A New Series of 1,3,4-Oxadiazole Linked Quinolinyl-Pyrazole/Isoxazole Derivatives: Synthesis and Biological Activity Evaluation

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Abstract—A series of 1,3,4-oxadiazole bridged pyrazole/isoxazole bearing quinoline derivatives has been designed and synthesized by a clean and convenient method. Structures of the newly synthesized compounds have been confirmed by FTIR, ^{1}H and ^{13}C NMR, and HRMS spectral data. The titled compounds have been evaluated for their molecular docking guided antimicrobial and anti-inflammatory activity. One of 1,3,4-oxadiazole bridged quinolinyl-pyrazole derivatives has interacted efficiently with *E. Coli* protein (PDB file: 1KZN), and has been characterized by good antimicrobial activity against the majority of the tested pathogens. Another product has exhibited excellent anti-inflammatory activity.

Keywords: 1,3,4-oxadiazole, pyrazole, isoxazole, quinoline, molecular docking, antimicrobial, anti-inflammatory

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INTRODUCTION

Quinoline and its derivatives are considered as versatile prodrugs, among those are antimalarial quinine (**A**) [1], chloroquine (**B**), and hydroxychloroquine (**C**) (Fig. 1). The latter one is currently under clinical trials as a repurposed drug for COVID-19 [2].

Currently, many quinoline-containing drugs are available on the market [3, 4]. Recently, three
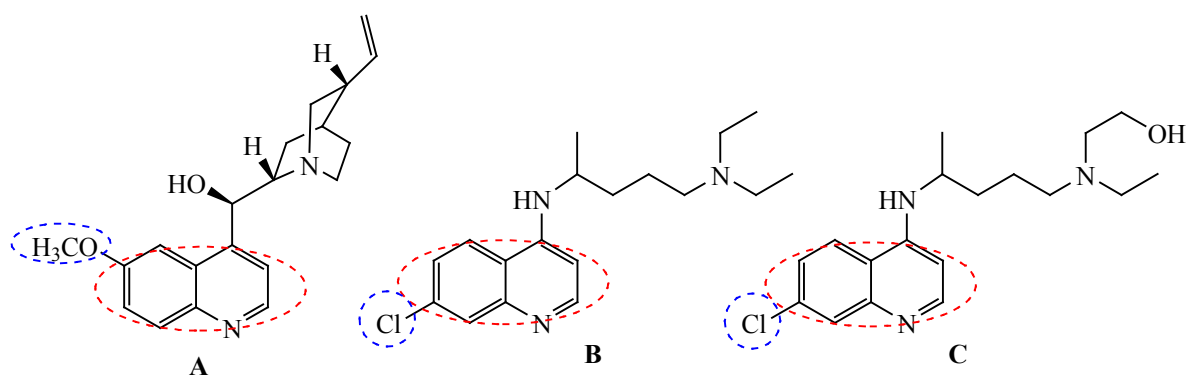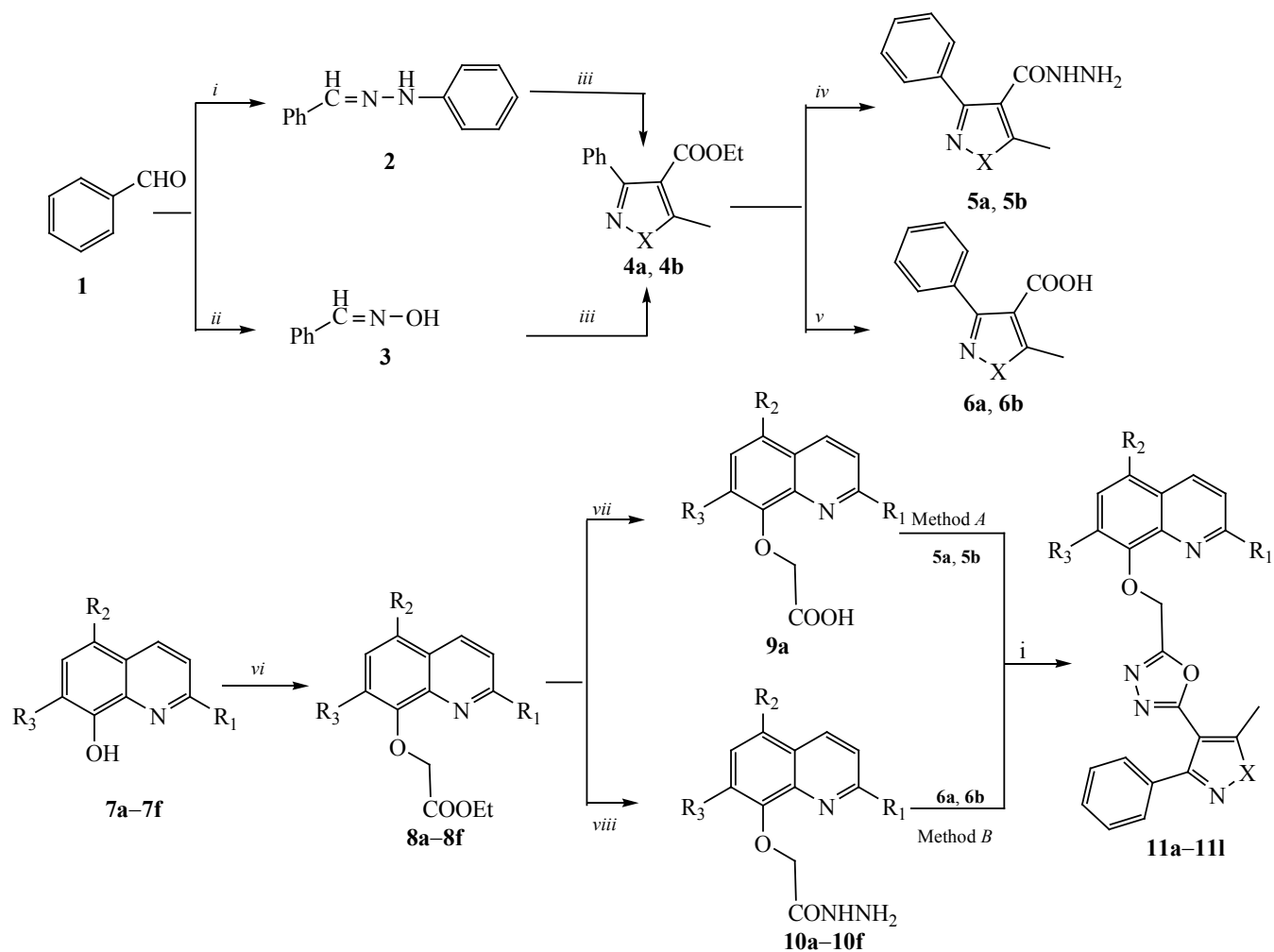

Fig. 1. Samples of promising quinoline alkaloids.

Scheme 1. Synthesis of target compounds **11a–11l**.

4a–6a = X = N–Ph, obtained from **2**; **4b–6b** = X = O, obtained from **3**; **9a** and **10a**: $R_1 = R_2 = R_3 = H$, **10b**: $R_1 = H$, $R_2 = Cl$, $R_3 = H$, **10c**: $R_1 = H$, $R_2 = R_3 = Cl$, **10d**: $R_1 = Me$, $R_2 = H$, $R_3 = H$, **10e**: $R_1 = Me$, $R_2 = R_3 = Cl$, **10f**: $R_1 = H$, $R_2 = R_3 = I$, **11a**: $X = -$, $R_1 = R_2 = R_3 = H$, **11b**: $X = -$, $R_1 = H$, $R_2 = Cl$, $R_3 = H$, **11c**: $X = -$, $R_1 = H$, $R_2 = R_3 = Cl$, **11d**: $X = -$, $R_1 = Me$, $R_2 = R_3 = H$, **11e**: $X = -$, $R_1 = Me$, $R_2 = R_3 = Cl$, **11f**: $X = -$, $R_1 = H$, $R_2 = R_3 = I$, **11g**: $X = O$, $R_1 = R_2 = R_3 = H$, **11h**: $X = O$, $R_1 = H$, $R_2 = Cl$, $R_3 = H$, **11i**: $X = O$, $R_1 = H$, $R_2 = R_3 = Cl$, **11j**: $X = O$, $R_1 = Me$, $R_2 = R_3 = H$, **11k**: $X = O$, $R_1 = Me$, $R_2 = R_3 = Cl$, **11l**: $X = O$, $R_1 = H$, $R_2 = R_3 = I$.

Reagents and conditions: (*i*) Phenyl hydrazine, ethanol, reflux, 3 h. (*ii*) Hydoxylamine hydrochloride, sodium acetate (1 eq.), ethanol, reflux, 12 h. (*iii*) Ethylacetoacetate, anhydrous zinc chloride (cat), reflux, 4 h, ethanol, (*iv*) Hydrazine hydrate, ethanol, reflux, 3 h, (*v*) Sodium hydroxide, tetrahydo furan : water (1 : 1), reflux, 1 h, (*vi*) Ethyl 2-chloroacetate, anhydrous potassium carbonate, dry acetone, reflux, 24 h, (*vii*) NaOH (10%), methanol, reflux, (*viii*) Hydrazine hydrate, ethanol, reflux, 1 h. (*ix*) POCl₃, 80°C, Method *A*: 24 h for the synthesis of **11a–11c**, Method *B*: 10 h for the synthesis of **11a–11l**.

protein kinase inhibitors (Lenvatinib, Bosutinib, and Cabozantinib) and one Farnesyl transferase inhibitor (Tipifarnib) were presented as anticancer agents [5]. 1,3,4-Oxadiazole derivatives are well known for their antioxidant, anticancer, anticonvulsant, tyrosinase inhibitory, cathepsin K inhibitors, P-glycoprotein inhibitors, antimicrobial, antitumor, anti-inflammatory, and antiretroviral activities [6].

Table 1. Optimization of reaction conditions of synthesis of titled compounds

Entry	Compound	Method A			Method B		
		time, h	weight, g	yield, %	time, h	weight, g	yield, %
1	**11a**	24	0.37	79	10	0.40	87
2	**11g**	24	0.33	84	10	0.35	92

Combination of quinoline and pyrazole structural blocks in molecules makes pharmacophores with respect to anti-cancer, interleukin, A3 adenosine receptor antagonists, anti-ulcer, and anti-inflammatory to be of certain potential [7]. Similarly, combination of quinoline and isoxazole heterocycles can result in formation of anti-TB [8, 9], cytotoxic [10], antibacterial, antifungal [11], anti-inflammatory, and analgesic [12] compounds. Quinoline containing 1,3,4-oxadiazole derivatives exhibit pronounced biological activities [13, 14].

According to the earlier reports [15], the position 8 in quinoline was determined to be decisive for its biological activity. Generally, quinoline and its derivatives are considered as highly potent among derivatives of other five-membered heterocycles [16]. All the above inspired us to design and synthesize quinoline based compounds containing azoles combined by 1,3,4-oxadiazole bridge.

RESULTS AND DISCUSSION

The compounds **2** and **3** synthesized from benzaldehyde were subjected to cyclization reaction with ethyl acetoacetate in presence of the catalytic amount of anhydrous zinc chloride without using an additional solvent to give intermediates **4a** and **4b**, respectively (Scheme 1).

In the following step, esters **4a**, **4b** were treated with hydrazine hydrate in ethanol to afford **5a** and **5b**, respectively. Subsequently, esters **4a**, **4b** were hydrolyzed by NaOH (10%) in THF–water (1 : 1) medium to afford **6a** and **6b**, respectively. Compounds **8a–8f** were synthesized by treatment of a variety of 8-hydroxyquinolines **7a–7f** by ethyl 2-chloroacetate in acetone in presence of potassium carbonate. The isolated product **8a** was subjected to hydrolysis by sodium hydroxide to afford acid **9a**. Refluxing of **8a–8f** with hydrazine hydrate in ethanol led to the key intermediates **10a–10f**.

In the first approach to the target compounds, the compounds **11a** and **11g** were synthesized by refluxing **9a** with **5a** or **5b**, respectively, in phosphorous oxychloride

(Scheme 1, method *A*). Alternatively, **10a** was treated with **6a** or **6b** with formation of the target compounds **11a** and **11g** under the similar reaction conditions (Scheme 1, method *B*).

Optimization of reaction conditions for both methods is presented in Table 1 and favored method *B*. Probably poor solubility of **5a** could lead to lower rate of the reaction. Accordingly, method *B* was applied for synthesis of the other derivatives. It is noteworthy that higher yield was achieved in case of isoxazole (84 and 92%) than pyrazole (79 and 87%) due to absence of the bulky N-phenyl group in the former one which minimized the partial positive charge on the carbonyl group.

Structures of the synthesized compounds were confirmed by FTIR, ^{1}H and ^{13}C NMR, and HRMS spectral data. Two singlets at 2.41 ppm (CH$_3$) and 5.35 ppm (O–CH$_2$), and a doublet of doublets at 8.88–8.90 ppm assigned to quinoline proton in ^{1}H NMR spectrum of compound **11a** indicated its formation. In IR spectra, presence of characteristic bands at 1065–1089 cm^{-1} (N–N), 1618–1643 cm^{-1} (C=N), and 1229–1256 cm^{-1} (C–O–C) also supported formation of compounds **11a–11l**.

Biological activity. *Molecular docking study.* The molecular docking data are summarized in Table 2. The majority of target compounds demonstrated significant binding energy (–11.57 to –7.97 kJ/mol) with the target sites possessing H-bonds.

Antimicrobial activity. Results of antimicrobial activity (Table 3, Fig. 2) indicated that majority of products were of significant activity against most of the tested microbes. Compound **11d** which contained the methyl group on quinoline ring was potent antimicrobial agent as well as **11a**. The other compounds showed moderate activity. Compound **11b** containing electron-withdrawing chlorine at C^5 of quinoline displayed significant activity against gram-negative bacterial strain and demonstrated moderate activity against other pathogens. Compounds **11c** and **11i** that contained Cl at C^5 and C^7 on quinoline ring connected

Table 2. Results of docking of compound **11a–11l** with protein *E. coli* (PDB file: 1KZN)

Comp. no.	Binding energy, kJ/mol	Ligand efficiency	Inhibition constant	vdW+H-bond + desolv energy	Number of H-bonds	Bonding residues	Bond length, Å
11a	−10.92	−0.38	9.94	−12.39	1	1KZN:A:GLY77:HN	2.050
11b	−7.97	−0.27	1.44	−9.51	1	1KZN:A:ASN46:HD21	1.793
11c	−10.27	−0.33	29.60	−11.85	–	–	–
11d	−11.57	−0.39	3.30	−13.07	1	1KZN:A:GLY77:HN	2.148
11e	−10.35	−0.32	25.69	−11.93	–	–	–
11f	−9.41	−0.30	126.89	−10.85	–	–	–
11g	−8.96	−0.26	268.80	−10.78	1	1KZN:A:ASN46:HD21	1.746
11h	−10.29	−0.29	28.60	−12.08	1	1KZN:A:GLN135:HE21	2.234
11i	−8.65	−0.23	453.37	−10.42	–	–	–
11j	−10.79	−0.30	12.40	−12.62	–	–	–
11k	−10.16	−0.27	35.42	−11.95	1	1KZN:A:ASN46:HD21	1.812
11l	−8.90	−0.24	298.46	−10.71	1	1KZN:A:GLU50:OE1	2.710

Table 3. Antimicrobial activity (MIC and MBC/MFC) of compounds 1,3,4-oxadiazole bridged quinolinyl-pyrazole/isoxazole analogues

Compound	Antibacterial activity										Antifungal activity			
	gram positive				gram negative									
	B. cereus		*S. aureus*		*E. coli*		*K. pneumonia*		*S. flexneri*		*C. albicans*		*A. niger*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC
11a	15	110	20	135	15	125	15	125	15	125	15	135	15	130
11b	40	245	50	260	15	125	20	140	15	105	45	265	40	255
11c	30	195	40	200	10	100	10	105	40	245	40	250	45	270
11d	10	115	15	135	15	145	20	180	10	110	10	165	5	105
11e	20	140	30	175	25	160	15	130	30	180	25	145	20	140
11f	50	245	30	180	50	250	40	230	35	195	20	150	15	215
11g	25	150	30	170	20	140	15	110	20	130	15	130	20	145
11h	40	235	55	280	20	130	10	105	15	120	55	255	55	275
11i	40	230	45	245	10	105	10	110	40	245	55	265	50	270
11j	20	135	30	160	25	140	30	150	25	140	15	125	20	140
11k	35	190	40	230	30	180	45	235	35	210	25	180	15	135
11l	55	250	60	300	40	225	50	245	50	250	25	185	20	135
Chloramphenicol	09	110	10	120	12	125	11	135	12	125	–		–	
Fluconazole	–		–		–		–		–		5		6	

by pyrazole/isoxazole bridge with 1,3,4-oxadiazole ring exhibited excellent activity against gram-negative bacterial strains (*E. coli* and *K. pneumonia*) at high concentration (100 μg/mL). So, compounds containing electron-withdrawing substituents demonstrated excellent activity against majority of the gram-negative bacteria, while compounds containing electron-donating groups displayed significant activity against both gram-positive and gram-negative bacterial strains. Compounds **11g** and **11h** were highly active against *S. flexneri* gram-negative

Table 4. *In vitro* anti-inflammatory activity of 1,3,4-oxadiazole bridged quinolinyl-pyrazole/isoxazole analogues **11a–11l**

Compound	Mean absorbance ± SD[a]	Inhibition of denaturation, %
11a	0.2539 ± 0.052	24.82
11b	0.3225 ± 0.022	58.55
11c	0.3621 ± 0.026	78.02
11d	0.2613 ± 0.021	28.46
11e	0.2757 ± 0.011	35.54
11f	0.2372 ± 0.056	16.61
11g	0.2421 ± 0.010	19.02
11h	0.3093 ± 0.008	52.06
11i	0.3158 ± 0.009	55.26
11j	0.2424 ± 0.021	19.17
11k	0.2538 ± 0.041	24.77
11l	0.2269 ± 0.023	11.15
Diclofenac sodium	0.3815 ± 0.008	87.56
Control	0.2034 ± 0.016	–

[a] S.D = standard deviation (average of three determinations).

bacteria but showed moderate activity against other tested strains which could be attributed to the presence of Cl on quinoline cycle in addition to isoxazole heterocycle. 1,3,4-Oxadiazole bridged pyrazolyl-quinoline compounds were more potent than isoxazolyl-quinoline compounds. The compounds containing iodine (**11f**, **11l**) exhibited the lowest antibacterial activity against all tested strains but showed good antifungal activity. Majority of the fungal strains were inhibited by compound **11a** and **11d** that contained the methyl group on quinoline ring while, other derivatives showed moderate antifungal activity.

Anti-inflammatory activity. Anti-inflammatory activity of the products were tested by inhibition of the albumin denaturation method according to Muzushima and Kabayashi with slight modification [17]. Diclofenac sodium was used as a standard (Table 4). The product **11c** which contained Cl at C^5 and C^7 of quinoline ring demonstrated potent anti-inflammatory activity comparable with that of the standard drug. Compound **11b** containing pyrazole and 1,3,4-oxadiazole and Cl at C^5 demonstrated promising activity as well as **11h** and **11i** containing isoxazole ring. Compounds **11f** and **11l** carrying iodine at C^5 and C^7 of quinoline ring were of the lowest activity. According to the data summarized in Table 4, anti-inflammatory activity of products was influenced by electronegative Cl substituent and pyrazole ring attached to quinoline via 1,3,4-oxadiazole heterocycle.

EXPERIMENTAL

All reagents were purchased from Sigma Aldrich. Melting points were determined on a Buchi oil melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a SHIMADZU 8400 S FTIR
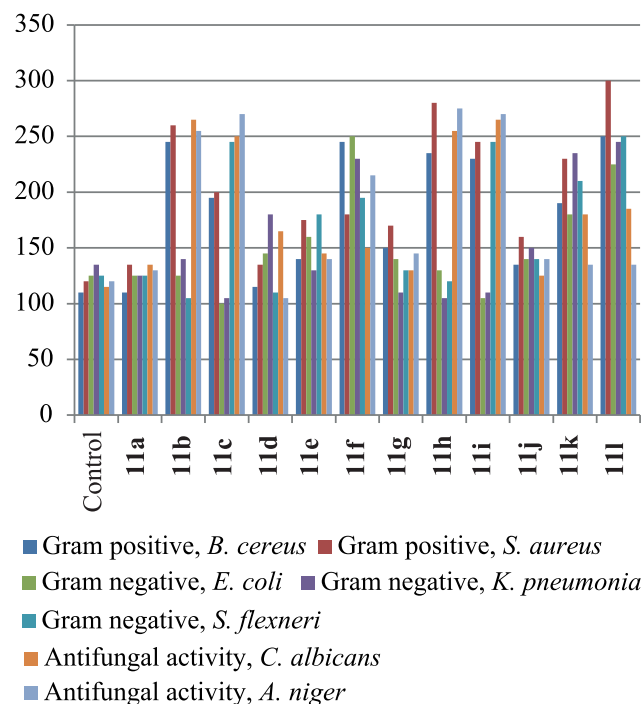
Fig. 2. Comparison of antimicrobial (MBC/MFC) activity of newly synthesized compounds **11a–11l**.

■ Gram positive, *B. cereus* ■ Gram positive, *S. aureus*
■ Gram negative, *E. coli* ■ Gram negative, *K. pneumonia*
■ Gram negative, *S. flexneri*
■ Antifungal activity, *C. albicans*
■ Antifungal activity, *A. niger*

spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance spectrometer (400 MHz for proton and 100 MHz for carbon) respectively using CDCl$_3$ as a solvent and TMS as an internal standard. High resolution mass spectra (HRMS) were measured by the direct infusion method on a MicroTOF-QII-ESI-Qq-TOF liquid chromatography mass spectrometer (Bruker Daltonics, Billerica, USA), electrospray ionization (ESI). Column chromatography was carried out using silica gel 60 (15–40 mm, Merck). TLC was performed on pre-coated (silica gel 60 F254, 0.2 mm, Merck) plates and visualized under UV light and/or by iodine vapor.

Synthesis of intermediates 10a–10f. Intermediate compounds **10a–10f** were synthesized by the reported earlier methods. 1-Benzylidene-2-phenylhydrazine (**2**) and benzaldehyde oxime (**3**) were synthesized from benzaldehyde [18, 19]. Ethyl 5-methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylate (**4a**) and ethyl 5-methyl-3-phenylisoxazole-4-carboxylate (**4b**) were synthesized by cyclization of compounds **2** and **3** with ethyl acetoacetate, respectively [20, 21]. 5-Methyl-1,3-diphenyl-1*H*-pyrazole-4-carbohydrazide (**5a**) and 5-methyl-3-phenylisoxazole-4-carbohydrazide (**5b**) were synthesized by treating compounds **4a** and **4b** with hydrazine hydrate [22, 23]. 5-Methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid (**6a**) and 5-methyl-3-phenylisoxazole-4-carboxylic acid (**6b**) were synthesized by hydrolysis in aqueous methanol [24, 25]. Ethyl 2-(quinolin-8-yloxy)-acetates **8a–8f** and 2-(quinolin-8-yloxy)acetohydrazides **10a–10f** were synthesized by treating the corresponding 8-hydoxyquinoline **7a–7f** with ethyl 2-chloroacetate followed by hydrazine hydrate [26, 27]. 2-(Quinolin-8-yloxy)acetic acid **9a** was obtained *via* hydrolysis of **8a** in aqueous methanol.

Typical procedure for synthesis of 2-(5-methyl-1,3-diphenyl-1*H*-pyrazol-4-yl)-5-[(quinolin-8-yloxy)-methyl]-1,3,4-oxadiazole (11a) and 2-(5-methyl-3-phenylisoxazol-4-yl)-5-[(quinolin-8-yloxy)methyl]-1,3,4-oxadiazole (11g). *Method A.* 5-Methyl-1,3-diphenyl-1*H*-pyrazole-4-carbohydrazide (**5a**, 0.29 g, 1.00 mmol) and 5-methyl-3-phenylisoxazole-4-carbohydrazide (**5b**, 0.21 g, 1.00 mmol) were placed in round bottom flasks fitted with a water cool condenser. To the flasks 2-(quinolin-8-yloxy)acetic acid (**9a**, 0.21 g, 1.00 mmol) was added followed by 10 mL of phosphorous oxychloride.

Method B. 5-Methyl-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid (**6a**, 0.27 g, 1.00 mmol) or 5-methyl-

3-phenylisoxazole-4-carboxylic acid (**6b**, 0.21 g, 1.00 mmol), 2-(quinolin-8-yloxy)acetohydrazide (**10a**, 0.22 g, 1.00 mmol) and 10 mL of phosphorous oxychloride were mixed together.

The other products were synthesized from the corresponding compounds following the method *B*.

The above reaction mixtures were refluxed on a water bath at 80°C upon monitoring by TLC, then reaction mixture was cooled down to 25°C and poured into crushed ice slowly with constant stirring. The resultant solution was neutralized by 20% KOH and extracted by dichloromethane (2×25 mL). The organic layer was washed with 5% hydrochloric acid (2× 25 mL), 5% sodium hydroxide (2×25 mL), brine solution (1×25 mL), and finally with water (25 mL). The organic layer obtained was dried by anhydrous Na$_2$SO$_4$ and the solvent was removed under reduced pressure. The product was purified by column chromatography using hexane : ethyl acetate (8 : 2) as an eluent to afford the corresponding pure product as a white amorphous solid.

2-(5-Methyl-1,3-diphenyl-1*H*-pyrazol-4-yl)-5-[(quinolin-8-yloxy)methyl]-1,3,4-oxadiazole (11a). IR spectrum, ν, cm^{-1}: 3261 (C–H), 1622 (C=N), 1241 (Ar–O–C), 1068 (N–N). ^{1}H NMR spectrum, δ, ppm: 2.41 s (3H, CH$_3$), 5.35 s (2H, CH$_2$), 7.40–7.60 m (14H, ArH), 8.79–8.80 d (1H, ArH), 8.88–8.90 d. d (1H, ArH, *J* = 1.6, 6.4 Hz). ^{13}C NMR spectrum, δ$_C$, ppm: 9.88 (C[8]), 71.03 (C[19]), 103.54 (C[2]), 106.53 (C[21]), 117.55 (C[23]), 120.24 (C[26]), 124.11 (C[9], C[13]), 126.80 (C[11]), 127.25 (C[22]), 127.59 (C[14], C[18]), 128.57 (C[16]), 129.14 (C[15], C[17]), 129.52 (C[24]), 129.92 (C[10], C[12]), 132.82 (C[7]), 135.13 (C[25]), 137.72 (C[3]), 139.34 (C[6]), 140.68 (C[28]), 147.48 (C[1]), 148.65 (C[27]), 153.41 (C[20]), 163.40 (C[5]), 165.10 (C[4]). HRMS: *m/z*: 460.1227. C$_{28}$H$_{21}$N$_5$O$_2$.

2-{[(5-Chloroquinolin-8-yl)oxy]methyl}-5-(5-methyl-1,3-diphenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (11b). Yield 79%. IR spectrum, ν, cm^{-1}: 3251 (C–H), 1635 (C=N), 1256 (Ar–O–C), 1064 (N–N). ^{1}H NMR spectrum, δ, ppm: 2.44 s (3H, CH$_3$), 5.31 s (2H, CH$_2$), 7.39–7.59 m (13H, ArH), 8.53–8.54 d (1H, ArH), 8.98–8.99 d. d (1H, ArH, *J* = 1.2, 5.2 Hz). ^{13}C NMR spectrum, δ$_C$, ppm: 9.90 (C[8]), 71.32 (C[19]), 103.33 (C[2]), 106.83 (C[21]), 118.90 (C[23]), 122.37 (C[26]), 122.77 (C[9], C[13]), 126.26 (C[11]), 127.39 (C[14], C[18]), 127.82 (C[22]), 128.41 (C[16]), 129.02 (C[15], C[17]), 129.39 (C[24]), 129.60 (C[10], C[12]), 131.94 (C[7]), 132.73 (C[25]), 134.00 (C[3]), 137.56 (C[6]), 139.79 (C[28]), 147.41 (C[1]), 152.72 (C[27]), 154.16

(C^{20}), 162.86 (C^5), 164.25 (C^4). HRMS: *m/z:* 494.0671. $C_{28}H_{20}ClN_5O_2$.

2-{[(5,7-Dichloroquinolin-8-yl)oxy]methyl}-5-(5-methyl-1,3-diphenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (11c). Yield 73%. IR spectrum, ν, cm⁻¹: 3238 (C–H), 1630 (C=N), 1251 (Ar–O–C), 1065 (N–N). ¹H NMR spectrum, δ, ppm: 2.53 s (3H, CH₃), 5.45 s (2H, CH₂), 7.41–7.61 m (12H, ArH), 8.54–8.55 d (1H, ArH), 8.99–9.01 d. d (1H, ArH, *J* = 1.2, 5.2 Hz). ¹³C NMR spectrum, $δ_C$, ppm: 10.01 (C^8), 71.40 (C^{19}), 103.78 (C^2), 107.11 (C^{21}), 120.85 (C^{23}), 122.48 (C^{26}), 122.88 (C^9, C^{13}), 126.37 (C^{11}), 127.47 (C^{14}, C^{18}), 127.90 (C^{22}), 128.52 (C^{16}), 129.11 (C^{15}, C^{17}), 129.54 (C^{24}), 129.72 (C^{10}, C^{12}), 132.04 (C^7), 132.92 (C^{25}), 134.24 (C^3), 137.73 (C^6), 139.80 (C^{28}), 147.63 (C^1), 152.92 (C^{27}), 154.28 (C^{20}), 162.90 (C^5), 164.45 (C^4). HRMS: *m/z:* 527.9884. $C_{28}H_{19}Cl_2N_5O_2$.

2-(5-Methyl-1,3-diphenyl-1*H*-pyrazol-4-yl)-5-{[(2-methylquinolin-8-yl)oxy]methyl}-1,3,4-oxadiazole (11d). Yield 74%. IR spectrum, ν, cm⁻¹: 3241 (C–H), 1628 (C=N), 1242 (Ar–O–C), 1089 (N–N). ¹H NMR spectrum, δ, ppm: 2.36 s (3H, CH₃), 2.68 s (3H, CH₃), 5.42 s (2H, CH₂), 7.41–7.62 m (13H, ArH), 8.79–8.80 d (1H, ArH), 8.87–8.88 d. d (1H, ArH, *J* = 1.6, 6.4 Hz). ¹³C NMR spectrum, $δ_C$, ppm: 9.96 (C^8), 25.32 (C^{29}), 71.12 (C^{19}), 103.63 (C^2), 106.73 (C^{21}), 117.81 (C^{23}), 120.37 (C^{26}), 124.31 (C^9, C^{13}), 126.92 (C^{11}), 127.36 (C^{22}), 127.64 (C^{14}, C^{18}), 128.68 (C^{16}), 129.35 (C^{15}, C^{17}), 129.74 (C^{24}), 130.04 (C^{10}, C^{12}), 133.05 (C^7), 135.35 (C^{25}), 137.67 (C^3), 139.42 (C^6), 140.71 (C^{28}), 147.57 (C^1), 148.81 (C^{27}), 153.62 (C^{20}), 163.58 (C^5), 165.27 (C^4). HRMS: *m/z:* 474.0633. $C_{29}H_{23}N_5O_2$.

2-{[(5,7-Dichloro-2-methylquinolin-8-yl)oxy]methyl}-5-(5-methyl-1,3-diphenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (11e). Yield 70%. IR spectrum, ν, cm⁻¹: 3252 (C–H), 1623 (C=N), 1233 (Ar–O–C), 1073 (N–N). ¹H NMR spectrum, δ, ppm: 2.54 s (3H, CH₃), 2.86 s (3H, CH₃), 5.56 s (2H, CH₂), 7.53–7.73 m (12H, ArH), 8.55–8.56 d (1H, ArH), 8.99–9.00 d. d (1H, ArH, *J* = 1.2, 5.2 Hz). ¹³C NMR spectrum, $δ_C$, ppm: 10.11 (C^8), 24.52 (C^{29}), 71.52 (C^{19}), 103.69 (C^2), 107.23 (C^{21}), 120.79 (C^{23}), 122.46 (C^{26}), 123.08 (C^9, C^{13}), 126.42 (C^{11}), 127.58 (C^{14}, C^{18}), 127.83 (C^{22}), 128.63 (C^{16}), 129.24 (C^{15}, C^{17}), 129.63 (C^{24}), 129.95 (C^{10}, C^{12}), 132.13 (C^7), 132.78 (C^{25}), 134.68 (C^3), 138.06 (C^6), 140.05 (C^{28}), 147.73 (C^1), 153.07 (C^{27}), 154.34 (C^{20}), 162.87 (C^5), 164.36 (C^4). HRMS: *m/z:* 542.0048. $C_{29}H_{21}Cl_2N_5O_2$.

2-{[(5,7-Diiodoquinolin-8-yl)oxy]methyl}-5-(5-methyl-1,3-diphenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole (11f). Yield 64%. IR spectrum, ν, cm⁻¹: 3241 (C–H), 1634 (C=N), 1245 (Ar–O–C), 1066 (N–N). ¹H NMR spectrum, δ, ppm: 2.64 s (3H, CH₃), 5.47 s (2H, CH₂), 7.55–7.75 m (12H, ArH), 8.60–8.61 d (1H, ArH), 8.98–9.99 d. d (1H, ArH, *J* = 1.2, 5.2 Hz). ¹³C NMR spectrum, $δ_C$, ppm: 9.82 (C^8), 71.53 (C^{19}), 76.34 (C^{21}), 85.78 (C^{23}), 102.54 (C^2), 121.62 (C^{26}), 123.11 (C^9, C^{13}), 126.44 (C^{11}), 127.69 (C^{14}, C^{18}), 128.79 (C^{22}), 128.78 (C^{16}), 129.47 (C^{15}, C^{17}), 129.68 (C^{24}), 129.93 (C^{10}, C^{12}), 132.11 (C^7), 132.86 (C^{25}), 134.56 (C^3), 137.88 (C^6), 139.92 (C^{28}), 147.77 (C^1), 153.05 (C^{27}), 154.35 (C^{20}), 162.83 (C^5), 164.67 (C^4). HRMS: *m/z:* 711.8631. $C_{28}H_{19}I_2N_5O_2$.

2-(5-Methyl-3-phenylisoxazol-4-yl)-5-[(quinolin-8-yloxy)methyl]-1,3,4-oxadiazole (11g). IR spectrum, ν, cm⁻¹: 3266 (C–H), 1631 (C=N), 1251 (Ar–O–C), 1069 (N–N). ¹H NMR spectrum, δ, ppm: 2.40 s (3H, CH₃), 5.13 s (2H, CH₂), 7.45–7.55 m (9H, ArH), 8.56–8.58 d. d (1H, ArH, *J* = 1.2, 5.2 Hz), 8.96–8.97 d. d (1H, ArH, *J* = 1.6, 5.6 Hz). ¹³C NMR spectrum, $δ_C$, ppm: 14.81 (C^6), 73.96 (C^{13}), 106.83 (C^{15}), 110.96 (C^2), 113.41 (C^{17}), 120.33 (C^{20}), 124.64 (C^{16}), 124.88 (C^8, C^{12}), 128.46 (C^{10}), 129.02 (C^9, C^{11}), 129.62, (C^{18}), 132.41 (C^7), 140.48 (C^{19}), 148.39 (C^{22}), 150.24 (C^{21}), 153.30 (C^1), 154.91 (C^{14}), 162.36 (C^3), 162.50 (C^5), 163.28 (C^4). HRMS: *m/z:* 385.0467. $C_{22}H_{16}N_4O_3$.

2-{[(5-Chloroquinolin-8-yl)oxy]methyl}-5-(5-methyl-3-phenylisoxazol-4-yl)-1,3,4-oxadiazole (11h). Yield 87% yield. IR spectrum, ν, cm⁻¹: 3258 (C–H), 1623 (C=N), 1243 (Ar–O–C), 1067 (N–N). ¹H NMR spectrum, δ, ppm: 2.13 s (3H, CH₃), 5.45 s (2H, CH₂), 7.46–7.59 m (8H, ArH), 8.57–8.59 d. d (1H, ArH, *J* = 1.6, 6.4 Hz), 9.00–9.01 d. d (1H, ArH, *J* = 1.6, 5.6 Hz). ¹³C NMR spectrum, $δ_C$, ppm: 13.72 (C^6), 72.57 (C^{13}), 106.38 (C^{15}), 110.38 (C^2), 118.46 (C^{17}), 122.34 (C^{20}), 126.16 (C^{16}), 127.61 (C^8, C^{12}), 127.88 (C^{10}), 128.48 (C^9, C^{11}), 128.72 (C^{18}), 131.75 (C^7), 132.87 (C^{19}), 139.84 (C^{22}), 152.80 (C^{21}), 155.19 (C^1), 155.44 (C^{14}), 163.29 (C^3), 164.34 (C^5), 165.59 (C^4). HRMS: *m/z:* 419.0205. $C_{22}H_{15}ClN_4O_3$.

2-{[(5,7-Dichloroquinolin-8-yl)oxy]methyl}-5-(5-methyl-3-phenylisoxazol-4-yl)-1,3,4-oxadiazole (11i). Yield 87%. IR spectrum, ν, cm⁻¹: 3259 (C–H), 1633 (C=N), 1238 (Ar–O–C), 1066 (N–N). ¹H NMR spectrum, δ, ppm: 2.20 s (3H, CH₃), 5.46 s (2H, CH₂), 7.47–7.59 m (7H, ArH), 8.61–8.62 d. d (1H, ArH, *J* = 1.6, 5.6 Hz),

9.03–9.05 d. d (1H, ArH, J = 1.6, 5.6 Hz). ^{13}C NMR spectrum, δ_C, ppm: 14.29 (C^6), 73.07 (C^{13}), 110.38 (C^{15}), 110.44 (C^2), 119.57 (C^{17}), 122.54 (C^{20}), 127.16 (C^{16}), 127.83 (C^8, C^{12}), 127.93 (C^{10}), 128.85 (C^9, C^{11}), 128.93 (C^{18}), 131.80 (C^7), 133.17 (C^{19}), 140.14 (C^{22}), 153.00 (C^{21}), 155.34 (C^1), 155.53 (C^{14}), 164.03 (C^3), 164.54 (C^5), 166.12 (C^4). HRMS: m/z: 452.5274. C$_{22}$H$_{14}$Cl$_2$N$_4$O$_3$.

2-(5-Methyl-3-phenylisoxazol-4-yl)-5-{[(2-methylquinolin-8-yl)oxy]methyl}-1,3,4-oxadiazole (11j). Yield 84%. IR spectrum, ν, cm^{-1}: 3251 (C–H), 1639 (C=N), 1243 (Ar–O–C), 1085 (N–N). ^{1}H NMR spectrum, δ, ppm: 2.22 s (3H, CH$_3$), 2.53 s (3H, CH$_3$), 5.51 s (2H, CH$_2$), 7.16–7.17 d (1H, ArH), 7.53–7.65 m (8H, ArH), 8.42–8.44 d. d (1H, ArH, J = 1.6, 5.6 Hz). ^{13}C NMR spectrum, δ_C, ppm: 12.00 (C^6), 24.42 (C^{23}), 71.89 (C^{13}), 107.12 (C^{15}), 111.13 (C^2), 113.76 (C^{17}), 120.13 (C^{20}), 124.00 (C^{16}), 124.97 (C^8, C^{12}), 128.56 (C^{10}), 128.89 (C^9, C^{11}), 132.76 (C^{18}), 136.57 (C^7), 140.63 (C^{19}), 148.68 (C^{22}), 150.00 (C^{21}), 153.52 (C^1), 155.06 (C^{14}), 162.44 (C^3), 162.86 (C^5), 163.47 (C^4). HRMS: m/z: 399.1336. C$_{23}$H$_{18}$N$_4$O$_3$.

2-{[(5,7-Dichloro-2-methylquinolin-8-yl)oxy]-methyl}-5-(5-methyl-3-phenylisoxazol-4-yl)-1,3,4-oxadiazole (11k). Yield 82%. IR spectrum, ν, cm^{-1}: 3263 (C–H), 1643 (C=N), 1229 (Ar–O–C), 1077 (N–N). ^{1}H NMR spectrum, δ, ppm: 2.23 s (3H, CH$_3$), 2.45 s (3H, CH$_3$), 5.55 s (2H, CH$_2$), 7.21–7.22 d (1H, ArH), 7.46–7.55 m (6H, ArH), 8.61–8.63 d. d (1H, ArH, J = 1.6, 5.6 Hz). ^{13}C NMR spectrum, δ_C, ppm: 14.37 (C^6), 25.26 (C^{23}), 73.12 (C^{13}), 110.47 (C^{15}), 111.14 (C^2), 118.76 (C^{17}), 122.72 (C^{20}), 127.34 (C^{16}), 128.02 (C^8, C^{12}), 128.01 (C^{10}), 128.98 (C^9, C^{11}), 128.79 (C^{18}), 131.91 (C^7), 133.27 (C^{19}), 140.42 (C^{22}), 153.13 (C^{21}), 155.47 (C^1), 155.67 (C^{14}), 164.42 (C^3), 164.97 (C^5), 166.32 (C^4). HRMS: m/z: 466.8572. C$_{23}$H$_{16}$Cl$_2$N$_4$O$_3$.

2-{[(5,7-Diiodoquinolin-8-yl)oxy]methyl}-5-(5-methyl-3-phenylisoxazol-4-yl)-1,3,4-oxadiazole (11l). Yield 77%. IR spectrum, ν, cm^{-1}: 3249 (C–H), 1639 (C=N), 1243 (Ar–O–C), 1072 (N–N). ^{1}H NMR spectrum, δ, ppm: 2.32 s (3H, CH$_3$), 5.39 s (2H, CH$_2$), 7.44–7.56 m (7H, ArH), 8.61–8.62 d. d (1H, ArH, J = 1.6, 5.6 Hz), 8.89–8.91 d. d (1H, ArH, J = 1.6, 5.2 Hz). ^{13}C NMR spectrum, δ_C, ppm: 13.81 (C^6), 73.23 (C^{13}), 76.97 (C^{15}), 86.78 (C^{17}), 110.56 (C^2), 122.76 (C^{20}), 127.86 (C^8, C^{12}), 127.26 (C^{10}), 128.90 (C^9, C^{11}), 128.87 (C^{18}), 131.77 (C^7), 133.47 (C^{19}), 140.38 (C^{22}), 147.42 (C^{16}), 153.15 (C^{21}), 155.47 (C^1), 155.68 (C^{14}), 164.17 (C^3), 164.46 (C^5), 166.23 (C^4). HRMS: m/z: 636.2477. C$_{22}$H$_{14}$I$_2$N$_4$O$_3$: 635.9155.

Molecular docking study. Docking study was performed using Autodock-4.2. For evaluating the best binding sites the study was carried out for the crystal structure of E. coli (complex with Clorobiocin, PDB file 1KZN) by the Lamarckian Genetic Algorithm computational method. To avoid interactions of solvent molecules, the software was calibrated by eliminating water and lower occupancy residues. Hydrogen bond with other docking constants represented the possible proximity of ligands with the receptor.

***In vitro* antibacterial and antifungal activity.** The antimicrobial assay was performed by well-known methods [28]. The target compounds were tested for their antimicrobial activity against Gram-positive (*Staphylococcus aureus* (MTCC 96) and *Bacillus cereus* (MTCC 8372)) and Gram-negative (*Escherichia coli* (MTCC 724), *Klebsiella pneumonia* (MTCC 3384) , and *Shigella flexneri* (MTCC 1457)) bacterial strains using nutrient agar medium by utilizing the paper disc diffusion method. Antifungal activity of synthesized compounds was tested on *Candida albicans* (MTCC 183) and *Aspergillus niger* (MTCC 281) fungal strains by the disc-diffusion method. Chloramphenicol and Fluconazole were used as standard drugs for antimicrobial and antifungal activity respectively. All experiments were done in triplicates and the results were represented in terms of diameter of the zone of inhibition. The MIC values were determined by the liquid dilution method against the same organisms, and absorbance of the test solutions was recorded on a spectrophotometer at 555 nm.

***In vitro* anti-inflammatory activity.** Anti-inflammatory study was carried out by Protein denaturation by bovine serum albumin method [29]. The procedure described by Mamata D N and coworkers was used for preparing the solutions with slight modification [30]. The bovine serum albumin solution was obtained using tris buffer saline and pH adjusted to 6.8 by acetic acid. Series of test samples were prepared in 0.5 mL DMSO by treating different concentrations of target compounds with 0.5 mL of bovine serum albumin. A standard solution containing *Diclofenac sodium* (0.05 mL) and bovine serum albumin (0.05 mL) were used for comparison. The sample solutions were incubated at 37°C (20 min) and then at 57°C (3 min), then cooled down to room temperature and 2.5 mL of phosphate buffer were added. The mixtures were shaken thoroughly, and absorbance

was measured on a UV-Vis spectrophotometer at 620 nm. Protein denaturation in terms of percentage inhibition (I, %) of precipitation was calculate using the following formula:

$$I = [V_c/V_s - 1] \times 100,$$

where V_c—absorbance of control and V_s—absorbance of sample solution.

CONCLUSIONS

The new 1,3,4-oxadiazole bridged quinoline linked pyrazole/isoxazole heterocyclic compounds have been synthesized. The molecular docking study of the final compounds against *E. coli* protein has indicated that those containing pyrazole heterocycle with methyl (CH_3) group at quinoline moiety have high binding energy and demonstrate excellent antimicrobial activity against the majority of the tested strains. Hence, the above templates can be considered as a useful lead for future development of potent drugs.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

SUPPLEMENTARY INFORMATION

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