Synthesis and evaluation of anti-inflammatory activity of 2-(4-isobutylphenyl)propionyl derivatives of amino acids and peptides

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A series of new 2-(4-isobutylphenyl)propionyl amino acids and peptide methyl esters have been synthesized by coupling ibuprofen with amino acid methyl esters and di- and tetra-peptide methyl esters. The structures of these compounds have been confirmed by elemental and spectral analyses. The anti-inflammatory activity of these compounds is also studied.

Ibuprofen is a widely used non-steroidal anti-inflammatory analgesic drug, mainly used in the treatment of spondylitis, juvenile arthritis, acute gout and all types of pains and trauma. However, it is contraindicated in gastro-intestinal disturbances and prolonged use leads to active peptic ulcers and gastro-intestinal bleeding due to the presence of free carboxylic group in its structure. This led us to search for a superior analogue of ibuprofen with less side effects.

Various heterocyclic and non-heterocyclic derivatives of amino acids and peptides were reported to exhibit a variety of biological activities. Since peptides are biocompatible, ibuprofen derivatives of amino acids and peptides were expected to be superior analogues of ibuprofen. Hence, an attempt was made towards the coupling of the carboxylic end of ibuprofen with the amino end of amino acid esters, di- and tetra-peptide methyl esters using DCC and triethylamine.

The amino acid methyl esters were prepared by refluxing the corresponding amino acids (L-tyrosine, L-valine, L-leucine, L-proline and glycine) with methanol and thionyl chloride. Di-tert-butyl pyrocarbonate was used for amino group protection of L-leucine, L-tryptophan, L-proline and L-threonine. The dipeptides Boc-Leu-Gly-OMe 1, Boc-Trp-Val-OMe 2, Boc-Pro-Leu-OMe 3 and Boc-Thr-Pro-OMe 4 were prepared by coupling the corresponding amino acid methyl esters with Boc-amino acids using DCC as the coupling reagent, triethylamine as the base and chloroform as the solvent and stirring the reaction mixture for 24 hr at room temperature.

The tetrapeptide Boc-Pro-Leu-Thr-Pro-OMe 5 was prepared by coupling the dipeptides Boc-Pro-Leu-OMe and Boc-Thr-Pro-OMe after proper deprotection. The Boc-group was removed using CF3COOH/CHCl3, and the ester group was removed with LiOH.

The Boc-group of the dipeptides, Boc-Leu-Gly-OMe and Boc-Trp-Val-OMe and the tetrapeptide, Boc-Pro-Leu-Thr-Pro-OMe was removed with CF3COOH/CHCl3 and the deprotected units were coupled with the carboxylic end of ibuprofen 6 using DCC to get 2-(4-isobutylphenyl)propionyl amino acid and peptide methyl esters 6-a-d. The coupling procedure was the same as that employed for the preparation of dipeptides (cf. Scheme 1).

The structures of the newly synthesized compounds were confirmed by IR, 1H NMR and elemental analysis.

Anti-inflammatory Activity

Winter’s hind paw method was used for the evaluation of the anti-inflammatory activity of compounds 6-a-d. Carragenin, an irritant, was injected subcutaneously into the hind paw of the albino rats at a concentration of 1 mg/mL to produce the oedema. 2% Acacia mucilage was given to one set of animals as control. Another set received the standard drug, ibuprofen (20 mg/kg body weight) and rest of the

Note

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\[ \text{H}_3\text{C} \text{CH}_{2}-\text{COOH} + \text{H}_{3}\text{N} \text{X-OMe} \xrightarrow{\text{a} \rightarrow \text{DCC, EtN, CHCl}_{3}, \text{Room Temperature, 24 hr}} \text{X} \rightarrow \text{Tyr-} \text{(}6\text{a)} \text{, Leu-Gly-} \text{(}6\text{b)} \text{, Trp-Val-} \text{(}6\text{c)} \text{, Pro-Leu-Thr-Pro-} \text{(}6\text{d)} \text{.}

Where \( \text{a} \rightarrow \text{DCC, EtN, CHCl}_{3}, \text{Room Temperature, 24 hr} \)
compounds 6a-d. Carrageenin, an irritant, was injected subcutaneously into the hind paw of the albino rats at a concentration of 1 mg/mL to produce the oedema. 2% Acacia mucilage was given to one set of animals as control. Another set received the standard drug, ibuprofen (20 mg/kg body weight) and rest of the animals received the test samples (20 mg/kg body weight) intraperitoneally. Increase in the paw volume was measured before and after 3 hr of administration and the results (% inhibition of oedema) were compared (cf. Table I).

Table I—Anti-inflammatory activity of compounds 6a-d

<table>
<thead>
<tr>
<th>Compd</th>
<th>Increase in paw volume (mL) ± S.E.</th>
<th>% Inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.91 ± 0.03</td>
<td>39.56</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.55 ± 0.03</td>
<td>38.46</td>
</tr>
<tr>
<td>6a</td>
<td>0.55 ± 0.03</td>
<td>39.54</td>
</tr>
<tr>
<td>6b</td>
<td>0.54 ± 0.04</td>
<td>40.66</td>
</tr>
<tr>
<td>6c</td>
<td>0.52 ± 0.02</td>
<td>42.86</td>
</tr>
</tbody>
</table>

All the compounds were found to possess potent anti-inflammatory activity as compared to the standard drug ibuprofen. The activity of compound 6a was slightly less than the standard drug, the activity of compound 6b was the same as that of ibuprofen. However, the activity of 6c and 6d respectively, was slightly more than that of ibuprofen.

Experimental Section

Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on a Perkin-Elmer 137 instrument (λ_{max} in cm⁻¹) and ¹H NMR in continuous wave mode on EM-390 (90 MHz) spectrometer for the peptide fragments and Bruker AC 300F (300 MHz) spectrometer for the final products, with TMS as an internal standard. Elemental analysis was done on Carlo Erba 1108 elemental analyser. Purity of all the compounds was checked by TLC on silica gel G plates.

Preparation of dipeptides. To a stirred solution of the amino acid methyl ester hydrochloride (10 mmoles) in CHCl₃ (30 mL), triethylamine (4 mL, 28.7 mmoles), Boc-Amino acid (10 mmoles) and DCC (10 mmoles) were added. The reaction mixture was stirred for 24 hr and then filtered. The filtrate was washed with 10% HCl (10 mL), 10% NaHCO₃ (10 mL) and saturated NaCl (10 mL). The organic layer was dried over anhydrous Na₂SO₄ and evaporated under vacuum. It was then purified by recrystallisation from CHCl₃/pet. ether.

N-tert-Butyloxy carbonyl-leucyl-glycine methyl ester 1: White crystals, m.p. 133°C (Theor. 133-35°C), yield 90.5%; IR (CHCl₃): 3250 (br s), 2950 (s), 1740 (s), 1700(s), 1655 (s), 1360 (s), 1090 (s), 895 (s) cm⁻¹; ¹H NMR (90 MHz, CDCl₃): δ 7.2 (1H, br s), 6.9 (1H, br s), 4.7-4.4 (1H, m), 4.2-4.0 (2H, m), 3.75 (3H, s), 2.3-1.7 (2H, m), 1.45 (9H, s), 1.3-1.1 (1H, m), 0.9 (6H, d, J = 6.5 Hz).

Boc-Tryptophanyl-valine methyl ester 2: Semi-solid mass, yield 72.4%; IR (CHCl₃): 3424 (br s), 2912 (br s), 1725 (s), 1680 (s), 1665 (s), 1488 (s), 1357 (br s), 1309 (s), 1046 (m), 992 (s), 890 (s) cm⁻¹; ¹H NMR (90 MHz, CDCl₃): δ 7.45-7.0 (5H, m), 6.5 (2H, br s), 5.3-5.2 (1H, br s), 4.8-4.7 (1H, m), 4.6-4.4 (1H, m), 3.75 (3H, s), 3.3-3.1 (2H, m), 1.4 (9H, s), 1.3-1.1 (1H, m), 0.9 (6H, d, J = 6.5 Hz).

Boc-prolyl-leucine methyl ester 3: White crystals, m.p. 93°C (Theor. 92-94°C), yield 75.4%; IR (CHCl₃): 3250 (br s), 2950 (s), 1710 (s), 1700 (s), 1670 (s), 1510 (m), 1240 (s), 1160 (m) 1020 (s), 1000 cm⁻¹ (m); ¹H NMR (90 MHz, CDCl₃): δ 6.5 (IH, br s), 4.6-4.4 (1H, m), 4.3-4.1 (1H, m), 3.7 (3H, s), 3.5-3.2 (2H, m), 2.0-1.6 (6H, m), 1.45 (9H, s), 1.3-1.1 (1H, m), 1.0 (6H, d, J = 6.0 Hz).

Boc-threonyl-proline methyl ester 4: Semi-solid mass, yield 71%; IR (CHCl₃): 3320 (br s), 2930 (s), 2850 (s), 1703 (s), 1645 (br s), 1533 (br s), 1450 (s), 1270 (br s), 1240 (s), 1090 (s) cm⁻¹; ¹H NMR (90 MHz, CDCl₃): δ 6.1 (1H, br s), 4.5-4.3 (2H, m), 3.7 (3H, s), 3.6-3.2 (2H, m), 2.3-1.6 (4H, m), 1.4 (9H, s), 1.3-1.1 (1H, m), 0.95 (3H, d, J = 6.5 Hz).

Preparation of Boc-Pro-Leu-Thr-Pro-OMe 5: The tetrapeptide (5) was prepared by coupling the dipeptides Boc-Pro-Leu-OMe and Boc-Thr-Pro-OMe after proper deprotection using the procedure similar to that of the dipeptides. It was obtained as a semi-solid mass, yield 80%; IR (CHCl₃): 3490 (br s), 2900 (s), 2850 (s), 1730 (s), 1690 (br s), 1675 (s), 1630 (s), 1460 (s), 1390 (br s), 1350 (s), 1210 (br s), 1180 (br s), 1100 (s), 1050 (s), 950 (br s), 900 (s) cm⁻¹; ¹H NMR (90 MHz, CDCl₃): δ 6.5 (1H, br s), 5.6 (1H, br s), 4.6-4.5 (1H, m), 4.4-4.3 (2H, m), 4.1-3.9 (1H, m), 3.75 (3H, s), 3.6-3.2 (4H, m), 2.4-1.6 (10H, m), 1.5 (9H, s), 1.4-1.1 (2H, m), 1.3 (3H, d, J = 6.5 Hz), 0.95 (6H, d, J = 6.5 Hz).
Coupling of ibuprofen with amino acids and peptides: To a stirred solution of amino acid/peptide methyl ester (10 mmoles) in CHCl₃ (30 mL), Et,N (28.7 mmoles), ibuprofen (10 mmoles) and DCC (10 mmoles) were added and stirred for 24 hr at room temperature. Further work-up was done as per the procedure for the preparation of dipeptides.

2-(4-Isothiobutylphenyl)proionyl-tyrosine methyl ester 6a: White solid, m.p. 153°C, yield 84% (Found: C, 71.99; H, 7.60; N, 3.68. C₁₂H₁₅N₂O₄ requires C, 72.02; H, 7.57; N, 3.65%); IR (CHCl₃): 3600 (s), 3300 (s), 1650 (s), 1570 (s), 1270 (s), 1160 (s), 1075 (s), 890 (s) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.7 (4H, d, J = 8.5 Hz), 7.06 (4H, d, J = 8.5 Hz), 6.9 (1H, br s), 4.1-3.9 (2H, m), 3.7 (3H, s), 3.3-3.1 (2H, m), 2.45 (2H, d, J = 6.5 Hz), 1.5 (3H, d, J = 6.0 Hz), 1.3-1.0 (2H, m), 0.9 (6H, d, J = 7.0 Hz).

2-(4-Isothiobutylphenyl) proionyl-leucyl-glycine methyl ester 6b: White solid, m.p. 151°C, yield 85% (Found: C, 67.65; H, 8.75; N, 7.18%); IR (CHCl₃): 3300 (s), 3200 (s), 1660 (s), 1530 (s), 1520 (br s), 1270 (s), 1075 (s), 1020 (s), 890 (s) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.7 (4H, d, J = 8.5 Hz), 7.05 (2H, d, J = 8.5 Hz), 6.7 (1H, br s), 6.5 (1H, br s), 4.6-4.4 (5H, m), 3.7 (3H, s), 3.4-3.3 (4H, m), 2.45 (2H, d, J = 6.5 Hz), 2.0-1.6 (10H, m), 1.5 (3H, d, J = 6.0 Hz), 0.9 (12H, d, J = 7.0 Hz).

2-(4-Isothiobutylphenyl) propionyl-tryptophanyl-tyrosine methyl ester 6c: Yellow solid, m.p. 157°C, yield 78.5% (Found: C, 71.35; H, 7.53; N, 8.32%); IR (CHCl₃): 3300 (s), 2920 (br s), 2750 (s), 1700 (s), 1650 (m), 1530 (br s), 1375 (s), 1290 (s), 1160 (s), 1075 (s), 1020 (s), 890 (s) cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 8.8 (1H, br s), 7.6 (1H, br s), 7.3 (1H, br s), 7.2-6.9 (9H, m), 4.1-3.9 (3H, m), 3.75 (3H, s), 3.4-3.0 (2H, m), 2.45 (2H, d, J = 6.5 Hz), 1.5 (3H, d, J = 6.0 Hz), 1.3-1.0 (2H, m), 0.9 (12H, d, J = 7.0 Hz).

References