Efficient one pot, three-component synthesis of new α-aminophosphonates and investigation of their antimicrobial activity

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ABSTRACT

A convenient one pot synthesis of new α-aminophosphonates (4a-h) containing ethyl acetate moiety was accomplished via Kabachnik-Fields reactions in good yield. All synthesized compounds were characterized by IR, 1H NMR, 13C NMR, 31P NMR and Mass spectroscopy and investigated their antimicrobial activity.

Key words: α- Aminophosphonates, one pot synthesis, Kabachnik-Fields reactions, Antimicrobial activity.

INTRODUCTION

Organophosphorus compounds have found a wide range of application in industry, agriculture and medicinal chemistry owing to their unique physical and biological properties [1]. Out of them α-aminophosphonates and related α-aminophosphonic acids were studied as the analogs of α-amino acids in which the carboxylic acid moieties are replaced by phosphonates or phosphonic acid groups [2]. α-Aminophosphonates are the key intermediates in the synthesis of naturally occurring α-aminophosphonic acids [3]. They are also used in medicinal chemistry as well as in agrochemistry. In medicinal chemistry α-aminophosphonates acts as HIV protease [4], enzyme inhibitors [5], anticancer agents [6], potential antibiotics [7], haptens for catalytic antibodies [8]. In agrochemistry α-aminophosphonates acts as herbicides [9], plant growth regulators [10].

In view of this several methods and modifications in the existing methods have been reported in the literature for the synthesis of α-aminophosphonates such as nucleophilic displacement of α-halo amino derivatives (Michaelis-Arbuzove reaction) [11], Pudovic reaction [12], oximation and reduction of α-oxophosphonates [13], Kabachnik-Fields reaction [14,15]. Recently various synthetic methods of cyclic α-aminophosphonic acids and α-aminophosphonates have been reviewed by Tarik Ali [16]. Zefirov and Matveeva reviewed the synthesis, characterization, stereochemistry and biological activities of acyclic α-aminophosphonates derivatives [17]. The most versatile and widely used approach is the Kabachnik-Fields reaction which is a one-pot, three-component method. In this method the reaction of carbonyl compound, amine and dialkyl or trialkyl phosphite results in the formation of α-aminophosphonates. Therefore we also utilized one pot Kabachnik-Fields reaction for the synthesis of new α-aminophosphonates (4a-h).

MATERIALS AND METHODS

All the chemicals and solvents used were of synthetic grade (Aldrich and s d fine chemicals). The purity of all the synthesized compounds was checked by TLC. Melting points of all the compounds were determined by open...
capillary method and are uncorrected. Thin layer chromatography was performed with pre-coated TLC sheets of silica gel (60 F254, Merck). IR spectra were recorded on Shimadzu IR Affinity-1 using KBr discs. 1H NMR spectra were scanned in CDCl₃ on Varian mercury plus (400MHz) and broker advance (300MHz) spectrometer taking TMS as an internal standard for both the spectrometers and 13C resonant frequency of 75MHz. 31P NMR spectra were recorded in CDCl₃ on Varian mercury plus/121MHz)spectrometer in which chemical shifts were referenced to 85% phosphoric acid. LCMS spectra were recorded on a Finnigan LCQ LC-MS system.

**Diethyl(2-((ethoxycarbonyl)methyl)-4,5-dimethoxyphenyl) (phenylamino) methyl phosphonates (4a).**

IR (KBr, cm⁻¹): 3292 (s-NH), 1718 (C=O), 1228 (P=O); 1H NMR (300MHz, CDCl₃, δ ppm): δ 7.04 (d, 2H, J = 8.6 Hz, ArH), 7.02 (s, 1H, ArH), 6.78 (s, 1H, ArH). 6.67 (d, 2H, J = 8.5 Hz, ArH), 5.50 (b, 1H, NH), 5.13 (d, 1H, Jp, = 20.9Hz, PCH), 4.03-4.21 (m, 4H, POCH₂CH₃), 3.79-3.86 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 3.58-3.71 (m, 2H, ArCH₂), 1.25 & 1.28 (t, 3H each, J = 7.0 Hz, 2 x P-OCH₂CH₃). 11.2 (t, 3H, 3H, OCH₂CH₃); 31P NMR (121 MHz, CDCl₃, δ ppm): δ 21.72; 13C NMR (75 MHz, CDCl₃, δ ppm): δ 171.27, 148.62, 148.46, 148.25, 138.93, 125.49, 125.39, 125.3, 113.92, 110.63, 63.77, 66.70, 55.84, 55.76. (Jc = 150.9Hz), 38.1, 38.3, 16.4, 16.6 (d, Jc,p = 5.7 Hz), 14; LC-MS (m/z): 522 (M+Na).

**Diethyl (2-((ethoxycarbonyl) methyl)-4,5-dimethoxyphenyl) (4-chlorophenyl amino) methyl phosphonates (4b).**

IR (KBr, cm⁻¹): 3294 (-NH), 1724 (C=O), 1230 (P=O); 1H NMR (300MHz, CDCl₃, δ ppm): δ 8.04 (d, 2H, J = 9.1 Hz, ArH), 6.97 (s, 1H, ArH), 6.80 (s, 1H, ArH), 6.67 (d, 2H, J = 9.1 Hz, ArH), 5.13 (b, 1H, NH), 5.13 (d, 1H, Jp, = 21.9Hz, PCH), 4.03-4.21 (m, 4H, POCH₂CH₃), 3.79-3.86 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 3.58-3.71 (m, 2H, ArCH₂), 1.26 & 1.30 (t, 3H each, J = 7.0 Hz, 2 x P-OCH₂CH₃). 11.2 (t, 3H, 3H, OCH₂CH₃); 13C NMR (75 MHz, CDCl₃, δ ppm): δ 171.1, 151.4, 148.6, 148.4, 138.9, 125.9, 125.3, 125, 113.9, 112.3, 110, 63.7, 63, 61.1, 51.93 (Jc,p = 152 Hz), 38.1, 16.29,16.18, 14; LC-MS (m/z): 533(M+Na).

**Diethyl (2-((ethoxycarbonyl) methyl)-4,5-dimethoxyphenyl) (4-nitrophenylamino) methyl phosphonate (4c).**

IR (KBr, cm⁻¹): 3273 (-NH), 1722 (C=O), 1230 (P=O); 1H NMR (300MHz, CDCl₃, δ ppm): δ 8.70 (d, 1H, J = 1.4 Hz, ArH), 7.0 (t, 2H, J = 8 Hz, ArH), 6.79 (s, 1H, ArH), 6.20-6.27 (m, 2H, ArH), 5.05 (d, 1H, J = 21.9Hz, PCH), 4.86 (brs, 1H, NH), 4.90-4.15 (m, 4H, POCH₂CH₃), 3.92-3.97 (m, 2H, OCH₂CH₃), 3.86 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃), 3.70 (s, 3H, OCH₃), 3.61-3.66 (m, 2H, ArCH₂), 1.25 & 1.28 (t, 3H each, J = 7.0 Hz, 2 x P-OCH₂CH₃). 11.2 (t, 2J, 3H, OCH₂CH₃); 13C NMR (75 MHz, CDCl₃, δ ppm): δ 171.28, 160.58, 148.26, 147.65, 147.48, 129.76, 126.54, 125.1 (d, J = 6.9Hz), 113.75, 110.3, 106.65, 103.54, 99.87, 63.24 (d, J = 6.9 Hz), 63.76, 60.95, 55.84, 55.78, 54.94, 52.29 (d, Jc,p = 150.9Hz), 38.2, 16.39 (d, J = 5.8 Hz, 16.19 (d, Jc,p = 4.6 Hz), 14; LC-MS (m/z): 496 (M+1), 518 (M+Na).

**Diethyl (2-((ethoxycarbonyl) methyl)-4,5-dimethoxyphenyl) (3-fluro phenyl amino) methyl phosphonates (4d).**

IR (KBr, cm⁻¹): 3290 (-NH), 1716 (C=O), 1224 (P=O); 1H NMR (300MHz, CDCl₃, δ ppm): δ 7.04 (s, 1H, ArH), 6.78-6.80 (m, 3H, ArH), 6.57 (s, 2H, ArH), 4.97 (d, 1H, Jp, = 22.3Hz, PCH), 4.60 (brs, 1H, NH), 4.90-4.15 (m, 4H, POCH₂CH₃), 3.92-3.97 (m, 2H, OCH₂CH₃), 3.78 (s, 3H, OCH₃), 3.58-3.71 (m, 2H, ArCH₂), 1.25 & 1.28 (t, 3H each, J = 7.0 Hz, 2 x P-OCH₂CH₃). 11.2 (t, 2J, 3H, OCH₂CH₃); 13C NMR (75 MHz, CDCl₃, δ ppm): δ 21.64; 31P NMR (121 MHz, CDCl₃, δ ppm): δ 171.41 (d, J = 2.3Hz), 156.32 (d, Jc,p = 236.6 Hz), 148.41 (d, J = 2.7Hz), 142.62 (d, J = 13.9Hz), 126.37 (d, J = 2.8Hz), 125.34 (d, J = 7.2 Hz), 115.56 (d, J = 22.7 Hz), 114.92 (d, J = 7.8 Hz), 113.92 (d, J = 2.8Hz), 110.45 (d, J = 4.5), 63.33 (d, J = 7.2Hz), 63.17 (d, J = 7.2Hz), 61.04, 257
Diethyl (2-((ethoxycarbonyl) methyl)-4, 5-dimethoxyphenyl) (3-chloro-4-fluoro phenylamino) methyl phosphonates (4g).

IR (KBr, cm\(^{-1}\)): 3318 (-NH), 1720 (P=O), 1317 (C-N), 1307 (C=O), 1238 (P=O). 1\(^{H}\) NMR (300MHz, CDCl\(_3\), ppm): \(\delta\) 7.18 (d, 2H, J = 7.2 Hz, Ar\(H^2\)), 7.04 (s, 1H, Ar\(H^1\)), 2.85 (t, 1H, J = 7.5 Hz, CH\(=\)) , 2.84 (t, 1H, J = 7.5 Hz, CH\(=\)) , 1.26 & 1.28 (t, 3H each, J = 7.2 Hz, CH\(3\)), 1.12 (t, 3H, J = 7.2 Hz, CH\(3\)).

\(\text{M+Na}\) m/z: 506 (M+Na).

RESULTS AND DISCUSSION

Antimicrobial Activity:
The synthesized compounds 4a-h were screen for antimicrobial activity by Agar disc-diffusion method [18]. Stock solutions of the synthesized compounds were prepared by dissolving in dimethyl sulfoxide (DMSO) to 100 ppm concentration. These solutions were added to each filter disc and DMSO was used as control. The anti-bacterial activity of 4a-h was assayed through the growth of gram-positive bacteria Staphylococcus aureus and gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa by the disc-fusion method in nutrient agar medium and antifungal activities were evaluated against Aspergillus niger and Candida albicans in Saboroud’s dextrose agar medium. After incubation for 24 h at 37 °C the zones of inhibition were measured in mm and compared with the activity of the standard drugs. The Ciprofloxacin was used as reference standard during screening of anti-bacterial activity and Amphotericin-B was used as the reference compound for anti-fungal activities.

RESULTS AND DISCUSSION

Chemistry:
Considering the importance of \(\alpha\)-aminophosphonates in Medicinal Chemistry as well as in Agrochemistry, we have developed \(\text{TiCl}_3\) catalyzed [19] (10 mol %) one pot three component Kabachnik-Fields reactions for the synthesis of new \(\alpha\)-aminophosphonates (4a-h) from ethyl 2-(2-formyl-4,5-dimethoxyphenyl) acetate (1), simple anilines (2a-h) and triethyl phosphate (3) in acetonitrile. In our previous work we have reported synthesis of 4e as a useful intermediate for the synthesis of N-phenyl isoquinolone-1-phosphonate using TFA as a catalyst [20]. The starting
compound, ethyl 2-(2-formyl-4,5-dimethoxyphenyl) acetate (1) was prepared from commercially available 3,4-dimethoxy phenyl acetic acid by esterification [21] followed by formylation with dichloromethyl methyl ether in presence of aluminium chloride and dichloromethane as a solvent [22]. The synthesis of α-aminophosphonate 4a involves reactions of ethyl 2-(2-formyl-4,5-dimethoxyphenyl) acetate (1), simple aniline (2a) and triethylphosphate (3) in acetonitrile using TiCl₄ (10 mol %) as a catalyst at room temperature for 3 h (Scheme). The progress of reaction was monitored by TLC and product was purified by column chromatography using hexane: ethyl acetate (70:30) as an eluent, gummy oily product was obtained, scratched it in hexane at 0 ºC to obtain a white solid of 4a in 85% yield, mp.110-112ºC. The structure of 4a was determined on the basis of IR, ¹H NMR, ¹³C NMR, ³¹P NMR and mass spectroscopy. IR spectrum of 4a showed presence of -NH, at 3292 cm⁻¹, band at 1718 cm⁻¹ indicates presence of C=O of ester, 1228 cm⁻¹ indicates presence of P=O and band at 1053 cm⁻¹ and 1024 cm⁻¹ indicates presence of –C-O stretching of methoxy and phosphate group. ¹H NMR spectrum showed doublet at 5.00 δ (J_H,H = 21.9 Hz) which indicates the presence of -P-CH, due to coupling with both phosphorus and the -NH proton. The broad singlet at 4.69 δ indicates presence of –NH proton. The methylene protons of two -P-OCH₂CH₃ showed a multiplet at 4.05-4.19 δ, and methyl protons of two -P-OCH₂CH₃ also gave multiplet in the region 1.23-1.31 δ. Due to asymmetric carbon at ortho position, the benzylic protons from –CH₂-COOC₂H₅ became non equivalent and exhibited multiplets at δ 3.61-3.70. Two methoxy protons appeared as two singlets at 3.81 and 3.87 δ. The methylene protons of -OCH₂CH₃ of ester group showed a multiplet at 3.90-3.97 δ. While methyl protons of -OCH₂CH₃ of ester appeared as a triplet at 1.12 δ (J = 6.9 Hz). Seven aromatic protons appeared in the region of 6.62–7.13 δ. In ³¹P NMR (121MHz, CDCl₃) one phosphorous of CH–P=O (OC₂H₅) appeared at 21.72 δ. In ¹³C NMR spectrum the carbon chemical shifts for P-CH observed at δ 52.3 (J_C,P = 150.9Hz), methylene carbons of two -P-OCH₂CH₃ were appeared at δ 63.12 and 63.21. Methyl carbon of two -P-OCH₂CH₃ in the title compound 4a was appeared at δ 16.16 and 16.40. The benzylic carbon from –CH₂-COOC₂H₅ appeared at δ 38.14, while carbonyl carbon, methylene carbon and methyl carbon of -CO- OCH₂CH₃ of ester group appeared at δ 171.26, 60.89 and 14.0 respectively. Two methoxy carbon of aromatic ring appeared at 55.73 and 55.81 δ. Aromatic carbons were found in the region 109.89 δ to 148.20 δ. LC-MS spectrum showed base peak of 488 (M + Na).

Scheme:

![Scheme](image)

Table-1: Synthesis of new α-aminophosphonates (4a-h)*.

<table>
<thead>
<tr>
<th>Compound #2 &amp; 4</th>
<th>R₁</th>
<th>R₂</th>
<th>Time (h)</th>
<th>Yield (%)</th>
<th>MP (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>H</td>
<td>H</td>
<td>3</td>
<td>85</td>
<td>110-112</td>
</tr>
<tr>
<td>b</td>
<td>H</td>
<td>Cl</td>
<td>4</td>
<td>83</td>
<td>98-100</td>
</tr>
<tr>
<td>c</td>
<td>H</td>
<td>NO₂</td>
<td>5</td>
<td>72</td>
<td>140-142</td>
</tr>
<tr>
<td>d</td>
<td>OCH₃</td>
<td>H</td>
<td>6</td>
<td>82</td>
<td>78-80</td>
</tr>
<tr>
<td>e</td>
<td>H</td>
<td>F</td>
<td>3</td>
<td>77</td>
<td>118-120</td>
</tr>
<tr>
<td>f</td>
<td>Cl</td>
<td>F</td>
<td>7</td>
<td>75</td>
<td>116-118</td>
</tr>
<tr>
<td>g</td>
<td>H</td>
<td>Br</td>
<td>4</td>
<td>80</td>
<td>102-104</td>
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<tr>
<td>h</td>
<td>H</td>
<td>I</td>
<td>5</td>
<td>79</td>
<td>104-106</td>
</tr>
</tbody>
</table>

*Standard reaction condition: 1 (1mmol), anilines 2a-h (1.1 mmol), 3 (1.2 mmol), TiCl₄ (10 mol %) at 25 ºC, Acetonitrile (10 mL). Isolated yield.

Similarly the compounds 4b-h was synthesized from ethyl 2-(2-formyl-4,5-dimethoxyphenyl) acetate (1) using substituted anilines which have either electron donating or electron withdrawing groups. The time required, yield obtained and melting point of compounds 4a-h was summarized in Table 1.

Antimicrobial Activity:
The synthesized new α-aminophosphonates (4a-h) showed moderate to low activity against gram-positive bacteria Staphylococcus aureus and gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa. The compounds 4a,
4c, 4e, and 4g exhibited appreciable anti-fungal activity against *Aspergillus niger* fungi as compared to *Candida albicans* while compound 4b and 4h showed moderate anti-fungal activity.

Table 2. Antimicrobial activity of α-aminophosphonates (4a-h).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of inhibition in mm</th>
<th>Anti-bacterial activity</th>
<th>Anti-fungal activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>E. coli</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>4a</td>
<td>12.01</td>
<td>12.5</td>
<td>16.7</td>
</tr>
<tr>
<td>4b</td>
<td>11.04</td>
<td>10.97</td>
<td>14.2</td>
</tr>
<tr>
<td>4c</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4d</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4e</td>
<td>9.59</td>
<td>11.34</td>
<td>9.45</td>
</tr>
<tr>
<td>4f</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4g</td>
<td>11.65</td>
<td>12.12</td>
<td>11.15</td>
</tr>
<tr>
<td>4h</td>
<td>12.96</td>
<td>13.14</td>
<td>10.34</td>
</tr>
<tr>
<td>Ciprofloxacin*</td>
<td>22.33</td>
<td>21.11</td>
<td>22.23</td>
</tr>
<tr>
<td>Amphotericine-B</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*The concentration of test compounds was 100µg/ml using solvent DMSO, '-' means no zone of inhibition, NA- Not applicable. *standard reference as anti-bacterial drug. *standard reference as anti-fungal drug.

CONCLUSION

In conclusion, an efficient, one pot synthesis of new α-aminophosphonates (4a-h) containing ethyl acetate moiety using TiCl₄ as a catalyst has been developed. These are useful intermediates in the synthesis of new N-Phenyl isoquinolone-1-phoshonates.²⁰ These new α-aminophosphonates (4a-h) showed low activity against bacteria and moderate to good activity on fungi. Among these, compounds 4a, 4c, 4e, and 4g showed significant antifungal activity against *Aspergillus niger* fungi.

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