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Novel 1,3,4-oxadiazole motifs bearing a quinoline nucleus: synthesis, characterization and biological evaluation of their antimicrobial, antitubercular, antimalarial and cytotoxic activities†

Gaurav G. Ladani and Manish P. Patel*

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A series of quinoline based 1,3,4-oxadiazole derivatives (**8a–l**) were synthesized by a chloro-amine coupling reaction approach with different catalysts and solvents. Substituted 1,3,4-oxadiazole intermediates **7a–c** were obtained from 2-substituted-*N*-phenylhydrazinecarbothioamides **6a–c** by cyclization with different cyclizing reagents such as mercuric acetate, lead dioxide, iodobenzenediacetate (IBD) and aqueous sodium hydroxide with iodine in aqueous potassium iodide to identify the most effective reaction conditions, in which iodobenzenediacetate was found to be an extremely good catalyst. The structures of the title compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The synthesized molecules were evaluated for their antibacterial, antifungal, antituberculosis and antimalarial activities. A brine shrimp bioassay was carried out to study the *in vivo* cytotoxic properties of the most highly active compounds of the *in vitro* biological evaluation.

1. Introduction

Malaria remains a significant worldwide health problem, with serious social and economic consequences in affected countries. The problem has been worsened by the emergence and spread of parasites that are resistant to well-established antimalarial drugs.[1] The swelling of malaria in the human body is through five different types of protozoan of the genus *Plasmodium*, but *Plasmodium falciparum* is responsible for most of the critical cases. However, the appearance of *P. falciparum* resistance to these drugs is a serious cause for concern. To reduce the variety of resistant parasites, the WHO has suggested the combined formulation of artemisinins with traditional antimalarial drugs such as lumefantrine, amodiaquine and mefloquine, and ACT (Artemisinin Combination Therapy) is currently approved in multiple countries.[2,3] For this reason, the development of new, effective, nontoxic, and inexpensive antimalarial drugs is a high priority in medicinal chemistry.

Tuberculosis, which is one of the deadliest infectious diseases caused predominantly by *Mycobacterium tuberculosis* (Mtb), kills 1.4 million people annually and has shown a rapid increase in cases resistant to multiple drugs. Furthermore, the increase of TB in conjunction with AIDS has exacerbated the problems associated with its cure, because both diseases (TB and AIDS) accelerate each other's progression.[4]The World Health Organization (WHO) has identified TB as one of the three priority diseases for drug research and development, underlining the importance of the discovery process in identifying new, urgently needed antibiotics against tuberculosis.

1,3,4-Oxadiazole is a bioactive motif in medicinal chemistry.[5] The general use of this motif as a core moiety in medicinal chemistry establishes it as a member of the privileged structures. Substituted 1,3,4-oxadiazoles are some of the most important heterocyclic compounds, and they have gained attention because of their remarkable biological and pharmacological properties.[6–15] Consequently, 1,3,4-oxadiazoles are the target of various drug discovery programs as analgesic, antimalarial, and antidepressant agents, and for other activities.[16,17] They have also attracted interest in medicinal chemistry as bioisosteres for carboxylic acids, esters, and carboxamides, which contribute substantially to increasing pharmacological activity by participating in hydrogen bonding interactions with receptors.[18] A number of molecules based upon this monocyclic heterocyclic template have been investigated for their anti-inflammatory activity.[19,20]

Quinolines represent an essential group of heterocyclic compounds as they are pivotal skeletons in many biologically active natural products as well as various pharmacologically interesting compounds.[21–24] Quinoline-containing compounds have been extensively used in medicinal chemistry and they have a broad range of biological activities, such as anti-inflammatory,[25]

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India. E-mail: patelmanish1069@yahoo.com; Tel: +91 02692 226856 ext. 212

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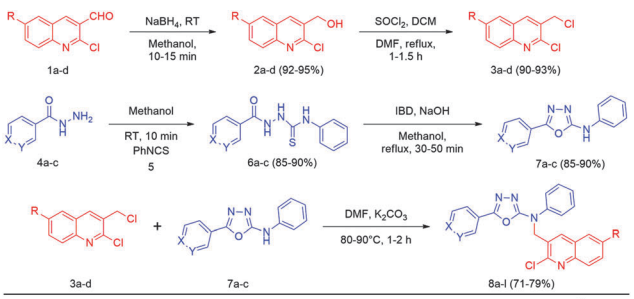
antimalarial,[26–31] anticancer,[32] analgesic,[33] and antifungal.[34] Quinoline scaffolds have been selected due to their diverse therapeutic and pharmacological properties, such as antitumor, antiatherosclerotic, vasodilator, geroprotective, bronchodilator and hepatoprotective activity.[35]

Based on these observations, the challenge of developing a novel series of bioactive molecules by coupling 1,3,4-oxadiazole with substituted quinoline moieties using a molecular hybridization approach has been proposed. We have synthesized quinoline based 1,3,4-oxadiazole scaffolds and evaluated their antibacterial, antifungal, antitubercular and antimalarial activities. Furthermore we also evaluated the effect of different substitutions on the biological activity.

2. Chemistry

The synthetic approach adopted to obtain the targeted quinoline nucleus incorporated 1,3,4-oxadiazole derivatives is depicted in Scheme 1. The starting materials 6-(un)substituted-2-chloro-3-(chloromethyl)quinolines **3a–d** were prepared by reduction followed by chlorination of 6-(un)substituted-2-chloroquinoline-3-carbaldehydes **1a–d**,[36] and the other starting materials, *N*-phenyl-5-(substituted)-1,3,4-oxadiazol-2-amines **7a–c**, were prepared by mixing different hydrazides **4a–c** with phenylisothiocyanate **5**, and then refluxing with different cyclizing reagents such as mercuric acetate, lead dioxide, chloramine-T, iodobenzene-diacetate and aqueous sodium hydroxide with iodine in aqueous potassium iodide.

For the screening of different cyclizing reagents we selected a model reaction between isonicotinohydrazide **4a** and phenyl-isothiocyanate **5** (Scheme 2). The results are summarized in Table 1. The final molecules *N*-((2-chloro-6-(un)substituted-quinolin-3-yl)methyl)-*N*-phenyl-5-(substituted)-1,3,4-oxadiazol-2-amines **8a–l** were synthesized using chloro-amine coupling reactions between **3a–d** and **7a–c**. The chloro-amine coupling reaction of **3a** and **7a** was used as a model reaction to optimize the reaction conditions (Scheme 3), and the results are summarized in Table 2.



Entry	R	X	Y	Yield[a] (%)
3a	H	-	-	93
3b	CH_3	-	-	91
3c	OCH_3	-	-	91
3d	Cl	-	-	90
7a	-	N	CH	85
7b	-	CH	N	87
7c	-	CH	CH	90
8a	H	N	CH	77
8b	H	CH	N	74
8c	H	CH	CH	78
8d	CH_3	N	CH	71
8e	CH_3	CH	N	78
8f	CH_3	CH	CH	74
8g	OCH_3	N	CH	72
8h	OCH_3	CH	N	75
8i	OCH_3	CH	CH	78
8j	Cl	N	CH	73
8k	Cl	CH	N	76
8l	Cl	CH	CH	79

[a] Isolated yields.

Scheme 1 Synthesis of *N*-((2-chloro-6-(un)substituted-quinolin-3-yl)methyl)-*N*-phenyl-5-(substituted)-1,3,4-oxadiazol-2-amines **8a–l**.

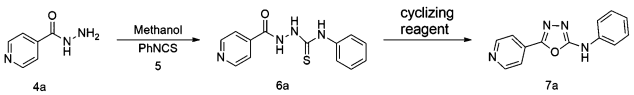
3. Pharmacology

3.1. *In vitro* antimicrobial activity

The *in vitro* antimicrobial screening of targeted compounds **8a–l** at minimal inhibitory concentration (MIC) in millimolar (mM) was carried out using the broth microdilution method according to the National Committee for Clinical Laboratory Standards (NCCLS).[37–39] The antibacterial activity was tested against three Gram positive (*Streptococcus pneumoniae* MTCC 1936, *Bacillus subtilis* MTCC 441 and *Clostridium tetani* MTCC 449) and three Gram negative (*Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 98, and *Vibrio cholera* MTCC 3906) bacteria using ampicillin, ciprofloxacin, norfloxacin and chloramphenicol as the reference antibacterial drugs. The antifungal activity was screened against two fungal species (*Candida albicans* MTCC 227 and *Aspergillus fumigatus* MTCC 3008) where nystatin and griseofulvin were used as the reference antifungal agents. The strains employed for the activity screening were gathered from the Institute of Microbial Technology, Chandigarh (MTCC – Micro Type Culture Collection). Mueller Hinton broth was used as the nutrient medium to grow and dilute the drug suspension for the test. The results of the antimicrobial screening are shown in Table 3.

3.2. *In vitro* antituberculosis activity

The encouraging results from the antibacterial activity screening provoked us to carry out preliminary screening of the title compounds for their *in vitro* antituberculosis activity. Primary screening of targeted compounds **8a–l** was performed at 250 μg mL^{-1} against the *Mycobacterium tuberculosis* H37Rv strain using Lowenstein–Jensen medium (the conventional method) as described by Rattan.[38–40] The acquired results are presented in Table 4 in the form of % inhibition. Rifampicin and isoniazid were used as the reference drugs. From these results the four compounds

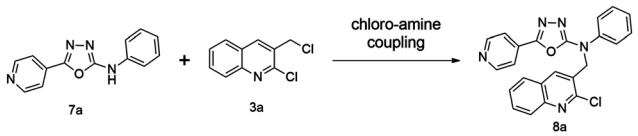


Scheme 2 Optimization of the reaction conditions for the synthesis of substituted 1,3,4-oxadiazole **7a**.

Table 1 The influence of different cyclizing reagents on the model reaction under different conditions

Entry[a]	Catalyst	Base	Solvent	Time[b] (h)	Yield[c] (%)
1	Conc. H_2SO_4	—	Acetic acid	5	40
2	Chloramine-T	—	Ethanol	5	60
3	IBD	—	MDC	4	45
4	IBD	—	Methanol	3	45
5	**IBD**	**NaOH**	**Methanol**	**1**	**85**
6	IBD	Na_2CO_3	Methanol	2	52
7	IBD	$NaHCO_3$	Methanol	4	45
8	IBD	Et_3N	Ethanol	3	70
9	IBD	K_2CO_3	Ethanol	3	65

[a] Reaction of isonicotinohydrazide **4a** (1 mmol) with phenylisothiocyanate **5** (1 mmol) in the presence of 1 mmol of catalyst. [b] Reaction progress monitored by TLC. [c] Isolated yield.



Scheme 3 Screening of catalysts for the chloro-amine coupling reaction.

Table 2 The influence of different reaction conditions on the chloro-amine coupling reaction

Entry[a]	Base	Solvent	Temperature (°C)	Time[b] (h)	Yield[c] (%)
1	NaH	THF	RT	12	Trace
2	NaH	DCM	RT	12	20
3	TEA	DCM	RT	12	30
4	K_2CO_3	Acetone	50–55	5	50
5	K_2CO_3	DMF	RT	12	45
6	**K_2CO_3**	**DMF**	**80–90**	**1**	**77**
7	TEA	Ethanol	70–75	4	55
8	—	*n*-Butanol	100	5	Trace
9	TEA	*n*-Butanol	100	5	40

[a] Reaction of 2-chloro-3-(chloromethyl)quinoline **3a** (1 mmol) with *N*-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine **7a** (1 mmol) in the presence of 2 mmol of catalyst. [b] Reaction progress monitored by TLC. [c] Isolated yield.

that exhibited the highest % inhibition were screened again to get their MIC values (Table 5).

3.3. *In vitro* antimalarial activity

All the target compounds (**8a–l**) were screened for their *in vitro* antimalarial activity against the *P. falciparum* strain using chloroquine and quinine as the reference compounds. The results of the antimalarial screening are expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth and are listed in Table 6.

3.4. Cytotoxicity (brine shrimp lethality bioassay)

An *in vivo* lethality test was done using brine shrimp eggs *i.e. Artemia* cysts. The brine shrimp lethality bioassay is well thought out as a useful tool for preliminary toxicity assessment of bioactive compounds. Active compounds, those which exhibited the highest % inhibition, were again screened for their cytotoxicity using the protocol of Meyer *et al.*[41] The corresponding LC_{50} values of all the compounds are listed in Table 7.

4. Results and discussion

4.1. Optimization of synthetic protocol

4.1.1. Screening of catalysts to optimize the reaction conditions for substituted 1,3,4-oxadiazoles 7a–c. We began our investigation with thiosemicarbazide **6a**, which was synthesized *via* the reaction of isonicotinohydrazide **4a** and phenylisothiocyanate **5**. Thiosemicarbazides **6a–c** were prepared in good to high yields (85–90%) by mixing equimolar amounts of the corresponding hydrazides and phenylisothiocyanate in methanol at room temperature for 30 minutes. When thiosemicarbazides are used as oxadiazole precursors, H_2S scavengers, such as stoichiometric mercuric salts[42] or lead oxide[43] can be used to affect the cyclization. Other desulfurization reagents including I_2/NaOH[44] and tosyl chloride[45] have been utilized, but they often lead to inconvenient handling and undesirable by-products. Different coupling reagents are used for the final cyclization including DIC and DCC as carbodiimides, and TBTU as a uronium coupling reagent.[46] From an industrial and environmental point of view, an effective coupling reagent should have the following advantages: (a) high efficiency, (b) ability to work in stoichiometric quantities, and (c) solubility in the solvents used. Considering these characteristics, IBD is one of the common coupling reagents and additives used in cyclization chemistry.[47–50]

For optimization of the reaction conditions, initially the reaction in acetic acid in the presence of concentrated H_2SO_4 as the catalyst afforded the desired product **7a** in a 40% yield (entry 1), but this is a lower yield than reported in the literature. Then we tried using chloramine-T as the cyclizing reagent for the 1,3,4-oxadiazole synthesis, as reported in the literature;[51] in this case we also obtained a lower yield (entry 2). After further investigation of various catalysts, IBD was found to be more effective than other catalysts such as chloramine-T, lead dioxide and mercuric acetate, and the reaction yield increased up to 85% when IBD was employed (entries 3–9). Several bases such as Na_2CO_3, $NaHCO_3$, NaOH, Et_3N and K_2CO_3 were examined, but NaOH was found to give the best result in the reaction media compared to other bases (entry 5). In addition, lower yields were observed if the reaction was performed without base or with weak bases (entries 3, 4, 6 and 7).

Due to these results, IBD was selected as the coupling reagent for the synthesis of the 1,3,4-oxadiazoles, and thus the reaction was carried out in a single step by heating different hydrazides **4a–c** and phenylisothiocyanate **5** at 50–60 °C in methanol in the presence of NaOH as the base and IBD (iodobenzenediacetate) as the coupling reagent, to produce the desired 2-amino-1,3,4-oxadiazoles **7a–c**. The results are summarized in Table 1.

4.1.2. Optimization of reaction conditions for quinoline incorporated 1,3,4-oxadiazoles 8a–l. The reaction of *N*-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine **7a** with 2-chloro-3-(chloromethyl)quinoline **3a** was selected as a model reaction to

Table 3 *In vitro* antimicrobial activity (MIC, mM) of compounds **8a–l**

	Gram-positive bacteria			Gram-negative bacteria			Fungi	
Entry	S.P. MTCC 1936	C.T. MTCC 449	B.S. MTCC 441	S.T. MTCC 98	V.C. MTCC 3906	E.C. MTCC 443	C.A. MTCC 227	A.F. MTCC 3008
8a	**0.241**	**0.604**	**0.604**	0.604	**0.241**	1.208	**1.208**	2.416
8b	**0.241**	**0.241**	**0.151**	1.208	**0.241**	0.604	>2.416	1.208
8c	0.484	**0.242**	**0.242**	0.484	**0.242**	0.605	2.422	>2.422
8d	0.467	1.168	1.168	**0.233**	0.467	**0.146**	**0.584**	2.337
8e	0.292	**0.467**	1.168	0.292	**0.233**	**0.233**	**0.584**	1.168
8f	0.468	**0.292**	**0.468**	0.585	0.292	0.585	2.342	2.342
8g	0.563	**0.450**	**0.225**	0.563	0.450	0.563	>2.252	2.252
8h	1.126	**0.563**	**0.450**	**0.225**	1.126	**0.140**	**1.126**	1.126
8i	0.451	**0.225**	**0.282**	0.451	0.451	0.451	2.257	>2.257
8j	0.446	**0.557**	**0.557**	0.557	0.446	0.557	>2.230	2.230
8k	0.446	**0.446**	**0.139**	**0.223**	0.557	**0.223**	>2.230	>2.230
8l	**0.223**	**0.447**	**0.223**	**0.279**	**0.223**	0.447	2.235	>2.235
A	0.286	0.715	0.715	0.286	0.286	0.286	n.t.[a]	n.t.
B	0.154	0.154	0.154	0.154	0.154	0.154	n.t.	n.t.
C	0.150	0.301	0.150	0.075	0.075	0.075	n.t.	n.t.
D	0.031	0.313	0.310	0.031	0.031	0.031	n.t.	n.t.
E	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.107	0.107
F	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	1.417	0.283

S.P.: *Streptococcus pneumoniae*, C.T.: *Clostridium tetani*, B.S.: *Bacillus subtilis*, S.T.: *Salmonella typhi*, V.C.: *Vibrio cholera*, E.C.: *Escherichia coli*, C.A.: *Candida albicans*, A.F.: *Aspergillus fumigatus*, MTCC: Microbial Type Culture Collection. A: ampicillin, B: chloramphenicol, C: ciprofloxacin, D: norfloxacin, E: nystatin, F: griseofulvin.[a] n.t.: not tested.

Table 4 *In vitro* antituberculosis activity (% inhibition) of quinoline based 1,3,4-oxadiazole derivatives **8a–l** against *M. tuberculosis* H37Rv (at a concentration of 250 μg mL^{-1})

Entry	Inhibition (%)	Entry	Inhibition (%)
8a	**98**	**8h**	32
8b	54	**8i**	89
8c	86	**8j**	**96**
8d	40	**8k**	**95**
8e	**96**	**8l**	80
8f	52	Isoniazid	99
8g	65	Rifampicin	98

Table 5 *In vitro* antituberculosis activity of the title compounds exhibiting higher % inhibition against *M. tuberculosis* H37Rv (MICs, mM)

Entry	Inhibition (%)	MIC (mM)
8a	98	0.060
8e	96	0.146
8j	96	0.111
8k	95	0.223
Rifampicin	98	0.048
Isoniazid	99	0.001

Table 6 *In vitro* antimalarial activity of compounds **8a–l**

Entry	IC_{50} (μM)	Entry	IC_{50} (μM)
8a	4.470	**8h**	**0.202**
8b	**0.386**	**8i**	3.793
8c	1.525	**8j**	**0.089**
8d	3.388	**8k**	**0.156**
8e	**0.467**	**8l**	1.630
8f	3.654	Chloroquine	0.062
8g	4.685	Quinine	0.826

optimize the reaction conditions (Scheme 2), and the results are summarized in Table 2. Subsequently, with the intention of finding suitable conditions for the reaction, we tested different catalysts and solvents for the synthesis of desired products **8a–l**. For the initial feasibility studies, **7a** was subjected to reaction with **3a** in THF using NaH as the catalyst at room temperature which produced **8a** in a trace amount (entry 1). Further to this, we tested the chloro-amine coupling reaction with NaH in DCM as the solvent which gave the required product in a 20% yield after 12 h (entry 2). Next, the same reaction was tested in DCM using TEA as the catalyst; in this case we got a 30% yield (entry 3). We also used TEA with ethanol or *n*-butanol as the solvent, and the reaction proceeded in a forward direction, but the yields were not much higher (entries 7 and 9). These observations forced us to screen different catalysts. In our attempts, we studied K_2CO_3 as the catalyst with different solvents, and the results are summarized in Table 2 (entries 4–6).

After the extensive screening of different catalysts, we concluded that K_2CO_3 was the most appropriate catalyst with DMF as the solvent for the chloro-amine coupling reaction, which resulted in a high yield of the product in the shortest reaction time (entry 6). Consequently, the present protocol avoids the use of expensive metallic catalysts and harsh reaction conditions.

4.2. Analytical results

The structures of all the synthesized compounds were confirmed by ^{1}H NMR, FT-IR, ^{13}C NMR, mass spectrometry and elemental analysis. The ^{1}H NMR spectra of compounds **8a–l** show signals for N–CH_2 protons (methylene protons at the quinoline ring) as a sharp singlet at around δ 5.39–5.48. The aromatic protons resonate as multiplets at around δ 7.23–8.99 ppm. The IR spectra of compounds **8a–l** exhibited a characteristic absorption band at around 1605–1659 cm^{-1} which was attributed to the

Table 7 Effect of compounds on the brine shrimp lethality bioassay

Entry	Concentration (μg mL^{-1})	Log (conc.)	No. of nauplii taken	No. of nauplii dead	% of mortality	LC_{50} (μg mL^{-1})	LC_{50} (mM)
8a	5	0.699	10	0	0	37.77	0.091
	10	1.000	10	1	10		
	20	1.301	10	2	20		
	30	1.477	10	4	40		
	40	1.602	10	5	50		
	50	1.699	10	7	70		
8b	5	0.699	10	1	10	19.78	0.047
	10	1.000	10	2	20		
	20	1.301	10	4	40		
	30	1.477	10	6	60		
	40	1.602	10	8	80		
	50	1.699	10	9	90		
8e	5	0.699	10	2	20	14.10	0.032
	10	1.000	10	4	40		
	20	1.301	10	6	60		
	30	1.477	10	7	70		
	40	1.602	10	8	80		
	50	1.699	10	9	90		
8h	5	0.699	10	0	0	35.60	0.080
	10	1.000	10	1	10		
	20	1.301	10	3	30		
	30	1.477	10	4	40		
	40	1.602	10	5	50		
	50	1.699	10	7	70		
8j	5	0.699	10	0	0	34.59	0.077
	10	1.000	10	1	10		
	20	1.301	10	2	20		
	30	1.477	10	4	40		
	40	1.602	10	6	60		
	50	1.699	10	7	70		
8k	5	0.699	10	3	30	11.76	0.026
	10	1.000	10	4	40		
	20	1.301	10	6	60		
	30	1.477	10	8	80		
	40	1.602	10	9	90		
	50	1.699	10	9	90		
Etoposide	—	—	—	—	—	7.46	0.012

presence of C═N stretching of the 1,3,4-oxadiazole ring. A strong absorption band was observed in the range of 1225–1242 cm^{-1} due to C–O–C stretching. The characteristic absorption band in the range of 1033–1096 cm^{-1} may be attributed to the symmetric stretching of nitrogen–nitrogen in the 1,3,4-oxadiazole ring. In the ^{13}C NMR spectra, the C-2 carbon of the 1,3,4-oxadiazole ring was displayed very downfield at δ 161.8–163.5 ppm for the reason that it was in between one oxygen atom and two nitrogen atoms. The signals at around δ 52.4–53.2 ppm were assigned to the methylene carbon of quinoline at the 3-position attached to the nitrogen of the 1,3,4-oxadiazole at the 2-position. The mass spectra of all the compounds showed molecular ion peaks at M^+ corresponding to their molecular weights, which confirmed the respective chemical structures.

4.3. Biological section

4.3.1. Antibacterial activity. Analysis of the antibacterial screening data (Table 3) revealed that all the compounds **8a–l** demonstrate moderate to very good inhibitory activity. Compounds **8b** (0.151 mM) and **8k** (0.139 mM) showed the highest activity against *B. subtilis*. The majority of the compounds displayed excellent activity towards Gram positive bacteria *B. subtilis* and *C. tetani* compared to ampicillin, while compounds **8a** and **8j** showed similar potency to that of the standard drugs against *C. tetani* and *B. subtilis*. Compounds **8a**, **8b** and **8l** displayed comparatively good activity against *S. pneumonia*. Compounds **8d**, **8h**, **8k** and **8l** showed similar potency to that of standard drugs against *S. typhi*, while compounds **8a**, **8b**, **8c**, **8e** and **8l** showed comparable potency to that of standard drugs against *V. cholera*. Compounds **8d** (0.143 mM) and **8h** (0.140 mM) showed the highest activity in inhibiting the Gram negative bacterium *E. coli*. The remaining compounds are moderately or less active against all the Gram positive and Gram negative bacteria.

4.3.2. Antifungal activity. The results of the antifungal study (Table 3) of the synthesized quinoline based 1,3,4-oxadiazole derivatives revealed that all the compounds have poor activity against *A. fumigatus*, while in comparison with the standard

fungicidal griseofulvin, compounds **8d** and **8e** displayed excellent antifungal activity against *C. albicans*. Although compounds **8a** and **8h** illustrated similar potency towards *C. albicans*, the remaining compounds showed weaker antifungal potency than nystatin and griseofulvin.

4.3.3. Antituberculosis activity. Obtaining some excellent results in the antimicrobial studies encouraged us to go for a preliminary screening of the title compounds for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv bacteria. The bioassay results obtained for the efficacy of all the synthesized analogues against *M. tuberculosis* H37Rv are summarized in Table 4. The outcome of the tests revealed that compounds **8a**, **8e**, **8j** and **8k** were found to possess outstanding activity (*i.e.* 98%, 96%, 96% and 95% at 250 μg mL^{-1}, respectively) against *M. tuberculosis* H37Rv. Compound **8a** shows moderate potency against *M. tuberculosis* (*i.e.* MIC = 0.060 mM) compared to rifampicin (*i.e.* MIC = 0.048 mM) at 98% inhibition. Compounds **8e** (MIC = 0.146 mM) and **8j** (MIC = 0.111 mM) at 96% inhibition and compound **8k** (MIC = 0.223 mM) at 95% inhibition displayed less activity towards *M. tuberculosis* (Table 5). Compound **8a** emerged as the most potent member of the series and opens up a new door to optimize this series as a new class of antituberculer agents.

4.3.4. Antimalarial activity. All the synthesized compounds (**8a–l**) were evaluated for their antimalarial activity against a chloroquine and quinine sensitive strain of *P. falciparum*. All experiments were performed in duplicate and mean values of IC_{50} are reported in Table 6. As shown in Table 6, compounds **8b**, **8e**, **8h**, **8j** and **8k** were found to have IC_{50} values in the range of 0.089 to 0.467 μM against the *P. falciparum* strain. These compounds displayed excellent activity against the *P. falciparum* strain compared to quinine (IC_{50} = 0.826 μM). Furthermore, compound **8j** was found to possess moderate activity (*i.e.* IC_{50} = 0.089 μM) compared to chloroquine. All the remaining compounds were found to be less active against chloroquine sensitive strains of *P. falciparum*.

4.3.5. Cytotoxicity (brine shrimp lethality bioassay). The LC_{50} values obtained for the six compounds that exhibited the highest anti tubercular activity and anti malarial activity are shown in Table 7. These six compounds were found to be less toxic than the standard drug etoposide. Compounds **8a** (LC_{50} = 0.091 mM), **8h** (LC_{50} = 0.080 mM) and **8j** (LC_{50} = 0.077 mM) exhibited comparatively less toxicity; compounds **8b** (LC_{50} = 0.047 mM), **8e** (LC_{50} = 0.032 mM) and **8k** (LC_{50} = 0.026 mM) possessed moderate toxicity when compared to all the tested compounds.

5. Conclusion

Some magnificent results have been obtained with the quinoline–oxadiazole hybridized scaffold. Furthermore, this work helps to validate the choice of the quinoline based 1,3,4-oxadiazole scaffold as a useful template for designing new antimicrobial, antitubercular and antimalarial compounds. This synthetic approach allows the inclusion of potent bioactive nuclei in a single scaffold in an easy way. Compounds **8a**, **8e**, **8j** and **8k** exhibited good antituberculosis activity. The majority of the compounds showed excellent activity against *P. falciparum* strains compared to quinine. Compound **8k** emerged as the most promising antimicrobial member of the series, showing better antitubercular and antimalarial activities, and lower toxicity. Consequently, such a type of compound would represent a fertile matrix for the further development of more biologically potent agents and deserves further investigation and derivatization in order to discover the scope and limitations of its biological activities.

6. Experimental section

6.1. Chemistry

All reactions were performed with commercially available reagents which were used without further purification. The solvents used were of analytical grade. All reactions were monitored by thin-layer chromatography (TLC) on aluminium plates coated with silica gel 60 F_{254}, of 0.25 mm thickness (Merck). Detection of the components was made by exposure to iodine vapour or UV light. Melting points were taken using the melting point apparatus μThermoCal$_{10}$ (Analab Scientific Pvt. Ltd, India) and are uncorrected. Mass spectra were recorded using a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under the PURSE program of DST at Sardar Patel University, Vallabh Vidyanagar, India. The IR spectra were recorded using a FTIR MB 3000 spectrometer (ABB Bomem Inc., Canada/Agaram Industries, Chennai) using Zn–Se optics (490–8500 cm^{-1}) and only the characteristic peaks are reported in cm^{-1}. ^1H and ^{13}C Nuclear Magnetic Resonance spectra were recorded in DMSO-d_6 using a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd, Switzerland) using the residual solvent signal as an internal standard at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). Splitting patterns were designated as follows: s, singlet; d, doublet; dd, doublet of doublets and m, multiplet. The elemental analysis was carried out using a Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) and all compounds are within ±0.4% of the theoretical compositions.

6.1.1. Synthesis of the *N*-phenyl-5-substituted-1,3,4-oxadiazol-2-amines (7a–c). Substituted hydrazides **4a–c** (10 mmol), phenylisothiocyanate **5** (10 mmol) and methanol (5 mL) were transferred to a 50 mL round bottom flask equipped with a mechanical stirrer and a condenser. The reaction mixture was stirred at ambient temperature for 10 min. After the addition of IBD (10 mmol) to the reaction mixture, it was refluxed for 30–50 min. After completion of the reaction, checked by TLC, the reaction mixture was cooled to room temperature to obtain a solid. The solid was filtered and washed with cold methanol and recrystallized from methanol to obtain substituted 1,3,4-oxadiazoles **7a–c**.

*6.1.1.1. N-Phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**7a**).* Yield 85%; white solid; m.p. 216 °C; IR (ν_{max}, cm^{-1}): 3263, 3058, 1617, 1586, 1501, 1242; ^1H NMR (400 MHz, DMSO-d_6): 7.03 (t, 1H, J = 7.6 Hz), 7.37 (t, 2H, J = 7.6 Hz), 7.62 (d, 2H, J = 7.6 Hz), 7.79 (d, 2H, J = 6.0 Hz), 8.77 (d, 2H, J = 6.0 Hz), 10.86 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 117.1, 118.9, 121.9, 128.8, 130.5,

138.1, 150.4, 155.9, 160.2; ESI-MS (*m*/*z*): calcd 238.24, found 238.08 (M^+); anal. calcd (%) for $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found: C, 65.82; H, 3.96; N, 23.41.

*6.1.1.2. N-Phenyl-5-(pyridin-3-yl)-1,3,4-oxadiazol-2-amine (**7b**).* Yield 87%; white solid; m.p. 220 °C; IR (ν_{max}, cm^{-1}): 3268, 3061, 1611, 1590, 1507, 1239; ^1H NMR (400 MHz, DMSO-d_6): 7.05 (t, 1H, *J* = 7.2 Hz), 7.40 (t, 2H, *J* = 8.0 Hz), 7.55–8.69 (m, 6H), 10.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 117.2, 122.0, 124.1, 124.6, 129.2, 131.1, 138.8, 150.1, 156.8, 160.5; ESI-MS (*m*/*z*): calcd 238.24, found 238.08 (M^+); anal. calcd (%) for $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found: C, 65.78; H, 4.02; N, 23.38.

*6.1.1.3. N,5-Diphenyl-1,3,4-oxadiazol-2-amine (**7c**).* Yield 90%; white solid; m.p. 213 °C; IR (ν_{max}, cm^{-1}): 3262, 3053, 1620, 1604, 1579, 1501, 1243; ^1H NMR (400 MHz, DMSO-d_6): 7.02 (t, 1H, *J* = 7.6 Hz), 7.37 (t, 2H, *J* = 7.6 Hz), 7.56–7.60 (m, 3H), 7.63 (d, 2H, *J* = 8.4 Hz), 7.89–7.92 (m, 2H), 10.69 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 117.1, 121.9, 123.9, 125.5, 129.1, 129.4, 131.0, 138.6, 157.7, 159.9; ESI-MS (*m*/*z*): calcd 237.26, found 237.05 (M^+); anal. calcd (%) for $C_{14}H_{11}N_3O$: C, 70.87; H, 4.67; N, 17.71. Found: C, 70.62; H, 4.11; N, 17.02.

6.1.2. Synthesis of *N*-((2-chloro-6-(un)substitutedquinolin-3-yl)methyl)-*N*-phenyl-5-substituted-1,3,4-oxadiazol-2-amines (8a–l). 2-Chloro-3-(chloromethyl)-6-(un)substitutedquinolines **3a–d** (1 mmol), *N*-phenyl-5-substituted-1,3,4-oxadiazol-2-amines **7a–c** (1 mmol) and anhydrous potassium carbonate (2 mmol) in dimethylformamide (5 mL) were transferred to a 50 mL round bottom flask equipped with a mechanical stirrer and a condenser. The reaction mixture was heated at 80–90 °C for 1–2 h and the progress of the reaction was monitored by TLC. After completion of the reaction (as evidenced by TLC), the reaction mixture was cooled to room temperature and then poured into ice cold water (50 mL) with continuous stirring followed by neutralization with 1 N HCl to pH 7. The separated precipitates of *N*-((2-chloro-6-(un)substitutedquinolin-3-yl)methyl)-*N*-phenyl-5-substituted-1,3,4-oxadiazol-2-amines **8a–l** were filtered, thoroughly washed with water, dried, and recrystallized from chloroform : methanol (1 : 1). The physicochemical and spectroscopic characterization data of the synthesized compounds **8a–l** are given below.

*6.1.2.1. N-((2-Chloroquinolin-3-yl)methyl)-N-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**8a**).* Yield 77%; brown solid; m.p. 238 °C; IR (ν_{max}, cm^{-1}): 1645 (C═N str.), 1235 (C–O–C str.), 1072 (N–N str.), 746 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 5.45 (s, 2H, N–CH_2), 7.27 (t, 1H, *J* = 7.2 Hz), 7.45 (t, 2H, *J* = 8.0 Hz), 7.61–7.73 (m, 5H), 7.80 (t, 1H, *J* = 7.8 Hz), 7.95 (d, 1H, *J* = 8.4 Hz), 8.02 (d, 1H, *J* = 8.4 Hz), 8.53 (s, 1H), 8.74 (d, 2H, *J* = 4.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 52.9 (N–CH_2), 119.5, 124.7, 126.9, 127.3, 127.9, 128.1, 128.5, 129.9, 131.2, 131.3, 138.1, 141.1, 146.7, 149.3, 151.2, 157.6, 163.5; ESI-MS (*m*/*z*): calcd 413.86, found 413.70 (M^+); anal. calcd (%) for $C_{23}H_{16}ClN_5O$: C, 66.75; H, 3.90; N, 16.92. Found: C, 66.55; H, 3.97; N, 16.95.

*6.1.2.2. N-((2-Chloroquinolin-3-yl)methyl)-N-phenyl-5-(pyridin-3-yl)-1,3,4-oxadiazol-2-amine (**8b**).* Yield 74%; light yellow solid; m.p. 244 °C; IR (ν_{max}, cm^{-1}): 1606 (C═N str.), 1236 (C–O–C str.), 1096 (N–N str.), 755 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 5.45 (s, 2H, N–CH_2), 7.25 (t, 1H, *J* = 7.2 Hz), 7.44 (t, 2H, *J* = 8.0 Hz), 7.55–7.69 (m, 4H), 7.78–7.82 (m, 1H), 7.95 (d, 1H, *J* = 8.4 Hz), 8.02 (d, 1H, *J* = 8.0 Hz), 8.16 (dd, 1H, *J* = 2.0 Hz, *J* = 6.4 Hz), 8.51 (s, 1H), 8.71 (dd, 1H, *J* = 1.6, *J* = 4.8 Hz), 8.99 (d, 1H, *J* = 2.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 52.7 (N–CH_2), 119.8, 124.1, 126.4, 127.6, 128.0, 128.4, 128.7, 129.2, 131.3, 131.9, 136.2, 142.5, 145.6, 147.8, 150.1, 152.4, 157.8, 163.1; ESI-MS (*m*/*z*): calcd 413.86, found 413.20 (M^+); anal. calcd (%) for $C_{23}H_{16}ClN_5O$: C, 66.75; H, 3.90; N, 16.92. Found: C, 66.61; H, 3.94; N, 16.85.

*6.1.2.3. N-((2-Chloroquinolin-3-yl)methyl)-N,5-diphenyl-1,3,4-oxadiazol-2-amine (**8c**).* Yield 78%; white solid; m.p. 249 °C; IR (ν_{max}, cm^{-1}): 1609 (C═N str.), 1230 (C–O–C str.), 1093 (N–N str.), 763 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 5.45 (s, 2H, N–CH_2), 7.31 (dd, 1H, *J* = 4.8 Hz, *J* = 9.2 Hz), 7.57–7.84 (m, 11H), 7.90–8.06 (m, 2H), 8.27 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 52.4 (N–CH_2), 119.6, 122.1, 122.3, 123.6, 124.3, 125.1, 125.4, 127.3, 128.9, 131.0, 131.8, 134.1, 136.1, 142.3, 144.1, 147.3, 158.5, 163.3; ESI-MS (*m*/*z*): calcd 412.87, found 412.30 (M^+); anal. calcd (%) for $C_{24}H_{17}ClN_4O$: C, 69.82; H, 4.15; N, 13.57. Found: C, 69.91; H, 4.07; N, 13.47.

*6.1.2.4. N-((2-Chloro-6-methylquinolin-3-yl)methyl)-N-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**8d**).* Yield 71%; pale yellow solid; m.p. 233 °C; IR (ν_{max}, cm^{-1}): 1605 (C═N str.), 1230 (C–O–C str.), 1096 (N–N str.), 749 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 2.45 (s, 3H, Ar–CH_3), 5.43 (s, 2H, N–CH_2), 7.26 (t, 1H, *J* = 7.2 Hz), 7.44 (t, 2H, *J* = 8.0 Hz), 7.62–7.68 (m, 3H), 7.72 (dd, 2H, *J* = 1.6 Hz, *J* = 4.8 Hz), 7.77 (s, 1H), 7.84 (d, 1H, *J* = 8.4 Hz), 8.40 (s, 1H), 8.74 (d, 2H, *J* = 6.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 21.9 (Ar–CH_3), 52.5 (N–CH_2), 123.5, 125.9, 126.2, 126.8, 127.3, 128.4, 129.1, 132.3, 134.0, 137.5, 139.2, 140.8, 145.5, 148.2, 153.6, 158.5, 162.4; ESI-MS (*m*/*z*): calcd 427.89, found 427.45 (M^+); anal. calcd (%) for $C_{24}H_{18}ClN_5O$: C, 67.37; H, 4.24; N, 16.37. Found: C, 67.42; H, 4.19; N, 16.22.

*6.1.2.5. N-((2-Chloro-6-methylquinolin-3-yl)methyl)-N-phenyl-5-(pyridin-3-yl)-1,3,4-oxadiazol-2-amine (**8e**).* Yield 78%; yellow solid; m.p. 231 °C; IR (ν_{max}, cm^{-1}): 1612 (C═N str.), 1239 (C–O–C str.), 1059 (N–N str.), 756 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 2.44 (s, 3H, Ar–CH_3), 5.42 (s, 2H, N–CH_2), 7.37 (t, 1H, *J* = 7.2 Hz), 7.43 (t, 2H, *J* = 8.0 Hz), 7.45–7.68 (m, 4H), 7.75 (s, 1H), 7.83 (d, 1H, *J* = 8.4 Hz), 8.15 (d, 1H, *J* = 8.0 Hz), 8.38 (s, 1H), 8.70 (d, 1H, *J* = 4.8 Hz), 8.98 (d, 1H, *J* = 1.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 22.1 (Ar–CH_3), 52.8 (N–CH_2), 122.8, 125.4, 125.8, 126.4, 128.2, 128.6, 129.4, 130.0, 131.1, 132.8, 133.9, 138.5, 140.4, 142.5, 144.1, 147.3, 158.1, 161.8; ESI-MS (*m*/*z*): calcd 427.89, found 428.10 (M^+); anal. calcd (%) for $C_{24}H_{18}ClN_5O$: C, 67.37; H, 4.24; N, 16.37. Found: C, 67.29; H, 4.33; N, 16.45.

*6.1.2.6. N-((2-chloro-6-methylquinolin-3-yl)methyl)-N,5-diphenyl-1,3,4-oxadiazol-2-amine (**8f**).* Yield 74%; white solid; m.p. 239 °C; IR (ν_{max}, cm^{-1}): 1611 (C═N str.), 1242 (C–O–C str.), 1054 (N–N str.), 764 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 2.45 (s, 3H, Ar–CH_3), 5.41 (s, 2H, N–CH_2), 7.24 (t, 1H, *J* = 7.6 Hz), 7.43 (t, 2H, *J* = 8.0 Hz), 7.54 (dd, 3H, *J* = 4.0 Hz, *J* = 6.8 Hz), 7.62–7.67 (m, 3H), 7.78–7.85 (m, 4H), 8.38 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 21.5 (Ar–CH_3), 52.7 (N–CH_2), 123.9, 124.3, 124.5, 125.8, 126.5, 127.1, 127.3, 127.6, 128.2, 129.7, 129.8, 131.3, 133.3, 137.2, 137.6, 141.4, 145.2, 148.4, 159.2, 162.9; ESI-MS (*m*/*z*): calcd

426.90, found 426.40 (M^+); anal. calcd (%) for $C_{25}H_{19}ClN_4O$: C, 70.34; H, 4.49; N, 13.12. Found: C, 70.15; H, 4.63; N, 13.25.

*6.1.2.7. N-((2-Chloro-6-methoxyquinolin-3-yl)methyl)-N-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**8g**).* Yield 72%; light yellow solid; m.p. 226 °C; IR (ν_{max}, cm^{-1}): 1615 (C═N str.), 1227 (C–O–C str.), 1069 (N–N str.), 742 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 3.84 (s, 3H, Ar–OCH_3), 5.44 (s, 2H, N–CH_2), 7.28 (t, 1H, J = 7.2 Hz), 7.30–7.48 (m, 4H), 7.67–7.72 (m, 4H), 7.84 (d, 1H, J = 9.6 Hz), 8.40 (s, 1H), 8.73 (d, 2H, J = 5.6 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 53.0 (N–CH_2), 56.1 (Ar–OCH_3), 93.2, 110.5, 123.7, 125.8, 126.2, 126.8, 127.0, 127.4, 128.7, 130.1, 130.5, 137.6, 142.2, 147.8, 150.4, 153.2, 158.0, 163.2; ESI-MS (m/z): calcd 443.89, found 443.40 (M^+); anal. calcd (%) for $C_{24}H_{18}ClN_5O_2$: C, 64.94; H, 4.09; N, 15.78. Found: C, 64.70; H, 4.21; N, 15.91.

*6.1.2.8. N-((2-Chloro-6-methoxyquinolin-3-yl)methyl)-N-phenyl-5-(pyridin-3-yl)-1,3,4-oxadiazol-2-amine (**8h**).* Yield 75%; off-white solid; m.p. 242 °C; IR (ν_{max}, cm^{-1}): 1659 (C═N str.), 1231 (C–O–C str.), 1078 (N–N str.), 753 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 3.85 (s, 3H, Ar–OCH_3), 5.42 (s, 2H, N–CH_2), 7.27 (t, 1H, J = 7.2 Hz), 7.40–7.47 (m, 4H), 7.57 (dd, 1H, J = 4.8 Hz, J = 8.0 Hz), 7.68 (d, 2H, J = 8.0 Hz), 7.85 (d, 1H, J = 8.8 Hz), 8.17 (d, 1H, J = 8.0 Hz), 8.44 (s, 1H), 8.72 (d, 1H, J = 3.6 Hz), 8.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 52.5 (N–CH_2), 55.8 (Ar–OCH_3), 90.5, 111.3, 123.1, 124.5, 125.6, 126.0, 126.5, 127.6, 129.2, 130.0, 133.1, 135.2, 137.9, 142.5, 147.3, 148.1, 159.7, 163.1; ESI-MS (m/z): calcd 443.89, found 443.50 (M^+); anal. calcd (%) for $C_{24}H_{18}ClN_5O_2$: C, 64.94; H, 4.09; N, 15.78. Found: C, 64.79; H, 4.22; N, 15.87.

*6.1.2.9. N-((2-Chloro-6-methoxyquinolin-3-yl)methyl)-N,5-diphenyl-1,3,4-oxadiazol-2-amine (**8i**).* Yield 78%; white solid; m.p. 237 °C; IR (ν_{max}, cm^{-1}): 1606 (C═N str.), 1237 (C–O–C str.), 1066 (N–N str.), 758 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 3.84 (s, 3H, Ar–OCH_3), 5.39 (s, 2H, N–CH_2), 7.26 (t, 1H, J = 7.2 Hz), 7.40–7.54 (m, 7H), 7.67 (d, 2H, J = 8.0 Hz), 7.79–7.86 (m, 3H), 8.38 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 52.8 (N–CH_2), 56.0 (Ar–OCH_3), 88.4, 106.3, 123.5, 124.2, 125.0, 125.8, 126.4, 127.2, 128.7, 129.7, 131.4, 136.4, 137.5, 141.6, 146.2, 148.8, 158.3, 162.5; ESI-MS (m/z): calcd 442.90, found 442.50 (M^+); anal. calcd (%) for $C_{25}H_{19}ClN_4O_2$: C, 67.80; H, 4.32; N, 12.65. Found: C, 67.93; H, 4.41; N, 12.78.

*6.1.2.10. N-((2,6-Dichloroquinolin-3-yl)methyl)-N-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine (**8j**).* Yield 73%; brown solid; m.p. 233 °C; IR (ν_{max}, cm^{-1}): 1616 (C═N str.), 1234 (C–O–C str.), 1033(N–N str.), 762 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 5.43 (s, 2H, N–CH_2), 7.29 (t, 1H, J = 7.2 Hz), 7.46 (t, 2H, J = 8.0 Hz), 7.68–7.73 (m, 4H), 7.81 (dd, 1H, J = 2.4 Hz, J = 9.2 Hz), 7.98 (d, 1H, J = 8.8 Hz), 8.17 (d, 1H, J = 2.4 Hz), 8.51 (s, 1H), 8.74 (dd, 2H, J = 1.6 Hz, J = 4.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 53.1 (N–CH_2), 119.9, 124.6, 126.8, 127.5, 128.0, 128.6, 129.9, 131.1, 131.6, 134.3, 136.9, 138.0, 141.5, 146.1, 149.6, 152.8, 162.5; ESI-MS (m/z): calcd 448.30, found 447.10 (M^+); anal. calcd (%) for $C_{23}H_{15}Cl_2N_5O$: C, 61.62; H, 3.37; N, 15.62. Found: C, 61.69; H, 3.31; N, 15.51.

*6.1.2.11. N-((2,6-Dichloroquinolin-3-yl)methyl)-N-phenyl-5-(pyridin-3-yl)-1,3,4-oxadiazol-2-amine (**8k**).* Yield 76%; brown solid; m.p. 233 °C; IR (ν_{max}, cm^{-1}): 1613 (C═N str.), 1225 (C–O–C str.), 1096 (N–N str.), 755 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 5.48 (s, 2H, N–CH_2), 7.27 (t, 1H, J = 7.6 Hz), 7.37–7.58 (m, 3H), 7.68 (d, 2H, J = 8.0 Hz), 7.79–7.84 (m, 1H), 7.98 (d, 1H, J = 8.8 Hz), 8.15–8.21 (m, 2H), 8.50–8.57 (m, 1H), 8.71 (dd, 1H, J = 1.2 Hz, J = 4.8 Hz), 8.99 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 53.0 (N–CH_2), 120.8, 124.4, 124.7, 126.7, 127.2, 128.2, 129.6, 129.8, 130.1, 131.7, 131.8, 132.2, 133.4, 133.7, 135.2, 136.9, 141.4, 145.1, 146.6, 149.8, 152.0, 162.2; ESI-MS (m/z): calcd 448.30, found 446.80 (M^+); anal. calcd (%) for $C_{23}H_{15}Cl_2N_5O$: C, 61.62; H, 3.37; N, 15.62. Found: C, 61.42; H, 3.49; N, 15.69.

*6.1.2.12. N-((2,6-Dichloroquinolin-3-yl)methyl)-N,5-diphenyl-1,3,4-oxadiazol-2-amine (**8l**).* Yield 79%; white solid; m.p. 241 °C; IR (ν_{max}, cm^{-1}): 1632 (C═N str.), 1229 (C–O–C str.), 1071 (N–N str.), 764 (C–Cl str.); ^1H NMR (400 MHz, DMSO-d_6): 5.41 (s, 2H, N–CH_2), 7.27 (t, 1H, J = 7.2 Hz), 7.45 (t, 2H, J = 8.0 Hz), 7.53–7.58 (m, 3H), 7.68 (d, 2H, J = 8.0 Hz), 7.78–7.81 (m, 3H), 7.98 (d, 1H, J = 9.2 Hz), 8.19 (d, 1H, J = 2.0 Hz), 8.51 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 53.2 (N–CH_2), 121.4, 123.7, 124.9, 126.0, 127.5, 128.4, 129.7, 131.5, 132.0, 132.6, 133.2, 133.7, 135.8, 137.1, 141.9, 145.5, 148.7, 153.5, 163.1; ESI-MS (m/z): calcd 447.32, found 446.40 (M^+); anal. calcd (%) for $C_{24}H_{16}Cl_2N_4O$: C, 64.44; H, 3.61; N, 12.53. Found: C, 64.31; H, 3.75; N, 12.45.

6.2 Biological evaluation

6.2.1. *In vitro* antimicrobial assay. The broth microdilution method was used for the screening of the *in vitro* antimicrobial activity of all the synthesized compounds. To obtain the desired concentration of the compounds, DMSO was used as the diluent for testing against standard bacterial strains. Mueller–Hinton broth and Sabouraud dextrose broth were used as nutrient media to grow and dilute the compound suspension for the test bacteria and fungi, respectively. The inoculum size of the test strain was adjusted to 10^8 CFU mL^{-1} (colony forming unit per millilitre) by comparing the turbidity (turbidimetric method). Serial dilutions were prepared in the primary and secondary screenings. Each synthesized compound and standard drug was diluted to obtain a concentration of 2000 μg mL^{-1} as a stock solution. The drugs which were found to be active in the primary screening (*i.e.* 500, 250 and 200 μg mL^{-1} concentrations) were further screened with their second set of dilutions at 100, 62.5, 50, 25 and 12.5 μg mL^{-1} concentration against all microorganisms. 10 microlitre suspensions were further inoculated on appropriate media and growth was noted after 24 and 48 h. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over an area of plate of medium suitable for the growth of the test organism. The tubes were then incubated at 37 °C overnight. The highest dilution preventing the appearance of turbidity after spot subculture was considered to be the minimal inhibitory concentration (MIC, μg mL^{-1}). All the tubes showing no visible growth (the same as the control tube) were subcultured and incubated overnight at 37 °C. These were compared with the amount of growth in the control tube before incubation. In this study ampicillin, norfloxacin and chloramphenicol were used as the standard antibacterial drugs. Nystatin and griseofulvin were used as the standard antifungal drugs. The results are summarized in Table 3.

6.2.2. *In vitro* antituberculosis assay. Drug susceptibility and determination of the antituberculosis activity of the test compounds against *M. tuberculosis* H37Rv were performed by

the Lowensteine–Jensen method[38–40] with a slight modification, where a 250 mg mL^{-1} dilution of each test compound was added to the liquid Lowenstein–Jensen medium and then the media were sterilized by the inspissation method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein–Jensen medium was collected in 0.85% saline in bijou bottles. Solutions of all the test compounds of 250 mg mL^{-1} concentration were prepared in DMSO. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. The tubes containing the compounds were compared with control tubes where the medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which no development of colonies occurred or <20 colonies developed was taken as the MIC concentration of the test compound. The screening results are summarized as % inhibition (Table 4) relative to the standard drugs isoniazid and rifampicin. The MIC values of the four compounds with the highest % inhibition *i.e.* **8a**, **8e**, **8j** and **8k** are shown in Table 5. The standard strain *M. tuberculosis* H37Rv was tested with the standard drugs isoniazid and rifampicin for comparison purposes.

6.2.3. *In vitro* antimalarial assay. All the synthesized compounds were screened for their antimalarial activity against the *P. falciparum* strain. The *P. falciparum* strain was cultivated by a modified method described by Trager and Jensen.[52] The compounds were dissolved in DMSO. The final concentration of DMSO used was not toxic and did not interfere with the assay. The antiparasitic effect of the compounds was measured by growth inhibition percentage as described by Carvalho and Krettli.[53] For experimental purposes, the cultures were synchronized with 5% D-sorbitol when the parasites were at the ring stage.[54] The parasitic suspension, consisting of predominately the ring stage parasites, was adjusted to a 1–2% parasitaemia and 2.5% haematocrit in hypoxanthine-free RPMI-1640 culture medium with 10% human plasma and was exposed to 7 concentrations of each compound for a single cycle of parasite growth for 48 h at 37 °C. Positive controls containing the standard antimalarial drugs chloroquine and quinine, in standard concentrations, were used in each experiment. The stock solutions were additionally diluted in whole medium (RPMI 1640 plus 10% human serum) to each of the used concentrations. The concentration that inhibited 50% of the parasite growth (IC_{50} value) was determined by interpolation using Microcal Origin software. The blood smears used were read blind and each duplicate experiment was repeated three times.

6.2.4. Cytotoxicity: brine shrimp lethality bioassay. The brine shrimp lethality bioassay technique was applied for the determination of the general toxic properties of the compounds. The *in vivo* lethality test has been carried out using brine shrimp eggs *i.e. Artemia* cysts. Brine shrimp eggs were hatched in a shallow rectangular plastic dish (22×32 cm), filled with artificial seawater, which was prepared with a commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs was sprinkled into the large compartment, which was darkened while the minor compartment was open to ordinary light. After two days, the nauplii were collected by a pipette from the lighter side. A stock solution of the test complex was prepared in DMSO. From this stock solution, solutions were transferred to the vials to make final concentrations of 5, 10, 20, 30, 40, and 50 mg mL^{-1} (dilutions were used in triplicate for each test sample and LC_{50} is the mean of three values) and three vials were kept as controls, containing only DMSO. After two days, when the nauplii were ready, 1 mL of seawater and 10 nauplii were added to each vial and the volume was adjusted with seawater to 2.5 mL per vial.[41] After 24 h each vial was observed using a magnifying glass and the number of survivors in each vial was counted and noted. The data were analysed by a simple logit method to determine the LC_{50} values, in which the log of the dose concentration of the samples was plotted against the percentage mortality of the nauplii.[38,39,55]

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