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

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ABSTRACT

*A simple and an efficient synthetic protocol was adopted for the synthesis of novel α-aminophosphonate derivatives containing quinoline moiety such as diethyl (2-chloro-6-methoxyquinolin-3-yl) (substituted phenylamino) methylphosphonates **(4a-j)** through one-pot three - component Kabachnik-Fields reaction. 2-Chloro-6-methoxyquinolin-3-carboxaldehyde **(1),** different substituted amines **(2a-j)** and diethylphosphite **(3)** were reacted in toluene under microwave irradiation without catalyst to obtain title compounds. The structures of the title compounds **(4a-j)** were confirmed by IR, ^{1}H, ^{13}C, ^{31}P NMR, mass spectral and elemental analysis. The newly synthesized compounds were screened for their antiviral activity against Tobacco mosaic virus (TMV) and antioxidant activity was evaluated by DPPH and SOD methods. The title compounds exhibited potent antiviral and antioxidant activities.*

Keywords: α-aminophosphonates, microwave irradiation, 2-chloro-6-methoxyquinolin-3-carboxaldehyde, antiviral activity, antioxidant activity.

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, $P(O)(OH)_2$, 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 quinolines and their derivatives are important constituents of pharmacologically active synthetic compounds. The quinoline nucleus is also frequently recognized in the structure of numerous naturally occurring alkaloids. They have been associated with broad spectrum of biological activities. Among quinolines, 2-choloro-6-methoxyquinolin-3-carboxaldehyde **(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]. Shingare et al. [25] reported the synthesis of α-hydroxy phosphonate and α-acetyloxy phosphonate derivatives of 2-chloroquinoline-3-carboxaldehyde and screened for their antibacterial activity. We herein report a series of novel bio-active 2-chloroquinoline moiety containing α-aminophosphonate derivatives **(4a-j)** with different pharmalogically active amines under microwave irradiation in toluene without catalyst and all the synthesized compounds were screened for their antiviral and antioxidant activities.

MATERIALS AND METHODS

Chemistry

Sigma-Aldrich, Merck and Lancaster Chemicals were used as such without further purification. Solvents used for spectroscopic and other physical studies were reagent grade and were further purified by literature methods [26]. The reactions were carried out in a Microwave Oven, CATALYST-4R, Research Model, Made in India. Melting points were determined by Guna Digital Melting Point apparatus using a calibrated centigrade thermometer and are uncorrected. IR spectra were obtained in KBr optics on a Perkin-Elmer Model 281-B spectrophotometer, in wave numbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker AVANCE III 500 MHz spectrometer operating at 500 MHz for ^{1}H, 125 MHz for ^{13}C and 202.4 MHz for ^{31}P NMR. The ^{1}H and ^{13}C chemical shifts were expressed in ppm with reference to tetramethylsilane and ^{31}P chemical shifts to 85% $H3PO_4$. ESI mass spectra were recorded on a Finnigan MAT 1020 mass spectrometer. Elemental analyses were performed at University of Hyderabad, India.

General Procedure for synthesis of title compounds (4a-j).

Method A: Conventional heating

2-Chloro-6-methoxyquinoline-3-carboxaldehyde **(1)** (0.002 mol), different aromatic/heterocyclic amines **(2)** (0.002 mol) were dissolved in dry toluene (15 mL) and stirred the contents for 10 min at room temperature to get homogenous solution. To this mixture diethyl phosphite **(3)** (0.002 mol) dissolved in 10 mL of dry toluene was added dropwise with stirring at room temperature. Later, the mixture was refluxed with stirring for 4-6 h at about 95-100 ^{o}C in oil both. Identification of the product and completion of the reaction was monitored by TLC using ethyl acetate: hexane (3:2). 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 structures of the title compounds **4a-j** were established by spectral and elemental analysis. The obtained yields of **4a-j** are in the range of 68-74%.

Method B: Microwave method

2-Chloro-6-methoxyquinoline-3-carboxaldehyde **(1)** (0.002 mol), different aromatic/heterocyclic amines **(2)** (0.002 mol) and diethyl phosphite **(3)** (0.002 mol) 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-j** in microwave method are in the range of 84-92%. These are the optimized conditions for the best results as compared with conventional method.

***Diethyl(2-chloro-6-methoxyquinolin-3yl)(2-chloropyridin-3-ylamino)methyl phosphonate* (4a).**
Dark brown solid. Mol.Wt: 469. mp 134-136 ^{o}C. IR (KBr) : 756 (P-C), 1212 (P=O), 3380 (NH) cm^{-1}; ^{1}H-NMR (DMSO- d_6) δ (ppm): 7.83-6.86 (m, 7H, Ar-H), 5.71-5.68 (dd, J = 14.2 Hz, J = 10.6 Hz, 1H, P-CH), 5.46 (t, 1H, N-Haliphatic), 3.86-3.82 (m, 4H, P-O-CH_2), 3.54 (s, 3H, Ar-OCH_3), 1.64-1.24 (m, 6H, P-O-CH_2-CH_3); ^{13}C NMR (DMSO-d_6) δ: 148.2 (C-2), 142.6 (C-5'), 142.4 (C-7), 136.8 (C-10), 134.2 (C-2'), 132.6 (C-3), 132.2 (C-6'), 128.6 (C-5), 126.8 (C-4'), 126.0 (C-9), 124.8 (C-4), 120.2 (C-3'), 112.8 (C-8), 104.2 (C-6), 67.4 (O-CH_2-CH_3, d, J = 7.2 Hz), 58.21 (P-CH), 54.2 (O-CH_3) 16.8 (OCH_2-CH_3, d, J = 8.0 Hz); ^{31}P-NMR (DMSO-d_6) δ: 24.75; E.S.I. mass m/z (%) 469 (100, M+.), 471 (65, M+2), 474 (11, M+4). Anal.cald.for $C_{20}H_{22}C_{l2}N_3O_4$ P: C, 51.08; H, 4.72; N, 8.94. Found: C, 51.02, H, 4.67; N, 8.89.

***Diethyl(2-chloro-6-methoxyquinolin-3yl)(pyridin-3-ylmethylamino)methyl phosphonate* (4b).**
yellow solid, yield 81%. Mol.Wt: 449. mp 156-158. IR (KBr) : 762 (P-C); 1208 (P=O), 3390 (N-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.52-6.76 (m, 8H, Ar -H), 5.78-5.70 (dd, J = 16.4 Hz, J = 12.2 Hz, 1H, P-CH), 5.44 (t, 1H, N-Haliphatic), 3.82 (d, 2H, Ar-CH_2, J = 6.0 Hz), 3.76-3.68 (m, 4H, P-O-CH_2), 3.48 (s, 3H, Ar-OCH_3), 1.58-1.42 (m, 6H, P-O-CH_2-CH_3); ^{13}C-NMR (DMSO-d6) δ: 152.4 (C-2), 144.2 (C-7), 136.8 (C-2'), 136.4 (C-5'), 134.8 (C-3), 131.2 (C-10), 128.2 (C-4'), 128.0 (C-6'), 126.4 (C-4), 126.2 (C-5), 124.0 (C-9), 121.2 (C-3'), 116.2 (C-8), 102.6 (C-6), 68.2 (O-CH2-CH3, d, J = 8.0 Hz), 60.4 (P-CH), 58.2 (Ar-CH_2), 54.2 (O-CH_3), 16.4 (OCH_2-CH_3, d, J = 6.4 Hz); ^{31}P-NMR (DMSO-d_6) δ: 23.86; ESI-MS m/z 449 (100, M+.), 451 (33, M+2). Anal. Calcd. for $C_{21}H_{25}ClN_3O_4$ P: C, 56.07; H, 5.60; N, 9.34. Found: C, 56.18, H, 5.53; N, 9.25.

***Diethyl(2-(1H-imidazol-4yl)ethylamino)(2-chloro-6-methoxyquinolin-3yl)methyl phosphonate* (4c).**
Dark brown yield 84%. Mol.Wt: 452. mp 122-124°C. IR (KBr) : 764 (P-C); 1220 (P=O), 3382 (N-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 11.2 (s, 1H, Ar-NH), 7.66-6.82 (m, 6H, Ar-H), 5.74-5.68 (dd, J = 14.2 Hz, J = 10.6 Hz, 1H, P-CH), 5.50 (t, 1H, N-Haliphatic), 3.64-3.52 (m, 4H, P-O-CH_2), 3.42 (s, 3H, Ar-OCH_3), 2.92-2.84 (m, 1H, CH_2-NH), 2.66 (t, 2H, Ar- CH_2), 1.62-1.54 (m, 6H, P-O-CH_2-CH_3); ^{13}C-NMR (DMSO-d_6) δ: 149.6 (C-2), 142.6 (C-7), 136.8 (C-5'), 132.6 (C-2'), 132.4 (C-3), 132.2 (C-10), 128.2 (C-5), 126.0 (C-9), 122.8 (C-4), 118.4 (C-8), 114.2 (C-4'), 104.2 (C-6), 69.8 (O-CH_2-CH_3, d, J = 6.8 Hz), 62.6 (P-CH), 56.8 (Ar-CH_2), 54.6 (O-CH_3), 42.6 (CH_2-NH), 15.8 (OCH_2-CH_3, d, J =7.2 Hz); ^{31}P-NMR (DMSO-d_6) δ: 23.55; ESI-MS m/z 452 (100, M+.], 454 (33, M+2). Anal. Calcd. for $C_{20}H_{26}ClN_4O_4$ P, C, 53.04; H, 5.79; N, 12.37. Found: C, 52.94, H, 5.73; N, 12.30.

***Diethyl (2-(1H-indol-3yl)ethylamino) (2-chloro-6-methoxyquinolin-3yl) methyl phosphonate* (4d).**
Red solid, yield 78%. Mol.Wt: 501. mp 144-146 °C. IR (KBr): 758 (P-C); 1216 (P=O), 3379 (N-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 10.6 (s, 1H, Ar-NH), 7.84-6.58 (m, 9H, Ar -H), 5.74-5.66 (dd, J = 12.6 Hz, J = 10.2 Hz, 1H, P-CH), 5.48 (t, 1H, N-Haliphatic), 3.72-3.64 (m, 4H, P-O-CH_2), 3.42 (s, 3H, Ar-OCH_3), 2.86-2.72 (m, 2H,CH_2-NH), 2.66 (t, 2H, Ar- CH_2), 1.66-1.54 (m, 6H, P-O-CH2-CH3); ^{13}C-NMR (DMSO-d_6) δ: 152.4 (C-2), 148.2 (C-7), 135.4 (C-10), 134.2 (C-2'), 132.4 (C-3), 130.4 (C-5), 128.0 (C-9), 126.8 (C-4), 124.6 (C-6'), 122.8 (C-4'), 122.4 (C-7'), 120.8 (C-9'), 116.8 (C-5'), 116.2 (C-8), 112.8 (C-3'), 106.8 (C-8'), 102.6 (C-6), 68.2 (O-CH_2-CH_3, d, J = 5.6 Hz), 61.2 (Ar-CH_2), 60.8 (P-CH), 57.4 (O-CH_3), 48.4 (CH_2-NH), 15.2 (OCH_2-CH_3, d, J = 10.4 Hz). ^{31}P-NMR (DMSO-d_6) δ: 23.08; ESI-MS m/z 501 (100, [M+.), 503 (33, M+2). Anal. Calcd. for $C_{20}H_{29}ClN_3O_4$ P, C, 59.75; H, 5.82; N, 8.37. Found: C, 59.75, H, 5.76; N, 8.30.

***Diethyl (2-chloro-6-methoxyquinolin-3yl) (3,4-dihydroxy- phenethylamino) methyl phosphonate* (4e).**
Black solid, yield 76%. Mol.Wt: 494. mp 201-203 °C. (KBr) : 764 (P-C), 1218 (P=O), 3392 (N-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.82-6.68 (m, 7H, Ar -H), 5.78-5.71 (dd, J = 18.2 Hz, J = 11.6 Hz, 1H, P-CH), 5.58-5.66 (s, 2H, Ar-OH), 5.45 (t, 1H, N-Haliphatic), 3.72-3.66 (m, 4H, P-O-CH_2), 3.50 (s, 3H, Ar-OCH_3), 2.82 (t, 2H, Ar-CH2), 2.74-2.68 (m, 2H,CH_2-NH), 1.52-1.46 (m, 6H, P-O-CH2-CH3); ^{13}C-NMR (DMSO-d_6) δ: 150.6 (C-2), 146.4 (C-7), 144.6 (C-4'), 142.4 (C-3'), 138.4 (C-10), 134.6 (C-5), 128.4 (C-1'), 126.4 (C-6'), 126.2 (C-3), 124.2 (C-8), 121.4 (C-4), 114.6 (C-2'), 112.8 (C-9), 112.4 (C-5'), 102.8 (C-6), 68.4 (O-CH_2-CH_3, d, J = 7.6 Hz), 61.2 (P-CH), 58.6 (Ar-CH_2), 54.2 (O-CH_3), 42.8 (CH_2-NH), 16.8 (OCH_2-CH_3, d, J = 8.2 Hz); ^{31}P NMR (DMSO-d_6) δ: 25.20; ESI-MS m/z 494 (100, M+.], 496 (33, M+2).

***Diethyl(4-chloro-2fluorophenylamino)(2-chloro-6-methoxyquinolin-3yl)methyl phosphonate* (4f).**
Brown solid, yield 86%. Mol.Wt: 501. mp 182-184 °C. (KBr) (ν_{max} cm^{-1}): 756 (P-C), 1211 (P=O), 3395 (N-H); 1H-NMR (DMSO-d_6) δ (ppm): 7.84-6.92 (m, 7H, Ar -H), 5.80-5.72 (dd, J = 18.2 Hz, J = 12.8 Hz, 1H, P-CH), 5.46 (t, 1H, N-Haliphatic), 3.82-3.74 (m, 4H, P-O-CH_2), 3.56 (s, 3H, Ar-OCH_3), 1.56-1.42 (m, 6H, P-O-CH_2-CH_3); ^{13}C-

NMR (DMSO-d_6) δ: 154.2 (C-2), 152.6 (C-2'), 148.6 (C-7), 134.8 (C-3), 129.8 (C-10), 128.4 (C-5), 126.8 (C-5'), 126.2 (C-4'), 125.2 (C-1'), 124.8 (C-9), 122.6 (C-4), 118.4 (C-8), 112.4 (C-3'), 104.2 (C-6), 67.8 (O-CH2-CH3, d, *J* = 6.8 Hz), 61.4 (P-CH), 54.6 (O-CH_3), 16.4 (OCH_2-CH_3, d, *J* = 8.6 Hz); ^{31}P NMR (DMSO-d_6) δ: 23.34; ESI-MS m/z 501 (100, M+.), 503 (64, M+2), 505 (10, M+4). Anal. Calcd. for $C_{21}H_{22}C_{l2}FN_2O_4P$: C, 51.76; H, 4.55; N, 5.75. Found: C, 51.67, H, 4.50; N, 5.69.

Diethyl(2-chloro-6-methoxyquinolin-3yl)(3,5-dichlorophenylamino)methyl phosphonate **(4g).**
Light red solid, yield 87%. Mol.Wt: 503. mp 162-164 °C. (KBr): 756 (P-C), 1210 (P=O), 3390 (N-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm); 8.00-6.55 (m, 7H, Ar-H), 5.71-5.68 (dd, *J* = 13.6 Hz, *J* = 11.2 Hz, 1H, P-CH), 5.50 (t, 1H, N-Haliphatic), 3.94-3.80 (m, 4H, P-O-CH_2), 3.56 (s, 3H, Ar-OCH_3), 1.32-1.26 (m, 6H, P-O-CH_2-CH_3); ^{13}C-NMR (DMSO-d_6) δ: 160.0 (C-2), 150.4 (C-7), 146.2 (C-1'), 136.4 (C-10), 136.2 (C-3), 126.4 (C-5), 126.2 (C-5'), 125.4 (C-3'), 124.2 (C-4), 122.6 (C-9), 116.4 (C-4'), 116.2 (C-8), 110.8 (C-6'), 108.8 (C-2'), 106.8 (C-6), 69.2 (O-CH_2-CH_3, d, *J* = 8.8 Hz), 62.2 (P-CH), 56.8 (O-CH_3), 16.2 (OCH_2-CH_3, d, *J* = 6.4 Hz); 31P NMR (DMSO-d_6) δ: 24.55; ESI-MS m/z 503 (100, M+.], 505 (95,M+2), 507 (32, M+4), 509 (9, M+6). Anal. Calcd. for $C_{21}H_{22}C_{l3}N_2O_4$ P: C, 50.07; H, 4.40; N, 5.56. Found: C, 50.05, H, 4.36; N, 5.53.

Diethyl(2-chloro-6-methoxyquinolin-3-yl)(2-fluoro-5-nitrophenylamino)methyl phosphonate **(4h).**
Light yellow, yield 80%. Mol.Wt: 497. mp 210-212 °C. (KBr): 754 (P-C); 1214 (P=O), 3388 (N-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.64-6.82 (m, 7H, Ar -H), 5.76-5.69 (dd, *J* = 15.6 Hz, *J* = 13.2 Hz, 1H, P-CH), 5.52 (t, 1H, N-Haliphatic), 3.62-3.68 (m, 4H, P-O-CH_2), 3.46 (s, 3H, Ar-OCH_3), 1.42-1.36 (m, 6H, P-O-CH_2-CH_3); ^{13}C NMR (DMSO-d_6) δ: 158.4 (C-2'), 151.6 (C-2), 145.4 (C-5'), 145.2 (C-7), 134.6 (C-5), 134.2 (C-10), 132.6 (C-1'), 129.2 (C-3), 120.8 (C-9), 118.2 (C-4), 116.2 (C-3'), 112.6 (C-8), 110.6 (C-4'), 104.6 (C-6'), 101.8 (C-6), 67.4 (O-CH_2-CH_3, d, *J* = 7.2 Hz), 61.8 (P-CH), 55.2 (O-CH_3), 16.4 (OCH_2-CH_3, d, *J* = 8.2 Hz); ^{31}P NMR (DMSO-d_6) δ: 22.41; ESI-MS m/z 497 (100, M+.), 499 (33,M+2).

Diethyl(2-chloro-5-(trifluromethyl)phenylamino)(2-chloro-6-methoxyquinolin-3-yl) methylphosphonate **(4i).**
Light orange, yield 79%. Mol.Wt: 537. mp 164-166 °C. (KBr): 762 (P-C); 1218 (P=O), 3380 (N-H) cm^{-1} ; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.68-6.62 (m, 7H, Ar -H), 5.80-5.74 (dd, *J* = 12.6 Hz, *J* = 9.8 Hz, ^{1}H, P-CH), 5.43 (t, 1H, N-Haliphatic), 3.68-3.52 (m, 4H, P-O-CH^2), 3.38 (s, 3H, Ar-OCH_3), 1.46-1.33 (m, 6H, P-O-CH_2-CH_3); ^{13}C NMR (DMSO-d_6) δ : 152.4 (C-2), 144.2 (C-7), 142.4 (C-1'), 134.8 (C-10), 134.6 (C-3), 128.6 (C-3'), 128.4 (C-5), 128.2 (C-5'), 124.2 (C-9), 122.6 (C-4), 120.4 (C-2'), 116.2 (C-6'), 112.6 (C-8), 112.4 (C-4'), 104.2 (C-6), 61.8 (P-CH), 66.8 (O-CH_2-CH_3, d, *J* = 8.6 Hz), 54.6 (O-CH_3), 26.8 (-CF_3) 15.8 (OCH_2-CH_3, d, *J* = 7.8 Hz); ^{31}PNMR (DMSO-d_6) δ: 21.76; ESI-MS m/z 537 (100, M+.], 539 (64, M+2), 541 (10, M+4). Anal. Calcd. for $C_{22}H_{22}Cl_2F_3N_2O_4$ P: C, 49.18; H, 4.13; N, 5.21. Found: C, 49.25, H, 4.06; N, 5.32.

Diethyl(2-chloro-6-methoxyquinolin-3yl)(3,5-dichloro-4-hydroxyphenylamino) methylphosphonate **(4j).**
Green solid, yield 81%. Mol.Wt: 518.mp 152-154 °C. (KBr): 768 (P-C), 1214 (P=O), 3382 (N-H) cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.74-6.82 (m, 6H, Ar -H), 5.78-5.68 (dd, *J* = 17.2 Hz, *J* = 14.2 Hz, 1H, P-CH), 5.62 (s,1H, Ar-OH), 5.48 (t, 1H, N-Haliphatic), 3.62-3.54 (m, 4H, P-O-CH_2), 3.48 (s, 3H, Ar-OCH_3), 1.54-1.42 (m, 6H, P-O-CH_2-CH_3); ^{13}CNMR (DMSO-d_6) δ: 153.2 (C-2), 145.8 (C-7), 142.8 (C-5'), 142.4 (C-1'), 136.4 (C-5), 136.2 (C-10), 132.8 (C-3'), 129.4 (C-3), 126.4 (C-9), 124.2 (C-2'), 121.2 (C-8), 118.4 (C-4), 114.6 (C-4'), 106.2 (C-6), 104.8 (C-6'), 68.4 (O-CH_2-CH_3, d, *J* = 8.2 Hz), 60.8 (P-C), 52.8 (O-CH_3), 16.2 (OCH_2-CH_3, d, *J* = 6.2 Hz); ^{31}P NMR (DMSO-d_6) δ: 26.05; ESI-MS m/z 518 (100, M+.], 520 (96, M+2), 522 (32, M+4), 524 (10, M+6]. Anal. Calcd. for $C_{21}H_{22}C_{l3}N_2O_5P$: C, 48.53; H, 4.27; N, 5.39. Found: C, 48.62, H, 4.21; N, 5.48.

Antiviral Bioassay

Purification of Tobacco Mosaic Virus (TMV)

Using Gooding's method [27], upper leaves of Nicotiana tobacum L inoculated with TMV were selected and ground in phosphate buffer, then filtered through a double layer pledget. The filtrate was centrifuged at 10,000 g, treated twice with PEG and centrifuged again. The whole experiment was carried out at 4 °C. Absorbance values were estimated at 260 nm using an ultraviolet spectrophotometer.
Virus concn = (A 260×dilution ratio)/E1cm $^{0.1\%,\ 260\ nm}$.

Curative effect of compounds against TMV *in vivo*.
Growing leaves of Nicotiana tobacum. L of the same ages were selected. TMV (concentration of 6×10^{-3} mg/mL) was dipped and inoculated on the whole leaves, and then the leaves were washed with water and dried. The compound solution was smeared on the left side and the solvent was smeared on the right side for control. The local lesion numbers were then counted and recorded 3-4 d after inoculation [28]. For each compound, three repetitions were measured. The inhibition rate of the compound was then calculated according to the following formula ('av' means average).

$$\text{Inhibition rate}(\%) = \frac{\text{av local lesion numbers of control (not treated with compound)} - \text{av local lesion numbers smeared with drugs}}{\text{av local lesion numbers of control (not treated with compound)}} \times 100$$

Antioxidant activity
Antioxidant activity was performed with two methods DPPH and Super Oxide radical scavenging activities. Scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm.

DPPH radical-scavenging activity
The DPPH radical scavenging activity was measured in a reaction mixture containing 1 mM DPPH radical solution 0.1 mL, 99% ethanol 0.8 mL, and 0.1 mL of each one of the title compounds prepared by dissolving the compound in methanol. The solution was rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm [29]. The antioxidant activity of test compounds was expressed as IC_{50}, which was defined as the concentrations of test compounds required for inhibition of the formation of DPPH radicals by 50%.

$$\text{DPPH radical scavenging activity (\%)} = 1 - \frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517nm}} \times 100$$

Superoxide radical scavenging activity
Superoxide radicals were identified by Spectrophotometric method to study the effect of various concentrations of test compounds on the reduction of nitroblue tetrazolium (NBT), according to a previously described procedure [30]. Superoxide radicals were generated in a non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS/NADH) system. The non-enzymatic generation of superoxide radicals was measured in reaction mixtures containing various concentrations of test compounds, PMS (15 µM), NADH (73 µM), and NBT (50 µM) in phosphate buffer (20 mM, pH 7.4). After incubation for 5 min at ambient temperature, the color was read at 560 nm against blank samples. The superoxide radical-scavenging activity was expressed as the IC_{50} value.

$$\text{Superoxide radical Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\cdot}$), hydroxyl radicals ($HO^{\cdot}$) and peroxy-radicals ($ROO^{\cdot}$) are produced as a part of normal metabolic processes. The compounds **4a-j** showed high antioxidant activity by scavenging the free radicals and superoxide radicals. The data are given in **Table 2**.

RESULTS AND DISCUSSION

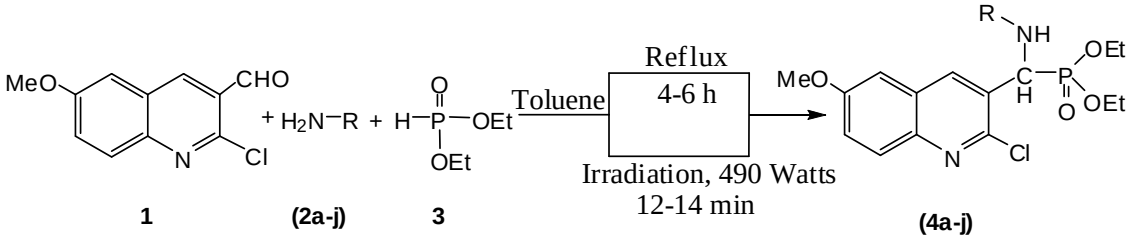
The title compounds were synthesized by reacting 2-chloro-6-methoxyquinolin-3-carboxaldehyde **(1),** with various amines **(2a-j)** and diethyl phosphite **(3)** in toluene (15 mL) in a one-pot three-component reaction by Kabachnik-Fields reaction under microwave and conventional methods. The schematic representation was underlined in **Scheme 1.** The results showed that the yields are high and the reaction times are very short for the synthesis of α-aminophosphonate derivatives **(4a-j)** under microwave conditions as compared with conventional method. After successful synthesis of target molecules **(4a-j),** they were screened for their antiviral and antioxidant activity. All the title compounds exhibited potent activity against *Tobacco mosaic virus* and antioxidant activity and the results are included in **Table 1** and **Table 2** respectively.

In conventional method, the target molecule **4a** was synthesized from 2-choloro-6-methoxyquinolin-3-carboxaldehyde **(1),** 2-chloropyridine-3-amine **(2a)** and diethyl phosphite **(3)** in dry toluene by refluxing the contents

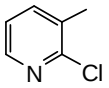
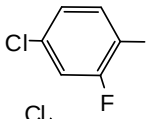
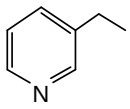
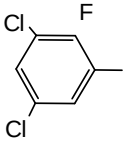
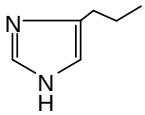
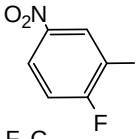
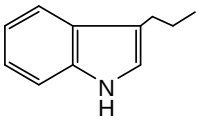
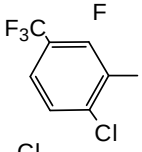
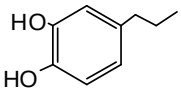
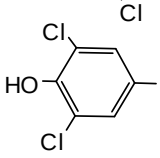
for 6 h at 90-98 ^{o}C. The reaction yield obtained was 68%. In microwave method, 2-chloro-6-methoxyquinolin-3-carboxaldehyde **(1),** 2-chloropyridine-3-amine **(2a)** and diethyl phosphite **(3)** were dissolved in dry toluene and irradiated at 490 Watts in microwave oven. The reaction was completed within 12 min with high yield 84%. The progress of the reactions was monitored by TLC. The chemical structures of the title compounds **(4a-j)** were deduced by IR, NMR, mass spectral and elemental analysis.

All the compounds **(4a-j)** exhibited infrared absorption bands for N-H, P=O and P-C aliphatic in the regions 3399-3378, 1222-1190 and 769-754 cm^{-1} respectively [31]. Chemical shifts for aromatic protons of the title compounds **(4a-j)** appeared as complex multiplets in the region δ 8.44-6.34, P-C-H proton signals appeared as doublet of doublet at δ 5.82-5.67 due to its coupling with phosphorus and neighbouring N-H proton [32-33]. The N-H proton gave a triplet in the range of δ 5.52-5.42 due to its coupling with neighbouring proton and phosphorus [34]. The ^{13}C NMR spectral data of **4a-j** showed characteristic chemical shifts for aromatic carbons. The carbon chemical shifts of P-O-CH_2 and P-CH-N appeared as a doublet at 69.4-66.9 ppm and singlet at 62.9-56.1 ppm respectively [35]. The ^{31}P NMR signals appeared as singlets in the region 26.1-20.8 ppm in all the compounds [36].

Scheme 1: Synthesis of α-aminophosphonates (4a-j).



1 **(2a-j)** **3** **(4a-j)**

Compound	R	Compound	R
3a		**3f**	
3b		**3g**	
3c		**3h**	
3d		**3i**	
3e		**3j**	

Pharmacology

Antiviral activity

The newly synthesized compounds **(4a-j)** were evaluated for their antiviral activity against Tobacco mosaic virus (TMV) by the Goodings method [27]. The bioassay results obtained at 500 µg/mL using Ningnanmycin as the control are presented in **Table 1**. It is clear that the title compounds **(4a-j)** showed a certain degree of antiviral activity against Tobacco mosaic virus. Amongst the compounds, **4b** and **4i** derivatives bearing heterocyclic pyridine,

trifluoromethyl groups respectively exhibited high TMV inhibition. The remaining compounds showed moderate antiviral activity.

Antioxidant activity

The title compounds exhibited good antioxidant activity, it was performed by using two methods DPPH and SOD. The title compounds **4e, 4a** and **4f, 4d** derivatives bearing heterocyclic hydroxyl substituted, pyridine, fluoro and chloro, indole groups respectively exhibited good antioxidant activity. The antioxidant activity of the title compounds was compared to the standard reference ascorbate.

Table 1. Viral inhibitory activity of title compounds (4a-j) against TMV.

Compound No.	Concentration (μg/mL)	Inhibition rate (%)
4a	0.5	42.8
4b	0.5	51.8
4c	0.5	48.4
4d	0.5	46.0
4e	0.5	44.0
4f	0.5	42.4
4g	0.5	46.2
4h	0.5	48.8
4i	0.5	50.2
4j	0.5	46.4
Ningnanmycin	0.5	52.4

Table 2. Antioxidant activities of the title compounds (4a-j)

Entry	DPPH Scavenging (%)	Superoxide dismutase (%)
3a	76.47±1.34	71.07±1.73
3b	67.34±1.36	66.12±1.55
3c	69.13±1.21	68.54±1.57
3d	72.48±1.84	69.93±1.86
3e	78.68±1.12	72.19±1.37
3f	75.02±1.45	70.64±1.09
3g	64.87±1.15	67.45±1.64
3h	64.94±1.73	61.08±1.88
3i	70.84±1.08	66.97±1.73
3j	65.69±1.58	64.96±1.01
Ascorbate	79.42 ± 1.65	72.51± 1.43

CONCLUSION

The synthesis of novel biologically active diethyl (2-chloro-6-methoxyquinolin-3-yl) (substituted phenylamino) methylphosphonate derivatives **(4a-j)** is accomplished through one-pot three-component Kabachnik-Fields reaction under microwave, catalyst-free and conventional methods. Microwave method offered the best results with respect of the reaction times and yields than conventional method. All the synthesized α-aminophosphonates **(4a-j)** were tested for their antiviral and antioxidant activities. The title compounds exhibited potent antiviral and antioxidant activity. Compounds **4b** and **4i** exhibited good antiviral activity against *Tobacco mosaic virus* and **4e, 4a** and **4f, 4d** showed good antioxidant activity when compared to ascorbate as a standard reference.

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