Synthesis of Some 4-Quinolinyl Pyridines and their Antimicrobial and Docking Studies

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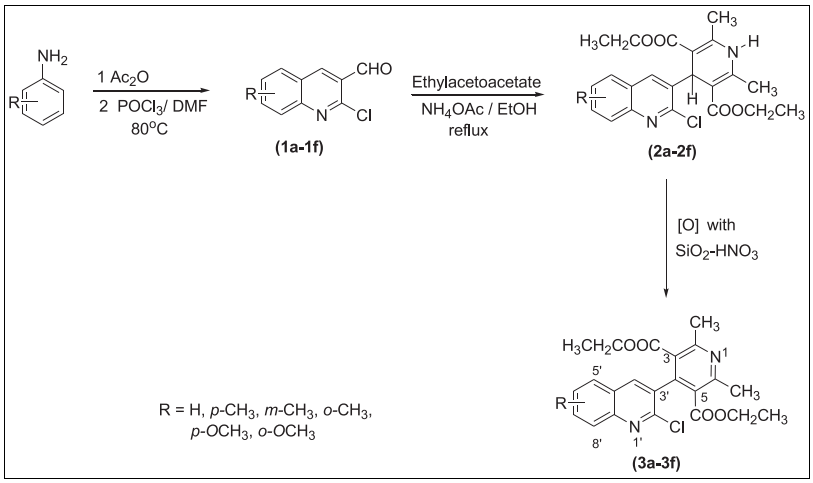

A series of some substituted diethyl 4-(2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates has been synthesized from substituted diethyl4-(2-chloroquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylates (1,4-DHPs) by treating the latter with SiO_2–HNO_3 which proved to be a better oxidant in terms of product yield, reaction time, and cost. Further, these compounds were screened for their antimicrobial activity. All the diethyl 4-(2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates exhibited more potent activities than the corresponding 1,4-DHPs. Further, docking simulation of the most active and least active compounds **3e** and **2e** into *Escherichia coli topoisomerase II DNA Gyrase B* was also performed.

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INTRODUCTION

Quinoline nucleus is of substantial significance as this ring is the key component in a variety of synthetic and naturally occurring biologically active compounds [1,2]. Quinoline alkaloids such as quinine, chloroquine, mefloquine, and amodiaquine are the drugs of choice for the cure of malaria [3]. The quinoline ring containing compounds exhibit various activities, such as antitubercular [4,5], antimalarial [6], anti-inflammatory [7], anticancer [8–10], antibiotic [11], antiprotozoic [12], antifungal [13], anti-trypanosomal [14], anti-herps [15], antihypertensive [16], anticonvulsant [17], tyrokinase PDGF-RTK inhibiting agents [18], and anti-HIV [19,20].

The pyridines, especially the polysubstituted pyridines, and their intermediate 1,4-dihydropyridines (1,4-DHPs) have gained great importance in the field of medicinal chemistry because they display a fascinating array of pharmacological properties such as antimalarial [21], antitumor [22,23], antihypertensive [24], antiproliferative [25], antitubercular [26], anti-inflammatory [27], and agrochemicals such as fungicide [28,29], pesticide [30], and herbicide [31]. Antimicrobial activity of quinoline derivative bearing pyrazoline and pyridine analogues have been reported [32]. Prompted by these biological properties of quinoline and pyridine derivatives [33], in the present study, we report the synthesis of substituted diethyl 4-(2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates, a hybrid organic scaffold in which both pyridine and quinoline units are linked together. The substituted quinoline ring has been taken to study the effect of substituents on antimicrobial activity. Herein, we use SiO_2–HNO_3 for oxidation, and it is a powerful oxidant for the aromatization of 1,4-DHPs and has earlier been established as a cost-effective, milder, and high yielding oxidant for obtaining the substituted benzothiazoles in our laboratory [34].

RESULT AND DISCUSSION

The synthetic protocol used for the synthesis of substituted diethyl-4-(2-chloroquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylates(**2a–2f)**

and substituted diethyl-4-(2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates (**3a–3f)** is described in Scheme 1. One pot condensation of quinolinecarbaldehydes (1 mol), ethylacetoacetate (2 mol), and ammonium acetate (2 mol) in ethanol gave the desired 1,4-DHP products (**2a–2f)** in good yield (Table 1) which were structurally in full agreement with the spectral data IR, ^{1}H NMR and ^{13}C NMR. The 1,4-DHPs were identified by the presence of characteristic N—H and C_4—H protons which resonate between δ 5.0 and 6.0 as a singlet in ^{1}H NMR. In the IR spectra, ester peak was observed between 1680 and 1690 cm^{-1}, and N—H peak appeared between 3100 and 3300 cm^{-1}. The aromatization of these 1,4-DHPs (**2a–2f)** was carried out with SiO_2–HNO_3 in dichloromethane which afforded (Table 2) the corresponding pyridine derivatives (**3a–3f)** whose formation was supported by the absence of two singlet between δ 5.0 and 6.0 for N—H and C_4—H protons in 1H-NMR spectra and disappearance of N—H stretching at 3100–3300 cm^{-1} in the IR spectra. The pattern of Rf values (TLC) and melting point of compounds **2a–2f** and **3a–3f** shows that the intermolecular hydrogen bonding exists in compounds **2a–2f,** resulting in higher melting points and lower Rf values of 1,4-DHPs (**2a–2f)** as compared to their respective oxidized pyridine derivatives **(3a–3f)** (Tables 1 and 2).

In an initial attempt, the oxidation of diethyl 4-(-2-chloroquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-

Scheme 1. Synthetic protocol of 1,4-DHPs and their oxidation.

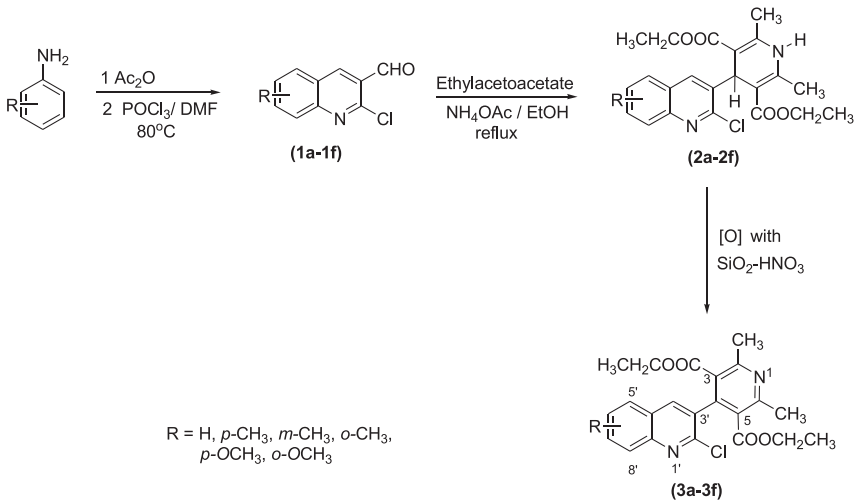

Table 1

Synthesis of 1,4-DHPs.

S. no.	R	Product	Time	Yield	M.p.	Rf value (1:4 ethyl acetate:pet ether)	Color of crystal
1	H	**2a**	60 min	75%	228–230°C	0.15	Cream yellow
2	*p*-CH_3	**2b**	80 min	70%	226–228°C	0.18	Cream yellow
3	*m*-CH_3	**2c**	60 min	72%	205–207°C	0.21	Cream yellow
4	*o*-CH_3	**2d**	65 min	70%	244–246°C	0.21	Cream yellow
5	*p*-OCH_3	**2e**	70 min	70%	238–240°C	0.09	Cream yellow
6	*o*-OCH_3	**2f**	65 min	75%	240–243°C	0.11	Cream yellow

Table 2

Oxidative aromatization of 1,4-DHPs with SiO_2–HNO_3.

			Oxidation with SiO2–HNO3				
S. no.	R	Product	Time	Yield	m.p.	Rf value (1:4 ethyl acetate: pet ether)	Color
1	H	3a	2 min	85%	128–130°C	0.30	Yellow
2	6′-CH3	3b	2 min	87%	105–108°C	0.45	Yellow
3	7′-CH3	3c	2 min	84%	97–99°C	0.48	Yellow
4	8′-CH3	3d	2 min	88%	113–115°C	0.51	Yellow
5	6′-OCH3	3e	2 min	87%	100–102°C	0.30	Yellow
6	8′-OCH3	3f	2 min	81%	104–106°C	0.32	Yellow

dicarboxylate **2a** (1 mol) was carried out with SiO_2–HNO_3 (2 wt %) in dichloromethane at room temp which yielded diethyl 4-(-2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylate **3a** (85%) in 1 min. When loading of SiO_2–HNO_3 was increased, then neither the rate of reaction nor the yield of the product was enhanced. But with decreasing amount of SiO_2–HNO_3 the yield of oxidized product was reduced. So, we have found that SiO_2–HNO_3 can serve as a mild and efficient oxidant for the aromatization of quinolinyl substituted 1,4-DHPs to their corresponding pyridine derivatives.

Biological discussion. All the synthesized compounds (**2a–2f)** and (**3a–3f)** were assessed *in vitro* for their antimicrobial activity against two Gram-positive bacterium strains, i.e. *Bacillus subtilis* and *Staphylococcus aureus*, two Gram-negative bacterium strains, i.e. *Escherichia coli* and *Pseudomonas aeruginosa* and antifungal activity against two yeasts named *Candida albicans* and *Saccharomyces cerevisiae* by agar well diffusion method. Their activities were compared with well-known commercially available standard drugs, i.e. ciprofloxacin and amphotericin-B. The widest antimicrobial activity was shown by diethyl 4-(2-chloro-6-methoxyquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates (**3e)**, which was found to be effective against Gram-positive and Gram-negative bacteria, and against the yeast as well. Among the whole series under test (Table 3), substituted diethyl 4-(2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates exhibited more potential activities than the corresponding 1,4-DHPs (Table 4).

Docking studies. To understand the reasonable mechanism of action of most potent compound **3e** and least active compound **2e**, docking simulation was executed against *E. coli*. Compound **3e** and **2e** were docked into active sites of *E. coli topoisomerase II DNA Gyrase B* complexed with the natural inhibitor clorobiocin (1KZN) [35]. Literature survey revealed that the *E. coli topoisomerase II DNA Gyrase B* [36,37] was a good target to study biological activity against this bacterium. The minimum energy binding mode of compound **3e** and **2e** was considered. The binding affinity for **3e** and **2e** was found −7.3 and −6.8, respectively. It was observed from *in silico* studies of compound **2e** (Fig. 1) that oxygen atom on ester linkage forms hydrogen bond with the asparagine (Asn^{46}) and quinoline ring forms pi-alkyl (hydrophobic interaction) with the Isoleucine (Ile^{78}). It was observed from *in silico* studies of compound **3e** that the sp^2 hybridized oxygen of ester group forms carbon hydrogen bond with proline (Pro^{79}) and isoleucine (Ile^{78}). The pyridine ring of compound **3e** forms pi-anion interaction with glutamic acid (Glu^{50}). The quinoline ring forms pi-alkyl interaction with the isoleucine (Ile^{78}) and pi-donor hydrogen bond with asparagine (Asn^{46}). The chlorine of quinoline ring shows alkyl interaction with isoleucine (Ile^{90}). From the docking analysis, it can be seen that the higher antimicrobial activity of compound **3e** than compound **2e** may be due to the pi-anion interaction with glutamic acid. Compounds **2e** and **3e** are shown along with co-crystallized ligand clorobiocin in the active site of *E. coli topoisomerase II DNA Gyrase B* (Fig. 2).

Table 3

In vitro antimicrobial activity of synthetic 1,4-DHPs and4-quinolinyl pyridines through agar well diffusion method.

	Diameter of growth of inhibition zone (mm)[a]					
	Gram-positive bacteria		Gram-negative bacteria		Yeast	
Compounds	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*S. cerevisiae*
2a	11	11	11	10	11	11
2b	10	11	10	11	11	10
2c	16	12	14	14	12	10
2d	10	11	10	10	11	10
2e	11	09	10	10	10	11
2f	10	11	10	10	10	11
3a	16	10	12	10	12	10
3b	10	12	14	10	10	10
3c	16	14	14	10	14	14
3d	10	12	14	12	12	14
3e	16	18	16	14	14	16
3f	14	12	14	14	14	14
Ciprofloxin	24	25.0	22.0	25.0	—	—
Amphotericin-B	—	—	—	—	16.6	19.3

— = no activity.
[a]Values, including diameter of the well (8 mm), are means of three replicates.

Table 4

MIC (in μg/mL) of synthetic 1,4-DHPs and 4-quinolinyl pyridines obtained using macro dilution method.

	Gram-positive bacteria		Gram-negative bacteria		Yeast	
Compounds	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*S. cerevisiae*
2a	200	200	200	200	200	200
2b	200	200	200	200	200	200
2c	50	100	50	100	50	200
2d	200	200	200	200	200	200
2e	200	200	200	200	200	200
2f	200	200	200	200	200	200
3a	50	200	100	200	100	200
3b	200	100	100	200	200	200
3c	50	100	100	200	100	100
3d	200	100	100	100	100	100
3e	50	50	50	100	100	50
3f	100	100	100	100	100	100
Ciprofloxin	6.25	6.25	6.25	12.5	—	—
Amphotericin-B	—	—	—	—	12.5	12.5

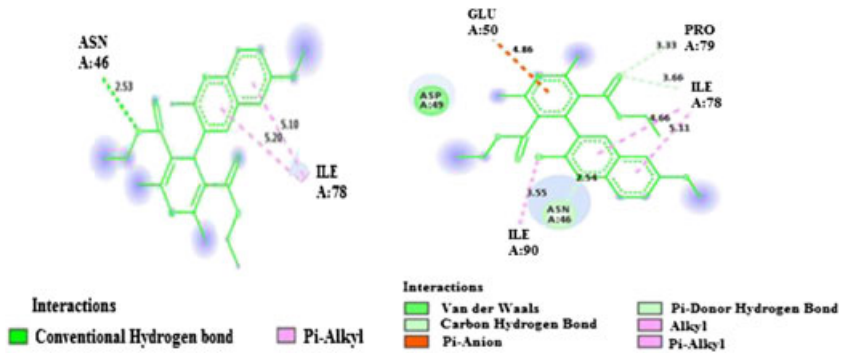

Figure 1. 2D schematic of compound **2e** and **3e** docked in active site of *DNA Gyrase B*. [Color figure can be viewed at wileyonlinelibrary.com]

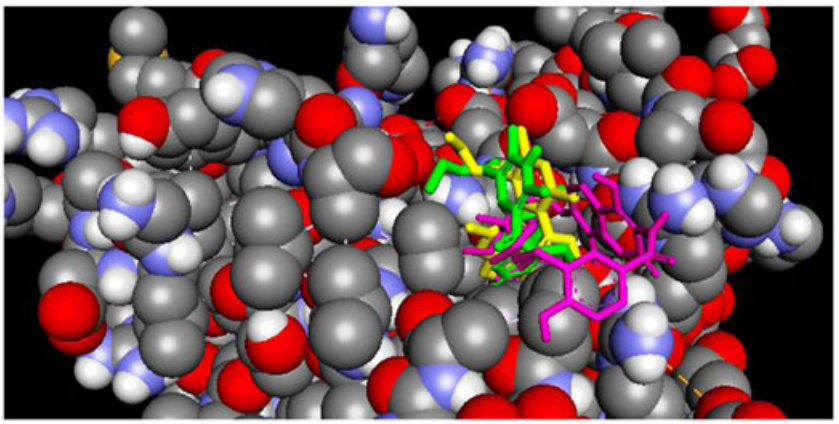

Figure 2. Surface diagram showing docked molecules **2e** and **3e** along with co-crystallized ligand clorobiocin in the active site of *E. coli topoisomerase II DNA Gyrase* (**2e** in green, **3e** in yellow and clorobiocin in magenta color). [Color figure can be viewed at wileyonlinelibrary.com]

CONCLUSION

A series of 4-quinolinyl pyridine based compounds has been synthesized with the hope of new lead molecules. The oxidation of 1,4-DHPs was carried out with SiO_2–HNO_3 which proved to be efficient oxidant in terms of yield, reaction time, easy workup, and cost. Within this study, the diethyl 4-(2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates exhibited more promising antimicrobial activities compared to 1,4-DHPs against all the microbial strains. And among all the derivatives, diethyl 4-(2-chloro-6-methoxyquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylates derivative **3e** was found to be the most biologically active compound. Further, docking studies showed that compounds **3e** (minimum inhibitory concentration (MIC), 50 μg/mL) inhibits *E. coli topoisomerase II DNA GyraseB*, respectively, through carbon hydrogen bond, pi-anion, pi-donor hydrogen bond, alkyl, and pi-alkyl interactions. These findings can be exploited toward the development and testing of pyridine and quinoline-based hybrid novel molecules for better antimicrobial activities.

EXPERIMENTAL

Melting points recorded are uncorrected and taken in open capillaries. IR spectra were recorded on a Perkin-Elmer IR spectrophotometer. ^1H and ^{13}C spectra were recorded in $CDCl_3$ on a 300 MHz/400 MHz and 75.4 MHz/100.6 MHz Bruker spectrometer using TMS as an internal standard, respectively. Mass spectra (DART-MS) were recorded on a JMS-T100LC Mass spectrometer having a DART (Direct Analysis in Real Time) source in ES^+ mode.

Synthesis of substituted 2-chloroquinolin-3-carbaldehyde (1a–1f): a general procedure. *N,N*-Dimethylformamide (2.2 mol) was cooled to 0°C in a flask equipped with a $CaCl_2$ guard tube, and phosphoryl chloride (4.0 mol) was added in to it dropwise with stirring. To this reaction mixture, acetanilide (1.0 mol) was added and kept the whole reaction mixture at 80°C for 6–8 h. The reaction mixture was cooled and poured into ice water (300 mL) and stirred for 1 h at 0–10°C. The precipitated 2-chloro-3-quinolincarbaldehyde **1a** was filtered, washed with water (100 mL), dried, and recrystallized from ethanol to give the title product as yellow needles with M.p. 150°C. Likewise, other carbaldehydes were obtained with M.p.: **1b**—110°C, **1c**—145°C, **1d**—140°C, **1e**—130°C, and **1f**—117°C.

Synthesis of substituted diethyl 4-(2-chloroquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (2a–2f): a general procedure. A mixture of 2-chloroquinolin-3-carbaldehyde (**1a–1e)** (1 mol), ethylacetoacetate (2 mol), and ammonium acetate (2 mol) was taken in a 250-mL round bottom flask separately and dissolved in ethanol. The reaction mixture was refluxed for about 60 min on water bath. Progress of the reaction was monitored by TLC (ethylacetate:pet ether). After the completion of reaction, the reaction mixture was cooled to room temperature to give solid diethyl 1,4-dihydro-2, 6-dimethyl-4-(2-chloroquinolin-3-carbaldehyde)pyridine-3,5-dicarboxylates (**2a–2f)** which was filtered and further purified by recrystallization from aqueous ethanol.

Diethyl 4-(2-chloroquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate [38] (2a, $C_{22}H_{23}ClN_2O_4$). IR (ν_{max}, cm^{-1}, KBr): 3271 (N—H stretch), 1697 (—COOEt); ^1H NMR[$CDCl_3$, δ (ppm), 400 MHz]: 8.134 (s, 1H, C_4'—H), 7.973–7.952 (d, J = 8.4 Hz, 1H, C_8'—H), 7.746–7.726 (d, J = 8.0 Hz, 1H, C_5'—H), 7.675–7.633 (m, J = 6.8 and 8.0 Hz, 1H, C_7'—H), 7.511–7.471 (m, J = 6.8 and 8.0 Hz, 1H, C_6'—H), 5.593 (s, 1H, N—H), 5.513 (s, 1H, C_4—H), 4.117-4.021 (q, J = 7.2 Hz, 4H, —OCH_2—CH_3), 2.357 (s, 6H, C_2 and C_6—CH_3), 1.203-1.167 (t, J = 7.2 Hz, 6H, —CH_2—CH_3); ^{13}C NMR [$CDCl_3$, δ (ppm), 75.4 MHz]: 167.2, 150.3, 146.3, 144.3, 140.3, 139.9, 129.8, 128.0, 127.7, 127.1, 126.5, 103.5, 59.9, 38.3, 19.6, 14.3; DART MS: *m/z* 414.23/416.14 (3:1), *Anal.* Calcd. C = 63.69, H = 5.59, Cl = 8.55, N = 6.75, O = 15.43; Found C = 63.66, H = 5.58, Cl = 8.59, N = 6.72, O = 15.45.

Diethyl 4-(2-chloro-6′-methylquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate [39] (2b, $C_{23}H_{25}ClN_2O_4$). IR (ν_{max}, cm^{-1}, KBr): 3269 (N—H stretch), 1690 (—COOEt); ^1H NMR[$CDCl_3$, δ (ppm), 300 MHz]: 8.052 (s, 1H, C_4'—H), 7.880–7.852 (d, J = 8.4 Hz, 1H, C_8'—H), 7.510–7.479 (m, 2H, C_5' and C_7'—H), 5.829 (s, 1H, N—H), 5.505 (s, 1H, C_4—H), 4.489–4.164 (q, 4H, —OCH_2—CH_3), 2.508 (s, 3H, C_6'—CH_3), 2.364 (s, 6H, C_2 and C_6—CH_3), 1.219–1.172 (t, J = 7.2 Hz, 6H, —CH_2—CH_3); ^{13}C NMR [$CDCl_3$, δ (ppm), 75.4 MHz]: 166.1, 148.2, 143.7, 143.1, 139.0, 138.1, 135.3, 130.9, 126.5, 124.8, 102.4, 58.7, 37.0, 20.3, 18.4, 13.1; DART MS: *m/z* 429.26/431.26 (3:1), *Anal.* Calcd. C = 64.41, H = 5.88, Cl = 8.27, N = 6.53, O = 14.92; Found C = 64.43, H = 5.84, Cl = 8.29, N = 6.51, O = 14.91.

Diethyl4-(2-chloro-7′-methylquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate [40] (2c, $C_{23}H_{25}ClN_2O_4$). IR (ν_{max}, cm^{-1}, KBr): 3294 (N—H stretch), 1690 (—COOEt); ^1H NMR[$CDCl_3$, δ (ppm), 300 MHz]: 8.080 (s, 1H, C_4'—H), 7.747 (s, 1H, C_8'—H), 7.643–7.615 (d, J = 8.4 Hz, 1H, C_5'—H), 7.345–7.317 (d, J = 8.4 Hz, 1H, C_6'—H), 5.888 (s, 1H, N—H), 5.505 (s, 1H, C_4—H), 4.159–4.024 (q, J = 7.2 Hz, 4H, —OCH_2—CH_3), 2.575 (s, 3H, C_7'—CH_3), 2.363 (s, 6H, C_2 and C_6—CH_3), 1.280–1.167 (t, J = 7.2 Hz, 6H, —CH_2—CH_3); ^{13}C NMR [$CDCl_3$, δ (ppm), 75.4 MHz]: 167.3, 150.2, 146.6, 144.2, 139.6, 139.2, 128.8, 127.0, 126.8, 125.8, 103.5, 59.8, 38.2, 21.8, 19.6, 14.3; DART MS: *m/z* 428.12/430.14 (3:1),

Anal. Calcd. C = 64.41, H = 5.88, Cl = 8.27, N = 6.53, O = 14.92; Found C = 63.38, H = 5.81, Cl = 8.25, N = 6.57, O = 14.93.

Diethyl 4-(2-chloro-8′-methylquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate [41] (2d, $C_{23}H_{25}ClN_2O_4$). IR (ν_{max}, cm^{-1}, KBr): 3294 (N—H stretch), 1690 (—COOEt); ^{1}H NMR[$CDCl_3$, δ (ppm), 300 MHz]: 8.091 (s, 1H, C_4'—H), 7.591–7.565 (d, *J* = 7.8 Hz, 1H, C_5'—H), 7.512–7.489 (d, *J* = 7.2 Hz, 1H, C_7'—H), 7.405–7.355 (t, *J* = 7.2, 7.8 Hz, 1H, C_6'—H), 5.806 (s, 1H, N—H), 5.533 (s, 1H, C_4—H), 4.167–4.023 (q, *J* = 7.2 Hz, 4H, —OCH_2—CH_3), 2.759 (s, 3H, C_8'—CH_3), 2.361 (s, 6H, C_2 and C_6—CH_3), 1.283–1.177 (t, *J* = 7.2 Hz, 6H, —CH_2—CH_3); ^{13}C NMR [$CDCl_3$, δ (ppm), 75.4 MHz]: 167.3, 149.2, 145.6, 144.2, 140.2, 139.5, 136.2, 129.8, 127.7, 126.2, 125.1, 103.4, 59.8, 38.4, 19.6, 17.8, 14.3; DART MS: *m/z* 428.23/430.14 (3:1), *Anal.* Calcd. C = 63.41, H = 5.88, Cl = 8.27, N = 6.53, O = 14.92; Found C = 63.44, H = 5.85, Cl = 8.28, N = 6.56, O = 14.94.

Diethyl 4-(2-chloro-6′-methoxyquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (2e, $C_{23}H_{25}ClN_2O_5$). IR (ν_{max}, cm^{-1}, KBr): 3202 (N—H stretch), 1682 (—COOEt); ^{1}H NMR[$CDCl_3$, δ (ppm), 300 MHz]: 8.034 (s, 1H, C_4'—H), 7.882–7.852 (d, 1H, C_8'—H), 7.416–7.330 (d, 2H, C_5' and C_7'—H), 5.850 (s, 1H, N—H), 5.504 (s, 1H, C_4—H), 4.110–4.069 (q, *J* = 5.4 Hz, 4H, —OCH_2—CH_3), 3.917 (s, 3H, C_6'—OCH_3), 2.551 (s, 6H, C_2 and C_6—CH_3), 1.309–1.206 (t, *J* = 5.1 Hz, 6H, —CH_2—CH_3); ^{13}C NMR [$CDCl_3$, δ (ppm), 100.6 MHz]: 167.4, 157.7, 147.7, 144.4, 142.3, 140.5, 138.8, 129.3, 128.8, 122.5, 104.7, 103.5, 59.9, 55.5, 38.1, 19.5, 14.3; DART MS: *m/z* 445.26/447.26 (3:1), *Anal.* Calcd. C = 62.09, H = 5.66, Cl = 7.97, N = 6.30, O = 17.98; Found C = 62.11, H = 5.63, Cl = 7.98, N = 6.26, O = 17.94.

Diethyl 4-(2-chloro-8′-methoxyquinolin-3-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (2f, $C_{23}H_{25}ClN_2O_5$). IR (ν_{max}, cm^{-1}, KBr): 3282 (N—H stretch), 1685 (—COOEt); ^{1}H NMR[$CDCl_3$, δ (ppm), 300 MHz]: 8.052 (s, 1H, C_4'—H), 7.880–7.776 (t, *J* = 6.3 Hz, 1H, C_6'—H), 7.510–7.479 (d, *J* = 6.3 Hz, 1H, C_5'—H), 6.854–6.795 (d, 2H, C_7'—H), 5.829 (s, 1H, N—H), 5.505 (s, 1H, C_4—H), 4.324–4.164 (m, 4H, —OCH_2—CH_3), 3.506 (s, 3H, C_8'—OCH_3), 2.364 (s, 6H, C_2 and C_6—CH_3), 1.467–1.381 (t, *J* = 7.2 Hz, 6H, —CH_2—CH_3); ^{13}C NMR [$CDCl_3$, δ (ppm), 75.4 MHz]: 167.3, 150.2, 145.6, 144.2, 139.5, 137.7, 128.8, 122.5, 104.7, 103.4, 59.8, 55.5, 38.1, 19.6, 14.3; DART MS: *m/z* 445.20/447.14 (3:1), *Anal.* Calcd. C = 62.09, H = 5.66, Cl = 7.97, N = 6.30, O = 17.98; Found C = 62.09, H = 5.64, Cl = 7.99, N = 6.28, O = 17.95.

Synthesis of substituted diethyl 4-(2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3a–3e). SiO_2–HNO_3 (2 wt % of 1,4-DHP) was added to a magnetically stirred solution of each 1,4-DHP (**2a–2e)** in dichloromethane at room temperature for 1–2 min separately. After the completion of reaction, indicated by TLC, reaction mixture was filtered and neutralized with saturated solution of sodium bicarbonate followed by the extraction of organic layer and dried it over anhydrous magnesium sulphate. Evaporation of the solvent afforded the pure final product diethyl-2,6-dimethyl-4-(2-chloroquinolin-3-carbaldehyde)pyridine-3,5-dicarboxylates (**3a–3e)**.

Diethyl 4-(2-chloroquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3a, $C_{22}H_{21}ClN_2O_4$). IR (ν_{max}, cm^{-1}, KBr): 1720 (—COOEt); ^{1}H NMR ($CDCl_3$, δppm, 400 MHz): 8.084 (s, 1H, C_4'—H), 7.979 (s, 1H, C_8'—H), 7.841–7.798 (m, 1H, C_5' and C_7'—H), 7.652–7.614 (t, *J* = 5.7 Hz, 1H, C_6'—H), 4.064–3.931 (q, *J* = 7.2 Hz, 4H, —OCH_2—CH_3), 2.796 (s, 6H, $C_2$and C_6—CH_3), 0.839–0.804 (t, *J* = 7.2 Hz, 6H, —CH_2—CH_3); ^{13}C NMR[$CDCl_3$, δppm, 75.4 MHz]:165.4, 156.4, 148.2, 147.2, 137.8, 131.4, 129.3, 128.4, 128, 127.8, 127.7, 125.8, 62.0, 22.0, 13.4; DART MS: *m/z* 413.23/415.23 (3:1), *Anal.* Calcd. C = 64.00, H = 5.13, Cl = 8.59, N = 6.79, O = 15.50; Found C = 64.02, H = 5.09, Cl = 8.59, N = 6.81, O = 15.48

Diethyl 4-(2-chloro-6′-methylquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3b, $C_{23}H_{23}ClN_2O_4$). IR (ν_{max}, cm^{-1}, KBr): 1728 (—COOEt); ^{1}H NMR ($CDCl_3$, δ ppm, 300 MHz): 7.990–7.636 (m, 4H, quinolinyl-H), 4.235-3.895 (q, 4H, —OCH_2—CH_3), 2.905 (s, 6H, C_2 and C_6—CH_3), 2.580 (s, 3H, C_6'—CH_3), 1.978–1.813 (t, 6H, —CH_2—CH_3); ^{13}C NMR[$CDCl_3$, δppm, 75.4 MHz]: 163.4, 154.6, 145.1, 136.3, 133.2, 128.5, 127.1, 125.7, 61.5, 20.7, 19.8, 12.5; DART MS: *m/z* 426.20/428.14 (3:1), *Anal.* Calcd. C = 64.71, H = 5.43, Cl = 8.30, N = 6.56, O = 14.99; Found C = 64.73, H = 5.41, Cl = 8.27, N = 6.59, O = 14.98.

Diethyl 4-(2-chloro-7′-methylquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3c, $C_{23}H_{23}ClN_2O_4$). IR (ν_{max}, cm^{-1}, KBr): 1720 (—COOEt); ^{1}H [$CDCl_3$, δ ppm, 300 MHz]: 7.917 (s, 1H, C_4'—H), 7.841 (s, 1H, C_8'—H), 7.717–7.690 (d, *J* = 8.1 Hz, 1H, C_5'—H), 7.453–7.427 (d, *J* = 8.1 Hz, 1H, C_6'—H), 4.162–3.932 (q, *J* = 6.9 Hz, 4H, —OCH_2—CH_3), 2.718 (s, 6H, C_2 and C_6—CH_3), 2.613 (s, 1H, C_7'—CH_3), 0.890–0.790 (t, *J* = 8.4 Hz, 6H, —CH_2—CH_3); ^{13}C NMR[$CDCl_3$, δ ppm, 75.4 MHz]:166.7, 157.1, 148.8, 147.4, 143.0, 141.7, 137.5, 129.8, 129.1, 127.4, 127.2, 126.6, 124.1, 61.5, 23.5, 21.9, 13.5; DART MS: *m/z* 427.25/429.25 (3:1), *Anal.* Calcd. C = 64.71, H = 5.43, Cl = 8.30, N = 6.56, O = 14.99; Found C = 64.68, H = 5.45, Cl = 8.26, N = 6.54, O = 14.99.

Diethyl 4-(2-chloro-8′-methylquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3d, $C_{23}H_{23}ClN_2O_4$). IR (ν_{max}, cm^{-1}, KBr): 1720 (—COOEt); ^{1} H NMR[$CDCl_3$, δ ppm, 300 MHz]: 7.936 (s, 1H, C_4'—H), 7.654–7.628 (m, 2H, C_5' and C_7'—H), 7.513–7.463 (t, *J* = 7.8 Hz, 1H,

C_6'—H), 4.121–3.905 (q, J = 7.2 Hz, 4H, —OC$\underline{H}_2$—C$\underline{H}_3$), 2.930 (s, 3H, C_8'—C$\underline{H}_3$), 2.805 (s, 6H, $C_2$and C_6—C$\underline{H}_3$), 0.891–0.812 (t, J = 6.6 Hz 6H, —C$\underline{H}_2$—C$\underline{H}_3$); ^{13}C NMR [$CDCl_3$, δ ppm, 100.6 MHz]: 166.2, 156.7, 147.4, 146.4, 144.2, 138.1, 136.6, 131.3, 129.4, 127.4, 127.2, 127.2, 126.0, 125.5, 61.7, 29.7, 22.9, 17.7, 13.4; DART MS: *m/z* 426.25/428.25 (3:1), *Anal.* Calcd. C = 64.71, H = 5.43, Cl = 8.30, N = 6.56, O = 14.99; Found C = 64.71, H = 5.46, Cl = 8.27, N = 6.56, O = 14.98

Diethyl 4-(2-chloro-6′-methoxyquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3e, $C_{23}H_{23}ClN_2O_5$). IR (ν_{max}, cm^{-1}, KBr): 1725 (—COOEt); ^{1}H NMR[$CDCl_3$, δ ppm, 400 MHz]: 7.958–7.935 (d, J = 6.9 Hz, 1H, C_8'—H), 7.849 (s, 1H, H-4′), 7.434–7.406 (d, J = 6.9 Hz, 1H, C_7'—H), 7.046 (s, 1H, C_5'—H), 4.074–3.985 (q, 4H, —OC$\underline{H}_2$—C$\underline{H}_3$), 3.960 (s, 6H, C_6'—H—OC$\underline{H}_3$), 2.714 (s, 6H, C_4 and C_6—C$\underline{H}_3$), 0.882–0.820 (t, J = 7.2 Hz, 6H, —C$\underline{H}_2$—C$\underline{H}_3$); ^{13}C NMR [$CDCl_3$, δ ppm, 100.6 MHz]: 166.6, 158.5, 157.1, 146.1, 143.1, 136.6, 130.2, 129.7, 127.2, 126.6, 123.8, 105.1, 61.6, 55.6, 23.4, 13.5; DART MS: *m/z* 444.25/446.25 (3:1), *Anal.* Calcd. C = 62.37, H = 5.23, Cl = 8.00, N = 6.33, O = 18.06; Found C = 62.39, H = 5.26, Cl = 7.98, N = 6.32, O = 18.03

Diethyl4-(2-chloro-8′-methoxyquinolin-3-yl)-2,6-dimethylpyridine-3,5-dicarboxylate (3f, $C_{23}H_{23}ClN_2O_5$). IR (ν_{max}, cm^{-1}, KBr): 1710 (—COOEt); ^{1}H NMR[$CDCl_3$, δ(ppm), 300 MHz]: 8.073 (s, C_4'—H), 7.774–7.751 (d, J = 6.9 Hz, 1H, C_5'—H), 7.643–7.592 (t, J = 6.9 and 8.4 Hz, 1H, C_6'—H), 7.346–7.318 (d, J = 8.4 Hz, 1H, C_7'—H), 4.164–4.003 (q, J = 8.4 Hz, 4H, —OC$\underline{H}_2$—C$\underline{H}_3$), 3.941 (s, 6H, C_8'—C$\underline{H}_3$), 2.774 (s, 6H, C_2 and C_6—C$\underline{H}_3$), 1.271–1.171 (t, J = 6.9 Hz, 6H, —C$\underline{H}_2$—C$\underline{H}_3$); ^{13}C NMR [$CDCl_3$, δ(ppm), 75.4 MHz]:166.7, 157.1, 148.8, 147.4, 146.1, 143.2, 141.5, 137.5, 129.6, 129.0, 127.2, 126.8, 124.0, 61.5, 55.7, 23.5, 13.5; DART MS: *m/z* 444.15/446.15 (3:1), *Anal.* Calcd. C = 62.37, H = 5.23, Cl = 8.00, N = 6.33, O = 18.06; Found C = 63.37, H = 5.21, Cl = 8.02, N = 6.31, O = 18.05.

Pharmacology. ***Test microorganisms.*** Total six microbial strains, i.e. two Gram-positive bacteria (*S. aureus* MTCC 96 and *B. subtilis* MTCC 121), two Gram-negative bacteria (*E. coli* MTCC 1652 and *P. aeruginosa* MTCC 741), and two yeasts (*C. albicans* MTCC 227 and *S. cerevisiae* MTCC 170) were screened for evaluation of antibacterial and antifungal activity of the chemical compounds. All the microbial cultures were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. The bacteria were subcultured on Nutrient Agar whereas yeast on Malt Extract Agar plates.

Antimicrobial assay (bacteria and yeasts). The antimicrobial activity and MIC value of 12 chemical compounds were evaluated by the agar well diffusion assay. The inoculum suspensions of the test microorganisms were prepared by using 16-h-old cultures adjusted to 10^8cfu/mL by referring the 0.5 McFarland standards. Twenty milliliters of agar medium (Nutrient Agar and Malt Extract Agar) was poured into each Petri plate, and plates were swabbed with 100-μL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8-mm diameter, wells were bored into the seeded agar plates, and these were loaded with a 100-μL volume with concentration of 4.0 mg/mL of each compound reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 h. Antimicrobial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). Dimethylsulphoxide was used as a negative control, whereas Ciprofloxacin was used as positive control for bacteria and Amphotericin-B for yeast. This procedure was performed in three replicate plates for each organism [42].

Determination of minimum inhibitory concentration (MIC) of chemical compounds. Minimum inhibitory concentration is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after incubation. Minimum inhibitory concentration of the 4-quinolinyl pyridines against bacterial and yeast strains was tested through a modified agar well diffusion method. In this method, a twofold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 4 to 0.0625 mg/mL. A 100-μL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 μL of standardized inoculum (10^8 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 h and observed for the inhibition zones. Minimum inhibitory concentration, taken as the lowest concentration of the chemical compound that inhibited the growth of the microbe, shown by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin and Amphotericin-B were used as positive control while DMSO as negative control.

Computational details. The crystal structure of topoisomerase II DNA Gyrase B was taken from Protein Data Bank (PDB entry: 1KZN) [35]. To execute docking studies, the 2D structure of most active ligand (**3e**) was drawn, converted to 3D, and its energy was minimized [43]. Ligand, **3e**, was prepared in pdb format with explicit hydrogen addition. Co-crystallized ligand was removed from pdb file,

1KZN, and protein molecule was prepared by deleting solvent molecules and non-complex ions using Chimera [44]. Incomplete side chains were replaced using Dun BrackRotamer library [45]. Hydrogens were added, and gasteiger charges were calculated using Antechamber [46], then prepared file was saved as pdb format. All pdb files were transformed into pdbqt format. Docking studies were executed by using Auto Dock Vina 1.1.2. Grid center was placed on the active site. The sizes and center of grid box were center-x = 18.1158019877, center-y = 30.1010002781, center-z = 35.8465211584, size-x = 25.0, size-y = 25.0, size-z = 25.0 for 1KZN. Exhaustiveness of the global search algorithm was set to be 8. Then, finally docking results were viewed using pdb and pdbqt files [47].

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