Synthesis and Antimicrobial Activity of 2-(Aminoacid ester)-3-(6-methyl-2-pyridyl)-3,4-dihydro-2H-1,3,2λ5-benzoazaphosphinin-2-thiones

M. Anil Kumar, K. Suresh Kumar, C. Devendranath Reddy, C. Naga Raju, C. Suresh Reddy and P. Hari Krishna

ABSTRACT

Synthesis of 2-(aminoacid ester)-3-(6-methyl-2-pyridyl)-3,4-dihydro-2H-1,3,2λ5-benzoazaphosphinin-2-thiones (3a–j) was accomplished through a two-step process. It involves the prior preparation of 2-chloro-3-(6-methyl-2-pyridyl)-3,4-dihydro-2H-1,3,2λ5-benzoazaphosphinin-2-thione monochloride (2) and its subsequent reaction with the aminoacid ester hydrochlorides in dry tetrahydrofuran-toluene in the presence of triethylamine at various temperatures. These compounds were characterized by IR, 1H, 13C, 31P NMR and mass spectral data.

KEYWORDS

2-[(6-methyl-2-pyridyl) amino] methylphenol, 1,3,2-benzoazaphosphinin-2-thione, antimicrobial activity.

1. Introduction

Organophosphorus compounds continue to receive widespread attention due to their ubiquity in biological systems and their potential to serve as possible pharmaceuticals, agrochemicals and chemical synthetic agents. Phosphorus derivatives have been found to display useful anti-neoplastic properties. The attachment of an aminoacid group to the phosphate moiety is expected to increase their cellular uptake and thus enhance their chemotherapeutic properties. In view of this we have synthesized new 6-membered heterocycles in which aminoacid esters are linked to the phosphorus atom. The antimicrobial activities of these compounds were studied and are reported below.

2. Results and Discussion

The synthesis of 2-(aminoacid ester)-3-(6-methyl-2-pyridyl)-3,4-dihydro-2H-1,3,2λ5-benzoazaphosphinin-2-thiones (3a–j) is accomplished in a two-step process. The synthetic route involves the condensation of 2-[(6-methyl-2-pyridyl) amino]methylphenol (1) with thiophosphoryl chloride in dry tetrahydrofuran in the presence of triethylamine at 40–45 °C to afford the corresponding intermediate 2-chloro-3-(6-methyl-2-pyridyl)-3,4-dihydro-2H-1,3,2λ5-benzoazaphosphinin-2-thione (2).

In the second step the intermediate 2 was reacted with the respective aminoacid ester hydrochlorides in dry tetrahydrofuran-toluene in the presence of triethylamine to obtain the title compounds 3a–j in good yields (Scheme 1). The second step of the reaction was completed at 40–50 °C with stirring for 3–5 h. The progress of the reaction was monitored by TLC analysis. Aliphatic aminoacid esters (3a–e and 1, j) reacted with the thione monochloride (2) more readily than with the aromatic aminoacid esters (3f–h). The tetrahydrofuran-toluene mixture was found to be a good solvent system for the second step of the reaction. The crude products obtained after removing the solvent were purified by column chromatography on silica gel. The synthetic and analytical data of title compounds 3a–j are given in the experimental part.

All the compounds 3a–j exhibited absorption bands for P=S and P-NH in the regions 805–736 cm–1 and 3402–3145 cm–1, respectively. The aromatic protons of 3a–j resonated as multiplets in the region δ 7.80–6.69 ppm. The C-4 methylene protons gave multiplets or triplets and pairs of doublets at δ 5.26–4.81 ppm, indicating their non-equivalence in the six-membered chair conformation of the benzoazaphosphinine ring. These protons couple with phosphorus and the coupling constants differ by as much as 1 Hz. The 31C NMR spectral data for 3a,3c,e,i,3f and 3j are given in the experimental part. The endocyclic oxygen-bonded C-8a gave signals as doublets at δ 150.7–149.9 ppm. The C-4 methylene carbon chemical shifts appeared in the region δ 46.7–46.5 ppm. The methyl carbon, which is linked to the pyridine ring, resonated in the region δ 24.4–24.1 ppm. The chemical shift of the carbon atom α to the aminoacid ester group appeared at δ 43.6–60.5 ppm. The remaining carbon signals are observed in the expected regions. Compounds 3a–j show a phosphorus-31 resonance signal in the range of δ 58.95–62.14 ppm. The high-resolution mass spectral data of 3a,3c,f,i and LCMS data of 3c,f,j are provided in the experimental section.

2.1. Bioactivity

Susceptibility of test organisms to the title compounds (3a–j) was determined by employing the standard disc diffusion technique. All the compounds (3a–j) were tested for their antifungal activity against the growth of Colletotrichum gloeosporioides and Sclerotium rolfsii along with the standard fungicide carbendazim at concentrations of 250 and 500 ppm (Table 1). Compounds 3a–j were also screened for their antibacterial activity against the growth of Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Klebsiella pneumoniae along with the standard gentamycin at concentrations of 100, 200 and 300 ppm (Table 2). The test...
compounds did not possess significant antifungal or antibacterial activity.

3. Experimental

Melting points were recorded on Buchi R-535 (Flawil, Switzerland) apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-c spectrophotometer (Waltham, MA, USA) using KBr optics. $^1$H, $^{13}$C and $^{31}$P NMR spectra were recorded on a Bruker 300 or AMX 400 MHz NMR spectrometers (Ettlingen, Germany) operating at 400 MHz for $^1$H, 100 MHz for $^{13}$C and 161.89 MHz for $^{31}$P. NMR data were recorded in CDCl$_3$ and were referenced to TMS ($^1$H and $^{13}$C) and 85 % H$_3$PO$_4$ ($^{31}$P). Mass spectra were recorded on a Finnigan MAT 1020/Micro-Mass Q-Tof micro AMPS MAX 10/6A, Hz 60/50 system (Ringoes, NJ, USA) fitted with a built-in inlet system. Elemental analyses were performed using a Perkin Elmer 2400 instrument at the Central Drug Research Institute (CDRI), Lucknow, India.

3.1. Synthesis of 2-Chloro-3-(6-methyl-2-pyridyl)-3,4-dihydro-2H-1,3,2-$\lambda^5$-benzoxazaphosphinin-2-thione (2)

A solution (0.002 mol) of thiophosphoryl chloride in 20 mL of dry THF was added dropwise over a period of 20 min to a stirred solution of 2-[(6-methyl-2-pyridyl)amino]methylphenol (1) (0.002 mol) and triethylamine (0.004 mol) in 25 mL of THF at 0–5℃. After stirring for 3 h at 40–45℃, formation of the intermediate, 2-chloro-3-(6-methyl-2-pyridyl)-3,4-dihydro-2H-1,3,2-$\lambda^5$-benzoxazaphosphinin-2-thione (2) was ascertained by TLC analysis run in a 3:7 mixture of ethyl acetate and hexane and the average $R_f$ value observed was 0.75. Triethylamine hydrochloride was removed by filtration. The filtrate was used for the next reaction step without further purification.

3.2. Typical Procedure for the Synthesis of 3a–j

To a stirred solution of aminoacid ester hydrochloride (0.002 mol) and triethylamine (0.004 mol) in dry toluene (10 mL) the intermediate monochloride (2), (0.002 mol) in dry tetrahydrofuran was added dropwise at 0℃. After completion of the addition, the temperature of the reaction was raised to 40–50℃ and the reaction mixture was stirred for 3–5 h. After completion of the reaction, as indicated by TLC conducted in 3:7 mixture of ethyl acetate and hexane, an average $R_f$ value of 0.60 was observed. The reaction mixture was filtered to remove solid triethylamine hydrochloride and the solvent was evaporated under reduced pressure to give the crude product. It was purified by column chromatography on silica gel (100–200 mesh, 150–200 mesh, 200–300 mesh).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Compound</th>
<th>R</th>
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<tbody>
<tr>
<td>3a</td>
<td>HN-CH$_2$-COOCH$_3$</td>
<td>3f</td>
<td>C=OCH$_3$</td>
</tr>
<tr>
<td>3b</td>
<td>HN-CH$_2$-COOC$_2$H$_5$</td>
<td>3g</td>
<td>COOCH$_3$</td>
</tr>
<tr>
<td>3c</td>
<td>COOC$_2$H$_4$</td>
<td>3h</td>
<td>NH-CH$_2$-C$_6$H$_5$</td>
</tr>
<tr>
<td>3d</td>
<td>NH-CH-CH$_3$-CH$_3$</td>
<td>3i</td>
<td>COOCH$_2$C$_6$H$_5$</td>
</tr>
<tr>
<td>3e</td>
<td>NH-CH$_2$-CH$_2$-CH$_3$</td>
<td>3j</td>
<td>COOCH$_3$</td>
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ethyl acetate:hexane, 1:9) to afford the pure compound. The compounds thus obtained were characterized by 1H NMR, 13C NMR, IR and mass spectrometry.

3.3. Spectral Data

3.3.1. Methyl 2-[3-(6-methyl-2-pyridyl)-2-thioxo-3,4-dihydro-2H-1,3,2λ2-benzoxazaphosphinin-2-yl]aminoacetate (3a)

Yield 78%; viscous liquid. δi (400 MHz, CDCl3) 7.47-7.13 (6H, m, Ar-H), 6.81 (1H, d, J 8 Hz, Ar-H), 5.21 (1H, t, J 15.6 Hz, -CH2-), 4.90 (1H, dd, J 9.7, 14.6 Hz, -CH2-), 4.08 (1H, brs, Ar-CH2), 3.83-3.73 (2H, m, O-CH2) and 2.48 ppm (3H, s, Ar-CH3); δj (100 MHz, CDCl3) 170.5 (d, J P-C 10 Hz, C=O), 156.8 (C-5'), 153.3 (d, J P-C 7 Hz, C-4a), 124.6 (C-6), 118.7 (d, J P-C 5 Hz, C-8), 117.5 (C-4'), 110.9 (C-2'), 52.4 (O-CH3), 46.7 (-CH2-), 43.6 (N-CH2) and 24.3 ppm (Ar-CH3); δk (CDCl3) 60.92 ppm; νmax (CHCl3) 764 cm–1 (P=S), 1744 (C=O) and 3325 cm–1 (P-NH). Calc. for C19H24N3O3PS: C, 56.29; H, 5.97; N, 11.13 %.

3.3.3. Methyl 2-[3-(6-(methyl-2-pyridyl)-2-thioxo-3,4-dihydro-2H-1,3,2λ2-benzoxazaphosphinin-2-yl]aminopropanoate (3d)

Yield 81%; viscous liquid. δi (400 MHz, CDCl3) 7.50–7.65 (7H, m, Ar-H), 5.25 (1H, t, J 15.4 Hz, -CH2-), 4.87 (1H, dd, J 9.0, 14.8 Hz, -CH2-), 4.14 (1H, brs, N-H), 3.80–3.71 (2H, m, N-CH2-), 4.08 (2H, q, J 7.0 Hz, O-CH2-), 2.46 (3H, s, Ar-CH3) and 1.21 ppm (3H, t, J 7.0 Hz, O-CH2-C); δj (CDCl3) 60.12 ppm; νmax (CHCl3) 758 cm–1 (P=S), 1751 (C=O) and 3351 cm–1 (P-NH). Calc. for C19H24N3O3PS: C, 56.29; H, 5.97; N, 10.36 %.

3.3.4. Methyl 3-methyl-2-[3-(6-(methyl-2-pyridyl)-2-thioxo-3,4-dihydro-2H-1,3,2λ2-benzoxazaphosphinin-2-yl]aminobutanoate (3d)

Yield 76%; viscous liquid. δi (400 MHz, CDCl3) 7.53–6.79 (7H, m, Ar-H), 5.26–4.87 (2H, m, -CH2-), 4.16–4.07 (2H, m, NH-CH2-CO), 3.69 (3H, s, O-CH3), 2.49 (3H, s, Ar-CH3) and 1.33 ppm (3H, d, J 6.7 Hz, NH-CH2-C=O); δj (100 MHz, CDCl3) 173.6 (d, J P-C 9 Hz, C=O), 156.8 (C-5'), 153.3 (d, J P-C 10 Hz, C-1'), 150.1 (d, J P-C 11 Hz, C-8a), 137.5 (C-3'), 129.2 (C-5), 127.0 (C-7), 126.6 (d, J P-C 7 Hz, C-4a), 124.6 (C-6), 118.8 (d, J P-C 5 Hz, C-8), 117.5 (C-4'), 110.9 (C-2'), 52.4 (O-CH3), 46.7 (-CH2-), 43.6 (N-CH2) and 24.3 ppm (Ar-CH3); δk (CDCl3) 69.22 ppm; νmax (CHCl3) 805 (P=S), 1744 (C=O) and 3328 cm–1 (P-NH). HRMS calc. for C19H24N3O3PSNa: 386.0704; found: 386.0695 (M+Na).
3.3.5. Ethyl 2-[(6-methyl-2-pyridyl)-2-thioxo-3,4-dihydro-2H-1,3,2-benzoxazaphosphinin-2-yl]amino-3-phenylpropanoate (3e)

Yield 71 %; viscous liquid. δi (400 MHz, CDCl3) 7.43–7.01 (6H, m, Ar-H), 6.74 (1H, d, J 7.2 Hz, Ar-H), 5.21–5.11 (1H, m, -CH2), 4.92–4.81 (1H, m, -CH2), 4.12–4.01 (3H, m, O-CH3, and NH-CH2). 3.92–3.81 (1H, m, NH-CH2-CO), 2.34 (3H, s, Ar-CH3), 1.59–1.41 (2H, m, CH2-CH2-CH3), 1.16 (3H, t, J 7.8 Hz, O-CH3-CH2-CH3), 1.11–1.05 (2H, m, CH2-CH2-CH3) and 0.80 ppm (3H, t, J 6.8 Hz, CH3-CH2-CH3); δc (100 MHz, CDCl3) 172.7 (2 δ15C 80 ppm, CH=O), 156.7 (C-S), 153.4 (δ31P 10 Hz, C-8a), 150.1 (δ13C 11 Hz, C-8a), 137.4 (C-3), 129.1 (C-5), 127.1 (C-7), 126.6 (δ31P 7 Hz, C-4a), 124.6 (C-6), 118.7 (δ31P 6 Hz, C-8), 117.3 (C-4), 113.1 (δ31P 2 Hz, C-2), 61.3 (O-CH3), 52.5 (NH-CH2-CO), 46.7 (δ31P 17 Hz, C-7), 36.5 (d, J 15 Hz, CH2-CH2-CH3), 24.3 (Ar-CH3), 18.1 (CH3-CH2-CH), 14.1 (O-CH3-CH2-CH3) and 13.6 ppm (CH2-CH2-CH3). δc (CDCl3) 60.43 ppm; νmax (CHCl3) 805 (P=S), 1735 (C=O) and 3310 cm–1 (P-NH).

5.56; N, 10.37 %.

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References