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Synthesis and Antimicrobial Activity of New α-Aminophosphonic Acid Esters

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ABSTRACT

Synthesis of new α-aminophosphonic acid esters (3a–l) was accomplished by the reaction of equimolar quantities of phenyl ethyl glycine and various aryl aldehydes with diethyl/ dimethylphosphite in dry toluene at reflux temperature. All the structures of the newly synthesized α-aminophosphonic acid esters (3a–l) were established by elemental analysis, and IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectral data. The antimicrobial and antifungal activities of these compounds were evaluated and they exhibited significant activity.

KEYWORDS

Phenyl glycine ethyl ester, aryl aldehydes, diethyl/dimethylphosphite, antimicrobial activity.

1. Introduction

The Kabachnik-Fields reaction is one of the most effective methods for the synthesis of biologically important α-aminophosphonic acid esters and it has been receiving a great deal of attention in recent years.[1,2] The addition of compounds containing phosphorus-hydrogen bonds to C-C or C-X (X = O,N) double bonds provides a method for the synthesis of organophosphorus derivatives. Amongst phosphorus compounds, (*R*)-amino phosphonic acids and their derivatives have received considerable attention in recent years because they exhibit intriguing biological activities.[3,4] Being considered as (*R*)-amino acid analogues,[5] they have found widespread use as biologically attractive peptide mimics which have been employed, for example, as inhibitors of protease[6] and as catalytic antibodies.[7] In addition, they have been used as antibacterial[8] and anti-HIV agents.[9] Keeping in view the importance of α-amino phosphonates, we report herein the synthesis, spectral characterization and antimicrobial activity of the title compounds.

2. Results and Discussion

Synthesis of new phenyl glycine ethyl ester-substituted phenyl ethyl/methylphosphonates (**3a–l**) was accomplished by the reaction of equimolar quantities of phenyl glycine ethyl ester (**1**), various aromatic aldehydes (**2a–l**) and diethyl/dimethyl phosphite in dry toluene at reflux temperature for 4–5 h in 80–90 % yields (Scheme 1).

The synthetic and analytical data of compounds **3a–l** are given in Table 1. All the compounds **3a–l** exhibited characteristic infrared absorption bands for the important functional groups, N-H, C = O, P = O and P-$C_{aliphatic}$ in the regions 3361–3435,1738–1752, 1256–1282 and 769–780 cm^{-1}, respectively.[10]

The ^{31}P NMR spectral data of **3a–l** are presented in Table 1. ^{31}P NMR signals[11] appeared in the region δ 23.37–26.75 ppm for all the compounds **3a–l**.

The proton NMR spectral data of compounds **3a–l** are presented in Table 2. The aromatic protons of the two benzene rings of α-aminophosphonic acid esters (**3a–l**) showed complex multiplets in the region δ 6.44–8.15 ppm and benzyl carbon protons (Ph-C*H*-N) appear as a doublet at δ 4.95–5.2 ppm. The P-C-H proton signal appears as a multiplet[12a] in the region δ 4.75–5.38 ppm due to its coupling with phosphorus and the neighbouring proton of N-H. The N-H proton gave a signal at δ 4.68–5.57 ppm as a multiplet.[12a] The proton signal of POCH_2CH$_3$ shows a multiplet and POCH$_2$CH_3 exhibited a triplet in the regions δ 4.45–4.50 ppm and δ 1.40–1.55 ppm, respectively, and POCH_3 resonates as a doublet of doublets in the regions δ 3.65–3.79 ppm and δ 3.46–3.67 ppm.[12b,c]

The ^{13}C NMR spectral data for a few α-aminophosphonic acid esters (**3a, 3b, 3g** and **3h**) are presented in Table 3.

The ^{13}C NMR chemical shifts for P-*C*-H appear in the region δ 49.5–51.7 ppm as a doublet (d, $^1J_{PC}$ 150 Hz, P-C). Ph-*CH*-CO$_2$Et resonates in the range of δ 60.1–64.8 ppm. The diethyl phosphite moiety carbon gave two doublets, one at δ 63.3–64.8 ppm (d, $^2J_{P\text{-}O\text{-}C}$ 6.9 Hz, P-O-*C*H$_2$) and the other at δ 15.8–15.9 ppm (d, $^3J_{P\text{-}O\text{-}C\text{-}C}$ 12.6 Hz, P-O-CH$_2$-*C*H$_3$). These values are in agreement with literature data.[12b] The methoxy carbon resonates as a doublet at δ 54.0–55.0 ppm (d, $^2J_{P\text{-}O\text{-}C}$ 6.5 Hz) due to coupling with phosphorus. The C = O carbon signal is observed in the region δ 172.2–175.3 ppm. FAB mass spectra were recorded for **3a** and **3h** (Table 4) and they exhibited $M^{+\bullet}$ values.

3. Experimental

Melting points were determined in open capillary tubes on a Mel-Temp apparatus (Tempo Instruments and Equip (Pvt.) Ltd., Mumbai, India) and were uncorrected.

Microanalysis was performed with a Thermo Finnigan (Courtaboeuf, France) Flash EA 1112 I instrument. IR spectra (KBr discs) were recorded on a Nicolet (San Diego, CA, USA) 380 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker (Ettlingen, Germany) AMX 400 MHz spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C and 161.9 MHz for ^{31}P. The compounds were dissolved in DMSO-d_6. The ^{1}H and ^{13}C chemical shifts were referenced to tetramethylsilane and ^{31}P chemical shifts to 85 % H_3PO_4. Mass spectra were recorded on a Jeol SX 102 DA/600 (Tokyo, Japan) mass spectrometer using argon/xenon (6 keV, 10 mA) as the FAB gas.

3.1. General Procedure for the Preparation of 3a–l

To a stirred solution of phenyl glycine ethyl ester (**1**) (0.005 mol),

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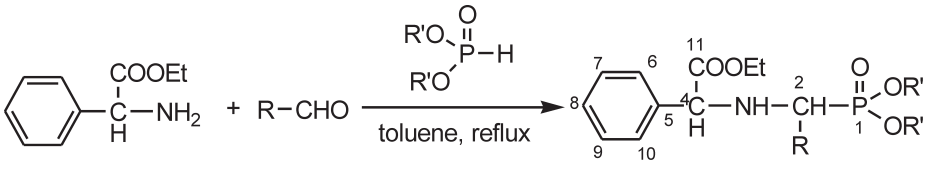
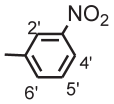
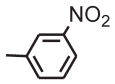
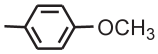
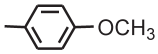
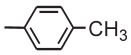
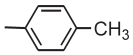
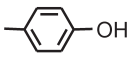
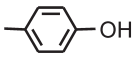


1 **2(a–l)** **3(a–l)**

Compound	R	R′	Compound	R	R′
3a		Me	3g		Et
3b		Me	3h		Et
3c		Me	3i		Et
3d		Me	3j		Et
3e		Me	3k		Et
3f		Me	3l		Et

Scheme 1

Table 1 Synthetic, analytical , infrared and ^{31}P spectral data of α-aminophosphonic acid esters **3a–l**.

Compound	Molecular formula	M.p./°C	Yield/%	Elemental analysis found (calculated)/%			$\bar{\nu}$/cm^{-1}				δ(^{31}P)/ppm [a,b]
				C	H	N	NH	P = O	C = O	P-C$_{(aliphatic)}$	
3a	$C_{19}H_{23}O_7N_2P$	157–159	81	54.03 (54.15	5.48 5.52	6.68 6.75)	3435	1282	1752	780	23.76
3b	$C_{19}H_{23}ClO_5NP$	163–165	74	55.41 (55.50	5.62 5.68	3.40 3.48)	3432	1278	1738	777	25.74
3c	$C_{20}H_{26}O_6NP$	120–122	77	58.96 (59.00	6.46 6.52	3.43 3.50)	3420	1275	1742	779	24.31
3d	$C_{20}H_{26}O_5NP$	178–179	65	61.35 (61.40	6.69 6.75	3.93 4.00)	3425	1277	1751	780	23.37
3e	$C_{19}H_{24}O_6NP$	198–199	79	58.01 (58.14	6.14 6.20	3.56 3.64)	3420	1281	1740	769	26.75
3f	$C_{21}H_{23}O_5FNP$	105–107	64	57.71 (57.80	5.86 5.90	3.55 4.00)	3423	1279	1739	772	24.81
3g	$C_{21}H_{27}O_7N_2P$	134–135	66	55.99 (56.05	6.08 6.15	6.21 6.30)	3424	1280	1740	770	25.57
3h	$C_{21}H_{27}O_5ClO_5NP$	150–152	72	57.34 (57.42	6.18 6.25	3.18 3.25)	3361	1256	1751	769	23.87
3i	$C_{22}H_{30}O_6NP$	160–162	65	61.64 (61.75	6.94 7.00	3.23 3.30)	3430	1282	1750	780	24.34
3j	$C_{22}H_{30}O_5NP$	134–136	76	62.97 (63.05	7.20 7.28	3.33 3.40)	3433	1281	1742	780	24.87
3k	$C_{21}H_{28}O_6NP$	125–127	62	59.80 (59.92	6.69 7.05	3.34 3.40)	3434	1278	1740	773	24.55
3l	$C_{21}H_{27}O_5FNP$	133–135	64	59.51 (59.62	6.44 6.50	3.30 3.38)	3425	1279	1751	775	24.67

[a] Recorded in DMSO-d_6.
[b] Referred to 85 % phosphonic acid.

Table 2 ^{1}H NMR spectral data [a,b] of α-aminophosphonic acid esters **3a–l**.

Compound	Ar-*H*	Ar-C*H*-N	P-C-*H*	N-*H*	C-O-CH_2CH$_3$ and C-O-CH$_2$CH_3	P-O-CH_3	P-O-CH_2CH$_3$ and P-O-CH$_2$CH_3	Ar-O-CH_3/Ar-CH_3
3a	6.45–7.47 (m, 9H)	4.95–5.10 (d, J = 6 Hz, 1H)	4.77–4.87 (m, 1H)	5.18–5.32 (m, 1H)	4.23 (q, J = 10.0 Hz, 2H) 1.50 (t, J = 8.0 Hz, 3H)	3.69 (d, J = 12.2Hz, 3H) 3.50 (d, J = 12.0 Hz, 3H)	–	–
3b	6.55–8.13 (m, 9H)	4.80–4.96 (d, J = 6 Hz, 1H)	4.77–4.87 (m, 1H)	4.98–5.12 (s, 1H)	4.25 (q, J = 10.0 Hz, 2H) 1.60 (t, J = 8.0 Hz, 3H)	3.65 (d, J = 12.0 Hz, 3H) 3.46 (d, J = 12.3 Hz, 3H)	–	–
3c	6.54–8.12 (m, 9H)	4.93–5.07 (d, J = 6 Hz, 1H)	4.81–4.95 (m, 1H)	4.65–4.86 (s, 1H)	4.13 (q, J = 10.0 Hz, 2H) 1.50 (t, J = 8.0 Hz, 3H)	3.70 (d, J = 12.1 Hz, 3H) 3.55 (d, J = 12.2 Hz, 3H)	–	3.89 (s, 3H)
3d	6.56–7.44 (m, 9H)	5.05–5.20 (d, J = 6 Hz, 1H)	5.25–5.35(m, 1H)	5.15–5.32 (s, 1H)	4.13 (q, J = 10.0 Hz, 2H) 1.20 (t, J = 8.0 Hz, 3H)	3.75 (d, J = 12.1 Hz, 3H) 3.56 (d, J = 12.4 Hz, 3H)	–	2.25 (s, 3H)
3e	6.55–8.12 (m, 9H)	4.81–4.97 (d, J = 6 Hz, 1H)	5.25–5.35 (m, 1H)	5.32–5.51 (s, 1H)	4.23 (q, J = 10.0 Hz, 2H) & 1.50 (t, J = 8.0 Hz, 3H)	3.79 (d, J = 12.3 Hz, 3H) 3.67 (d, J = 12.4 Hz, 3H)	–	–
3f	6.44–7.46 (m, 9H)	4.90–5.04 (d, J = 6 Hz, 1H)	4.78–4.89 (m, 1H)	5.41–5.57 (s, 1H)	4.20 (q, J = 10.0 Hz, 2H) & 1.25 (t, J = 8.0 Hz, 3H)	3.72 (d, J = 12.6 Hz, 3H) 3.55 (d, J = 12.2 Hz, 3H)	–	–
3g	6.46–7.49 (m, 9H)	4.85–5.01 (d, J = 6 Hz, 1H)	4.79–4.90 (m, 1H)	5.12–5.31 (s, 1H)	4.21 (q, J = 10.0 Hz, 2H) & 1.49 (t, J = 8.0 Hz, 3H)	–	4.50 (m, 12.0 Hz, 2H) & 1.55 (t, 9.0 Hz, 3H)	–
3h	6.54–8.13 (m, 9H)	4.87–5.02 (d, J = 6 Hz, 1H)	4.76–4.86 (m, 1H)	4.87–5.04 (s, 1H)	4.27 (q, J = 10.0 Hz, 2H) & 1.55 (t, J = 8.0 Hz, 3H)	–	4.45 (m, 12.0 Hz, 2H) & 1.40 (t, 9.0 Hz, 3H)	–
3i	6.45–7.48 (m, 9H)	4.98–5.12 (d, J = 6 Hz, 1H)	4.75–4.85 (m, 1H)	5.32–5.50 (s, 1H)	4.10 (q, J = 10.0 Hz, 2H) & 1.45 (t, J = 8.0 Hz, 3H)	–	4.49 (m, 12.0 Hz, 2H) & 1.50 (t, 9.0 Hz, 3H)	3.88 (s, 3H)
3j	6.56–8.14 (m, 9H)	5.02–5.18 (d, J = 6 Hz, 1H)	4.79–4.89 (m, 1H)	5.29–5.48 (s, 1H)	4.15 (q, J = 10.0 Hz, 2H) & 1.24 (t, J = 8.0 Hz, 3H)	–	4.48 (m, 12.0 Hz, 2H) & 1.48 (t, 9.0 Hz, 3H)	2.27 (s, 3H)
3k	6.54–8.11 (m, 9H)	4.90–5.04 (d, J = 6 Hz, 1H)	5.23–5.33(m, 1H)	5.38–5.56(s, 1H)	4.22(q, J = 10.0 Hz, 2H) & 1.51 (t, J = 8.0 Hz, 3H)	–	4.50 (m, 12.0 Hz, 2H) & 1.55 (t, 9.0 Hz, 3H)	–
3l	6.57–8.15 (m, 9H)	4.97–5.12 (d, J = 6 Hz, 1H)	5.28–5.38 (m, 1H)	5.34–5.53 (s, 1H)	4.25 (q, J = 10.0 Hz, 2H) & 1.22 (t, J = 8.0 Hz, 3H)	–	4.48 (m, 12.0 Hz, 2H) & 1.47 (t, 9.0 Hz, 3H)	–

[a] Chemical shifts in ppm.
[b] Recorded in DMSO-d_6

Table 3 ^{13}C NMR spectral data[a,b] of α-aminophosphonic acid esters **3a–c**, **3g** and **3h**.

	3a	**3b**	**3c**	**3g**	**3h**
C_2	51.7 (d, J = 152 Hz)	50.5 (d, J = 154 Hz)	49.5 (d, J = 151 Hz)	51.0 (d, J = 154 Hz)	51.5 (d, J = 152 Hz)
C_4	61.4	63.1	60.1	63.1	64.8
C_5	133.0	134.2	135.4	136.2	133.5
C_6 & C_{10}	132.4	130.4	131.4	134.2	130.6
C_7 & C_9	122.3	129.2	130.2	131.6	130.7
C_8	122.0	124.3	123.1	123.5	123.3
C_{11}	172.3	172.2	175.3	174.6	174.2
O-CH_2CH_3	61.4	62.3	61.5	60.3	62.4
O-CH_2CH_3	13.8	13.7	13.4	14.2	14.7
P-O-CH_3	54.0 (d, J = 7.4 Hz)	54.2 (d, J = 7.3 Hz)	55.0 (d, J = 7. 3 Hz)	–	–
P-O-CH_2CH_3	–	–	–	63.3 (d, J = 6.9 Hz)	64.8 (d, J = 6.7 Hz)
P-O-CH_2CH_3	– (d, J = 12.6 Hz)	– (d, J = 12.6 Hz)	–	15.8	15.9
C_1^1	148.8	139.3	139.5	140.3	147.3
C_2^1	122.0	129.8	130.8	122.8	128.9
C_3^1	149.7	142.3	149.7	149.7	148.1
C_4^1	122.0	123.9	152.9	120.7	122.2
C_5^1	131.7	131.1	149.7	131.6	131.2
C_6^1	133.0	129.4	130.8	138.4	128.9

[a] Recorded in DMSO-d_6.
[b] Chemical shifts in ppm.

Table 4 Mass spectral data of α-aminophosphonic acid esters **3a** and **3h**.

Compound	m/z (% relative abundance)
3a	422 ($M^{+\bullet}$, 48), 421 (100), 419 (12), 403 (06), 312(8), 299(18), 298 (28), 297 (14), 150 (26), 122(12)
3h	438 ($M^{+\bullet}$, 10), 437 (12), 436.5 (05), 403 (06), 361.5 (14), 359.5(36), 327 (10), 326 (86), 324 (100), 289(08), 205(06), 166(09), 132(10), 108(05)

various aromatic aldehydes (**2**) (0.005 mol) in anhydrous toluene (15 mL) were added dropwise with stirring at room temperature. Then diethyl/dimethylphosphite (0.005 mol) in anhydrous toluene (15 mL) was added dropwise. Stirring was continued at room temperature for half an hour. The reaction mixture was then heated to gentle reflux and stirring and heating continued for 5–6 h. After completion of the reaction (as indicted by TLC) the solvent was removed in a rota-evaporator. The resulting residue was purified by column chromatography on silica gel (60–120 mesh) using petroleum ether-ethyl acetate (8:2) as eluent (typical rf value 0.75). Physical, analytical and IR spectral data of **3a–l** are given in Table 1.

3.2. Antibacterial Activity

Antibacterial activity of all the title compounds **3a–l** was assayed[13] against *Staphylococcus aureus* ATCC-25923 (Gram-positive) and *Escherichia coli* ATCC-25922 (Gram-negative) at three different concentrations (100, 50 and 25 ppm) in DMF (Table 5). The compounds were diluted in DMF for bioassay. Solvent control was included although no antibacterial activity has been noted in the solvent employed. Penicillin (Hi-media) controls (20 μg mL^{-1}) were included to compare with compounds **3a–l**. All samples were tested in triplicate and average results were recorded.

Disc diffusion bioassay: For the bioassay a suspension of approximately 1.5×10^8 bacterial cells per mL was used. 1.5 mL of the bacterial suspension was uniformly spread on nutrient agar (Hi-media) in 12 × 1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded. For the agar disc diffusion method, the test compound was introduced onto the disc and then allowed to dry. Thus the disc was completely saturated with the test compound. Then the

Table 5 Antibacterial activity of α-aminophosphonic acid esters **3a–l**.

Compound	Zone of inhibition/mm					
	Escherichia coli			*Staphylococcus aureus*		
	100 ppm[a]	50 ppm[a]	25 ppm[a]	100 ppm[a]	50 ppm[a]	25 ppm[a]
3a	14	8	4	11	8	6
3b	10	7	5	8	6	–
3c	13	8	4	10	8	6
3d	12	6	6	6	5	4
3e	15	12	8	12	9	5
3f	15	12	7	15	10	8
3g	9	8	4	7	4	
3h	10	6	5	10	8	5
3i	10	5	3	12	11	8
3j	12	8	6	11	8	5
3k	8	5	5	–	–	–
3l	8	7	6	9	7	5
Penicillin[b]	12	8	–	9	6	–

[a] In DMF.
[b] Reference compound.

disc was introduced onto the upper layer of the medium seeded with bacteria. The Petri dishes were incubated at 35 °C for 24 h. Bioactivity was determined by measuring the diameters of the inhibition zones in mm. The compounds **3a-l** were made up at 25, 50 and 100 μg mL^{-1} concentrations for bio-activity screening by the disc method. Each test was done in triplicate and the mean diameter of the inhibition zones was calculated. Controls included the use of solvent without test compounds although no antibacterial activity was noted for the solvent employed in the test. The minimum inhibitory concentration (MIC) was determined for the compounds **3a–l** used at concentrations of 0.1–5.6 mg mL^{-1}. Specifically 0.1 mL of standardized inoculum (1–2 × 10^7 CFU mL^{-1}) was added to each tube. The tubes were incubated aerobically at 35 °C for 24 h. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing the growth medium without inoculum) and organism control (tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the compounds **3a–l** that produced no visible bacterial growth (no turbidity) when compared with the control tubes was regarded as MIC. The highlight is that the majority of compounds exhibited high activity against both bacteria and two compounds (**3e** and **3f)** were more effective than the standard compound. Penicillin was tested as a standard reference to compare the activities of these compounds.

3.3. Antifungal Activity

The compounds **3a–l** were screened for their antifungal activity (Table 6) against *Aspergillus niger* and *Helminthosporium oryzae* species along with standard fungicide Griseofulvin at three different concentrations (100, 50 and 25 ppm) in DMF.[14]

All the compounds **3a–l** exhibited moderate to high antifungal activity when compared with that of the reference compound. The majority of the compounds exhibited high activity against fungi. The compounds **3d** and **3i** showed higher activity against *H. oryzae* and *A. niger,* respectively, compared with the standard and warrant further testing to determine their minimum inhibitory concentrations as well as their cytotoxicity.

4. Conclusions

In conclusion, we have developed a convenient method for the synthesis of new α-aminophosphonates by using various aryl aldehydes with diethyl and dimethyl phosphites involving the Kabachnik-Fields reaction. These compounds exhibited significant activity against the growth of both bacteria and fungi.

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Table 6 Antifungal activity of α-aminophosphonic acid esters **3a–l**.

Compound	Zone of inhibition/mm					
	Aspergillus niger			*Helminthosporium oryzae*		
	100 ppm[a]	50 ppm[a]	25 ppm[a]	100 ppm[a]	50 ppm[a]	25 ppm[a]
3a	10	7	5	11	6	5
3b	11	8	4	11	9	5
3c	13	9	6	13	10	7
3d	12	10	8	15	9	4
3e	10	7	5	12	8	7
3f	9	5	3	13	11	9
3g	10	6	4	9	8	–
3h	9	8	6	11	9	5
3i	14	10	9	13	12	8
3j	8	9	6	9	7	4
3k	13	9	8	10	9	7
3l	13	10	8	11	9	5
Griseofulvin[b]	12	10	5	12	10	5

[a] In DMF.
[b] Reference compound.

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