

Synthesis, characterization and antimicrobial evaluation of novel diethyl (2-phenylquinazolin-4-ylamino) (phenyl) methyl phosphate

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ABSTRACT: A convenient and efficient one-pot reaction has been employed for the synthesis of new α -aminophosphonates **4a-4l** via Kabachnik-Fields reaction in 89-95 % yields. In the procedure developed, equimolar quantities of 2- phenylquinazoline-4-amine **1**, various aromatic aldehydes **2a-2l** and triethyl phosphite **3** was carried out under solvent-free conditions using Alum (KAl (SO₄)₂.12H₂O). The products were characterized by IR, ¹H and ¹³C spectra and elemental analysis. All the synthesized compounds were screened for in vitro antibacterial activity (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*) and antifungal activity (*Candida albicans*, *Aspergillus niger*).

KEY WORDS: α -Aminophosphonate, Kabachnik-fields reaction, Antibacterial and antifungal activities

I. INTRODUCTION

Organophosphorus compounds have wide range of applications in the industrial, agricultural and medicinal chemistry owing to their unique physicochemical and biological properties. Their utility as reagents and potential synthons in organic synthesis is gaining increased attention [1]. Synthesis of α -aminophosphonates, structural analogues of natural amino acids, is receiving great attention due to their applications in agriculture as plant growth regulators, herbicides [2] and virucides [3] and in medicine as anti-cancer agents [4], enzyme inhibitors [5], peptide mimetics [6], antibiotics and pharmacological agents [7]. As a result, a variety of synthetic approaches [8] have been developed for the synthesis of α -aminophosphonates. Of them, Kabachnik-Fields three-component reaction, in which an aldehyde, an amine and a dialkyl phosphite are reacted in one-pot set-up, is an important one. In some reports, this reaction was carried out in straight-forward one-pot procedures without any catalysts [9, 10]. While, in most cases it was performed using catalysts, such as LiClO₄ [11, 12], TaCl₅-SiO₂ [13], InCl₃ [14], lanthanide-triflate [15] and CF₃COOH [16]. The key step in this synthesis is the nucleophilic addition of an amine to carbonyl compound followed by addition of a trialkyl phosphite to the resulting imine. Formation of hydroxy phosphonates and product of rearrangement frequently accompany this reaction. In view of greater applicability of α -aminophosphonates, an easy one-pot synthetic procedure for them by Kabachnik-Fields reaction under mild conditions using Alum (KAl (SO₄)₂) as a catalyst has been developed.

II. RESULTS AND DISCUSSION

2.1 Chemistry

The synthetic route involves reaction of equimolar quantities of 2- phenylquinazoline-4-amine **1**, various aromatic aldehydes **2a-2l** results in the formation of imine intermediates which subsequently reacts with trialkyl phosphites using Alum (KAl (SO₄)₂) as a catalyst to produce the corresponding α -aminophosphonates (**Scheme 1**). Structurally diverse aldehydes, amines and phosphites were used to afford the corresponding α -aminophosphonates in high to excellent yields (**Table 2**) 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 under solvent-free conditions (**Scheme 1**).

2.2 Microbiology

The antimicrobial activities of these compounds were evaluated by minimum inhibitory concentration (MIC) against Gram-positive and Gram-negative test bacteria *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 1650), *Pseudomonas aeruginosa*, (MTCC 1688), and fungi *Aspergillus niger* (MTCC 1789) and *Candida albicans* (MTCC 227) *in vitro*. Streptomycin and nystatin as reference drug, by agar diffusion method, [17-18] the results of *in vitro* antibacterial activities and MIC's of compound **4 a-l** against various bacterial strains are summarized in **Table 1**, compound **4 a**, **4 c**, shows excellent activity against all bacteria with showing more than 12 mm zone of inhibition at 25 μ g/mL MIC value. Compound **4 f** is found to be potent activity against *Bacillus subtilis* and *Escherichia coli* even at very low

concentration where as compound **4 i** also point out excellent activity against *Pseudomonas aeruginosa*, with showing above 15 mm zone of inhibition even at 25 MIC value in competition with standard drug, where as compound **4 f** is found to be almost equally potent active against *Escherichia coli* in comparison with streptomycin even at lowest MIC (25 µg/ml). Compound **4 b**, **4 d**, **4 e**, and **4 h** are also found good active but at double concentration. Compound **4 a** shows considerable activity against *Aspergillus niger* where as compound **4 c** and **4 f** shows good activity against both fungal species. Compound **4 i** found to most potent active towards *Aspergillus niger* and *Candida albicans* with showing more than 12 mm zone of inhibition at 25 µg/ mL MIC value in comparison with standard drug. Likewise remaining compound **4 b**, **4 d**, **4 e**, **4 g** and, **4 h** are also found active but above 25 µg/ mL.

III. EXPERIMENTAL

Chemistry : All chemicals and solvents used were laboratory grade and directly used. Melting points were determined by open capillary method and are uncorrected. ¹H 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 = multiplets 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-SHIMADZU-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 (2-phenylquinazolin-4-ylamino) (phenyl) methyl phosphate (4 a-l)

The mixture of aldehyde (2.5 mmol), 2- phenylquinazoline-4-amine (2.5 mmol), triethyl phosphite (3 mmol) and powdered alum (20 mol %) was stirred magnetically at room temperature. After the completion of reaction as monitored by TLC; 20 mL ice-cold water was added to the reaction mixture and product was extracted by chloroform (2 mL × 25 mL). The organic layer washed by brine (2 mL × 20 mL) and dried over anhydrous sodium sulphate. The solvent was distilled out on Rota-evaporator under reduced pressure to afford the α-aminophosphonates which on passing through a column of silica gel (1.5 gm) and elution with petroleum ether–ethyl acetate (7:3) to give pure α-aminophosphonates.

Spectral data of selected compounds

- [1] Yellow crystals: Yield was found to be 95%, m.p. 109-111^oC. IR (KBr) ν cm⁻¹: 3281, 1228, 753. ¹H-NMR(DMSO-*d*₆): δ 8.01-7.06 (m, 14H, Ar-H), 4.93 (s, 1H, NH), 4.72 (d, *J* = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH₂), 1.32 (t, *J* = 7Hz, 3H, P-O-CH₂- CH₂), 1.14 (t, *J* = 7 Hz, 3H, P-O-CH₂- CH₃), ³¹P-NMR (DMSO-*d*₆): δ 22.61. FAB MS: *m/z* (%) 447 (M+K), Anal. C₂₅H₂₆N₃O₃P. 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.
- [2] Yellow crystals: Yield was found to be 94%, m.p. 178-180^oC. IR (KBr) ν cm⁻¹: 3281, 1228, 753. ¹H-NMR(DMSO-*d*₆): δ 8.10-7.09 (m, 13H, Ar-H), 4.93 (s, 1H, NH), 4.72 (d, *J* = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH₂), 1.32 (t, *J* = 7Hz, 3H, P-O-CH₂- CH₂), 1.14 (t, *J* = 7 Hz, 3H, P-O-CH₂- CH₃), ³¹P-NMR (DMSO-*d*₆): δ 22.61. FAB MS: *m/z* (%) 465 (M+K), Anal. C₂₅H₂₅FN₃O₃P. Calcd: C, 64.53; H, 5.43; F, 4.10; N, 9.06, O, 10.32; P, 6.67, Found: C, 64.51; H, 5.41; F, 4.08; N, 9.03, O, 10.31; P, 6.65.
- [3] Yellow crystals: Yield was found to be 90%, m.p. 155-157^oC. IR (KBr) ν cm⁻¹: 3281, 1228, 753. ¹H-NMR(DMSO-*d*₆): δ 7.88-6.53 (m, 13H, Ar-H), 4.93 (s, 1H, NH), 5.95 (s, 1H, OH), 4.72 (d, *J* = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH₂), 1.32 (t, *J* = 7Hz, 3H, P-O-CH₂- CH₂), 1.14 (t, *J* = 7 Hz, 3H, P-O-CH₂- CH₃), ³¹P-NMR (DMSO-*d*₆): δ 22.61. FAB MS: *m/z* (%) 465 (M+K), Anal. C₂₅H₂₆N₃O₄P. 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.
- [4] Yellow crystals: Yield was found to be 89%, m.p. 140-142^oC. IR (KBr) ν cm⁻¹: 3281, 1228, 753. ¹H-NMR(DMSO-*d*₆): δ 7.88-6.53 (m, 13H, Ar-H), 4.93 (s, 1H, NH), 4.72 (d, *J* = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH₂), 1.32 (t, *J* = 7Hz, 3H, P-O-CH₂- CH₂), 1.14 (t, *J* = 7 Hz, 3H, P-O-CH₂- CH₃), ³¹P-NMR (DMSO-*d*₆): δ 22.61. FAB MS: *m/z* (%) 492 (M+K), Anal. C₂₅H₂₆N₄O₅P. 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.
- [5] Yellow crystals: Yield was found to be 93%, m.p. 166-168^oC. IR (KBr) ν cm⁻¹: 3281, 1228, 753. ¹H-NMR(DMSO-*d*₆): δ 7.88-6.53 (m, 13H, Ar-H), 2.93 (s, 3H, CH₃), 4.93, (s, 1H, NH), 4.72 (d, *J* = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH₂), 1.32 (t, *J* = 7Hz, 3H, P-O-CH₂- CH₂), 1.14 (t, *J* = 7 Hz, 3H, P-O-CH₂- CH₃), ³¹P-NMR (DMSO-*d*₆): δ 22.61. FAB MS: *m/z* (%) 461 (M+K), Anal. C₂₆H₂₈N₃O₃P. 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.
- [6] Yellow crystals: Yield was found to be 92%, m.p. 168-170^oC. IR (KBr) ν cm⁻¹: 3281, 1228, 753. ¹H-NMR(DMSO-*d*₆): δ 7.88-6.53 (m, 13H, Ar-H), 2.93 (s, 3H, CH₃), 4.93, (s, 1H, NH), 4.72 (d, *J* = 24 Hz, 1H, P-C- H), 3.70-4.21(m, 4H, 2×OCH₂), 1.32 (t, *J* = 7Hz, 3H, P-O-CH₂- CH₂), 1.14 (t, *J* = 7 Hz, 3H, P-O-

CH₂-CH₃), ³¹P-NMR (DMSO-d₆): δ 22.61. FAB MS: m/z (%) 515 (M+K), Anal. C₂₅H₂₄Cl₂N₃O₃P. 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

3.3.1. Antibacterial 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 spread 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. Inoculated plates were then incubated at 37°C for 24 h and the resulting zones of inhibition (in mm) were measured. The minimum inhibitory concentrations at which no growth was observed was taken as the MIC value.

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.

4. Figures and Tables

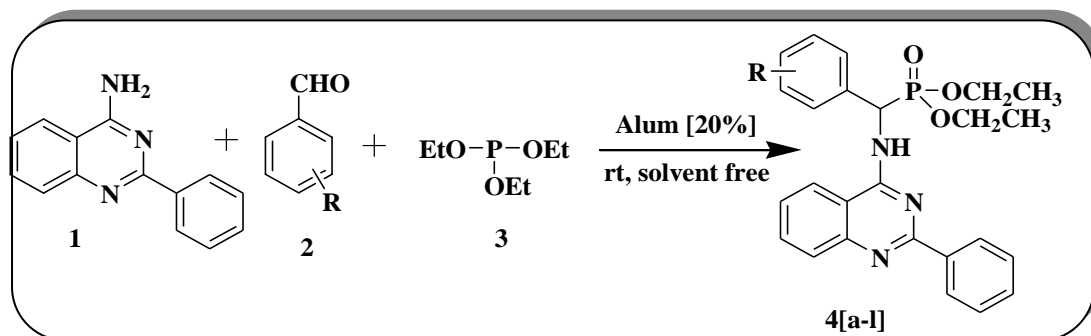


Table: 1 Preparation of diethyl (2-phenylquinazolin-4-ylamino) (phenyl) methyl phosphate **4 [a-l]**

Entry	Compound	Aldehyde [R]	Time [Min]	Yield [%]	M. P. [°C]
1	4a	C ₆ H ₅	10	95	109-111
2	4b	4-OH- C ₆ H ₄	15	90	112-114
3	4c	4-Cl- C ₆ H ₄	12	92	165-167
4	4d	4-F- C ₆ H ₄	12	94	178-180
5	4e	2-OH- C ₆ H ₄	16	90	112-114
6	4f	3-OH- C ₆ H ₄	15	90	155-157
7	4g	4-NO ₂ - C ₆ H ₄	25	89	145-147
8	4h	3-NO ₂ - C ₆ H ₄	22	89	140-142
9	4i	4-OCH ₃ - C ₆ H ₄	15	90	178-180
10	4j	4-CH ₃ - C ₆ H ₄	20	93	166-168
11	4k	3,4,5-OCH ₃ - C ₆ H ₂	18	90	175-177
12	4l	2,4- Cl- C ₆ H ₃	15	92	168-170

Table: 2 Antibacterial Activity of synthesized Compounds (4 a-l)

Comp. No	MIC in µg/mL [Zone of inhibition, mm]					
	Antibacterial activity				Antifungal activity	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
4a	25 (14)	25 (12)	25 (15)	25 (12)	25 (10)	25 (14)
4b	25 (6)	25 (7)	50 (8)	25 (10)	25 (10)	25 (10)
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 (18)	25 (14)	25 (17)	25 (18)	25 (16)	25 (14)
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 (18)	25 (14)	25 (17)	25 (18)	25 (16)	25 (14)
4l	25 (13)	25 (15)	25 (17)	25 (12)	25 (13)	25 (13)
Streptomycin	25 (13)	25 (15)	25 (17)	25 (12)	ND	ND
Nystatin	ND	ND	ND	ND	6.58(18)	25 (14)

IV. CONCLUSION

In our endeavor, a simple procedure for the synthesis of new α -aminophosphonates **4a-4l** in high yields by Kabachnik-Fields reaction using Alum ($KAl(SO_4)_2$) as a catalyst is described. 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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