Synthesis and antibacterial activity of new aryl / alkyl phosphonates via Michaelis-Arbuzov rearrangement

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Abstract

Synthesis of new aryl / alkyl phosphonates **3a-j** has been accomplished via a Michaelis-Arbuzov-type rearrangement by the reaction of aryl / alkyl halide (**1a-j**) with triethyl phosphite (**2**) in dry toluene at reflux temperature. Products **3a-j** were characterized by IR, ¹³C and ³¹P NMR and their antibacterial activity was evaluated.

Keywords: Aryl / alkyl phosphonates, antibacterial

Introduction

Phosphorus compounds containing the P-C bond are not particularly abundant in nature. Their diverse biological activity,^{1,2} has for a long time attracted considerable synthetic³ and pharmacological interest.⁴ The Michaelis-Arbuzov rearrangement, also known as the Arbuzov reaction, is very versatile way to form P-C bond from the reaction of an aryl / alkyl halide and trialkyl phosphite.⁵ This reaction is one of the most extensively investigated and is widely used to prepare phosphonates, phosphinates and phosphine oxides.⁶ Michaelis-Arbuzov reaction on the solid surface assisted by microwave heating for synthesis of organophosphorus compounds⁷⁻⁹ and also for the phosphorylation of aromatic compounds has been realized under the catalytical conditions.¹⁰⁻¹² Without catalyst, Michaelis-Arbuzov rearrangement can only be carried out with highly activated benzene compounds by heating them with phosphites to yield the corresponding phosphonates.¹³ In our work we synthesized aryl / alkyl phosphonates without catalyst and under the mild conditions.

Results and Discussion

The synthetic route involves reaction of aryl / alkyl halides (**1a-j**) with triethyl phosphite (**2**) in toluene at reflux temperature (Scheme 1) and the formation of new aryl / alkyl phosphonates **3a-j** involved Michaelis-Arbuzov rearrangement. The chemical structures of all the new compounds were confirmed by elemental analysis, IR^{14,15} (Table 1), ¹H NMR¹³ (Table 1) and ¹³C NMR (Table 2) and ³¹P NMR^{16a} (Table 2) spectral data.

Table 1. Physical and IR spectral data of **3a-j**

Compd. ^a	Yield ^b	Mol. Formula (Mol. Wt)	Elemental analysis (%) found (Calcd.)			IR (cm ⁻¹)	
			C	H	N	P=O	P-C
3a	68	C ₁₀ H ₁₅ O ₄ P (230.20)	52.12 (52.18)	6.47 (6.57)	-	1211	981
3b	84	C ₁₀ H ₁₃ N ₂ O ₇ P (304.19)	39.37 (39.48)	4.27 (4.31)	9.09 (9.21)	1240	960
3c	68	C ₆ H ₁₃ O ₅ P (196.14)	36.62 (36.74)	6.49 (6.68)	-	1229	991
3d	65	C ₁₁ H ₁₅ O ₅ P (258.21)	50.97 (51.17)	5.72 (5.86)	-	1209	1021
3e	63	C ₁₁ H ₁₅ O ₅ P (258.21)	51.03 (51.17)	5.78 (5.86)	-	1215	1015
3f	82	C ₁₀ H ₁₄ NO ₅ P (259.20)	46.14 (46.34)	5.29 (5.44)	5.24 (5.40)	1245	1014
3g	62	C ₁₁ H ₁₅ O ₄ P (242.21)	54.37 (54.55)	6.10 (6.24)	-	1232	1015
3h	60	C ₁₁ H ₁₇ O ₄ P (244.22)	53.96 (54.10)	6.85 (7.02)	-	1237	1009
3i	64	C ₁₁ H ₁₇ O ₄ P (244.22)	53.92 (54.10)	6.86 (7.02)	-	1232	967
3j	67	C ₇ H ₁₆ NO ₇ P (257.18)	32.55 (32.69)	6.09 (6.27)	5.26 (5.45)	1248	960

^a Obtained viscous liquids that decompose on attempted vacuum distillation.

^b After separation from the column chromatography.

The ³¹P NMR spectral data for **3a-j** are given in the Table 2. The ³¹P NMR signals for **3b**, **3c**, **3e** and **3g-i** appeared as two distinct signals in the range of -1.28 to -2.08 and 5.74 to 20.71 ppm. This may be due to the presence of two isomers^{16b,16c} in the solution state with sufficient internal energy difference and considerable stability that enable measurement of their ³¹P NMR resonance. The other compounds **3a**, **3d**, **3f** and **3j** gave only one ³¹P NMR signal in the range of -1.29 to -1.50 and 7.09 ppm.

Table 2. (^1H , ^{13}C and ^{31}P) NMR spectral data (δ , CDCl_3) of **3a-j**

Compd.	^1H NMR	^{13}C NMR	^{31}P NMR
3a	Ar-H : 7.69 (d=7.4 Hz, 2H) 6.75 (d = 7.8 Hz, 2H) OCH ₂ : 3.71-4.16 (m, 4H) CH ₃ : 1.21-1.35 (m, 6H)	155.54, 132.14, 117.28, 111.82, 62.49, 16.02	7.09
3b	Ar-H : 7.31-8.68 (m, 6H) OCH ₂ : 4.08-4.36 (m, 4H) CH ₃ : 1.33-1.40 (m, 6H)	--	-1.28, 7.97
3c	P-CH ₂ : 4.25(d, $J=7.14\text{Hz}$, 2H) OCH ₂ : 3.71-4.28 (m, 4H) CH ₃ : 1.22-1.36 (m, 6H) COOH: 9.32 (s, 1H)	--	-2.08, 6.93
3d	Ar-H : 7.16-7.89 (m, 4H) OCH ₂ : 3.88-4.32 (m, 4H) CH ₃ : 1.24-1.38 (m, 6H)	--	-1.29
3e	Ar-H : 7.26-8.03 (m, 4H) OCH ₂ : 3.60-4.21 (m, 4H) CH ₃ : 1.22-1.42 (m, 6H)	--	-1.72, 10.64
3f	Ar-H : 7.17-8.33 (m, 4H) OCH ₂ : 4.09-4.15 (m, 4H) CH ₃ : 1.32-1.37 (m, 6H)	--	-1.29
3g	Ar-H : 7.29-7.83 (m, 4H) OCH ₂ : 4.01-4.13 (m, 4H) CH ₃ : 1.21-1.36 (m, 6H) CHO : 9.96 (s, 1H)	190.64, 135.69, 133.21, 128.25 (d, $J=4.7\text{ Hz}$), 127.86, 62.83 (d, $J=7.1\text{ Hz}$), 15.95	-1.50, 20.71
3h	Ar-H : 7.21-7.35 (m, 4H) OCH ₂ : 3.62-4.09 (m, 4H) CH ₃ : 1.16-1.32 (m, 6H) CH ₂ : 4.57 (s, 2H)	--	-1.66, 5.74
3i	Ar-H : 7.67 (d, $J=7.1\text{ Hz}$, 2H) 7.35 (d, $J=7.3\text{ Hz}$, 2H) OCH ₂ : 4.08-4.19 (m, 4H) CH ₃ : 1.21-1.39 (m, 6H) OCH ₃ : 3.77 (s, 3H)	154.63, 128.16, 118.04, 114.28, 63.30 (d, $J=4.5\text{ Hz}$), 57.32, 15.66 (d, $J=5.7\text{ Hz}$)	-1.38, 7.04
3j	CH ₂ : 4.31 (s, 4H) OH : 4.82 (brs, 2H) OCH ₂ : 4.08-4.19 (m, 4H) CH ₃ : 1.33-1.37 (m, 6H)	66.22, 63.51 (d, $J=4.1\text{ Hz}$), 48.18, 15.81 (d, $J=5.4\text{ Hz}$)	-1.50

Scheme 1

Antibacterial activity

The compounds were diluted in DMSO for bioassay. Solvent control was included although no antibacterial activity has been noted for the solvent employed. Ciprofloxacin (Hi-media) controls were included to compare with compounds **3a-j**. All samples were tested in triplicate and average results were recorded.

The compounds were assayed for antibacterial activity against six registered bacterial isolates which were obtained from the NCIM (National Collection of Industrial Microorganisms, National Chemical Laboratories, Pune-411 003, India). The bacteria included Gram positive bacterial isolates-*Staphylococcus aureus* (NCIM No: 5021, ATCC No. 25923), *Bacillus faecalis* (NCIM No: 2063, ATCC No. 6633) and four Gram negative bacteria-*Escherichia coli* (NCIM No: 2931, ATCC No. 25922), *Pseudomonas aeruginosa* (NCIM No: 5029, ATCC No: 27853), *Salmonella typhimurium* (NCIM No: 2501, ATCC No: 23564) and *Klebsiella pneumoniae* (NCIM No: 2957). The bacteria were grown on Hi-media nutrient agar and sub cultured as needed.

Experimental Section

General Procedures. All chemicals were commercial products and distilled / recrystallised before use. Elemental analyses were performed by the Central Drug Research institute, Lucknow, India. IR spectra were recorded as KBr pellets and Nujol mulls on a Perkin Elmer 283 unit. The ^1H , ^{13}C and ^{31}P NMR spectra were taken on AMX 400 MHz spectrometer operating at 400 MHz for ^1H , 100 MHz for ^{13}C and 161.9 MHz for ^{31}P . All these compounds were dissolved in $\text{DMSO}-d_6$. The chemical shifts in δ were referenced to TMS (^1H and ^{13}C) and 85% H_3PO_4 (^{31}P).

General procedure for products 3a-j. In a flame-dried three-necked flask the appropriate aryl / alkyl halide (0.001 mol) was mixed with triethyl phosphite (0.249 g, 0.0015 mol) and stirred at reflux temperature for 6-8 hrs and protected with a CaCl_2 -tube, respectively. After the completion of reaction (monitored by TLC), the oily product was obtained. The product was purified by column chromatography on silica gel using petroleum ether-ethylacetate (7:3) as eluent.

Table 3. Antibacterial activity of compounds 3a-j in terms of DIZ in mm

Compd.	<i>Staphylococcus aureus</i>		<i>Bacillus faecalis</i>		<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>		<i>Salmonella typhimurium</i>		<i>Klebsiella pneumoniae</i>	
	20 $\mu\text{g}/\text{mL}$	40 $\mu\text{g}/\text{mL}$	20 $\mu\text{g}/\text{mL}$	40 $\mu\text{g}/\text{mL}$	20 $\mu\text{g}/\text{mL}$	40 $\mu\text{g}/\text{mL}$	20 $\mu\text{g}/\text{mL}$	40 $\mu\text{g}/\text{mL}$	20 $\mu\text{g}/\text{mL}$	40 $\mu\text{g}/\text{mL}$	20 $\mu\text{g}/\text{mL}$	40 $\mu\text{g}/\text{mL}$
3a	+	++	-	+	+	++	+	++	-	+	+	++
3b	+	++	-	+	-	+	-	+	-	+	-	+
3c	-	++	-	+	-	++	+	++	-	+	+	++
3d	+	++	-	+	-	+	-	+	-	+	-	+
3e	+	++	+	++	+	++	+	++	+	++	+	++
3f	-	+	-	+	-	+	-	+	+	++	-	+
3g	-	+	-	+	-	+	-	+	-	+	-	+
3h	+	++	+	++	+	++	+	++	-	+	+	++
3i	+	++	-	+	-	+	-	+	-	+	-	+
3j	+	++	-	+	-	+	-	+	-	+	-	+
Ciprofloxacin	22		24		30		25		28		25	

‘+’ indicates 10-12 mm. ‘++’ indicates 12-15 mm. ‘-’ indicates no activity.

Disc diffusion bioassay. For bioassay suspension of approximately 1.5×10^8 bacterial cells per mL were used. In sterile normal saline was prepared as described by Forbes *et al*¹⁷ and 1.5 mL of it was uniformly spread on Nutrient Agar (Hi-media) in 12 x 1.2 cm glass Petri dishes, left aside for 15 min and excess of suspension was then drained and discarded properly. 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 disc was introduced onto the upper layer of the medium with the bacteria. The petri dishes were incubated overnight at 37 °C for 24 hrs. Bioactivity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm. The compounds' **3a-j** concentrations were taken as 20 and 40 µg / mL were evaluated for disc method. Each test was done in triplicate and the mean of the 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 test¹⁸ (Table 3).

Determination of minimum inhibitory concentration (MIC). Minimum inhibitory concentration (MIC) was determined for the compounds **3a-j**. The concentration at which there was no visually detectable bacterial growth was taken as MIC. Compounds **3a-j** concentrations of 0.1-5.6 mg / mL in steps of 100 µg / mL were evaluated. Specifically 0.1 mL of standardized inoculum ($1-2 \times 10^7$ CFU / mL) was added to each tube. The tubes were incubated aerobically at 37 °C for 18-24 hrs. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing compounds **3a-j** and the growth medium without inoculum) and organism control (the tube containing the growth medium, physiological saline and the inoculum). The lowest concentration (highest dilution) of the compounds **3a-j** that produced no visible bacterial growth (no turbidity) when compared with the control tubes was regarded as MIC¹⁸ (Table 4).

Table 4. Minimum inhibitory concentration (MIC) mg / mL

Compd.	<i>Staphylococcus aureus</i>	<i>Bacillus faecalis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella typhimurium</i>	<i>Klebsiella pneumoniae</i>
3a	3.6	4.0	3.3	3.6	4.0	3.8
3b	3.8	4.1	4.0	4.1	4.2	4.0
3c	4.0	4.2	3.9	3.8	4.4	3.7
3d	5.4	5.3	4.2	4.6	4.0	4.3
3e	2.8	2.6	2.7	3.0	2.8	3.2
3f	4.4	4.3	4.2	4.6	3.9	4.0
3g	5.2	5.0	4.9	4.8	5.4	4.2
3h	3.6	3.2	3.8	3.4	4.4	3.9
3i	3.0	5.0	4.0	4.4	5.6	4.2
3j	3.7	3.9	4.2	4.4	4.0	4.1

Conclusions

We synthesized bioactive and novel phosphonates **3a-j** in high yield by Michaelis-Arbuzov reaction without any catalyst. They showed moderate antibacterial activity against selected bacteria. Among all these compounds **3e** showed highest antibacterial activity at lower

concentration against both Gram negative and Gram positive bacteria. Compounds **3g**, **3i** showed lowest activity even at highest concentrations. Compound **3d** showed highest activity against Gram positive bacteria when compared with Gram negative bacteria.

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