tissues such as skeletal muscle, selectively quenches unsaturated aldehydes both in vitro and in vivo.[18,19] Despite this promising finding, the therapeutic use of L-carnosine is limited because it is rapidly hydrolyzed in human plasma by carnosinase.[20]

Use of NSAIDs can cause major gastrointestinal and hepatotoxicity side effects whereas carnosine is used for the treatment of gastrointestinal and hepatotoxicity. Amino acids have frequently been used as carriers for drugs because of their ability to transplant into mammalian tis-

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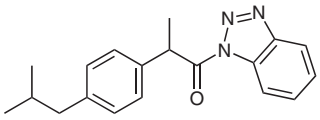
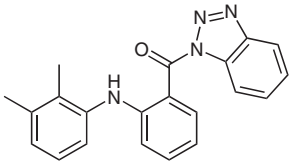
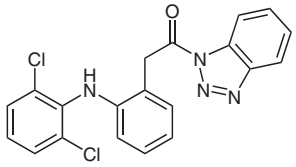
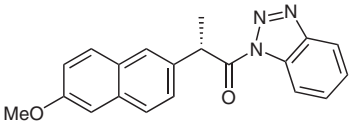
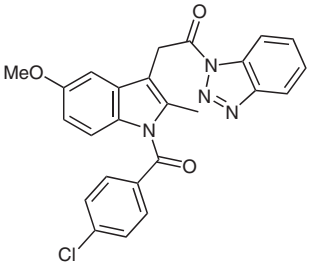
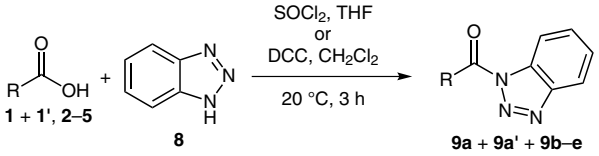
Table 1 Preparation of NSAID Benzotriazolides **9a–e**

Entry	Product **9**	Yield (%)	Mp (°C)
1	 **9a** + **9a′**	63	75–77[23]
2	 **9b**	78	111–112
3	 **9c**	66	128–130
4	 **9d**	67	183–185[23]
5	 **9e**	76	79–81

sue.[21] In the present work, we have synthesized novel NSAID conjugates with carnosine as well as with amino acids as potential anti-inflammatory agents.

Synthesis of NSAID Benzotriazolides 9a + 9a′, 9b–e

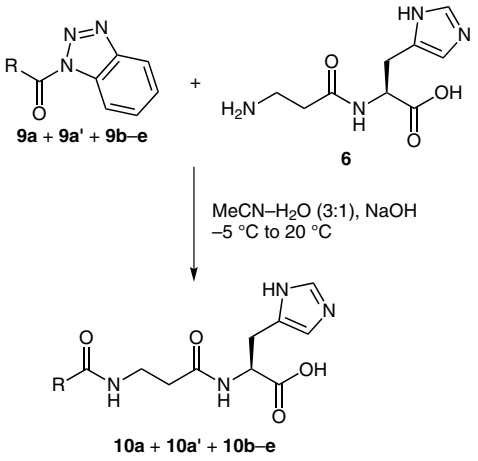
Using previously reported methods (Scheme 1, Table 1),[22] the carboxylic groups of NSAIDs were activated with 1*H*-benzotriazole (**8**) in yields of 64–78%. Compound **9a**,**b** and **9d**,**e** were prepared by treating NSAIDs **1**,**2**,**4**, and **5** with 1*H*-benzotriazole in the presence of $SOCl_2$ in THF at room temperature, whereas compound **9c** was prepared by treating diclofenac (**3**) with 1*H*-benzotriazole in the presence of DCC as coupling agent in dichloromethane at room temperature.



Scheme 1 Synthesis of NSAID benzotriazolides **9a–e**

Synthesis of NSAID Conjugates 10a + 10a′, 10b–e with Carnosine

NSAID-carnosine conjugates **10a** + **10a′, 10b–e** were obtained by coupling **9a** + **9a′** and **9b–e**, respectively, with carnosine (**6**) in aqueous acetonitrile in the presence of sodium hydroxide at –5 to 20 °C in 55–72% yields (Scheme 2, Table 2).



Scheme 2 Synthesis of NSAID conjugates **10a–e** with carnosine

Table 2 Preparation of NSAID Conjugates **10a** + **10a′**, **10b–e** with Carnosine

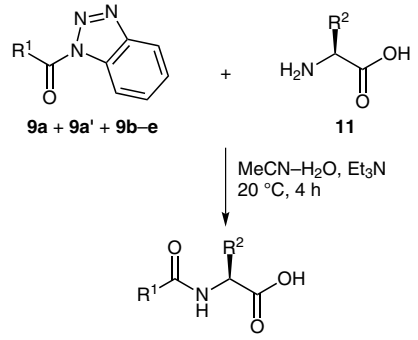
Entry	Reactant **9**	Product **10**	Yield (%)	Mp (°C)
1	**9a** + **9a′**	DL-ibuprofen-carnosine-OH (**10a** + **10a′**)	57	175–177
2	**9b**	mefenamic-carnosine-OH (**10b**)	72	108–110
3	**9c**	diclofenac-carnosine-OH (**10c**)	55	193–195
4	**9d**	naproxen-carnosine-OH (**10d**)	56	230–233
5	**9e**	indomethacin-carnosine-OH (**10e**)	57	163–165

Table 3 Preparation of NSAID Conjugates **12a–i** with Amino Acids

Reactant **9**	Product **12**	Yield (%)	Mp (°C)
9a + **9a′**	DL-ibufrofen-L-His-OH (**12a** + **12a′**)	57	76–77
9a + **9a′**	DL-ibufrofen-L-Thr-OH (**12b** + **12b′**)	63	206–207
9b	mefenamic Acid-L-Ala-OH (**12c**)	97	54–55
9b	mefenamic Acid-DL-Ala-OH (**12c** + **12c′**)	55	55–56
9b	mefenamic Acid-L-Trp-OH (**12d**)	79	115–116
9d	naproxen-L-His-OH (**12e**)	65	111–112
9d	naproxen-L-Thr-OH (**12f**)	67	145–146
9d	naproxen-DL-Thr-OH (**12f** + **12f′**)	50	172–173
9d	naproxen-L-Ala-OH (**12g**)	76	151–153
9e	indomethacin-L-His-OH (**12h**)	54	125–126
9e	indomethacin-L-Thr-OH (**12i**)	61	93–94
9e	indomethacin-DL-Thr-OH (**12i** + **12i′**)	50	92–93

Synthesis of NSAID Conjugates 12a–i with Amino Acids

NSAID amino acid conjugates were obtained by coupling **9a** + **9a′** and **9b–e**, respectively, with amino acids **11** in aqueous acetonitrile in the presence of triethylamine at 25 °C in 53–97% yields (Scheme 3, Table 3). Histidine, threonine, tryptophan, and alanine were used because these amino acids help directly or indirectly in the treatment of inflammation.[23–25] The chirality of starting materials was preserved in the products, as we can see duplication of peaks in ^{13}C NMR spectra in the case of racemic mixtures only.



Scheme 3 Synthesis of NSAID conjugates **12a–l** with amino acids

In summary, benzotriazole-activated nonsteroidal anti-inflammatory drugs are sufficiently reactive coupling reagents to form amides under mild conditions, which offer an efficient preparation of chirally pure nonsteroidal anti-inflammatory drug-conjugates with carnosine and amino acids in good yields. The chirality of starting materials was preserved in the products, as evidenced by NMR spectra.

Commercial reagents were purchased from Sigma-Aldrich and were used without purification. Solvents were purified by distillation. Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in $CDCl_3$ or CD_3OD on Mercury or Gemini NMR spectrometers operating at 300 MHz for ^1H (with TMS as an internal standard) and 75 MHz for ^{13}C. Elemental analyses were performed on a Carlo Erba-EA1108 instrument. Analytical TLC was performed on E. Merck silica gel 60 F254 plates and visualized by UV and $KMnO_4$ staining. Yields refer to chromatographically and spectroscopically pure compounds. Mass spectrometry was done with electrospray ionization (ESI).

Benzotriazole Derivatives of NSAIDs 9a + 9a′, 9b–e; General Procedure

$SOCl_2$ (2.64 mmol) was added to a solution of 1*H*-benzotriazole (**8**; 1.03 g, 8.7 mmol) in anhydrous CH_2Cl_2 (60 mL) at r.t. and the reaction mixture was stirred for 20 min. Ibuprofen (**1** + **1′**; 453 mg, 2.2 mmol), mefenamic acid (**2**; 530 mg, 2.2 mmol), naproxen (**4**; 506 mg, 2.2 mmol), or indomethacin (**5**; 787 mg, 2.2 mmol) was added and the mixture was stirred for 3 h at r.t. The white precipitate formed was filtered off and the filtrate was concentrated under reduced pressure. Each residue was diluted with EtOAc (50 mL) and each solution was washed with aq 4 M HCl (3 × 15 mL) and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave the desired products **9a** + **9a′**, **9b**, **9d**, and **9e**. For the preparation of compound **9c**, diclofenac (**3**; 651 mg, 2.2 mmol) was treated with 1*H*-benzotriazole (**8**; 1.03 g, 8.7 mmol) in the presence of DCC (453 mmol, 2.2 mmol) in anhydrous CH_2Cl_2 (60 mL) at r.t. and the reaction mixture was stirred for 3 h. The white precipitate formed was filtered off through Celite and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (50 mL) and the solution was washed with aq 10% Na_2CO_3 (3 × 15 mL) and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave the desired product.

1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-2-(4-isobutylphenyl)propan-1-one (Ibuprofen-Bt, 9a + 9a′)

Yield: 0.47 g (63%); white microcrystals; mp 75–77 °C.

^1H NMR ($CDCl_3$): δ = 8.27 (d, *J* = 8.1 Hz, 1 H), 8.06 (d, *J* = 8.1 Hz, 1 H), 7.60 (t, *J* = 7.5 Hz, 1 H), 7.48–7.38 (m, 3 H), 7.08 (d, *J* = 7.8

Hz, 2 H), 5.39–5.37 (m, 1 H), 2.39 (d, *J* = 7.2 Hz, 2 H), 1.81–1.72 (m, 4 H), 0.84 (d, *J* = 6.3 Hz, 6 H).

^{13}C NMR ($CDCl_3$): δ = 173.8, 146.4, 141.3, 136.6, 131.6, 130.5, 129.8, 127.8, 126.3, 120.3, 114.8, 45.2, 44.6, 30.3, 22.6, 18.9.

Anal. Calcd for $C_{19}H_{21}N_3O$: C, 74.24; H, 6.89; N, 13.67. Found: C, 73.96; H, 6.97; N, 13.70.

(1*H*-Benzo[*d*][1,2,3]triazol-1-yl){2-[(2,3-dimethylphenyl)amino]phenyl}methanone (Mefenamic acid-Bt, 9b)
Yield: 0.55 g (78%); yellow solid; mp 111–112 °C.

^{1}H NMR ($CDCl_3$): δ = 9.06 (s, 1 H), 8.29 (d, *J* = 9.0 Hz, 1 H), 8.18 (d, *J* = 9.0 Hz, 1 H), 8.09 (d, *J* = 8.1 Hz, 1 H), 7.69 (t, *J* = 8.4 Hz, 1 H), 7.54 (t, *J* = 7.6 Hz, 1 H), 7.38 (t, *J* = 7.9 Hz, 1 H), 7.21 (d, *J* = 8.7 Hz, 1 H), 7.14 (t, *J* = 7.8 Hz, 1 H), 7.06 (d, *J* = 6.3 Hz, 1 H), 6.89 (d, *J* = 8.7 Hz, 1 H), 6.81 (t, *J* = 7.9 Hz, 1 H), 2.35 (s, 3 H), 2.24 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 167.8, 151.0, 146.0, 138.6, 138.3, 135.6, 134.9, 132.9, 132.4, 130.1, 127.4, 126.4, 126.1, 123.1, 120.4, 116.7, 114.8, 114.7, 112.6, 20.9, 14.3.

Anal. Calcd for $C_{21}H_{18}N_4O$: C, 73.67; H, 5.30; N, 16.36. Found: C, 73.27; H, 5.40; N, 16.36.

1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-2-{2-[(2,6-dichlorophenyl)amino]phenyl}ethan-1-one (Diclofenac-Bt, 9c)
Yield: 0.44 g (66%); white microcrystals; mp 128–130 °C.

^{1}H NMR ($CDCl_3$): δ = 8.23 (d, *J* = 8.1 Hz, 1 H), 8.06 (d, *J* = 8.4 Hz, 1 H), 7.58 (t, *J* = 7.6 Hz, 1 H), 7.46–7.41 (m, 1 H), 7.30–7.26 (m, 2 H), 7.10–7.05 (m, 1 H), 6.97–6.89 (m, 3 H), 6.53 (d, *J* = 8.1 Hz, 1 H), 4.84 (s, 2 H).

^{13}C NMR ($CDCl_3$): δ = 171.0, 146.5, 143.1, 137.9, 131.5, 131.4, 130.8, 129.8, 129.1, 128.6, 126.6, 124.4, 123.2, 122.6, 120.5, 118.9, 114.8, 38.9.

(*S*)-1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-2-(6-methoxynaphthalen-2-yl)propan-1-one (Naproxen-Bt, 9d)
Yield: 0.48 g (67%); white microcrystals; mp 183–185 °C.

^{1}H NMR ($CDCl_3$): δ = 8.25 (d, *J* = 9 Hz, 1 H), 8.02 (d, *J* = 9 Hz, 1 H), 7.86 (s, 1 H), 7.67 (d, *J* = 6.0 Hz, 2 H), 7.61–7.55 (m, 2 H), 7.41 (t, *J* = 9.0 Hz, 1 H), 7.10–7.03 (m, 2 H), 5.55–5.49 (m, 1 H), 3.85 (s, 3 H), 1.80 (d, *J* = 9.0 Hz, 3 H).

^{13}C NMR ($CDCl_3$): δ = 173.7, 157.9, 146.4, 134.5, 134.1, 131.5, 130.5, 129.5, 129.1, 127.6, 127.1, 126.6, 126.3, 120.3, 119.3, 114.7, 105.7, 55.5, 44.9, 18.8.

Anal. Calcd for $C_{20}H_{17}N_3O_2$: C, 72.49; H, 5.17; N, 12.68. Found: C, 72.05; H, 5.08; N, 12.76.

1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethan-1-one (Indomethacin-Bt, 9e)
Yield: 0.49 g (76%); yellow microcrystals; mp 79–81 °C.

^{1}H NMR ($CDCl_3$): δ = 8.27 (d, *J* = 8.1 Hz, 1 H), 8.14 (d, *J* = 7.8 Hz, 1 H), 7.69–7.61 (m, 3 H), 7.56–7.54 (m, 3 H), 7.14 (d, *J* = 2.4 Hz, 1 H), 6.87 (d, *J* = 9.0 Hz, 1 H), 6.67 (d, *J* = 9.0 Hz, 1 H), 4.79 (s, 2 H), 3.84 (s, 3 H), 2.53 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 169.4, 165.4, 156.3, 146.5, 139.6, 137.1, 133.9, 131.4, 130.8, 129.3, 126.6, 120.5, 115.2, 114.7, 112.2, 111.1, 101.5, 55.9, 31.7, 13.9.

HRMS: *m*/*z* calcd for $C_{25}H_{19}ClN_4O_3$ $[M + H]^+$: 459.1218; found: 459.1201.

NSAID-Carnosine Conjugates 10a + 10a′, 10b–e; General Procedure
To a solution of carnosine (**6**; 1 equiv) in H_2O (1 mL) and NaOH (1.5 equiv) was added a solution of the respective NSAID benzotriazole **9** (1 equiv) in MeCN (3 mL) at –5 °C and the mixture was stirred for 12 h at r.t. After completion of the reaction, MeCN was evaporated and the mixture was poured over crushed ice followed by the addition of citric acid (1 mL) maintaining the pH at 4–5. The precipitate obtained was then dried and washed with Et_2O (5 mL) and then with EtOAc (5 mL) to obtain the product in pure form.

{3-[2-(4-Isobutylphenyl)propanamido]propanoyl}-L-histidine (Ibuprofen-carnosine, 10a + 10a′)
Yield: 0.15 g (57%); white microcrystals; mp 175–177 °C.

^{1}H NMR (DMSO-d_6): δ = 8.13 (d, *J* = 7.8 Hz, 1 H), 7.94 (br s, 1 H), 7.58 (s, 1 H), 7.18 (d, *J* = 8.1 Hz, 2 H), 7.05 (d, *J* = 7.8 Hz, 1 H), 6.81 (s, 1 H), 4.40–4.39 (m, 1 H), 3.52–3.50 (m, 2 H), 3.21–3.12 (m, 2 H), 2.89–2.81 (m, 2 H), 2.38 (d, *J* = 7.2 Hz, 2 H), 2.24–2.20 (m, 2 H), 1.81–1.76 (m, 1 H), 1.27 (d, *J* = 7.2 Hz, 3 H), 0.84 (d, *J* = 6.6 Hz, 6 H).

^{13}C NMR (DMSO-d_6): δ = 176.2, 172.9, 170.3, 139.5, 139.2, 134.5, 132.6, 128.7, 126.9, 116.8, 71.7, 52.1, 44.3, 35.4, 35.2, 29.7, 28.4, 22.2, 18.6.

Anal. Calcd for $C_{22}H_{30}N_4O_4$: C, 63.75; H, 7.29; N, 13.52. Found: C, 63.48; H, 7.57; N, 13.24.

(3-{2-[(2,3-Dimethylphenyl)amino]benzamido}propanoyl)-L-histidine (Mefenamic Acid-carnosine, 10b)
Yield: 0.19 g (72%); white microcrystals; mp 108–110 °C.

^{1}H NMR (DMSO-d_6): δ = 9.58 (s, 1 H), 8.59–8.52 (m, 1 H), 8.26 (d, *J* = 7.2 Hz, 1 H), 7.91 (d, *J* = 7.5 Hz, 1 H), 7.83 (s, 1 H), 7.61 (d, *J* = 7.5 Hz, 1 H), 7.42 (s, 1 H), 7.23 (t, *J* = 7.6 Hz, 1 H), 7.11–7.04 (m, 2 H), 6.93–6.90 (m, 2 H), 6.83 (d, *J* = 8.1 Hz, 1 H), 6.72 (t, *J* = 7.0 Hz, 1 H), 4.48–4.45 (m, 1 H), 3.50–3.37 (m, 2 H), 3.10–2.86 (m, 2 H), 2.46–2.39 (m, 2 H), 2.26 (s, 3 H), 2.10 (s, 3 H).

^{13}C NMR (DMSO-d_6): δ = 176.2, 173.1, 171.4, 170.4, 169.1, 146.1, 139.3, 137.7, 134.5, 132.7, 131.9, 129.3, 128.7, 125.8, 125.4, 125.0, 119.6, 117.0, 116.8, 113.9, 59.8, 44.0, 40.3, 35.1, 28.5, 20.3.

HRMS: *m*/*z* calcd for $C_{24}H_{27}N_5O_4$ $[M + H]^+$ 450.2136; found: 450.2150.

[3-(2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetamido)propanoyl]-L-histidine (Diclofenac-carnosine, 10c)
Yield: 0.14 g (55%); white microcrystals; mp 193–195 °C.

^{1}H NMR (DMSO-d_6): δ = 8.43 (d, *J* = 9.9 Hz, 2 H), 8.20 (d, *J* = 5.7 Hz, 1 H), 7.67 (s, 2 H), 7.50 (d, *J* = 10.5 Hz, 2 H), 7.43 (s, 1 H), 7.20–7.10 (m, 2 H), 7.01 (s, 1 H), 6.85 (s, 2 H), 6.28 (d, *J* = 10.5 Hz, 1 H), 4.44–4.42 (m, 1 H), 3.56 (s, 2 H), 3.26–3.20 (m, 2 H), 3.04–2.83 (m, 2 H), 2.30–2.28 (m, 2 H).

^{13}C NMR (DMSO-d_6): δ = 173.0, 171.6, 170.1, 142.9, 137.2, 134.6, 133.1, 130.4, 129.3, 129.1, 127.1, 125.5, 125.3, 124.9, 120.6, 116.7, 115.9, 114.8, 52.2, 43.9, 35.4, 35.0, 28.8.

HRMS: *m*/*z* calcd for $C_{23}H_{23}Cl_2N_5O_4$ $[M + Na]^+$: 504.1187; found: 504.1199.

{3-[(*S*)-2-(6-Methoxynaphthalen-2-yl)propanamido]propanoyl}-L-histidine (Naproxen-carnosine, 10d)
Yield: 0.15 g (56%); white microcrystals; mp 230–233 °C.

^{1}H NMR (DMSO-d_6): δ = 8.13 (d, *J* = 7.5 Hz, 1 H), 8.02 (t, *J* = 5.7 Hz, 1 H), 7.78–7.68 (m, 2 H), 7.60 (s, 1 H), 7.41 (d, *J* = 8.6 Hz, 1 H), 7.26–7.23 (m, 1 H), 7.12 (d, *J* = 8.8 Hz, 1 H), 6.80 (s, 1 H), 4.46–4.33 (m, 1 H), 3.85 (s, 3 H), 3.70–3.63 (m, 1 H), 3.25–3.13 (m, 2 H), 2.93–2.77 (m, 2 H), 2.30–2.17 (m, 2 H), 1.38 (d, *J* = 6.9 Hz, 3 H).

^{13}C NMR (TFA-d_4): δ = 182.0, 176.8, 174.1, 133.4, 133.0, 129.4, 128.8, 128.1, 126.4, 125.5, 119.9, 118.2, 117.6, 116.1, 112.3, 108.6, 55.2, 52.0, 45.8, 36.9, 34.1, 26.0, 15.7.

Anal. Calcd for $C_{23}H_{26}N_4O_5$: C, 63.00; H, 5.98; N, 12.78. Found: C, 62.62; H, 5.98; N, 12.49.

(3-{2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetamido}propanoyl)-L-histidine (Indomethacin-carnosine, 10e)
Yield: 0.14 g (57%); yellow microcrystals; mp 163–165 °C.

^{1}H NMR (DMSO-d_6): δ = 8.19 (d, *J* = 7.8 Hz, 1 H), 8.09 (s, 1 H), 7.82 (s, 1 H), 7.42–7.60 (m, 4 H), 7.10 (s, 1 H), 6.94–6.88 (m, 2 H), 6.69 (d, *J* = 8.4 Hz, 1 H), 4.45–4.39 (m, 1 H), 3.75 (s, 3 H), 3.48 (s, 2 H), 3.25–3.17 (m, 2 H), 3.00–2.80 (m, 2 H), 2.30–2.24 (m, 2 H), 2.21 (s, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.9, 171.3, 170.3, 169.3, 155.5, 137.5, 135.1, 134.5, 134.2, 132.6, 131.1, 130.2, 129.0, 116.7, 114.5, 114.3, 11.3, 101.8, 55.4, 52.0, 43.6, 35.4, 35.1, 31.1, 28.4, 13.4.

HRMS: *m*/*z* calcd for $C_{28}H_{28}ClN_5O_6$ [M + H]$^+$: 566.1728; found: 566.1800.

NSAID-Amino Acid Conjugates 12a–i; General Procedure
To a solution of the amino acid **11** (1 equiv) in H_2O (1 mL) and Et_3N (1.5 equiv) was added a solution of the respective NSAID benzotriazole **9** (1 equiv) in MeCN (3 mL) at 20 °C and the mixture was stirred for 4 h at r.t. After completion of the reaction, MeCN was evaporated and the mixture was poured over crushed ice followed by the addition of citric acid (1 mL) maintaining the pH at 4–5. The precipitate obtained was then dried and washed with Et_2O (5 mL) and then with EtOAc (5 mL) to afford the product in pure form.

[2-(4-Isobutylphenyl)propanoyl]-L-histidine (Ibuprofen-L-histidine, 12a + 12a′)
Yield: 0.13 g (57%); white microcrystals; mp 76–77 °C.

^{1}H NMR (DMSO-d_6): δ = 8.21 (s, 1 H), 7.69 (d, *J* = 15 Hz, 2 H), 7.18–7.04 (m, 5 H), 6.84 (s, 1 H), 7.75 (s, 1 H), 4.41 (m, 1 H), 3.61 (s, 1 H), 2.94–2.84 (m, 1 H), 2.58–2.55 (m, 1 H), 2.39 (s, 2 H), 1.79 (br s, 1 H), 1.26 (d, *J* = 15.3 Hz, 3 H), 0.84 (br s, 6 H).

^{13}C NMR (DMSO-d_6): δ = 173.2, 139.0, 134.4, 132.8, 128.6, 127.0, 126.9, 116.5, 52.0, 44.25, 29.6, 29.3, 22.2, 18.8, 18.2.

HRMS: *m*/*z* calcd for $C_{19}H_{25}N_3O_3$ [M – H]$^-$: 342.1823; found: 342.1839.

[2-(4-Isobutylphenyl)propanoyl]-L-threonine (DL-Ibuprofen-L-threonine, 12b + 12b′)
Yield: 0.13 g (63%); white microcrystals; mp 206–207 °C.

^{1}H NMR (DMSO-d_6): δ = 7.20 (d, *J* = 7.2 Hz, 3 H), 7.06 (d, *J* = 7.5 Hz, 2 H), 3.85 (br s, 1 H), 3.71 (t, *J* = 7.3 Hz, 2 H), 2.39 (d, *J* = 6.9 Hz, 2 H), 1.81–1.77 (m, 2 H), 1.28 (d, *J* = 6.9 Hz, 3 H), 0.83 (d, *J* = 6.9 Hz, 6 H), 0.66 (d, *J* = 6.9 Hz, 2 H).

^{13}C NMR (DMSO-d_6): δ = 172.8, 172.2, 139.9, 139.0, 128.6, 127.2, 66.4, 57.4, 44.7, 44.3, 22.2, 20.4, 20.1, 19.0, 18.3.

Anal. Calcd for $C_{17}H_{25}NO_4$: C, 66.43; H, 8.20; N, 4.56. Found: C, 66.79; H, 8.57; N, 4.29.

{2-[(2,3-Dimethylphenyl)amino]benzoyl}-L-alanine (Mefenamic Acid-L-alanine, 12c)
Yield: 0.18 g (97%); yellow solid; mp 54–55 °C.

^{1}H NMR (CD_3OD): δ = 7.51 (d, *J* = 7.5 Hz, 1 H), 7.07 (t, *J* = 7.5 Hz, 1 H), 6.95–6.87 (m, 2 H), 6.79 (d, *J* = 6.0 Hz, 1 H), 6.67–6.57 (m, 2 H), 4.48–4.44 (m, 1 H), 2.17 (s, 3 H), 2.02 (s, 3 H), 1.37 (d, *J* = 7.2 Hz, 3 H).

^{13}C NMR (CD_3OD): δ = 176.5, 171.9, 148.2, 141.0, 139.2, 133.4, 132.0, 129.9, 127.0, 126.9, 122.3, 118.7, 118.2, 115.6, 20.8, 17.7, 14.1.

Anal. Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45; N, 8.97. Found: C, 68.99; H, 6.64; N, 8.84.

{2-[(2,3-Dimethylphenyl)amino]benzoyl}-DL-alanine (Mefenamic Acid-DL-alanine, 12c + 12c′)
Yield: 0.10 g (55%); yellow solid; mp 55–56 °C.

^{1}H NMR (DMSO-d_6): δ = 9.01 (br s, 1 H), 7.47(d, *J* = 8.1 Hz, 1 H), 7.21 (t, *J* = 7.9 Hz, 1 H), 7.14 (d, *J* = 7.8 Hz, 1 H), 7.06 (t, *J* = 7.6 Hz, 1 H), 6.95 (d, *J* = 6.9 Hz, 1 H), 6.87 (d, *J* = 8.1 Hz, 1 H), 6.75 (d, *J* = 6.9 Hz, 1 H), 6.69 (t, *J* = 7.5 Hz, 1 H), 4.82–4.75 (m, 1 H), 2.31 (s, 3 H), 2.17 (s, 3 H), 1.57 (d, *J* = 7.2 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 177.7, 169.7, 147.7, 139.4, 138.3, 133.0, 131.4, 127.9, 126.1, 126.0, 121.6, 117.0, 115.8, 115.1, 48.5, 20.9, 18.4, 14.1.

Anal. Calcd for $C_{18}H_{20}N_2O_3$: C, 69.21; H, 6.45; N, 8.97. Found: C, 69.57; H, 6.55; N, 8.86.

{2-[(2,3-Dimethylphenyl)amino]benzoyl}-L-tryptophan (Mefenamic Acid-L-tryptophan, 12d)
Yield: 0.20 g (79%); orange solid; mp 115–116 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.97 (br s, 1 H), 8.10 (s, 1 H), 7.58 (d, *J* = 7.5 Hz, 1 H), 7.24 (d, *J* = 8.4 Hz, 1 H), 7.17–7.02 (m, 6 H), 6.99–6.91 (m, 2 H), 6.82 (d, *J* = 8.7 Hz, 1 H), 6.71 (d, *J* = 6.9 Hz, 1 H), 6.51 (t, *J* = 7.5 Hz, 1 H), 5.09–5.05 (m, 1 H), 3.50–3.30 (m, 2 H), 2.27 (s, 3 H), 2.11 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 176.4, 169.9, 147.5,139.5, 138.3, 136.3, 132.9, 131.3, 128.1, 127.8, 126.1, 126.1, 123.4, 122.4, 121.5, 120.0, 118.7, 117.1, 116.0, 115.1, 111.6, 109.7, 53.7, 27.4, 20.8, 14.1.

HRMS: *m*/*z* calcd for $C_{26}H_{25}N_3O_3$ [M + H]$^+$: 428.1969; found: 428.1963.

[(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoyl]-L-histidine (Naproxen-L-histidine, 12e)
Yield: 0.14 g (65%); white solid; mp 111–112 °C.

^{1}H NMR (DMSO-d_6): δ = 8.28 (d, *J* = 7.2 Hz, 1 H), 7.83–7.63 (m, 3 H),7.63 (s, 1 H), 7.43 (d, *J* = 8.7 Hz, 1 H), 7.27 (s, 1 H), 7.14 (d, *J* = 9.3 Hz, 1 H), 6.83 (s, 1 H), 4.50–4.37 (m, 1 H), 3.87–3.72 (m, 4 H), 3.02–2.78 (m, 2 H), 1.35 (d, *J* = 6.9 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 173.2, 173.0, 156.9, 137.1, 134.6, 133.1, 129.1, 128.3, 126.7, 126.5, 125.4, 118.5, 116.9, 105.7, 55.2, 52.2, 44.7, 28.9, 18.7.

HRMS: *m*/*z* calcd for $C_{20}H_{21}N_3O_4$ [M + H]$^+$: 368.1605; found: 368.1602.

[(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoyl]-L-threonine (Naproxen-L-threonine, 12f)
Yield: 0.13 g (67%); white solid; mp 145–146 °C.

^{1}H NMR (DMSO-d_6): δ = 7.87 (d, *J* = 8.7 Hz, 1 H), 7.79–7.72 (m, 3 H), 7.48 (d, *J* = 8.7 Hz, 1 H), 7.27 (s, 1 H), 7.13 (d, *J* = 8.7 Hz, 1 H), 4.26–4.22 (m, 1 H), 4.16–4.11 (m, 1 H), 4.04–3.97 (m, 1 H), 3.86 (s, 3 H), 1.43 (d, *J* = 7.2 Hz, 3 H), 1.08 (d, *J* = 6.3 Hz, 2 H).

^{13}C NMR (DMSO-d_6): δ = 174.5, 172.6, 157.5, 137.7, 133.6, 129.6, 128.9, 127.3, 127.0, 126.0, 119.0, 106.2, 67.0, 58.1, 55.7, 44.9, 20.9, 19.4.

HRMS: *m*/*z* calcd for $C_{18}H_{21}NO_5$ [M + Na]$^+$: 354.1312; found: 354.1218.

[(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoyl)-DL-threonine (Naproxen-DL-threonine, 12f + 12f′)
Yield: 0.10 g (50%); white solid; mp 172–173 °C.

^{1}H NMR (DMSO-d_6): δ = 7.89 (d, *J* = 8.6 Hz, 1 H), 7.79–7.73 (m, 3 H), 7.49 (d, *J* = 8.7 Hz, 1 H), 7.20 (s, 1 H), 7.14 (d, *J* = 8.7 Hz, 1 H), 4.27–4.19 (m, 1 H), 4.16–4.08 (m, 1 H), 4.05–3.98 (m, 1 H), 3.86 (s, 3 H), 1.45–1.38 (m, 3 H), 1.08 (d, *J* = 6.0 Hz, 2 H), 0.84 (d, *J* = 6.3 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 174.6, 172.7, 157.6, 137.8, 133.7, 129.7, 129.0, 127.4, 127.1, 126.1, 119.1, 106.3, 67.1, 58.2, 55.8, 45.1, 21.1, 20.8, 19.5, 19.0.

Anal. Calcd for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.36; H, 6.64; N, 3.99.

[(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoyl)-L-alanine (Naproxen-L-alanine, 12g)
Yield: 0.14 g (76%); white solid; mp 151–153 °C.

^{1}H NMR (DMSO-d_6): δ = 8.34 (d, *J* = 7.2 Hz, 1 H), 7.78–7.72 (m, 3 H), 7.46 (d, *J* = 7.8 Hz, 1 H), 7.26 (s, 1 H), 7.13 (d, *J* = 7.2 Hz, 1 H), 4.25–4.20 (m, 1 H), 3.86–3.60 (m, 4 H), 1.39 (d, *J* = 7.2 Hz, 3 H), 1.28 (d, *J* = 6.9 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 174.1, 173.1, 156.9, 137.2, 133.1, 129.1, 128.3, 126.0, 126.4, 125.3, 118.5, 105.6, 55.1, 47.5, 44.4, 18.7, 17.3.

Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 68.10; H, 6.48; N, 4.37.

{2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetyl}-L-histidine (Indomethacin-L-histidine, 12h)
Yield: 0.12 g (54%); yellow solid; mp 125–126 °C.

^{1}H NMR (DMSO-d_6): δ = 8.44 (s, 1 H), 8.04 (s, 1 H), 7.71–7.65 (br s, 4 H), 7.07 (s, 1 H), 6.94 (s, 2 H), 7.68 (s, 1 H), 3.74 (s, 3 H), 3.54 (s, 2 H), 3.00–2.95 (m, 2 H), 2.16 (s, 3 H).

^{13}C NMR (DMSO-d_6): δ = 175.8, 172.9, 171.5, 169.6, 167.9, 155.6, 137.7, 135.2,134.5,134.3, 132.4, 131.2, 130.9, 129.1, 116.7, 114.5, 114.2, 111.5, 101.8, 55.5, 52.2, 43.6, 30.8, 13.4.

HRMS: *m*/*z* calcd for $C_{25}H_{23}ClN_4O_5$ $[M + Na]^+$: 517.1249; found: 517.1263.

[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-carbonyl]-L-threonine (Indomethacin-L-threonine, 12i)
Yield: 0.12 g (61%); white solid; mp 93–94 °C.

^{1}H NMR (DMSO-d_6): δ = 7.87 (s, 2 H), 7.66–7.57 (br s, 2 H), 7.49–7.42 (m, 3 H), 7.03–6.89 (m, 2 H), 6.68–6.57 (m, 1 H), 4.41 (s, 1 H), 4.30 (s, 1 H), 3.69 (s, 1 H), 3.62 (s, 1 H), 3.26 (s, 1 H), 2.89–2.73 (m, 2 H), 1.24 (s, 1 H), 1.10 (s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 167.4, 165.4, 164.1, 160.3, 148.1, 130.5, 127.5, 127.2, 126.1, 122.8, 122.5, 120.6, 117.5, 106.4, 105.2, 103.6, 103.0, 92.8, 92.6, 64.6, 58.7, 49.7, 46.6, 34.4, 22.6, 11.12.

HRMS: *m*/*z* calcd for $C_{23}H_{23}ClN_2O_6$ $[M - H]^-$: 457.1245; found: 457.1169.

[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-carbonyl]-DL-threonine (Indomethacin-DL-threonine, 12i + 12i′)
Yield: 0.10 g (50%); white solid; mp 92–93 °C.

^{1}H NMR (DMSO-d_6): δ = 12.52 (br s, 1 H), 8.09 (d, *J* = 8.7 Hz, 1 H), 7.69–7.60 (m, 4 H), 7.21 (s, 1 H), 6.95 (d, *J* = 9.3 Hz, 1 H), 6.69 (d, *J* = 8.4 Hz, 1 H), 5.01 (s, 1 H), 4.25–4.14 (m, 2 H), 3.78 (s, 3 H), 3.70–3.60 (m, 2 H), 2.24 (s, 3 H), 1.04 (d, *J* = 6.0 Hz, 3 H).

^{13}C NMR (DMSO-d_6): δ = 172.1, 171.2, 169.9, 167.8, 155.5, 137.5, 135.0, 134.2, 131.1, 130.8, 130.1, 129.0, 114.6, 114.4, 111.5, 101.8, 66.2, 57.6, 55.4, 30.7, 20.5, 13.4.

Anal. Calcd for $C_{23}H_{23}ClN_2O_6$: C, 60.20; H, 5.05; N, 6.10. Found: C, 60.06; H, 5.01; N, 5.70.

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