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One-pot and catalyst-free synthesis of novel α - aminophosphonates under microwave irradiation and their Bio-activity

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Abstract: *A simple and an efficient synthetic protocol were adopted for the synthesis of novel α-aminophosphonates derivatives containing pyrazine moiety such as diethyl phenyl (pyrazin-2-yl-amino) methylphosphonates* ***(4a-l)*** *through one-pot three - component Kabachnik-Fields reaction. Pyrazino-2-amine* ***(1),*** *different substituted aldehydes and triethyl phosphite* ***(3)*** *were reacted in toluene under microwave irradiation without catalyst to obtain title compounds. The newly synthesized compounds were screened for their in vitro antibacterial activity (Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, and Salmonella typhimurium) and antifungal activity (Candida albicans, Aspergillus niger).*

Keywords: *α-aminophosphonates, microwave irradiation, diethyl phenyl (pyrazin-2-yl-amino) methylphosphonates, antiviral activity.*

I. Introduction

Organophosphorus compounds are important substrates in the study of biochemical processes and tetracoordinated pentavalent phosphorus compounds are widely used as biologically active compounds and their utility as synthetic intermediates and they have found in a wide range of applications in the areas of industry, agriculture and medicinal chemistry [1–5]. Literature survey reveals that the analogues with C-P bond show broad spectrum of biological activity such as antifungal and insecticidal activity [6-7]. Phosphonates functionalized with hydroxyl, amine groups and α-substituted phosphonates [8] in particular the quinquavalent organophosphorus compounds find application in biological relevant processes. Recently, new vinyl phosphates have been reported to have potent mechanism based inhibitors of phosphatase [9-11] or phosphodiesterase [12-13]. For a long time the so-called 'phosphorus analogues' of the amino acids, in which the carboxylic acid group is replaced by a phosphonic, or phosphinic acid group, P(O)(OH)R (in which R may be H, alkyl, or aryl), as well as a phosphonate group, $P(O)(OR)_2$ (in which R may be alkyl, or aryl), have attracted particular interest in the preparation of isosteric or bioisosteric analogues of numerous natural products [14,15]. Therefore α-aminophosphonates have attracted considerable attention since they are considered as structural analogues of α-amino acids and they act as enzyme inhibitory neuroactive agents, HIV protease antagonists, collagenase inhibitors, peptide mimics [16], antibiotics, herbicides [17], pharmacological agents [18] and exhibited pesticidal [19] and antiviral [20] activity. The ubiquitous nature of α-aminophosphonates in the biological system stimulated the researchers to develop various methods to synthesize novel bio-active α-aminophosphonate derivatives. Nowadays, microwave irradiation is used to accomplish certain unsuccessful or low-yielding reactions, reducing the reaction time from days to minutes, and improving yields [21-22]. The Kabachnik-Fields reaction under microwave irradiation without catalyst 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. To the best of literature knowledge pyrazines and their derivatives are important constituents of pharmacologically active synthetic compounds. The pyrazine nucleus is also frequently recognized in the structure of numerous naturally occurring alkaloids. They have been associated with broad spectrum of biological activities. Among pyrazine, Pyrazino-2-amine **(1)** occupy a prominent position, as it is a key intermediate for further annelation of a wide variety of ring and for various functional groups inter conversions [23-24]. We herein report a series of novel bio-active pyrazine moiety containing α-aminophosphonate derivatives **(4a-l)** with different pharmalogically active aldehydes under microwave irradiation in toluene without catalyst and the newly synthesized compounds were screened for their in vitro antibacterial activity.

II. Results and Discussion

2.1 Chemistry

In continuation of our work to develop environmentally benign and green protocols for the synthesis of heterocyclic molecules, herein, we developed a one pot, mild and efficient method for the synthesis of diethyl phenyl (pyrazin-2-yl-amino) methylphosphonates derivatives from Pyrazino-2-amine **1**, various aromatic

aldehydes **2** and tri ethyl phosphite **3** using microwave irradiation in higher yields. Structurally diverse aldehydes, amines and phosphites were used to afford the corresponding α-aminophosphonates in high to excellent yields (**Table 1**) herein; we would like to report that alum is an efficient catalyst for the formation of α -aminophosphonates by one-pot three component reaction of an aldehyde, amine and triethyl phosphite in toluene under microwave irradiation conditions in higher yields **(Scheme 1).**

2.2 Microbiology

Bioassay- Agar well bioassay was employed for testing antibacterial and antifungal activity of **4a-4l**. Diluted inoculums 0.1 mL (105 CFU/mL) of bacteria was spread on nutrient agar and fungi on potato dextrose agar plates (PDA). Wells of 8 mm were punched into the agar medium and filled with the title compounds at the concentration of 25 and 50 µg in each well. The plates were incubated for 24 h at 37 °C for test bacteria and the fungi plates were incubated for 72 h at28 °C. The antimicrobial activity was evaluated by measuring the zone of inhibition against test organisms. Chloramphenicol and Ketoconazole were used as commercial standards. Controls were maintained with dimethyl sulphoxide (DMSO) [25].

Determination of MIC- Minimum inhibitory concentration (MIC) was determined for the compounds that showed total growth inhibition using the protocol described below. The minimum concentration, at which there was no visually detectable bacterial growth, was taken as MIC. The compound concentration of 0.1 mg to 0.50 mg/mL in steps of 0.1 mg/mL was evaluated. Specifically 0.1 mL of standardized inoculums (1-2 x 107 CFU/mL) was added to each test tube. Two controls (DMSO with bacteria and antibiotics with bacteria) were maintained for each test sample. The tubes were incubated aerobically at 37 °C for 24 h. The method followed for antifungal bioassay is similar to that followed for antibacterial assay where in the medium is PDA and incubation temperature is 28 °C for 72 h [26].

III. Experimental

3.1. Chemistry

All chemicals and solvents used were laboratory grade and directly used. Melting points were determined by open capillary method and are uncorrected. ^{1}H NMR spectra were recorded (in DMSO-d^{6}, ppm) on AVANCE-300 MHz spectrometer using TMS as an internal standard (s = singlet, d = doublet, t = triplet, m = multiplates and br = brod). Coupling constant (J) are given in (Hz). IR spectra were recorded (in KBr pallets) on SCHIMADZU spectrophotometer. Mass spectra were recorded on EI-SHIMDZU-GC-MS spectrometer. All reactions and purity of isolated product was monitored by using thin layer chromatography (TLC) using 0.2 mm silica gel plates 60 F254 (MERCK) and mobile phase petroleum ether and ethyl acetate (80:20). Reaction components were visualized in UV (255 and 365 nm) and iodine chamber.

3.2. General procedure for Preparation of diethyl phenyl (pyrazin-2-yl-amino) methylphosphonates derivatives (4 a-l)

The mixture of aromatic aldehyde (2.5 mmol), pyrazin-2-yl-amino (2.5 mmol), triethyl phosphite (3 mmol) were dissolved in dry toluene (15 mL). The reaction mixture was irradiated in a microwave oven at 490 Watts for 10 - 14 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was concentrated in a rota-evaporator and the residue was purified by column chromatography on silica gel (100–200 mesh) using petroleum ether-ethyl acetate (7:3) as eluent. The obtained yields of **4a-l** in microwave method are in the range of 84-92%. These are the optimized conditions for the best results as compared with conventional method to give pure a-aminophosphonates**.**

Spectral data of selected compounds

[1] Diethyl phenyl (pyrazin-2-yl-amino) methylphosphonates: Yield was found to be 90%, m.p. 120-121^{O}C. IR (KBr) v cm-^{1}: 3281, 1228, 753. ^{1}H-NMR(DMSO-d$_6$): □ 8.01-7.06 (m, 8H, Ar-H), 4.93 (s, 1H, NH), 4.72 (d, J = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH$_2$), 1.32 (t, J = 7Hz, 3H, P-O-CH$_2$- CH$_2$), 1.14 (t, J = 7 Hz, 3H, P-O-CH$_2$- CH$_3$), ^{31}P-NMR (DMSO-d$_6$): d 22.61., m/z (70 ev): M^{+}= 321 (100%)., Anal. $C_{15}H_{20}N_3O_3P$. Calcd: C, 67.12; H, 5.88; N, 9.39, O, 10.75; P, 6.97, Found: C, 67.10; H, 5.86; N, 9.39, O, 10.73; P, 6.92.

[3] Diethyl (4-chlorophenyl) (pyrazin-2-yl-amino) methylphosphonates Yield was found to be 90%, m.p. 175-176. IR (KBr) v cm-^{1}: 3281, 1228, 753. ^{1}H-NMR(DMSO-d$_6$): □ 7.88-6.53 (m, 7, Ar-H), 4.93 (s, 1H, NH), 4.72 (d, J = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH$_2$), 1.32 (t, J = 7Hz, 3H, P-O-CH$_2$- CH$_2$), 1.14 (t, J = 7 Hz, 3H, P-O-CH$_2$- CH$_3$), ^{31}P-NMR (DMSO-d$_6$): d 22.61., m/z (70 ev): M^{+}= 355 (100%)., Anal. $C_{15}H_{19}ClN_3O_3P$. Calcd: C, 64.80; H, 5.67; N, 9.09, O, 10.86; P, 6.69, Found: C, 64.79; H, 5.65; N, 9.07, O, 10.81; P, 6.68.

[6] Diethyl (4-hydroxyphenyl) (pyrazin-2-yl-amino) methylphosphonates, Yield was found to be 87%, m.p. 155-156. IR (KBr) v cm-^{1}: 3281, 1228, 753. ^{1}H-NMR(DMSO-d$_6$): □ 7.88-6.53 (m, 7, Ar-H), 4.93 (s, 1H, NH), 4.72 (d, J = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH$_2$), 1.32 (t, J = 7Hz, 3H, P-O-CH$_2$- CH$_2$), 1.14 (t, J = 7 Hz, 3H, P-O-CH$_2$- CH$_3$), ^{31}P-NMR (DMSO-d$_6$): d 22.61., m/z (70 ev): M^{+}= 377.3(100%)., Anal.

$C_{15}H_{20}ClN_3O_4P$. Calcd: C, 64.80; H, 5.67; N, 9.09, O, 10.86; P, 6.69, Found: C, 64.79; H, 5.65; N, 9.07, O, 10.81; P, 6.70.

[8] Diethyl (3-nitrophenyl) (pyrazin-2-yl-amino) methylphosphonates, Yield was found to be 89%, m.p. 140-142^{O}C. IR (KBr) ν cm-^{1}: 3281, 1228, 753. ^{1}H-NMR(DMSO-d_6): □ 7.88-6.53 (m, 7H, Ar-H), 4.93 (s, 1H, NH), 4.72 (d, J = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH_2), 1.32 (t, J = 7Hz, 3H, P-O-CH_2- CH_2), 1.14 (t, J = 7 Hz, 3H, P-O-CH_2- CH_3), ^{31}P-NMR (DMSO-d_6): d 22.61., m/z (70 ev): M^+= 366.3(100%), Anal. $C_{15}H19_{26}N_4O_5P$. Calcd: C, 60.99; H, 5.15; N, 11.39, O, 16.27; P, 6.30, Found: C, 60.97; H, 5.12; N, 11.38, O, 16.24; P, 6.29.

[10] Diethyl (4-methylphenyl) (pyrazin-2-yl-amino) methylphosphonates, Yield was found to be 91%, m.p. 166-168^{O}C. IR (KBr) ν cm-^{1}: 3281, 1228, 753. ^{1}H-NMR(DMSO-d_6): □ 7.88-6.53 (m, 7H, Ar-H), 2.93 (s, 3H, CH_3) ,4.93, (s, 1H, NH), 4.72 (d, J = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH_2), 1.32 (t, J = 7Hz, 3H, P-O-CH_2-CH_2), 1.14 (t, J = 7 Hz, 3H, P-O-CH_2- CH_3), ^{31}P-NMR (DMSO-d_6): d 22.61., m/z (70 ev): M^+= 335.3(100%), Anal. $C_{16}H_{22}N_3O_3P$. Calcd: C, 67.69; H, 6.13; N, 9.13, O, 10.42; P, 6.75, Found: C, 67.67; H, 6.12; N, 9.11, O, 10.40; P, 6.71.

[12] Diethyl (2, 4-dichlorophenyl) (pyrazin-2-yl-amino) methylphosphonates: Yield was found to be 89%, m.p. 158-159^{O}C. IR (KBr) ν cm-^{1}: 3281, 1228, 753. ^{1}H-NMR(DMSO-d_6): □ 7.88-6.53 (m, 6H, Ar-H), 2.93 (s, 3H, CH_3) ,4.93, (s, 1H, NH), 4.72 (d, J = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH_2), 1.32 (t, J = 7Hz, 3H, P-O-CH_2- CH_2), 1.14 (t, J = 7 Hz, 3H, P-O-CH_2- CH_3), ^{31}P-NMR (DMSO-d_6): d 22.61., m/z (70 ev): M^+= 390.3(100%), Anal. $C_{15}H_{18}Cl_2N_3O_3P$. Calcd: C, 58.17; H, 4.69; Cl, 13.75; N, 8.16, O, 9.32; P, 6.04, Found: C, 58.15; H, 4.68; Cl, 13.73; N, 8.14, O, 9.30; P, 6.00.

3.3. Determination of Antimicrobial Activity

The antibacterial activities of the synthesized compounds **4 a-l** were determined by agar diffusion method as recommended by the National Committee for Clinical Laboratory Standards, (NCCLS) [19-21], against selected Gram-positive bacteria viz. Bacillus subtilis (MTCC 441) and Staphylococcus aureus (MTCC 96) and Gram-negative bacteria viz. Pseudomonas aeruginosa (MTCC 1688), and Escherichia coli (MTCC 1650) strains by the agar well diffusion method. Briefly, 0.1 mL of overnight grown respective bacterial culture was spreaded over the nutrient agar plates. The wells of 6 mm diameter were prepared on the nutrient agar plates and filled with diluted test compounds separately. For comparison, DMSO and antibiotic Streptomycin were used as a solvent control and as reference antibacterial agent, respectively.

3.3.2. Antifungal activity

The compounds were screened for their antifungal activity on the fungal strains Aspergillus niger (MTCC 1789) and Candida albicans (MTCC 227). Fungal suspension (0.1 mL) was spread on Sabourauds agar plates. The wells of 6 mm diameter were prepared on the inoculated plates and filled with diluted test compounds separately. For comparison, DMSO and antibiotic Nystatin were used as solvent control and reference antifungal agent, respectively. Inoculated plates were then incubated at 30°C for 2-3 days and the resulting zones of inhibition (in mm) were measured. The minimum inhibitory concentrations at which no fungal growth observed was recorded as the MIC value.

IV. Figures and Tables

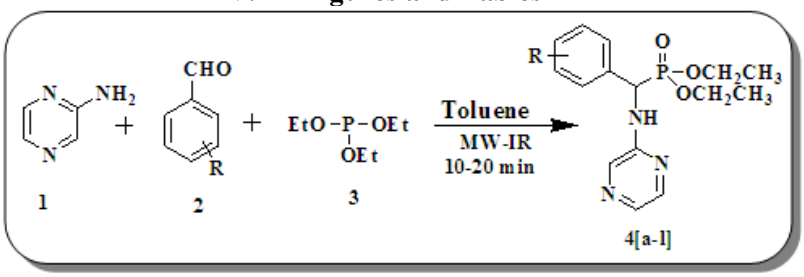

Table: 1 Preparation of diethyl phenyl (pyrazin-2-ylamino) methyl phosphate derivatives 4 [a-l]

Entry	Compound	Aldehyde [R]	Time [Min]	Yield [%]	M. P. [^{O}C]
1	4a	C_6H_5	10	90	120-121
2	4b	4-OH- C_6H_4	16	85	132-133
3	4c	4-Cl- C_6H_4	12	90	175-176
4	4d	4-F- C_6H_4	12	89	168-169
5	4e	2-OH- C_6H_4	16	90	112-114
6	4f	3-OH- C_6H_4	15	87	155-156
7	4g	4-NO_2- C_6H_4	20	84	145-147
8	4h	3-NO_2- C_6H_4	18	89	140-142

9	4i	4-OCH_3- C_6H_4	15	90	170-171
10	4j	4-CH_3- C_6H_4	18	91	166-168
11	4k	3,4,5-OCH_3- C_6H_2	20	92	175-177
12	4l	2,4- Cl- C_6H_3	13	89	158-159

Table: 2 Antibacterial Activity of diethyl phenyl (pyrazin-2-ylamino) methyl phosphate derivatives (4 a-l)

Comp. No	**MIC in µg/mL [Zone of inhibition, mm]**					
	Antibacterial activity				**Antifungal activity**	
	B. subtilis	S. aureus	E. coli	P.aeruginosa	A. niger	C. albicans
4a	25 (16)	25 (13)	25 (16)	25 (14)	25 (11)	25 (15)
4b	25 (8)	25 (5)	50 (9)	25 (12)	25 (13)	25 (12)
4c	25 (15)	25 (13)	25 (16)	25 (15)	25 (12)	25 (14)
4d	25 (13)	25 (15)	25 (17)	25 (12)	25 (13)	25 (13)
4e	50 (10)	50 (14)	50 (17)	50 (18)	50 (16)	50 (18)
4f	50 (10)	50 (14)	50 (13)	50 (12)	50 (14)	50 (12)
4g	25 (16)	25 (13)	25 (16)	25 (14)	25 (11)	25 (15)
4h	25 (18)	25 (14)	25 (17)	25 (18)	25 (16)	25 (14)
4i	50 (14)	50 (16)	50 (17)	50 (19)	50 (13)	50 (18)
4j	50 (10)	50 (14)	50 (17)	50 (18)	50 (16)	50 (18)
4k	25 (16)	25 (13)	25 (16)	25 (14)	25 (11)	25 (15)
4l	25 (8)	25 (5)	50 (9)	25 (12)	25 (13)	25 (12)
Streptomycin	25 (13)	25 (15)	25 (17)	25 (12)	ND	ND
Nystatin	ND	ND	ND	ND	6.58(18)	25 (14)

V. Conclusion

The synthesis of novel biologically active diethyl phenyl (pyrazin-2-ylamino) methyl phosphate derivatives (**4a-l**) is accomplished through one-pot three-component Kabachnik-Fields reaction under microwave irradiation and catalyst-free. Microwave method offered the best results with reaction times and yields. All the synthesized α-aminophosphonates **(4a-l)** were tested for their antibacterial and antifungal activities. Compound **4 a, 4 c, 4 i** found to be potent antibacterial agents against Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa respectively, where as compound **4 c, 4f** and **4 i** shows predominant activity against Aspergillus niger and Candida albicans fungal species. All the synthesized compounds showed moderate antibacterial activity against both gram negative and gram positive bacteria.

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