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A facile synthesis, spectral characterization and antimicrobial activity of novel dialkylheteroaryl phosphonates

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ABSTRACT

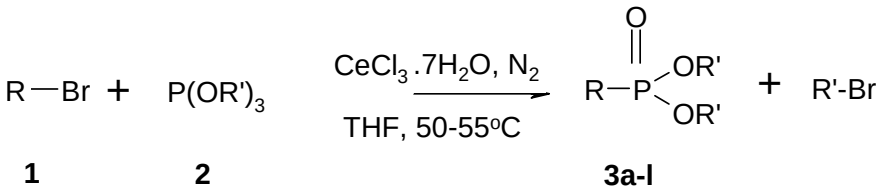
*Synthesis of a series of novel dialkylheteroaryl phosphonates (**3a-l**) was accomplished via Michaelis-Arbuzov rearrangement in high yields (70-80%) by the reaction of various heteroaryl halides (**1**) with trialkyl phosphite (**2**) at 50-55^{o}C in dry tetrahydrofuran (THF) under N_2 atmosphere by using $CeCl_3.7H_2O$ as a catalyst. The structures of the title compounds were established by elemental analyses and spectral data (IR, ^{1}H, ^{13}C and ^{31}P NMR and LC-MS). All the titled compounds (**3a-l**) showed promising antimicrobial activity.*

Keywords: Trialkyl phosphites, Dialkylheteroaryl phosphonates, Michaelis-Arbuzov rearrangement, $CeCl_3.7H_2O$

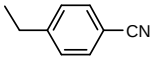
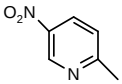
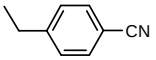
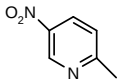
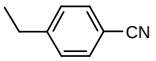
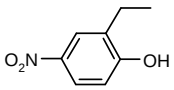
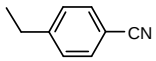
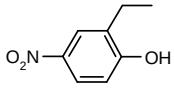
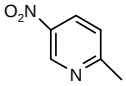
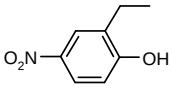
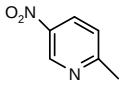
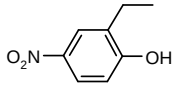
INTRODUCTION

The Michaelis-Arbuzov rearrangement is one of the most versatile routes to synthesize phosphonates, phosphinates and phosphine oxides, containing a phosphorus-carbon bond by the reaction of trialkyl phosphite and alkyl halides which are particularly scarce in nature [1, 2]. Their diverse biological activity has attracted considerable synthetic and pharmacological interest [3]. A phosphonate motif is present in biomolecules which can act as inhibitors of certain biosynthetic pathways and can be degraded only by some prokaryotic microorganisms [4]. Microwave assisted solid surface Michaelis-Arbuzov synthesis accomplishes the phosphonylation of aromatic compounds under catalytical conditions of organophosphorus compounds [5, 6]. The catalytic Arbuzov rearrangement involves iodine [7], alkalimetal iodide [8] and Ni [II] chloride [9] as catalysts. Synthetic procedures for phosphonates require very high temperature, longer reaction time and pressure [10].

In view of the above reports, we herein report the synthesis of novel dialkylheteroaryl phosphonates (**3a-l**) via Michaelis-Arbuzov rearrangement and their antimicrobial activities. Their structures were characterized by elemental analyses, spectral data (IR, ^{1}H, ^{13}C and ^{31}P NMR and LC-MS).



1 **2** **3a-l**

Compound	R	R'	Compound	R	R'
3a		Me	**3g**		But
3b		Et	**3h**		i-Pr
3c		But	**3i**		Me
3d		i-Pr	**3j**		Et
3e		Me	**3k**		But
3f		Et	**3l**		i-Pr

Scheme 1

MATERIALS AND METHODS

Chemistry

Sigma-Aldrich, Merck and Lancaster Chemicals were used as such. Solvents used for spectroscopic and other physical studies were reagent grade and were further purified by standard procedures and techniques. The IR spectra (KBr pellets) were recorded on a Thermo Nicolet 380 double beam spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) with TMS as an internal standard. ^{31}P NMR spectra were measured using 85% H_3PO_4 (*ortho* phosphoric acid) as external reference on a 300 MHz. Mass spectra were recorded on LCMS-2010A, Shimadzu spectrometer. Melting points were determined in an open capillary tube on Mel-temp apparatus, Tempo instruments, India and were uncorrected.

General procedure for the preparation of the title compounds (3a-l):

Synthesis of dialkylheteroaryl phosphonates (3a-l):

A mixture of heteroaryl halide (1) (0.001 mole), trialkyl phosphite (2), $CeCl_3.7H_2O$ (catalytic amount) were placed in a 50 mL round-bottomed flask in THF (20 mL) and the mixture was stirred at 50-55^{o}C for 4-6 hours under N_2 atmosphere **(Scheme 1)** to obtain the products (**3a-l).** The reaction progress was monitored by TLC (silica gel) using hexane and ethyl acetate (3:1) as a mobile phase, the solvent was removed in a rotaevaporator and the crude product obtained was purified by column chromatography on silica gel (60-120 mesh) using hexane and ethyl acetate (3:1) as an eluent.

Synthesis of dimethyl (4-cyanobenzyl)phosphonate (3a):
IR (KBr) 1413, 1228, 1020 cm^{-1}; ^{1}H NMR δ: 7.4 (d, 4H, 2 CH_2), 7.6 (d, 4H, 2 CH_2), 4.4 (s, 2H, CH_2), 3.7 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): 142.8, 132.5, 129.1, 118.3, 112.3, 44.9, 31.4; ^{31}P NMR ($CDCl_3$): δ 22.06. Melting point: 196-198 ^{o}C; Yield 76 %; LC-MS (m/z): 226 $(M+H)^{+}$. Elemental Analysis for $C_{10}H_{12}NO_3P$: Calcd (Found): C: 53.34 (53.21); H: 5.37 (5.43); N: 6.22 (6.32).

Synthesis of diethyl (4-cyanobenzyl)phosphonate (3b):
IR (KBr): 1440, 1269, 1082 cm^{-1}; ^{1}H NMR δ: 7.4 (d, 2H, 2 CH_2), 7.6 (d, 2H, 2 CH_2), 3.4 (s, 2H, CH_2), 4.4 (q, 2H, CH_2), 1.6 (t, 3H, CH_3); ^{31}P NMR ($CDCl_3$): δ 19.68. Melting point: 200-202 ^{o}C; Yield 72 %; LC-MS (m/z): 254 $(M+H)^{+}$. Elemental Analysis for $C_{12}H_{16}NO_3P$: Calcd (Found): C: 56.92 (56.85); H: 6.37 (6.29); N: 5.53 (5.48).

Synthesis of dibutyl (4-cyanobenzyl)phosphonate (3c):
IR (KBr): 1413, 1252, 1018 cm^{-1}; ^{1}H NMR δ: 7.4 (d, 2H, 2 CH_2), 7.6 (d, 2H, 2 CH_2), 4.4 (s, 2H, CH_2), 3.3 (q, 1H, -CH), 1.2 (d, 3H, CH_3), 1.6 (m, 2H, CH_2), 0.96 (t, 3H, CH_3); ^{31}P NMR ($CDCl_3$): δ 22.3. Melting point: 180-182 ^{o}C; Yield 70 %.

Synthesis of diisopropyl (4-cyanobenzyl)phosphonate (3d):
IR (KBr): 1439, 1228, 1020 cm^{-1}; ^{1}H NMR δ: 7.4 (d, 2H, 2 CH_2), 7.6 (d, 2H, 2 CH_2), 4.4 (s, 2H, $-CH_2$), 3.5 (m, 1H, -CH), 1.6 (d, 6H, 2 $-CH_3$); ^{31}P NMR ($CDCl_3$): δ 19.7. Melting point: 171-173 ^{o}C; Yield 78 %.

Synthesis of dimethyl (5-nitro-pyridin-2-yl)phosphonate (3e):
IR (KBr): 1440, 1251, 1012 cm^{-1}; ^{1}H NMR δ: 9.2 (s, 1H, -CH), 8.4 (d, 1H, -CH), 7.5 (d, 1H, -CH), 3.7 (s, 3H, CH_3); ^{31}P NMR ($CDCl_3$): δ 24.3. Melting point: 193-195 ^{o}C; Yield 73 %.

Synthesis of diethyl (5-nitro-pyridin-2-yl)phosphonate (3f):
IR (KBr): 1442, 1222, 1034 cm^{-1}; ^{1}H NMR δ: 9.2 (s, 1H, -CH), 8.4 (d, 1H, -CH), 7.5 (d, 1H, -CH), 3.3 (q, 2H, CH_2), 2.0 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$): 157.2, 148.3, 145.5, 133.0, 128.7, 19.3, 8.2; ^{31}P NMR ($CDCl_3$): δ 21.74. Melting point: 180-182 ^{o}C; Yield 74 %; LC-MS (m/z): 261$(M+H)^{+}$. Elemental Analysis for $C_9H_{13}N_2O_5P$: Calcd (Found): C: 41.55 (41.65); H: 5.04 (5.12); N: 10.77 (10.68).

Synthesis of dibutyl (5-nitro-pyridin-2-yl)phosphonate (3g):
IR (KBr): 1442, 1235, 1080 cm^{-1}; ^{1}H NMR δ: 9.2 (s, 1H, -CH), 8.4 (d, 1H, -CH), 7.5 (d, 1H, -CH), 3.3 (q, 1H, -CH), 1.2 (d, 3H, CH_3), 1.4 (m, 2H, CH_2), 0.94 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$): 157.1, 148.2, 145.6, 133.0, 128.7, 32.3, 18.6, 7.4; ^{31}P NMR ($CDCl_3$): δ 21.2. Melting point: 173-175 ^{o}C; Yield 78 %.

Synthesis of diisopropyl (5-nitro-pyridin-2-yl)phosphonate (3h):
IR (KBr): 1442, 1248, 1012 cm^{-1}; ^{1}H NMR δ: 9.2 (s, 1H, -CH), 8.4 (d, 1H, -CH), 7.5 (d, 1H, -CH), 3.5 (m, 1H, -CH), 1.6 (d, 6H, 2 CH_3); ^{31}P NMR ($CDCl_3$): δ 25.4. Melting point: 191-193 ^{o}C; Yield 76 %.

Synthesis of dimethyl (2-hydroxy-5-nitrobenzyl)phosphonate (3i):
IR (KBr): 1427, 1231, 1080 cm^{-1}; ^{1}H NMR δ: 6.8 (d, 1H, -CH), 7.2 (d, 1H, -CH), 7.7 (s, 1H, -CH), 5.9 (s, 1H, OH), 3.6 (s, 3H, CH_3); ^{31}P NMR ($CDCl_3$): δ 23.5. Melting point: 176-178 ^{o}C; Yield 70 %.

Synthesis of diethyl (2-hydroxy-5-nitrobenzyl)phosphonate (3j):
IR (KBr): 1427, 1254, 1080 cm^{-1}; ^{1}H NMR δ: 6.9 (d, 1H, -CH), 7.2 (d, 1H, -CH), 7.6 (s, 1H, -CH), 5.9 (s, 1H, OH), 3.3 (q, 2H, CH_2), 2.6 (t, 3H, CH_3); ^{13}C NMR ($CDCl_3$): 161.7, 140.1, 125.8, 119.5, 115.7, 27.3, 14.8; ^{31}P NMR ($CDCl_3$): δ 23.70; Melting point: 186-188 ^{o}C; Yield 78 %; LC-MS (m/z): 262 $(M+H)^{+}$. Elemental Analysis for $C_9H_{12}NO_6P$: Calcd (Found): C: 41.39 (41.28); H: 4.63 (4.69); N: 5.36 (5.28).

Synthesis of dibutyl (2-hydroxy-5-nitrobenzyl)phosphonate (3k):
IR (KBr): 1427, 1223, 1080 cm^{-1}; ^1H NMR δ: 6.8 (d, 1H, -CH), 7.2 (d, 1H, -CH), 7.7 (s, 1H, -CH), 5.8 (s, 1H, OH), 3.7 (q, 1H, -CH), 1.3 (d, 3H, CH_3), 1.5 (m, 2H, CH_2), 0.90 (t, 3H, CH_3); P^{31} NMR ($CDCl_3$): δ 24.2. Melting point: 195-197 ^oC; Yield 79 %.

Synthesis of diisopropyl (2-hydroxy-5-nitrobenzyl)phosphonate (3l):
IR (KBr): 1427, 1230, 1080 cm^{-1}; ^1H NMR δ: 6.9 (d, 1H, -CH), 7.6 (d, 1H, -CH), 7.7 (s, 1H, -CH), 5.9 (s, 1H, OH), 3.1 (m, 1H, -CH), 1.7 (d, 6H, 2 CH_3); ^{31}P NMR ($CDCl_3$): δ 22.1. Melting point: 203-204 ^oC; Yield 71 %.

Biological assay
Antibacterial activity
A standard inoculum (1-2 × 10^7 c.f.u/cm^3 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatmann no.1 filter paper and sterilized by dry heat at 140 ˚C for 1 h. The sterile disc previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 ˚C. The inhibition zones were measured and compared with the controls. Minimum inhibitory concentration (MIC) was determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 5 × 10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 ˚C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (*MIC*). Streptomycin was used as a standard drug. The diameter of the zone of inhibition and minimum inhibitory concentration (MIC) values are given in Table 1.

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Bacillus subtilis and Streptococcus bovis* (recultured) bacterial strains by disc diffusion method. The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The compounds **3a-l** showed very good activity against all the bacterial strains.

Antifungal activity
Sabourauds agar media was prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 cm^3 distilled water, and adjusting pH to 5.7 using buffer. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 cm^3 saline to get a suspension of corresponding species. 20 cm^3 of agar media was poured in to each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 ˚C for 1 h. By using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 ˚C for 3-4 d. The inhibition zones in diameter were measured and compared with the controls. The Nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately 1.6 × 10^4-6 × 10^4 c.f.u cm^{-3}. The cultures were incubated for 48 h at 35 ˚C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentrations (*MIC*). Bovastin was used as the standard drug. The diameter of zone of inhibition and minimum inhibitory concentration values are given in Table 2.

Newly prepared compounds were screened for their antifungal activity against *Fusarium oxyporum*, *Aspergillus flavus*, *Aspergillus niger* (recultured) in DMSO by serial plate dilution method. The antifungal screening data showed moderate to good activity. Compounds **3a-l** emerged as very active against all the fungal strains.

Table 1. Antibacterial activity of dialkylheteroaryl phosphonates 3a-l against Gram positive and Gram negative bacteria

Compound	Zone of inhibition (mm)								
	Escherichia coli			*Bacillus subtilis*			*Streptococcus bovis*		
	150 µg	250 µg	350 µg	150 µg	250 µg	350 µg	150 µg	250 µg	350 µg
3a	9	12	16	--	--	8	--	12	15
MIC	63 µg			180 µg			160 µg		
3b	6	11	15	6	12	17	8	11	16
MIC	70 µg			80 µg			55 µg		
3c	8	15	18	--	10	14	--	11	15
MIC	51 µg			165 µg			175 µg		
3d	5	11	17	7	13	16	9	15	18
MIC	55 µg			75 µg			52 µg		
3e	10	16	20	7	14	17	--	8	15
MIC	35 µg			68 µg			175 µg		
3f	7	13	16	10	14	19	8	12	15
MIC	62 µg			30 µg			80 µg		
3g	--	5	11	4	11	16	--	8	13
MIC	180 µg			95 µg			175 µg		
3h	7	13	17	6	13	17	--	6	11
MIC	95 µg			65 µg			180 µg		
3i	4	9	13	8	13	15	6	13	16
MIC	180 µg			60 µg			90 µg		
3j	--	--	7	--	6	13	--	--	8
MIC	190 µg			170 µg			200 µg		
3k	9	14	19	7	15	17	6	11	15
MIC	40 µg			55 µg			85 µg		
3l	6	9	13	4	9	13	5	12	15
MIC	110 µg			85 µg			90 µg		
Streptomycin (Standard)	22			23			21		

MIC=Minimum inhibitory concentration

RESULTS AND DISCUSSION

The dialkylheteroaryl phosphonates (**3a-l**) were synthesized by the reaction of heteroaryl halides (**1**) with various trialkyl phosphites (**2**) at 50-55^{o}C in dry tetrahydrofuran (THF) under N_2 atmosphere in the presence of $CeCl_3.7H_2O$ as a catalyst. The progress of the reaction was monitored by thin layer chromatography (TLC) analysis and the products were purified by column chromatography using hexane: ethyl acetate (3:1) as an eluent.

The structures of the title compounds **3a-l** were established by their spectroscopic data. The characteristic IR stretching absorptions were observed in the regions 1256-1224 (P=O), 1081-1012 (P-O-C aliphatic) and 1470-1420 (P-C aromatic). In ^{1}H NMR, the 4-cyanobenzyl moiety appeared as a doublet at 7.4 (d, *J*=6.2-6.6 Hz) and 7.6 (d, *J*= 7.2-7.4 Hz), 5-nitro-pyridin-2-yl moiety resonated as a doublet at 8.4 (d, *J*=7.2-7.4 Hz) and 7.53 (d, *J*=8.2-8.3 Hz) and for 2-hydroxy-5-nitrobenzyl moiety appeared as a doublet at δ 6.8 (d, *J*=6.2-6.4 Hz) and 7.2 (d, *J*=8.2-8.3 Hz). The remaining protons of the title compounds **3a-l** appeared in the expected region [11].

The ^{13}C NMR spectral data of **3a**, **3f**, **3g** and **3j** compounds showed characteristic chemical shifts for aromatic carbons. The data of other carbon signals were observed in the expected region [11]. ^{31}P NMR chemical shifts of these compounds **3a-l** appeared in the expected region 19.2-26.2 ppm [11]. Liquid chromatography/mass spectrometry (LC-MS) data were recorded for **3a**, **3b**, **3f** and **3j** and they gave protonated $M^{+\cdot}$ ions at their respective *m/z* values.

Table 2. Antifungal activity of dialkylheteroaryl phosphonates 3a-l

Compound	Zone of inhibition (mm)								
	Fusarium oxysporum			*Aspergillus flavus*			*Aspergillus niger*		
	150 µg	250 µg	350 µg	150 µg	250 µg	350 µg	150 µg	250 µg	350 µg
3a	7	11	14	--	7	13	--	--	11
MIC	75 µg			190 µg			200 µg		
3b	8	13	16	5	11	14	7	12	16
MIC	60 µg			120 µg			95 µg		
3c	7	13	17	3	9	16	6	14	17
MIC	65 µg			170 µg			110 µg		
3d	6	12	18	4	8	14	--	--	12
MIC	80 µg			130 µg			170 µg		
3e	9	16	20	7	15	18	6	13	18
MIC	50 µg			70 µg			115 µg		
3f	6	12	15	9	11	17	7	14	19
MIC	85 µg			55 µg			95 µg		
3g	3	13	15	6	12	16	4	12	18
MIC	140 µg			95 µg			125 µg		
3h	8	13	18	6	13	16	--	--	8
MIC	65 µg			105 µg			170 µg		
3i	--	6	13	6	11	16	6	9	14
MIC	140 µg			120 µg			120 µg		
3j	--	--	--	2	8	12	--	7	11
MIC	------			135 µg			180 µg		
3k	7	13	15	9	16	19	11	16	19
MIC	85 µg			50 µg			45 µg		
3l	2	8	13	--	9	15	6	14	17
MIC	120 µg			170 µg			95 µg		
Bovastin (Standard)	24			23			24		

MIC=Minimum inhibitory concentration

Biological activity

Antibacterial activity

All the synthesized compounds were screened against Gram positive bacteria and Gram negative bacteria by the disc diffusion method and the results were compared with the standard drug (Streptomycin). The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. In general, the compounds **3c, 3e** and **3k** were more effective towards *Eschrichia coli*, **3d** and **3i** were more effective towards *Streptococcus bovis* and the compounds **3f, 3i** and **3k** were more effective towards *Bacillus subtilis.*

Minimum inhibitory concentration (MIC) was determined for the compounds **3a-l** by broth dilution technique. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (MIC). Streptomycin was used as a standard drug. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in Table 1.

Antifungal activity

All the titled compounds **3a-l** were tested for antifungal activity and the results were compared with the standard drug, bovastin. Among them, the compounds **3e, 3f** and **3k** were more effective towards *Aspergillus flavus*, **3d, 3f** and **3k** compounds were more effective towards *Aspergillus niger* and the compounds **3e** and **3k** were more effective towards *Fusarium oxysporum.* The inhibition zones in diameter were measured and compared with the controls. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). Bovastin was used as the standard drug. The diameter of zone of inhibition and minimum inhibitory concentration (MIC) values are given in Table 2.

CONCLUSION

We have successfully synthesized a series of novel dialkylheteroaryl phosphonates in higher yields by one-pot, two-component reaction between heteroarylhalides and trialkyl phosphites in the presence of $CeCl_3.7H_2O$ as a catalyst in THF at 50-55^oC via Michaelis-Arbuzov rearrangement. $CeCl_3.7H_2O$ was acted as an efficient heterogeneous Lewis acid catalyst. The advantages are shorter reaction times, low cost of the starting chemicals and simple experimental procedure. All the title compounds exhibited promising antibacterial and antifungal activities, and a few of the compounds showed low MIC values.

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