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Original article

Synthesis, in vitro antibacterial and antifungal evaluations of new α-hydroxyphosphonate and new α-acetoxyphosphonate derivatives of tetrazolo [1, 5-a] quinoline

Amol H. Kategaonkar [a], Rajkumar U. Pokalwar [a], Swapnil S. Sonar [a], Vaibhav U. Gawali [b], Bapurao B. Shingate [a], Murlidhar S. Shingare [a,*]

[a] Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431004, Maharashtra, India
[b] Pharmacology Laboratory, Maharashtra Institute of Pharmacy, Pune 411 038, Maharashtra, India

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ABSTRACT

A series of new α-hydroxyphosphonate and α-acetoxyphosphonate derivatives have been synthesized for the first time of tetrazolo [1, 5-a] quinoline derivatives. Elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data elucidated the structures of the all newly synthesized compounds. In vitro antimicrobial activities of the synthesized compounds were investigated against Gram-positive *Bacillus subtilis*, Gram-negative *Escherichia coli* and fungi *Candida albicans and Aspergillus niger*. Some of the tested compounds showed significant antimicrobial activity.

1. Introduction

Knowledge of phosphorous compounds has expanded so rapidly that it constitutes now a major branch of chemistry, organic molecules containing phosphorous offer fascinating possibilities for structural, synthetic and mechanistic study [1,2]. A wide range of natural phosphorus based biologically active compounds which plays important roles as metabolic intermediates as common regulatory switches for proteins and as a backbone for the genetic information [3]. However, aside from prodrug applications, phosphate esters are normally considered impractical functional group for drug design because they are subject to cleavage by digestive phosphatases.

The syntheses of α-hydroxy phosphonates have received an increasing amount of attention due to significant biological interests. They showed potential biological activities, such as antiviral, antibacterial, anticancer, pesticides, renin inhibitors, HIV protease, and enzyme inhibitor properties [4–13]. Much of these activities has been attributed to the relatively inert nature of the C–P bond and to the physical and structural similarity of phosphonic and phosphinic acids to the biologically important phosphate ester and carboxylic acid functionality [14]. In addition, α-hydroxy phosphonates are useful precursors for the preparation of α-functionalized phosphonates, such as amino, keto, halo, and acetoxy phosphonates [15–18].

Quinolines and their derivatives are important constituents of pharmacologically active synthetic compounds. The quinoline nucleus can also be frequently recognized in the structure of numerous naturally occurring alkaloids. They have been associated with broad spectrum of biological activities [19,20]. The fusion of quinoline to the tetrazole ring is known to increase the biological activity. The tetrazole group which is considered as analogues to carboxylic group as a pharmacore possesses wide range of biological activities. Several substituted tetrazoles have been shown to possess anticonvulsant [21], anti-inflammatory [22], CNS dispersant [23], antimicrobial [24], anti-AIDS [25], and antifertility agents [26,27].

By considering all above aspects, for the first time we have synthesized the title compounds in regards to develop our on going research work [28–31].

* Corresponding author. Tel.: +91 240 2403313; fax: +91 240 2403335.
E-mail address: prof_msshingare@rediffmail.com (M.S. Shingare).

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2. Results and discussion

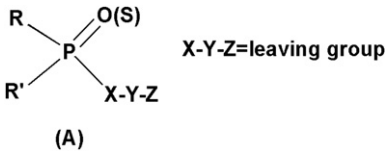
2.1. Chemistry

Synthesis of these new compounds was carried out in a straightforward manner. The key intermediates tetrazolo [1, 5-a] quinoline-4 carbaldehyde derivatives (**2a–i**) were prepared by the reaction of 2-chloroquinoline-3-carbaldehyde derivatives (**1a–i**) with sodium azide in DMSO/AcOH mixture. [32] Furthermore, we have synthesized new α-hydroxyphosphonate compounds (**3a–i**) by reacting tetrazolo[1,5-a] quinoline derivatives (**2a–i**), triethylphosphite and trimethylsilyl chloride (TMSCl) at room temperature stirring. Additionally, α-acetoxyphosphonate compounds (**4a–i**) were synthesized by reacting compounds (**3a–i**) in acetic anhydride and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at room temperature stirring. (Scheme 1). The time required for this protocol is less than the previously reported methods [29] on other than this nucleus.

Structures of compounds (**3a–i**) and (**4a–i**) were confirmed by analytical and spectral data (see Section 5 for details). IR spectra of compounds (**3a–i**) showed intense band in the region of 3205–3253 cm^{-1} of the ν (O–H) stretch, 1592–1617 cm^{-1} corresponding to ν (C═N) stretch, 1205–1249 cm^{-1} of the ν (P═O) stretch and 1022–1058 cm^{-1} of ν (P–O–C) stretch, which confirms the formation of desired compounds (**3a–i**). IR spectra compounds (**4a–i**) showed intense band in the region of 1750–1757 cm^{-1} corresponding to ν (O–CO–CH_3) stretch, 1593–1617 cm^{-1} of the ν (C═N) stretch, 1210–1231 cm^{-1} of ν (P═O) stretch and 1020–1042 cm^{-1} due to ν (P–O–C) stretch. The absence of bands in the region 3205–3253 cm^{-1} and the presence of 1750–1757 cm^{-1} are regarded as positive evidence for the formation of compounds (**3a–i**) and (**4a–i**).

^{1}H NMR spectra showed doublet due to (CH–P) proton in compounds (**3a–i**) was observed in the range of 5.86–5.90 ppm and also doublet due to (CH–P) proton in compounds (**4a–i**) 6.65–6.84 ppm, The variation in values of proton at (CH–P) position confirms the formation of desired compounds. Carbon at (CH–P) position showed peak around 67.04–67.58 ppm and 66.73–67.97 ppm regions for the compounds (**3a–i**) and (**4a–i**) respectively.

2.2. Antimicrobial activity

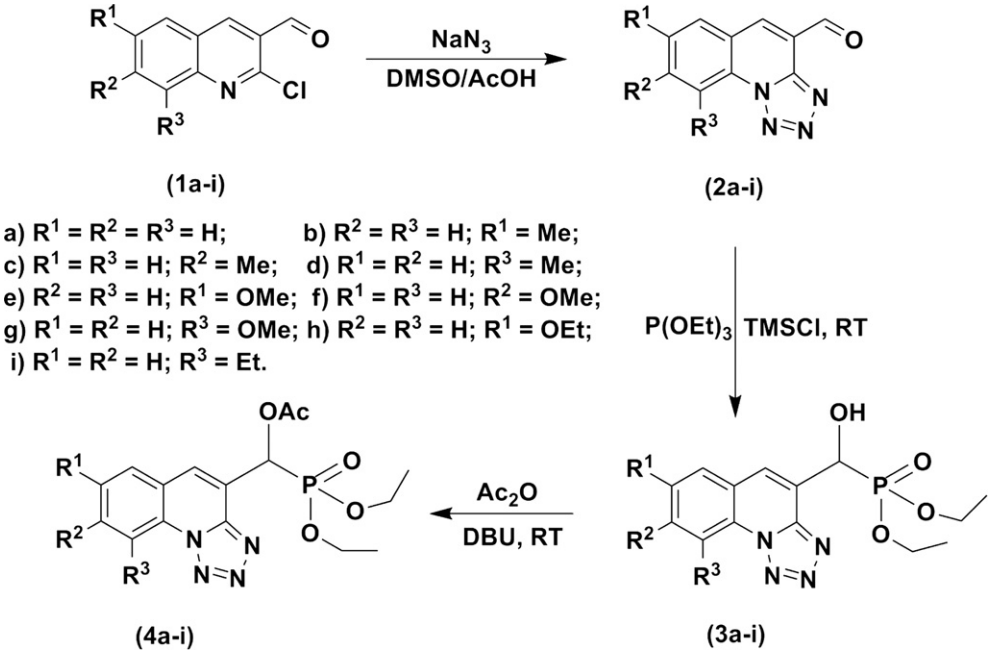


(A)

Schrader-Clark [33] proposed that organophosphorus compounds containing the general structure (A) may have significant biological activity. All organophosphorus compounds are inherently good phosphorylating agents of enzymes by virtue of the group P-XYZ in the general structure (A). Slight variation in structure can have very dramatic effects on the efficiency of organophosphorus compounds in bio-activity. These chemically and biologically variable parameters which are hard to estimate are involved in deciding "structure–activity" relationship of these compounds.

All the compounds synthesized in the present work were screened for their potential biological activities such as antibacterial activity against *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative) bacterial strains. Streptomycin was used as a reference standard in antibacterial study. Compounds were also screened for their potential antifungal activity against *Candida albicans* and *Aspergillus niger*. Griseofluvin was used as reference standard in antifungal study. The results of the antibacterial and antifungal activity screening of the tested compounds are summarized in Table 1 and Table 2, respectively.

Most of the compounds tested were found to have good antibacterial and antifungal activity, whereas compounds **3b**, **3h**, **3i**, **4c** and **4i** were acted as excellent antibacterial agents against *B. subtilis*. Likewise compounds **3b**, **3i** and **4i** showed better activity against *E. coli*. The zone of inhibition of bacterial growth with these



Scheme 1. Synthesis of new α-hydroxyphosphonates and α-acetoxyphosphonates.

Table 1
Antibacterial activity of compounds (**3a–i**) and (**4a–i**).

Compound	*Bacillus subtilis*	*Escherichia coli*
	ZI[a](MIC)[b]	ZI(MIC)
3a	12.0(10)	13.4(20)
3b	15.2(10)	15.0(10)
3c	13.6(10)	12.0(15)
3d	12.3(10)	13.7(25)
3e	12.4(10)	13.3(15)
3f	12.0(10)	13.6(15)
3g	12.5(10)	13.3(15)
3h	15.4(10)	13.5(15)
3i	14.5(10)	16.2(20)
4a	11.2(15)	12.5(10)
4b	12.0(10)	11.4(15)
4c	14.1(10)	13.2(15)
4d	13.1(20)	13.4(10)
4e	13.2(10)	12.4(10)
4f	12.5(10)	11.1(15)
4g	11.4(15)	13.9(25)
4h	12.1(10)	13.8(10)
4i	15.1(10)	14.2(10)
Streptomycin	16.4(05)	16.2(05)

[a] Zone of inhibition in mm.
[b] Minimum inhibitory concentration in μg/mL.

mentioned compounds was very close to the standard. Excellent antifungal activities were also observed with compounds **3e**, **4a** and **4h** against *C. albicans* and compounds **3b**, **3f**, **3i**, **4b**, **4c**, **4e** and **4h** were also acted as good antifungal agents against *A. niger*.

The data (Tables 1 and 2) indicate that a change in the substituent might also affect the antimicrobial activity of synthesized compounds (**3a–i**) and (**4a–i**). Comparison of biological activities among (**3a–i**) shows functional groups at $R^1 = Me/OEt$ and $R^3 = Et$ exhibit potent antibacterial activity against *B. subtilis* of corresponding compounds. Against *E. coli* functional groups at $R^3 = Me$ and $R^2 = OMe$ shows more activity. Higher activity of **3i** ($R^3 = Et$) than other compounds might be the indication of Et group somehow playing a role in killing mechanism. Functional groups at $R^1 = OMe/OEt$, $R^2 = Me$ and $R^3 = OMe$ has positional interference, as results indicate the antifungal activity of the corresponding compounds against *Candida albicans*. Positional changes of functional groups at $R^1 = Me$, $R^2 = OMe$ and $R^3 = Et$ exhibit the potent antifungal activity against *A. niger* of the corresponding compounds.

Also the comparison of the biological activities among (**4a–i**) shows as follows, against *B. subtilis* functional groups at $R^3 = Et$ and $R^2 = Me$ exhibit potent antibacterial activity of the corresponding compounds. $R^3 = Et/OMe$ and $R^1 = OEt$ functionalities might affect the antibacterial activity of compounds against *Escherichia coli*. We also found that functional groups at $R^1 = OEt$ and $R^2 = OMe$ interferes in the antifungal activity of the corresponding compounds against *Candida albicans*. At $R^1 = OMe/Me$ and $R^2 = Me$ positions affect the potency regarding antifungal activity of the corresponding compound.

Table 2
Antifungal activity of compounds (**3a–i**) and (**4a–i**).

Compound	*Candida albicans*	*Aspergillus niger*
	ZI[a](MIC)[b]	ZI(MIC)
3a	13.6(10)	12.1(15)
3b	13.2(10)	14.2(10)
3c	13.8(10)	13.2(15)
3d	13.0(10)	13.1(15)
3e	14.1(10)	12.2(15)
3f	13.4(10)	14.4(10)
3g	13.8(15)	11.5(15)
3h	13.8(10)	12.8(15)
3i	13.4(10)	14.2(10)
4a	14.2(10)	13.4(20)
4b	13.1(10)	14.1(10)
4c	13.4(10)	15.2(10)
4d	12.2(10)	12.2(15)
4e	13.4(10)	14.2(10)
4f	13.8(10)	12.5(25)
4g	12.1(15)	13.4(15)
4h	14.1(10)	14.5(10)
4i	12.1(10)	13.5(15)
Griseofluvin	16.4(05)	16.5(05)

[a] Zone of inhibition in mm.
[b] Minimum inhibitory concentration in μg/mL.

3. Conclusion

We have synthesized new α-hydroxyphosphonate and α-acetoxyphosphonate derivatives and their antimicrobial activities have been evaluated. All compounds demonstrated potent inhibition against all the strains tested. The importance of such work lies in the possibility that the new compounds might be more efficacious drugs against bacteria and fungi, which could be helpful in designing more potent antibacterial and antifungal agents for therapeutic use.

4. Experimental section

All chemicals and solvents were purchased from Merck, Spectrochem and S. D. Fine-chem. (India). Melting points were determined in open capillaries on Kumar's melting point apparatus (India) and are uncorrected. IR spectra were recorded on JASCO FT-IR 4100, Japan using KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRK-300 and NMR Spectrometer AC200. Mass spectra were recorded on Single-Quadrupole Mass Detector 3100, Waters. Elemental analyses were performed on CHNS analyzer Flash 1112, Thermo Finnigan. The progress of the reactions was monitored by TLC on Merck silica plates. Results are presented as, chemical shift δ in ppm, multiplicity, J values in Hertz (Hz), number of protons, proton's position. Multiplicities are shown as the abbreviations: s (singlet), brs (broad singlet), d (doublet), t (triplet), m (multiplet). Solvents were commercially available materials of reagent grade.

4.1. Diethyl hydroxy(tetrazolo[1,5-a]quinolin-4-yl)methylphosphonate (*3a*)

General procedure

A mixture of **2** (5 mmol), triethylphosphite (10 mmol) and TMSCl (10 mmol) were stirred magnetically at room temperature. The progress of reaction was monitored on TLC. After completion of reaction (5 min), to the reaction mixture was poured on crushed ice. The solid crude product was extracted with chloroform (2×50 mL) and washed with water (2×10 mL), brine (2×20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The obtained crude product was purified by column chromatography on silica gel by hexane: ethyl acetate (8:2) as an eluent. Yield 94%, m.p. 178–180 °C. IR (KBr, ν_{max}/cm^{-1}): 3205 (O–H), 1614 (C═N), 1215 (P═O), 1058 (P–O–C). ^{1}H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.25 (t, 6H, $J = 6$, 8 Hz, $2 \times CH_3$), 2.13 (s, 1H, OH), 4.12–4.33 (m, 4H, $2 \times CH_2$), 5.87 (d, 1H, $J = 14$ Hz, CH–P), 7.70 (t, 1H, $J = 6$, 8 Hz, Ar–H), 7.87 (t, 1H, $J = 6$, 8 Hz, Ar-H), 8.0 (d, 1H, $J = 6$ Hz, Ar-H), 8.27 (d, 1H, $J = 4$ Hz, Ar–H), 8.65(d, 1H, $J = 8$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 16.31 (CH_3), 16.35 (CH_3), 63.87 (O–CH_2), 65.42 (O–CH_2), 67.04 (CH–P), 116.67, 123.41, 124.05, 128.09, 129.24, 130.09, 131.11, 139.15, 146.53 (Ar-C). MS: m/z 337.1 (m + 1).

Elemental analysis: $C_{14}H_{17}N_4O_4P$ Calcd.: C: 50.00%; H: 5.10%; N: 16.66%. Found: C: 49.96%; H: 5.05%; N: 16.60%.

4.2. Diethyl hydroxy(7-methyltetrazolo[1,5-a]quinolin-4-yl)methylphosphonate (**3b**)

Yield 95%, m.p. 216–218 °C. IR (KBr, ν_{max}/cm^{-1}): 3237 (O–H), 1610 (C═N), 1205 (P═O), 1022 (P–O–C). 1H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.25 (t, 6H, $J = 6$, 8 Hz, $2 \times CH_3$), 1.81 (brs, 1H, OH), 3.17 (s, 3H, Ar–CH_3), 4.12–4.37 (m, 4H, $2 \times CH_2$), 5.89 (d, 1H, $J = 14$ Hz, CH–P), 7.55 (t, 1H, $J = 8$ Hz, Ar–H), 7.66 (d, 1H, $J = 8$ Hz, Ar–H), 7.81 (d, 1H, $J = 8$ Hz, Ar–H), 8.21 (d, 1H, $J = 4$ Hz, Ar–H), ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 16.31 (CH_3), 16.37 (CH_3), 21.40 (Ar–CH_3), 63.84 (O–CH_2), 65.75 (O–CH_2), 67.35 (CH–P), 116.43, 123.03, 124.05, 128.24, 128.72, 130.98, 132.51, 138.43, 145.91 (Ar–C). MS: m/z 351.2(m + 1). Elemental analysis: $C_{15}H_{19}N_4O_4P$ Calcd.: C: 51.43%; H: 5.47%; N: 15.99%. Found: C: 51.36%, H: 5.41%, N: 15.96%.

4.3. Diethyl hydroxy(8-methyltetrazolo[1,5-a]quinolin-4-yl)methylphosphonate (**3c**)

Yield 90%, m.p. 176–178 °C. IR (KBr, ν_{max}/cm^{-1}): 3247 (O–H), 1615 (C═N), 1229 (P═O), 1038 (P–O–C). ^1H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.25 (t, 6H, $J = 6$, 8 Hz, $2 \times CH_3$), 1.78 (brs, 1H, OH), 2.66 (s, 3H, Ar–CH_3), 4.10–4.31 (m, 4H, $2 \times CH_2$), 5.86 (d, 1H, $J = 12$ Hz, CH–P), 7.51 (d, 1H, $J = 8$ Hz, Ar–H), 7.87 (d, 1H, $J = 8$ Hz, Ar–H), 8.20 (d, 1H, $J = 4$ Hz, Ar–H), 8.46 (s, 1H, Ar–H). MS: m/z 351.2(m + 1). Elemental analysis: $C_{15}H_{19}N_4O_4P$ Calcd.: C: 51.43%; H: 5.47%; N: 15.99%. Found: C: 51.37%, H: 5.41%, N: 15.95%.

4.4. Diethyl hydroxy(9-methyltetrazolo[1,5-a]quinolin-4-yl)methylphosphonate (**3d**)

Yield 85%, m.p. 200–202 °C. IR (KBr, ν_{max}/cm^{-1}): 3239 (O–H), 1617 (C═N), 1249 (P═O), 1033 (P–O–C). ^1H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.26 (t, 6H, $J = 6$, 8 Hz, $2 \times CH_3$), 1.84 (brs, 1H, OH), 3.16 (s, 3H, Ar–CH_3), 4.12–4.36 (m, 4H, $2 \times CH_2$), 5.90 (d, 1H, $J = 12$ Hz, CH–P), 7.54 (t, 1H, $J = 6$, 8 Hz, Ar–H), 7.65 (d, 1H, $J = 6$ Hz, Ar–H), 7.80 (d, 1H, $J = 8$ Hz, Ar–H), 8.22 (d, 1H, $J = 4$ Hz, Ar–H). MS: m/z 351.2(m + 1). Elemental analysis: $C_{15}H_{19}N_4O_4P$ Calcd.: C: 51.43%; H: 5.47%; N: 15.99%. Found: C: 51.32%, H: 5.42%, N: 15.92%.

4.5. Diethyl hydroxy(7-methoxytetrazolo[1,5-a]quinolin-4-yl)methylphosphonate (**3e**)

Yield 90%, m.p. 190–192 °C. IR (KBr, ν_{max}/cm^{-1}): 3251 (O–H), 1614 (C═N), 1211 (P═O), 1040 (P–O–C). ^1H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.26 (t, 6H, $J = 6$, 8 Hz, $2 \times CH_3$), 1.80 (brs, 1H, OH), 3.97 (s, 3H, O–CH_3), 4.14–4.31 (m, 4H, $2 \times CH_2$), 5.80 (d, 1H, $J = 14$ Hz, CH–P), 7.35 (d, 1H, $J = 2$ Hz, Ar–H), 7.45 (d, 1H, $J = 4$ Hz, Ar–H), 7.48 (d, 1H, $J = 4$ Hz, Ar–H), 8.14 (d, 1H, $J = 4$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 16.33 (CH_3), 16.38 (CH_3), 55.88 (O–CH_3), 63.85 (O–CH_2), 65.98 (O–CH_2), 67.58 (CH–P), 109.76, 118.11, 120.76, 123.41, 124.71, 129.6, 130.75, 145.54, 159.10 (Ar–C). MS: m/z 367.1(m + 1). Elemental analysis: $C_{15}H_{19}N_4O_5P$ Calcd.: C: 49.18%; H: 5.23%; N: 15.29%; Found: C: 49.12%, H: 5. 18%, N: 15.22%.

4.6. Diethyl (7-ethoxytetrazolo[1,5-a]quinolin-4-yl)(hydroxy)methylphosphonate (**3h**)

Yield 87%, m.p. 182–184 °C. IR (KBr, ν_{max}/cm^{-1}): 3253 (O–H), 1612 (C═N), 1207 (P═O), 1044 (P–O–C). ^1H NMR (75 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.20–1.37 (m, 6H, $2 \times CH_3$), 1.51 (t, 3H, $J = 6.9$, 7.2 Hz, CH_3), 1.84 (brs, 1H, OH), 4.08–4.32 (m, 6H, $3 \times CH_2$), 5.83 (d, 1H, $J = 12.9$ Hz, CH–P), 7.28 (d, 1H, $J = 7.5$ Hz, Ar–H), 7.43 (d, 1H, $J = 7.5$ Hz, Ar–H), 8.13 (d, 1H, $J = 3.3$ Hz, Ar–H), 8.54 (d, 1H, $J = 9.3$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 14.64 (CH_3), 16.29 (CH_3), 16.36 (CH_3) 63.85 (O–CH_2), 64.27 (O–CH_2), 65.38 (O–CH_2), 67.53 (CH–P), 110.32, 117.98, 120.99, 123.49, 124.52, 125.41, 130.73, 145.81, 158.43 (Ar–C). MS: m/z 381.2(m + 1). Elemental analysis: $C_{16}H_{21}N_4O_5P$ Calcd.: C: 50.53%; H: 5.57%; N: 14.73%. Found: C: 50.49%, H: 5. 51%, N: 14.67%.

4.7. Diethyl (9-ethyltetrazolo [1,5-a]quinolin-4-yl)(hydroxy)methylphosphonate (**3i**)

Yield 88%, m.p. 140–142 °C. IR (KBr, ν_{max}/cm^{-1}): 3245 (O–H, 1592 (C═N), 1215 (P═O), 1052 (P–O–C). ^1H NMR (75 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.20 (t, 3H, $J = 7.2$ Hz, CH_3), 1.30–1.39 (m, 6H, $2 \times CH_3$), 1.69 (brs, 1H, OH), 3.24 (q, 2H, $J = 7.2$ Hz, CH_2) 4.08–4.26 (m, 4H, $2 \times CH_2$), 5.67 (d, 1H, $J = 11.4$ Hz, CH–P), 7.46 (t, 1H, $J = 7.2$ Hz, Ar–H), 7.57 (d, 1H, $J = 6.9$ Hz, Ar–H), 7.67 (d, 1H, $J = 7.8$ Hz, Ar–H), 8.52 (d, 1H, $J = 2.4$ Hz, Ar–H), ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 14.73 (CH_3), 16.28 (CH_3), 16.35 (CH_3), 24.11 (Ar–CH_2) 63.54 (O–CH_2), 65.93 (O–CH_2), 67.55 (CH–P), 125.67, 126.97, 127.27, 128.88, 129.33, 138.47, 142.13, 145.71, 148.2 (Ar-C). MS: m/z 365.1(m + 1). Elemental analysis: $C_{16}H_{21}N_4O_4P$ Calcd.: C: 52.75%; H: 5.81%; N: 15.38%. Found: C: 52.69%, H: 5. 75%, N: 15.34%.

4.8. (Diethoxyphosphoryl)(7-methyltetrazolo[1,5-a]quinolin-4-yl)methyl acetate (**4b**)

General procedure

To the stirring solution of (**3**) (1.5 mmol) in acetic anhydride (4.5 mmol) and DBU (1.5 mmol) was added at room temperature. The reaction mixture was stirred at room temperature. Progress of reaction was monitored on TLC. After completion of reaction (5 min), the reaction mixture was poured on crushed ice and stirred to get a solid product. The obtained solid was filtered and washed with water, dried in oven at 60 °C. This was purified by crystallization. Yield 85%, m.p. 120–122 °C. IR (KBr, ν_{max}/cm^{-1}): 1757 (O–CO–CH_3), 1615 (C═N), 1210 (P═O), 1020 (P–O–C). ^1H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.16 (t, 3H, $J = 6$, 8 Hz, CH_3), 1.32 (t, 3H, $J = 6$, 8 Hz, CH_3), 2.23 (s, 3H, $COCH_3$), 2.58 (s, 3H, Ar–CH_3), 4.02–4.40 (m, 4H, $2 \times CH_2$), 6.84 (d, 1H, $J = 14$ Hz, CH–P), 7.69 (d, 1H, $J = 8$ Hz, Ar–H), 7.76 (s, 1H, Ar–H), 8.06 (d, 1H, $J = 0.4$ Hz, Ar–H), 8.55 (d, 1H, $J = 8$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 16.17 (CH_3), 16.37 (CH_3), 20.64 (O═C–CH_3), 21.35 (Ar–CH_3), 63.71 (O–CH_2), 64.08 (O–CH_2), 66.73 (CH–P), 116.47, 120.45, 123.73, 128.46, 128.79, 131.58, 132.88, 138.42, 146.12 (Ar–C), 168.85 (C═O). MS: m/z 393.2(m + 1). Elemental analysis: $C_{17}H_{21}N_4O_5P$ Calcd.: C: 52.04%; H: 5.39%; N: 14.28%. Found: C: 52.00%, H: 5. 35%, N: 14.24%.

4.9. (Diethoxyphosphoryl)(7-methoxytetrazolo[1,5-a]quinolin-4-yl)methyl acetate (**4e**)

Yield 90%, m.p. 156–158 °C. IR (KBr, ν_{max}/cm^{-1}): 1755 (O–CO–CH_3), 1616 (C═N), 1226 (P═O), 1032 (P–O–C). ^1H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.15 (t, 3H, $J = 6$, 8 Hz, CH_3), 1.32(t, 3H, $J = 6$, 8 Hz, CH_3), 2.23 (s, 3H, $COCH_3$), 3.96 (s, 3H, OCH_3), 4.05–4.41 (m, 4H, $2 \times CH_2$), 6.84 (d, 1H, $J = 14$ Hz, CH–P), 7.34 (d, 1H, $J = 0.4$ Hz, Ar–H), 7.46 (d, 1H, $J = 10$ Hz, Ar–H), 8.08 (d, 1H, $J = 0.4$ Hz, Ar–H), 8.57 (d, 1H, $J = 10$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 16.24 (CH_3), 16.26 (CH_3), 20.69 (O═C–CH_3), 55.58 (O–CH_3), 63.84 (O–CH_2), 64.31 (O–CH_2), 66.76 (CH–P), 109.82, 118.21, 121.12, 129.92, 131.45. 138.42, 143.41, 146.13, 159.06, (Ar–C), 168.92 (C═O). MS: m/z 409.2(m + 1). Elemental analysis: $C_{17}H_{21}N_4O_6P$ Calcd.: C: 50.00%; H: 5.18%; N: 13.72%. Found: C: 49.94%, H: 5. 11%, N: 13.65%.

*4.10. (Diethoxyphosphoryl)(7-ethoxytetrazolo[1,5-a]quinolin-4-yl)methyl acetate (**4h**)*

Yield 84%, m.p. 98–100 °C. IR (KBr, ν_{max}/cm^{-1}): 1755 (O–CO–CH_3), 1593 (C═N), 1231 (P═O), 1042 (P–O–C). ^1H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.18 (t, 3H, $J = 6$, 8 Hz, CH_3), 1.35(t, 3H, $J = 6$, 8 Hz, CH_3), 1.48 (t, 3H, $J = 6$, 8 Hz, CH_3), 2.19 (s, 3H, OCH_3), 3.8–4.6 (m, 6H, $3 \times CH_2$), 6.65 (d, 1H, $J = 14$ Hz, CH–P), 7.08 (s, 1H, Ar–H), 7.40 (d, 1H, $J = 8$ Hz, Ar–H), 7.89 (d, 1H, $J = 8$ Hz, Ar–H), 8.33 (d, 1H, $J = 0.4$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 14.67 (CH_3), 16.26 (CH_3), 16.46 (CH_3), 20.74 (O═C–CH_3), 63.56 (O–CH_2), 63.80 (O–CH_2), 65.65 (O–CH_2), 67.95 (CH–P), 105.90, 124.31, 126.70, 128.10, 129.66. 137.69, 143.38, 146.57, 157.71 (Ar–C), 168.80 (C═O). MS: m/z 423.2(m + 1). Elemental analysis: $C_{18}H_{23}N_4O_6P$ Calcd.: C: 51.19%; H: 5.49%; N: 13.26%. Found: C: 51.14%, H: 5. 42%, N: 13.23%.

*4.11. Diethoxyphosphoryl)(9-ethyltetrazolo[1,5-a]quinolin-4-yl)methyl acetate (**4i**)*

Yield 78%, m.p. 78–80 °C. IR (KBr, ν_{max}/cm^{-1}): 1750 (O–CO–CH_3), 1617 (C═N), 1218 (P═O), 1041 (P–O–C). ^1H NMR (200 MHz, $CDCl_3$, Me_4Si, δ ppm): 1.20 (t, 3H, $J = 6$, 8 Hz, CH_3), 1.31–1.42(m, 6H, $2 \times CH_3$), 2.18 (s, 3H, $COCH_3$), 3.21 (q, 2H, $J = 6$, 8 Hz, CH_2), 3.96–4.31 (m, 4H, $2 \times CH_2$), 6.69 (d, 1H, $J = 14$ Hz, CH–P), 7.48 (t, 1H, $J = 8$ Hz, Ar–H), 7.58 (d, 1H, $J = 8$ Hz, Ar–H), 7.68 (d, 1H, $J = 8$ Hz, Ar–H), 8.40 (d, 1H, $J = 2$ Hz, Ar–H). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 15.16 (CH_3), 16.20 (CH_3), 16.40 (CH_3), 20.67 (O═C–CH_3), 24.12 (CH_3) 63.55 (O–CH_2), 64.09 (O–CH_2), 67.97 (CH–P), 125.66, 126.1, 127.39, 129.41, 133.04, 135.45, 139.11, 145.95, 148.06 (Ar–C), 168.84 (C═O). MS: m/z 407.2(m + 1). Elemental analysis: $C_{18}H_{23}N_4O_5P$ Calcd.: C: 53.20%; H: 5.70%; N: 13.79%. Found: C: 53.16%, H: 5. 65%, N: 13.73%.

5. Biological assays

The antimicrobial activity of the synthesized compounds was screened by Agar cup plate method against a panel of human pathogenic microorganisms: one Gram positive (*B. subtilis* NCIM 2250), one Gram negative (*E. coli* ATCC 25922) were used for the antibacterial assay, while for the antifungal assay, *C. albicans* MTCC 277and *A. niger* NCIM 545 were used for the studies. Microorganisms were maintained at 37 °C on Mueller Hinton (MH) agar slants. MH agar and Czapek Dox broth were used to evaluate antibacterial and antifungal activity respectively. All compounds were dissolved in dimethylformamide (DMF) with required concentrations. Commercial antibiotics such as Streptomycin (Strept.) and Griseofluvin (Gris.) in DMF served as reference standards to compare inhibition of growth. The plate containing bacterial organism were incubated at 37 ± 0.5 °C and plates containing fungal organism were incubated at 28 ± 0.5 °C for 48 h. The zone of inhibition was calculated by measuring the diameter zone of inhibition of bacterial and fungal growth around the disc. An average of three independent determinations was recorded.

The minimum inhibitory concentration (MIC) of the samples by Cup plate method on MH agar plates containing the following concentrations (μg/mL): 0 (control), 1, 2, 3, 5, 10, 15, 20, 30 and 40 μg/mL. MH was molted and poured in Petri dishes according to National Committee for Clinical Laboratory Standards (NCCLS) (M7-A5 January 2000). The plates were incubated at 37 °C, examined after 24 h and incubated further for 72 h, where necessary. The lowest concentration of the drug in a plate that failed to show any visible macroscopic growth was considered as its MIC. The MIC determination was performed in triplicate for each organism and the experiment was repeated where necessary.

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