Synthesis and Evaluation of Antioxidant, Anti-inflammatory and Antiulcer Activity of Conjugates of Amino Acids with Nifedipine

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A new series of novel (2S)-2-[2-[1,4-dihydro-3,5-bis(methoxy-carbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxyethyl]amino)-3-(4-hydroxyphenyl) propanoic acid (3a) and its analogues 3b—j has been synthesized. These compounds were evaluated for their in vitro antioxidant activity, anti-inflammatory activity and antiulcer activity. Compounds 3b and f exhibited significant antioxidant action comparable with that of standard. Efficacy against inflammation and ulceration was also found to be significant. The chemical structures of these compounds were confirmed on the basis of spectral data.

Key words nifedipine; amino acid; conjugate; antioxidant; anti-inflammatory; antiulcer

Gastric acid secretion is a calcium dependent process and calcium channel blockers including nifedipine have exhibited good potential to reduce gastric secretion.1—3 The role of free radicals in inflammation of gastric mucosa and use of antioxidants for protection against ulceration has been widely suggested.4,5 Several amino acids6 and their conjugates with antioxidants for protection against ulceration has been widely suggested.

Antioxidant Study

In-Vitro Antioxidant Study The free radical scavenging activity of synthesized compounds was evaluated by the method first employed by Blois(10) using 1,1-diphenyl-2-picrylhydrazyl (DPPH). To 1 ml of each compound of different concentrations (1, 2, 3, 4, 5 mg/ml) 1 ml of 0.1 mmol DPPH was added and incubated in the dark room for 35 min. The absorbance was measured at 517 nm and percentage quenching of DPPH was calculated. For all the compounds and standard half inhibition concentration (IC50) was calculated and showed in Table 1. Conjugation of amino acids increased the antioxidant potential of nifedipine as evident from the lower IC50 value of compounds. The IC50 value of compound 3b was lowest among the test compounds followed by that of 3f indicating their good radical scavenging potential. The DPPH radical scavenging action of 3b was found to be better than ascorbic acid (Fig. 1).

Anti-inflammatory Activity of Synthesized Compounds

Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenin in normal saline in the right hind paw of the rats. Paw volume was measured plethysmometrically11) at 0 and 4 h after carrageenin injection. The animals were treated with the synthesized compounds (50 mg/kg). Saline (3 ml/kg, orally) treated animals served as control and acetyl salicylic acid (100 mg/kg, orally) was administered as standard drug. The drugs were administered simultaneously with carrageenin injection. Mean increase in paw volume was measured and reported in Table 1. All the test compounds reduced the paw volume. The anti-inflammatory activity was found to be significant (p<0.001) for compounds 3b, c and f (Fig. 2).

Antiulcer Activity of Synthesized Compounds

The antiulcer activity of the synthesized compounds were evaluated by pyloric ligature induced gastric ulcers in rat model using parameters including volume, pH, ulcer index, free acidity and total acidity.12) The test compounds exhibited variable antiulcer activity. Reduction in acid secretion was more significant (p<0.001) in animals administered with compounds 3b, c and f. The acid neutralizing ability of 3b and 3f were more significant (p<0.001) and comparable with omeprazole. The reduction in free acidity by 3b and f was close to that of the standard. The reduction in total acidity by both compounds was significant (p<0.001) but less than omeprazole. The ulcer healing capacity as indicated by ulcer index was significant (p<0.001) for compounds 3a, d, f, h and i. This study shows that antioxidant action complements the antiseretary properties of the compounds as evident from activities of compounds 3b and f. Compound 3b showed significant antiulcer activity.
better antioxidant action than 3f but the anti-inflammatory and antiulcer actions were comparable. Thus it can be suggested that antioxidant action of 3b has not been able to remarkably potentiate its antisecretory properties. From this study it can be proposed that conjugation of amino acids to nifedipine enhanced the antioxidant action that complemented the antisecretory action of the compounds resulting in increased anti-inflammatory and antiulcer potential. This improvement was more significant in phenyl alanine and methionine conjugates of nifedipine.

**Experimental**

**General** All reagents and solvents were used as purchased without further purification. Melting points were determined on a Sisco melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60—120 mesh. IR spectra were obtained on a JASCO FTIR-4100 spectrometer using KBr pellet. NMR spectra were recorded on BRUKER AVANCE-II-400 MHz spectrometer for 1H-NMR. The chemical shifts were reported as ppm down field using tetramethylsilane (TMS) as an internal standard. Elemental analysis was carried out with PERKIN ELMER-2400 analyser. Mass spectra were recorded on a MICRO-MASS Q-TOF MICRO spectrometer operating at 70 eV.

**Typical Procedure**

**Dimethyl 1,4-Dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate (1)** A solution of 2-nitro benzaldehyde (0.2 mol), methyl acetoacetate (0.2 mol) and ammonium hydroxide (8 ml) in ethanol (60 ml) was heated under reflux for 3 h. To the resulting mixture warm water (40 ml) was added and then allowed to cool. The separated product was filtered off, washed with 60% aqueous ethanol and recrystallised from ethanol. The purity of the compound was checked with TLC.

**Dimethyl-1-(2-chloroacetyl)-1,4-dihydro-2,6-dimethyl-4-(2-nitrophosphorylpyridine-3,5-dicarboxylate (2)** Chloroacetyl chloride (0.2 mol) and ammonium hydroxide (8 ml) in ethanol (60 ml) was heated under reflux for 3 h. To the resulting mixture warm water (40 ml) was added and then allowed to cool. The separated product was filtered off, washed with 60% aqueous ethanol and recrystallised from ethanol. The purity of the compound was checked with TLC.
Table 1. Antioxidant, Anti-inflammatory and Anticancer Activity of 2(S)-2-((2-[1,4-Dihydro-3,5-bis(methoxycarbonyl)]-2,6-dimethyl-4-[2-nitrophenyl]pyridin-1-yl]-2-oxoethyl)amino)-3-(4-hydroxyphenyl)propanoic Acid (3a) and Its Analogs 3b—j

<table>
<thead>
<tr>
<th>Group</th>
<th>RSA</th>
<th>Anti-inflammatory activity</th>
<th>Anticancer activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC_{50} (µg/ml)</td>
<td>Volume (ml)</td>
<td>pH</td>
</tr>
<tr>
<td>Cont.</td>
<td>—</td>
<td>6.4±0.3141</td>
<td>1.912±0.2693</td>
</tr>
<tr>
<td>Standard 50</td>
<td>0.1500±0.0222***</td>
<td>2.167±0.283***</td>
<td>5.050±0.3354***</td>
</tr>
<tr>
<td>1</td>
<td>272</td>
<td>0.3124±0.0210***</td>
<td>4.85±1.2</td>
</tr>
<tr>
<td>3a</td>
<td>326</td>
<td>0.3333±0.0210***</td>
<td>4.78±0.2587</td>
</tr>
<tr>
<td>3b</td>
<td>10</td>
<td>0.1833±0.0167***</td>
<td>2.38±0.25**</td>
</tr>
<tr>
<td>3c</td>
<td>180</td>
<td>0.3667±0.04216***</td>
<td>3.83±0.40**</td>
</tr>
<tr>
<td>3d</td>
<td>169</td>
<td>0.3500±0.02236***</td>
<td>5.85±0.37</td>
</tr>
<tr>
<td>3e</td>
<td>381</td>
<td>0.3167±0.04773***</td>
<td>4.81±0.365</td>
</tr>
<tr>
<td>3f</td>
<td>65</td>
<td>0.2000±0.02582***</td>
<td>2.23±0.2951*</td>
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<tr>
<td>3g</td>
<td>254</td>
<td>0.400±0.06009*</td>
<td>4.50±0.2569**</td>
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<tr>
<td>3h</td>
<td>90</td>
<td>0.35±0.03651</td>
<td>3.98±0.5474**</td>
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<tr>
<td>3i</td>
<td>81</td>
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<td>4.53±0.5542</td>
</tr>
<tr>
<td>3j</td>
<td>332</td>
<td>0.5833±0.03073***</td>
<td>4.08±0.2344**</td>
</tr>
</tbody>
</table>

RSA: Radical (DPPH) scavenging activity. Data are represented as mean±S.E.M. Statistical analysis was done with one way analysis of variance (ANOVA). ***p<0.001, **p<0.01 and *p<0.05 as compared to control (n=6 in each group).
1678, 1132. 1H-NMR (CDCl3) δ: 10.4 (s, 1H, OH), 7.6—7.8 (m, Ar-H), 4.3 (s, 1H, –CH–), 3.5 (s, 1H, OH), 2.1—2.6 (m, –CH2–), 3.4 (s, 6H, –CH3) .

(2R)-2-{(3,5-Bis(methoxy carbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridine-1(4H)-3-oxoacetyl)amino}-4-(methylthio)butanoic Acid (3f): Deep yellow solid, yield 51%, mp 153—155 °C. IR (KBr) cm⁻¹: 3415, 3122, 3025, 2955, 7748, 1128. 1H-NMR (CDCl3) δ: 11 (s, 1H, OH), 7.5—7.8 (m, Ar-H), 4.6 (s, 1H, –CH), 3.8 (s, 6H, –CH3), 2.1—2.7 (m, –CH2–). Anal. Caled for C24H30N3O9S: C, 53.82; H, 5.64. Found: C, 53.87; H, 5.47. [α]D25 85.

(2S)-2-{(3-(3,5-Bis(methoxycarbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridine-1(4H)-3-oxoacetyl)amino)-3-(1H-indol-3-yl)propanoic Acid (3g): Brownish yellow solid, yield 56%, mp 122—124 °C. IR (KBr) cm⁻¹: 3426, 3125, 3045, 1731, 2960. 1H-NMR (CDCl3) δ: 2 (1H, s, NH), 2.28 (s, 6H, CH3), 2.3—2.8 (m, –CH2–), 3.6 (s, 6H, –CH3), 4.8 (s, 1H, –CH–), 7.2—7.9 (m, Ar-H), 11 (s, 1H, OH). Anal. Caled for C30H31N4O9: C, 61.01; H, 5.29. Found: C, 60.561; H, 5.12. MS m/z: 590 (M+) . [α]D25 68.

(2S)-2-{(2-[1,4-Dihydro-3,5-bis(methoxy carbonyl)-2,6-dimethyl-4-(2-nitrophenyl)pyridin-1-yl]-2-oxoethyl)amino)-4-carbamoylbutanoic Acid (3h): Brown solid, yield 56%, mp 150—152 °C. IR (KBr) cm⁻¹: 3437, 3245, 3160, 3046, 2950, 1748, 1148. 1H-NMR (CDCl3) δ: 11 (s, 1H, OH), 7.1 (1H, NH), 7.4—7.8 (m, Ar-H), 4.7 (s, 1H, –CH–), 3.8 (s, 6H, CH3), 2.2—2.8 (m, –CH2–). Anal. Caled for C24H29N4O10: C, 54.13; H, 5.48. Found: C, 54.42; H, 5.13. [α]D25 44.

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References