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Synthesis and *in vitro* Antimicrobial Activity of New Ethyl 2-(Ethoxyphosphono)-1-cyano-2-(substituted tetrazolo-[1,5-*a*]quinolin-4-yl)ethanoate Derivatives

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A series of new ethyl 2-(ethoxyphosphono)-1-cyano-2-(substituted tetrazolo[1,5-*a*]quinolin-4-yl)ethanoate derivatives have been synthesized for the first time of tetrazolo[1,5-*a*]quinoline derivatives. Elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and mass spectral data elucidated the structures of the all newly synthesized compounds. *In vitro* antimicrobial activities of synthesized compounds have been investigated against Gram-positive *Bacillus subtilis*, Gram-negative *Escherichia coli* and two fungi *Candida albicans* and *Aspergillus niger* in comparison with standard drugs. Significantly microbiological behavior of these newly synthesized derivatives possesses significant antibacterial and antifungal activity.

Keywords tetrazolo[1,5-*a*]quinoline, Knoevenagel condensation, α-phosphonate, antibacterial, antifungal

Introduction

The phosphonate (PO_3^{2-}) moiety is a common structural fragment present in a wide range of biologically active compounds. A wide range of natural phosphorus based biologically active compounds which play important roles as metabolic intermediates, as common regulatory switches for proteins and as a backbone for the genetic information.[1] 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. Despite structural and electronic differences between phosphonate and carboxylic functionalities (in terms of size, shape, acidity and geometry), the phosphonate functionality is regarded as a bioisostere of the carboxylic acids.[2] Many of them can serve as haptens in catalytic enzyme antibody generation and as transition state analogue inhibitors of different proteolytic enzymes exhibiting a wide spectrum of biological properties including antimicrobial, antitumor, antihypertensive and antibacterial activities.[3]

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.[4] 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,[5] anti-inflammatory,[6] CNS dispersant,[7] antimicrobial,[8] anti-AIDS,[9] and antifertility agents.[10]

The Knoevenagel reaction continues to be important in organic synthesis in converting carbonyl groups into alkenes.[11] Preparation of ethyl α-cyanocinnamates was usually completed via Knoevenagel condensation between ethyl cyanoacetate and various aromatic aldehydes.[12] Ethyl α-cyanocinnamates show fungicidal, herbicidal and insecticidal activity.[13]

By considering all above aspects, for the first time we have synthesized the titled compounds in regards to develop our on going research work.[14]

Results and discussion

The key intermediate tetrazolo[1,5-*a*]quinoline-4-carbaldehyde derivatives **2a**—**2i** were prepared by the reaction of 2-chloroquinoline-3-carbaldehyde derivatives (**1a**—**1i**) with sodium azide in DMSO/AcOH mixture.[15] Further, we have synthesized ethyl 2-cyano-3-(tetrazolo[1,5-*a*]quinolin-4-yl)acrylate derivatives **3a**—**3i** via Knoevenagel condensation reaction of ethyl cyano acetate and tetrazolo[1,5-*a*]quinoline derivatives **2a** — **2i** in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) under room temperature condition

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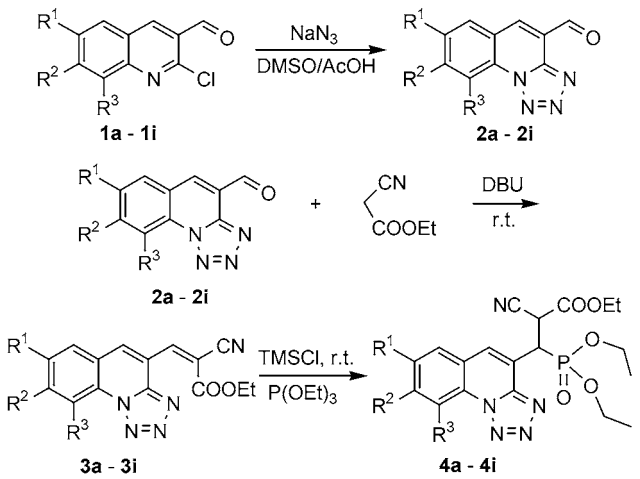
in excellent yields. The synthesized compounds **3a**—**3i** further treated with triethyl phosphite in the presence of TMSCl at room temperature afforded the compounds **4a**—**4i** which are outlined in Scheme 1.

Scheme 1 Synthesis of ethyl 2-(ethoxyphosphono)-1-cyano-2-(tetrazolo[1,5-*a*]quinolin-4-yl)ethanoates derivatives



a: $R^1 = R^2 = R^3 = H$; **b**: $R^1 = Me$, $R^2 = R^3 = H$; **c**: $R^1 = R^3 = H$, $R^2 = Me$; **d**: $R^1 = R^2 = H$, $R^3 = Me$; **e**: $R^1 = OMe$, $R^2 = R^3 = H$; **f**: $R^1 = R^3 = H$, $R^2 = OMe$; **g**: $R^1 = R^2 = H$, $R^3 = OMe$; **h**: $R^1 = OEt$, $R^2 = R^3 = H$; **i**: $R^1 = R^2 = H$, $R^3 = Et$

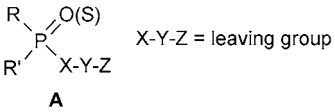
Structural determination

Structures of the compounds **3a**—**3i** and **4a**—**4i** were elucidated on the basis of the FT-IR, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and mass spectroscopic analysis. Assignments of selected characteristic IR band positions provide significant indication for the formation of compounds **3a**—**3i** and **4a**—**4i**. All the compounds **3a**—**3i** showed intense bands in the region of 2224—2257 cm^{-1} corresponding to ν(C≡N) stretch, 1735—1748 cm^{-1} of ν(COOEt) stretch and 1587—1634 of ν(C=N) stretch, which confirms the formation of desired compounds (**3a**—**3i**). Also the compounds **4a**—**4i** showed intense bands in the region of 2238—2269 cm^{-1} corresponding to ν(C≡N) stretch, 1725—1751 cm^{-1} of ν(COOEt) stretch, 1598—1637 cm^{-1} of ν(C=N), 1238—1267 cm^{-1} of ν(P=O) stretch and 1014—1048 cm^{-1} of ν(P—O—C) stretch, which confirms the formation of desired compounds **4a**—**4i**. The aromatic protons of the compounds **3a**—**3i** and **4a**—**4i** are in the region of δ 7.15—9.05 and 7.26—8.87, respectively. The protons at (CHP) and (CHCN) for all **4a**—**4i** compounds were observed in the region of δ 4.16—4.90. The aromatic carbons of compounds **3a**—**3i** and **4a**—**4i** are in the region of δ 117.41—149.61 and 118.03—149.50, respectively. For the compounds **3a**—**3i** the region between δ 156.2—158.68 represents the C=CH carbons, which confirms C=C bond formation. All the compounds **3a**—**3i** and **4a**—**4i** showed the peaks in the region of δ 160.84—161.20 and 163.40—164.13, respectively for the carbonyl carbons. The carbon at (CHP) for all **4a**—**4i** compounds has been observed in the region of δ 63.33—63.78.

Antimicrobial screening

Schrader-Clark[16] proposed that organophosphorus compounds containing the general structure **A** (Scheme 2) 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.

Scheme 2



A

In vitro antibacterial and antifungal activity was screened by considering zone of inhibition of growth. The synthesized compounds **4a**—**4i** were screened with their different concentrations with standard antibiotics such as streptomycin (10 μg/mL) and Griseofluvin (10 μg/mL) (Table 1). The results showed that most of our designed compounds had moderate antibacterial and antifungal activities in between 10—25 μg/mL MIC values against standard antibiotics *in vitro* as shown in Table 1. Compounds **4e** ($R^2=R^3=H$; $R^1=OMe$) and **4h** ($R^2=R^3=H$; $R^1=OEt$) have the zone of inhibition 17.0 and 16.0 mm respectively, comparable to that of the standard Streptomycin (16.4 mm) against *Bacillus subtilis*. Against *Escherichia coli* the compounds **4c** ($R^1=R^3=H$; $R^2=Me$) and **4e** ($R^2=R^3=H$; $R^1=OMe$) showed 15.0 and 16.0 mm zone of inhibition respectively, comparable to that of the standard Streptomycin (16.9 mm). The data indicate that a change in the substituent might also affect the antibacterial activity of title compounds **4a**—**4i**. Comparison of biological activities among **4a**—**4i** shows functional groups as R^1OMe/OEt to be potentially more active against *Bacillus subtilis.* Also antibacterial potency of compounds among **4a**—**4i** shows that functional groups as $R^1=$ OMe and $R^2=$ Me shows more active against *Escherichia coli*.

In antifungal activity, compounds **4c** ($R^1=R^3=H$; $R^2=Me$) have showed 16.0 mm zone of inhibition against *Candida albicans*, which might indicate that the functional group $R^2=Me$ involves in the antifungal potency of respective compound as that of standard Griseofluvin (16.8 mm). Against *Aspergillus niger* (16.2, 16.4 and 16.1 mm) zone of inhibition of compounds **4b** ($R^2=R^3=H$; $R^1=Me$), **4e** ($R^2=R^3=H$; $R^1=OMe$) and **4h** ($R^2=R^3=H$; $R^1=OEt$) respectively indicate that the functional groups at $R^1=$Me, OMe or OEt position

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Table 1 Antibacterial and antifungal activity of compounds **4a**—**4i**[a]

Compound	*Bacillus subtilis*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
	ZI (MIC)	ZI (MIC)	ZI (MIC)	ZI (MIC)
4a	—	12.0 (20)	12.0 (25)	—
4b	12.0 (20)	12.0 (15)	—	16.2 (10)
4c	—	15.0 (10)	16.0 (10)	—
4d	—	13.0 (15)	14.0 (15)	13.8 (10)
4e	17.0 (10)	16.0 (10)	14.0 (10)	16.4 (10)
4f	12.0 (20)	—	12.0 (20)	—
4g	13.0 (20)	13.0 (20)	—	—
4h	16.0 (10)	13.0 (20)	—	16.1 (10)
4i	—	12.0 (20)	12.0 (10)	—
Strept.	16.4 (10)	16.9 (10)	n.t.[c]	n.t.
Gris.	n.t.	n.t.	16.8 (10)	16.7 (10)

[a] ZI: zone of inhibition in mm; MIC: minimum inhibitory concentration in μg/mL; n.t.: not tested.

interfere in the antifungal potency of title compounds **4a**—**4i**.

Experimental

Apparatuses and materials

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, ^{13}C NMR and ^{31}P NMR spectra were recorded on Varian NMR spectrometer, Model Mercury Plus (400), Bruker DRX-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. Solvents were commercially available materials of reagent grade.

Biological evaluation procedure

The compounds were diluted in dimethylformamide (DMF) with required concentrations for bioassay. Antimicrobial activity was evaluated by screening of the compounds by standard method, *i.e.* agar cup plate method against a panel of human pathogenic microorganisms: one Gram positive (*Bacillus subtilis* NCIM 2250), one Gram negative (*Escherichia coli* ATCC 25922) were used for the antibacterial assay, while for the antifungal assay, *Candida albicans* MTCC 277 and *Aspergillus niger* NCIM 545 were used for the studies. Microorganisms were maintained at 37 ℃ on Mueller Hinton (MH) agar slants. Moreover the MH agar and Czapek Dox broth were used to evaluate antibacterial and antifungal activity, respectively. To make a judgment of antibacterial and antifungal potency of the synthesized compounds **4a**—**4i**, commercial antibiotics such as Streptomycin (Strept.) and Griseofluvin (Gris.) in DMF served as reference standards to compare inhibition of growth. The plates containing bacterial organisms were incubated at (37±0.5) ℃ and plates containing fungal organisms were incubated at (28±0.5) ℃ 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 were measured 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. 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 ℃, examined after 24 h and incubated further for 72 h, where necessary. The MIC was defined as the lowest concentration resulting in inhibition of visible bacterial and fungal growth. The MIC determination was performed in triplicate for each organism and the experiment was repeated wherever necessary.

Preparation and analysis data

7-Methoxytetrazolo[1,5-*a*]quinoline-4-carbaldehyde (2e) ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.63 (s, 3H, OCH_3), 7.84—7.94 (m, 2H, Ar-H), 8.54 (s, 1H, Ar-H), 8.64 (d, J=10 Hz, 1H, Ar-H), 10.72 (s, 1H, CHO); MS *m*/*z*: 229 (M^++1). Anal. calcd for $C_{11}H_8N_4O_2$: C 57.89, H 3.53, N 24.55; found C 57.74, H 3.47, N 24.67.

7-Ethoxytetrazolo[1,5-*a*]quinoline-4-carbaldehyde (2h) ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.24 (t, J=6 Hz, 3H, $OCH_2C\mathbf{H}_3$), 3.68 (q, J=6 Hz, 2H, $OC\mathbf{H}_2CH_3$), 7.70—7.82 (m, 2H, Ar-H), 8.57 (s, 1H, Ar-H), 8.70 (s, 1H, Ar-H), 10.75 (s, 1H, CHO); MS *m*/*z*: 243 (M^++1). Anal. calcd for $C_{12}H_{10}N_4O_2$: C 59.50, H 4.16, N 23.13; found C 59.44, H 4.27, N 23.17.

9-Ethyltetrazolo[1,5-*a*]quinoline-4-carbaldehyde (2i) ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.36 (t, J=8 Hz, 3H, $CH_2C\mathbf{H}_3$), 2.89 (q, J=8 Hz, 2H, $C\mathbf{H}_2CH_3$), 7.59—

7.78 (m, 2H, Ar-H), 8.52 (s, 1H, Ar-H), 8.64 (s, 1H, Ar-H), 10.54 (s, 1H, CHO); MS *m/z*: 227 (M^++1). Anal. calcd for $C_{12}H_{10}N_4O$: C 63.71, H 4.46, N 24.76; found C 63.64, H 4.41, N 24.83.

General procedure for the synthesis of ethyl 2-cyano-3-(substituted tetrazolo[1,5-*a*]quinolin-4-yl)-acrylate 3 To the stirred solution of tetrazolo[1,5-*a*]-quinoline-4-carbaldehyde **2** (1.02 g, 5 mmol) and ethyl cyano acetate (0.6 g, 5.2 mmol) was added DBU (2 to 3 drops) at room temperature. The progress of the reaction was monitored by TLC. After completion of reaction (8 min), the reaction mixture was poured on crushed ice. The solid obtained was extracted with chloroform (50 mL×2), washed with water (10 mL×2) and brine (20 mL×2), and the separated organic layer was 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 (V∶V=8∶2) as an eluent.

Ethyl 2-cyano-3-(tetrazolo[1,5-*a*]quinolin-4-yl)-acrylate (3a) Solid (yield 85%); m.p. 168—170 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.44 (t, J=7.2 Hz, 3H, $OCH_2C\mathbf{H}_3$), 4.44 (q, J=7.2 Hz, 2H, $OC\mathbf{H}_2CH_3$), 7.81 (t, J=6 Hz, 1H, Ar-H), 8.02 (t, J=6 Hz, 1H, Ar-H), 8.18 (d, J=8 Hz, 1H, Ar-H), 8.72 (d, J=8 Hz, 1H, Ar-H), 9.05 (s, 1H, Ar-H), 9.10 (s, 1H, C=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.12 (CH_3), 39.84 (OCH_2), 107.80 (CHCN), 117.00 (CN), 117.59, 123.31, 128.81, 130.87, 131.37, 133.82, 134.17, 144.88, 146.77 (Ar-C), 157.2 (C=C), 160.90 (C=O); IR (KBr) v: 2257 (C≡N), 1744 (COOEt), 1597 (C=N) cm^{-1}; MS *m/z*: 294.2 (M^++1). Anal. calcd for $C_{15}H_{11}N_5O_2$: C 61.43, H 3.78, N 23.88; found C 61.40, H 3.80, N 23.84.

Ethyl 2-cyano-3-(7-methyltetrazolo[1,5-*a*]quinolin-4-yl)acrylate (3b) Solid (yield 78%); m.p. 190—192 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.44 (t, J=7.2 Hz, 3H, $OCH_2C\mathbf{H}_3$), 2.62 (s, 3H, Ar-CH_3) 4.43 (q, J=6 Hz, 2H, $OC\mathbf{H}_2CH_3$), 7.88 (dd, J=2, 6 Hz, 1H, Ar-H), 7.94 (d, J=2 Hz, 1H, Ar-H), 8.60 (d, J=10 Hz, 1H, Ar-H), 9.04 (s, 2H, Ar-H and C=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.11 (CH_3), 21.33 (Ar-CH_3), 40.25 (OCH_2), 107.52 (CHCN), 116.71 (CN), 117.41, 123.35, 129.51, 130.31, 134.01, 135.30, 139.30, 145.04, 146.58 (Ar-C), 156.2 (C=C), 160.96 (C=O); IR (KBr) v: 2252 (C≡N), 1735 (COOEt), 1603 (C=N) cm^{-1}; MS *m/z*: 308.3 (M^++1). Anal. calcd for $C_{16}H_{13}N_5O_2$: C 62.53, H 4.26, N 22.79; found C 62.48, H 4.32, N 22.84.

Ethyl 2-cyano-3-(8-methyltetrazolo[1,5-*a*]quinolin-4-yl)acrylate (3c) Solid (yield 84%); m.p. 200—202 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.34 (t, J=8 Hz, 3H, $OCH_2C\mathbf{H}_3$), 2.66 (s, 3H, Ar-CH_3) 4.38 (q, J=8 Hz, 2H, $OC\mathbf{H}_2CH_3$), 7.71 (d, J=8 Hz, 1H, Ar-H), 8.28 (d, J=8 Hz, 1H, Ar-H), 8.49 (s, 1H, Ar-H), 8.75 (s, 1H, Ar-H), 9.01 (s, 1H, CH=C); IR (KBr) v: 2250 (C≡N), 1741 (COOEt), 1617 (C=N) cm^{-1}; MS *m/z*: 308.3 (M^++1). Anal. calcd for $C_{16}H_{13}N_5O_2$: C 62.53, H 4.26, N 22.79; found C 62.44, H 4.21, N 22.86.

Ethyl 2-cyano-3-(9-methyltetrazolo[1,5-*a*]quinolin-4-yl)acrylate (3d) Solid (yield 86%); m.p. 194—196 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.34 (t, J=8 Hz, 3H, $OCH_2C\mathbf{H}_3$), 3.09 (s, 3H, Ar-CH_3) 4.39 (q, J=8 Hz, 2H, $OC\mathbf{H}_2CH_3$), 7.76 (t, J=8 Hz, 1H, Ar-H), 7.96 (d, J=8 Hz, 1H, Ar-H), 8.21 (d, J=8 Hz, 1H, Ar-H), 8.77 (s, 1H, Ar-H), 9.01 (s, 1H, CH=C); IR (KBr) v: 2253 (C≡N), 1738 (COOEt), 1609 (C=N) cm^{-1}; MS *m/z*: 308.3 (M^++1). Anal. calcd for $C_{16}H_{13}N_5O_2$: C 62.53, H 4.26, N 22.79; found C 62.47, H 4.23, N 22.74.

Ethyl 2-cyano-3-(7-methoxytetrazolo[1,5-*a*]quinolin-4-yl)acrylate (3e) Solid (yield 82%); m.p. 142—144 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.44 (t, J=7.2 Hz, 3H, CH_3), 4.00 (s, 3H, OCH_3), 4.43 (q, J=6 Hz, 2H, CH_2), 7.45 (d, J=6 Hz, 1H, Ar-H), 7.58 (dd, J=4, 6 Hz, 1H, Ar-H), 8.60 (d, J=8 Hz, 1H, Ar-H), 9.04 (d, J=4 Hz, 2H, Ar-H and C=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.00 (CH_3), 38.31 (OCH_2), 55.70 (OCH_3) 107.43 (CHCN), 117.48 (CN), 118.12, 123.79, 124.30, 125.73, 129.71, 133.49, 144.88, 145.99, 146.83 (Ar-C), 158.68 (C=C), 160.84 (C=O); IR (KBr) v: 2224 (C≡N), 1748 (COOEt), 1625 (C=N) cm^{-1}; MS *m/z*: 324.2 (M^++1). Anal. calcd for $C_{16}H_{13}N_5O_3$: C 59.44, H 4.05, N 21.66; found C 59.48, H 4.10, N 21.58.

Ethyl 2-cyano-3-(8-methoxytetrazolo[1,5-*a*]quinolin-4-yl)acrylate (3f) Solid (yield 83%); m.p. 212—214 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.34 (t, J=6 Hz, 3H, $OCH_2C\mathbf{H}_3$), 4.08 (s, 3H, OCH_3), 4.39 (q, J=6 Hz, 2H, $OC\mathbf{H}_2CH_3$), 7.48 (dd, J=4, 6 Hz, 1H, Ar-H), 8.05 (d, J=4 Hz, 1H, Ar-H), 8.35 (d, J=4 Hz, 1H, Ar-H), 8.76 (s, 1H, Ar-H), 9.03 (s, 1H, CH=C); IR (KBr) v: 2225 (C≡N), 1739 (COOEt), 1634 (C=N) cm^{-1}; MS *m/z*: 324.2 (M^++1). Anal. calcd for $C_{16}H_{13}N_5O_3$: C 59.44, H 4.05, N 21.66; found C 59.41, H 4.14, N 21.58.

Ethyl 2-cyano-3-(9-methoxytetrazolo[1,5-*a*]quinolin-4-yl)acrylate (3g) Solid (yield 76%); m.p. 132—134 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.34 (t, J=6 Hz, 3H, $OCH_2C\mathbf{H}_3$), 3.98 (s, 3H, OCH_3), 4.38 (q, J=6 Hz, 2H, $OC\mathbf{H}_2CH_3$), 7.41 (dd, J=4, 6 Hz, 1H, Ar-H), 8.12 (d, J=4 Hz, 1H, Ar-H), 8.39 (d, J=4 Hz, 1H, Ar-H), 8.79 (s, 1H, Ar-H), 9.04 (s, 1H, CH=C); IR (KBr) v: 2228 (C≡N), 1737 (COOEt), 1626 (C=N) cm^{-1}; MS *m/z*: 324.2 (M^++1). Anal. calcd for $C_{16}H_{13}N_5O_3$: C 59.44, H 4.05, N 21.66; found C 59.31, H 4.14, N 21.75.

Ethyl 2-cyano-3-(7-ethoxytetrazolo[1,5-*a*]quinolin-4-yl)acrylate (3h) Solid (yield 87%); m.p. 128—130 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.32 (t, J=7.2 Hz, 3H, CH_3), 1.44 (t, J=7.2 Hz, 3H, CH_3), 4.15 (q, J=6 Hz, 2H, $ArOCH_2$), 4.42 (t, J=7 Hz, 2H, CH_2), 7.15 (d, J=4 Hz, 1H, Ar-H), 7.45 (dd, J=4 Hz, 1H, Ar-H), 7.92 (d, J=10 Hz, 1H, Ar-H), 8.74 (s, 1H, Ar-H) 8.99 (s, 1H, C=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.36 (CH_3), 14.48 (Ar-CH_3), 38.11 (OCH_2), 48.40 (OCH_3) 106.49 (CHCN), 116.92 (CN), 122.73, 123.66, 127.30, 128.45, 137.54, 141.84, 144.29, 146.82, 149.46 (Ar-C), 157.50 (C=C), 161.11 (C=O); IR (KBr) v: 2235 (C≡N), 1742 (COOEt), 1614 (C=N) cm^{-1}; MS *m/z*: 338.3

(M^++1). Anal. calcd for $C_{17}H_{15}N_5O_3$: C 60.53, H 4.48, N 20.76; found C 60.48, H 4.40, N 20.82.

Ethyl 2-cyano-3-(9-ethyltetrazolo[1,5-*a*]quinolin-4-yl)acrylate (3i) Solid (yield 84%); m.p. 112—114 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.36 (t, J=7.2 Hz, 3H, Ar-CH_3), 1.43 (t, J=7.2 Hz, 3H, CH_3), 3.26 (q, J=6 Hz, 2H, Ar-CH_2), 4.41 (t, J=7 Hz, 2H, CH_2), 7.52—7.81 (m, 3H, Ar-H), 8.76 (s, 1H, Ar-H), 9.04 (d, J=12 Hz, 1H, C=CH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.60 (CH_3), 14.89 (Ar-CCH_3), 23.69 (Ar-CH_2) 38.12 (OCH_2), 107.17 (CHCN), 116.73 (CN), 123.39, 125.50, 126.40, 128.30, 130.36, 135.30, 142.36, 144.75, 149.61 (Ar-C), 158.20 (C=C), 161.20 (C=O); IR (KBr) v: 2250 (C≡N), 1745 (COOEt), 1587 (C=N) cm^{-1}; MS m/z: 322.2 (M^++1). Anal. calcd for $C_{17}H_{15}N_5O_2$: C 63.54, H 4.71, N 21.79; found C 63.48, H 4.78, N 21.82.

General procedure for the synthesis of ethyl 2-cyano-3-(diethoxyphosphoryl)-3-(substituted tetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4) The mixture of ethyl 2-cyano-3-(tetrazolo[1,5-*a*]quinolin-4-yl)acrylate **3** (4 mmol), triethylphosphite (10 mmol) and TMSCl (10 mmol) was stirred magnetically at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction (5 min), the reaction mixture was poured on crushed ice. The solid obtained was extracted with chloroform (50 mL×2), and washed with water (10 mL×2) and brine (20 mL×2). The separated organic layer was 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 (V∶V=8∶2) as an eluent.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(tetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4a) Solid (yield 84%); m.p. 76—78 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.18—1.36 (m, 9H, 3×CH_3), 4.12—4.36 (m, 6H, 3×CH_2), 4.68—4.88 (m, 2H, CHP and CHCN), 7.77 (t, J=6 Hz, 1H, Ar-H), 7.91 (t, J=6 Hz, 1H, Ar-H), 8.09 (t, J=6 Hz, 1H, Ar-H), 8.39 (d, J=4 Hz, 1H, Ar-H), 8.70 (d, J=6 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.69 (CH_3), 16.16 (2×CH_3), 36.83 (C-CN), 38.75 (OCH_2), 39.85 (2×OCH_2), 63.74 (CHP), 116.70 (CN), 123.28, 123.74, 128.46, 129.56, 130.26, 131.67, 132.29, 133.49, 134.25 (Ar-C), 163.89 (C=O); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 20.24; IR (KBr) v: 2250 (C≡N), 1749 (COOEt), 1608 (C=N), 1262 (P=O), 1025 (P—O—C) cm^{-1}; MS m/z: 432.2 (M^++1). Anal. calcd for $C_{19}H_{22}N_5O_5P$: C 52.90, H 5.14, N 16.23; found C 52.84, H 5.18, N 16.29.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(7-methyltetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4b) Solid (yield 89%); m.p. 120—122 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.17—1.39 (m, 9H, 3×CH_3), 2.61 (s, 3H, Ar-H), 4.11—4.26 (m, 6H, 3×CH_2), 4.62—4.90 (m, 2H, CHP and CHCN), 7.72 (d, J=8 Hz, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 8.33 (d, J=4 Hz, 1H, Ar-H), 8.56 (d, J=8 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.53 (CH_3), 15.90 (2×CH_3), 20.75 (Ar-CH_3), 35.76 (CCN) 36.27 (OCH_2), 37.65 (OCH_2), 38.27 (OCH_2), 63.33 (CHP), 116.03 (CN), 118.12, 119.10, 123.38, 127.53, 129.08, 132.72, 133.38, 138.83, 147.15 (Ar-C), 164.13 (C=O); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 20.34; IR (KBr) v: 2248 (C≡N), 1738 (COOEt), 1598 (C=N), 1258 (P=O), 1048 (P—O—C) cm^{-1}; MS m/z: 446.3 (M^++1). Anal. calcd for $C_{20}H_{24}N_5O_5P$: C 53.93, H 5.43, N 15.72; found C 53.98, H 5.48, N 15.65.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(8-methyltetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4c) Solid (yield 84%); m.p. 134—136 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.17—1.38 (m, 9H, 3×CH_3), 2.59 (s, 3H, Ar-H), 4.13—4.35 (m, 6H, 3×CH_2), 4.59—4.78 (m, 2H, CHP and CHCN), 7.66 (d, J=8 Hz, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 8.36 (d, J=4 Hz, 1H, Ar-H), 8.72 (d, J=8 Hz, 1H, Ar-H); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 17.62; IR (KBr) v: 2241 (C≡N), 1729 (COOEt), 1609 (C=N), 1258 (P=O), 1045 (P—O—C) cm^{-1}; MS m/z: 446.3 (M^++1). Anal. calcd for $C_{20}H_{24}N_5O_5P$: C 53.93, H 5.43, N 15.72; found C 53.88, H 5.57, N 15.69.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(9-methyltetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4d) Solid (yield 89%); m.p. 140—142 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.16—1.42 (m, 9H, 3×CH_3), 2.18 (s, 3H, Ar-H), 4.09—4.23 (m, 6H, 3×CH_2), 4.62—4.82 (m, 2H, CHP and CHCN), 7.72 (d, J=8 Hz, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 8.41 (d, J=4 Hz, 1H, Ar-H), 8.83 (d, J=8 Hz, 1H, Ar-H); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 17.58; IR (KBr) v: 2238 (C≡N), 1739 (COOEt), 1637 (C=N), 1257 (P=O), 1043 (P—O—C) cm^{-1}; MS m/z: 446.3 (M^++1). Anal. calcd for $C_{20}H_{24}N_5O_5P$: C 53.93, H 5.43, N 15.72; found C 53.98, H 5.38, N 15.69.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(7-methoxytetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4e) Solid (75%); m.p. 102—104 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.16—1.39 (m, 9H, 3×CH_3), 4.00 (s, 3H, OCH_3), 4.14—4.24 (m, 6H, 3×CH_2), 4.68—4.88 (m, 2H, CHP and CHCN), 7.45—7.51 (m, 2H, Ar-H), 8.34 (d, J=4 Hz, 1H, Ar-H), 8.58 (d, J=8 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.71 (CH_3), 16.22 (2×CH_3), 21.37 (Ar-CH_3), 35.70 (CCN) 36.82 (OCH_2), 37.86 (OCH_2), 38.17 (OCH_2), 48.25 (OCH_3), 63.78 (CH-P), 116.48 (CN), 118.03, 123.83, 127.52, 128.43, 129.15, 133.09, 133.31, 135.25, 138.69 (Ar-C), 163.86 (C=O); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 20.46; IR (KBr) v: 2258 (C≡N), 1725 (COOEt), 1605 (C=N), 1247 (P=O), 1024 (P—O—C) cm^{-1}; MS m/z: 462.3 (M^++1). Anal. calcd for $C_{20}H_{24}N_5O_6P$: C 52.06, H 5.24, N 15.18; found C 52.13, H 5.18, N 15.25.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(8-methoxytetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4f) Solid (yield 84%); m.p. 121—124 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.17—1.32 (m, 9H, 3×CH_3), 3.91 (s, 3H, OCH_3), 4.12—4.22 (m, 6H, 3×CH_2), 4.69—4.88 (m, 2H, CHP and CHCN), 7.44—7.60 (m, 2H, Ar-H), 8.32 (d, J=4 Hz, 1H, Ar-H), 8.76 (d, J=8 Hz, 1H, Ar-H); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 17.61; IR (KBr) v: 2267 (C≡N), 1747 (COOEt), 1626 (C=N), 1257 (P=O),

1014 (P—O—C) cm^{-1}; MS *m/z*: 462.3 (M^++1). Anal. calcd for $C_{20}H_{24}N_5O_6P$: C 52.06, H 5.24, N 15.18; found C 52.13, H 5.18, N 15.25.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(9-methoxytetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4g) Solid (yield 89%); m.p. 96—98 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.17—1.34 (m, 9H, 3×CH_3), 3.62 (s, 3H, OCH_3), 4.18—4.31 (m, 6H, 3×CH_2), 4.61—4.87 (m, 2H, CHP and CHCN), 7.41—7.63 (m, 2H, Ar-H), 8.45 (d, *J*=4 Hz, 1H, Ar-H), 8.87 (d, *J*=8 Hz, 1H, Ar-H); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 20.25; IR (KBr) *v*: 2269 (C≡N), 1751 (COOEt), 1634 (C=N), 1267 (P=O), 1037 (P—O—C) cm^{-1}; MS *m/z*: 462.3 (M^++1). Anal. calcd for $C_{20}H_{24}N_5O_6P$: C 52.06, H 5.24, N 15.18; found C 52.18, H 5.14, N 15.29.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(7-ethoxytetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4h) Liquid (yield 68%); b.p. 125—127 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.07—1.44 (m, 12H, 4×CH_3), 4.04—4.16 (m, 8H, 4×CH_2), 4.16—4.66 (m, 2H, CHP and CHCN), 6.99—7.04 (dd, *J*=2, 6 Hz, 1H, Ar-H), 7.26—7.32 (dd, *J*=2, 8 Hz, 1H, Ar-H), 7.77 (d, *J*=10 Hz, 1H, Ar-H), 8.55 (d, *J*=2 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 13.64 (CH_3), 15.80 (CH_3), 16.18 (CH_3), 21.25 (Ar-CH_3), 36.60 (C-CN), 36.78 (OCH_2), 37.87 (OCH_2), 38.65 (OCH_2), 40.25 (OCH_2), 63.58 (CHP), 116.50 (CN), 118.15, 123.75, 127.38, 128.45, 129.21, 133.21, 133.48, 135.69, 139.02 (Ar-C), 163.40 (C=O); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 17.57; IR (KBr) *v*: 2246 (C≡N), 1745 (COOEt), 1604 (C=N), 1245 (P=O), 1027 (P—O—C) cm^{-1}; MS *m/z*: 476.3 (M^++1). Anal. calcd for $C_{21}H_{26}N_5O_6P$: C 53.05, H 5.51, N 14.73; found C 53.12, H 5.56, N 14.89.

Ethyl 2-cyano-3-(diethoxyphosphonyl)-3-(9-ethyltetrazolo[1,5-*a*]quinolin-4-yl)propanoate (4i) Liquid (yield 84%); b.p. 144—146 ℃; ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.07—1.30 (m, 12H, 4×CH_3), 3.17 (q, *J*=8 Hz, 2H, Ar-CH_2) 4.03—4.17 (m, 6H, 3×CH_2), 4.44—4.71 (m, 2H, CHP and CHCN), 7.40—7.52 (m, 2H, Ar-H), 7.66 (t, *J*=8 Hz, 1H, Ar-H), 8.64 (d, *J*=4 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 14.60 (CH_3), 15.00 (CH_3), 15.99 (CH_3), 23.88 (Ar-CH_3), 28.05 (Ar-CH_2), 37.43 (CCN), 38.37 (OCH_2), 39.29 (OCH_2), 40.12 (OCH_2), 63.46 (CH-P), 116.50 (CN), 123.72, 125.57, 126.76, 127.40, 129.51, 139.58, 142.90, 145.40, 149.50 (Ar-C), 163.70 (C=O); ^{31}P NMR ($CDCl_3$, 160 MHz) δ: 17.24; IR (KBr) *v*: 2251 (C≡N), 1748 (COOEt), 1624 (C=N), 1238 (P=O), 1024 (P—O—C) cm^{-1}; MS *m/z*: 460.3 (M^++1). Anal. calcd for $C_{21}H_{26}N_5O_5P$: C 54.90, H 5.70, N 15.24; found C 54.85, H 5.76, N 15.31.

Conclusion

We have synthesized new ethyl 2-(ethoxyphosphono)-1-cyano-2-(tetrazolo[1,5-*a*]quinolin-4-yl)-ethanoate 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.

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