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ARTICLE

Novel 1,3,4-oxadiazole motifs bearing quinoline nucleus: synthesis, characterization and their biological evaluation for antimicrobial, antitubercular, antimalarial and cytotoxic activity

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A series of quinoline based 1,3,4-oxadiazole derivatives **8a-l** were synthesized by chloro-amine coupling reaction approach with different catalyst and solvents. The substituted 1,3,4-oxadiazole intermediates **7a-c** were obtained from 2-sustituted-*N*-phenylhydrazinecarbothioamide **6a-c** by cyclization with different cyclizing reagents like mercuric acetate, lead dioxide, iodobenzenediacetate (IBD) and aqueous sodium hydroxide with iodine in aqueous potassium iodide to isolate the most effective reaction condition by using iodobenzenediacetate as extremely good catalyst. The structure 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. The brine shrimp bioassay was carried out to study the *in vitro* cytotoxic properties for the highly active compounds of *in vitro* biological evaluation.

1. Introduction

Malaria remains a significant worldwide health difficulty, with poor social and economic consequences in countries. The problem has been improved by the emergence and spread of parasites that have resistant to well-established antimalarial drugs[1]. The swelling of malaria in human body through five different types of protozoans of the species *plasmodium*, but *plasmodium falciparum* is responsible for most of the critical cases. However, appearance of *P. falciparum* resistance to these drugs is a serious cause of anxiety. To reduce the variety of resistant parasites, WHO has suggested the formulations of artemisinins with traditional antimalarial drugs such as lumefantrine, amodiaquine and mefloquine and ACT (Artemisinin Combination Therapy) in combine form are currently approved in widespread countries[2, 3]. For that reason, 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), killing 1.4 million people annually and showing a rapid increase in cases resistant to multiple drugs. Furthermore, expansion of TB in conjunction with AIDS has more strengthened the problems associated with its cure, because both diseases (TB and AIDS) accelerate each other succession[4]. World Health Organization (WHO) identified as one of the three priority diseases for drug research and development, underling the importance of the discovery process in identifying new antibiotics against tuberculosis are urgently needed.

1,3,4-Oxadiazole is a bioactive motifs in medicinal chemistry[5]. The general use of them as the core moiety in medicinal chemistry establishes as a member of the privileged structures. Substituted 1,3,4-oxadiazoles are one of the most important heterocyclic compounds, which 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, antidepressant agents and 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 the 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 with a broad range of biological activities, such as anti-inflammatory[25], 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].

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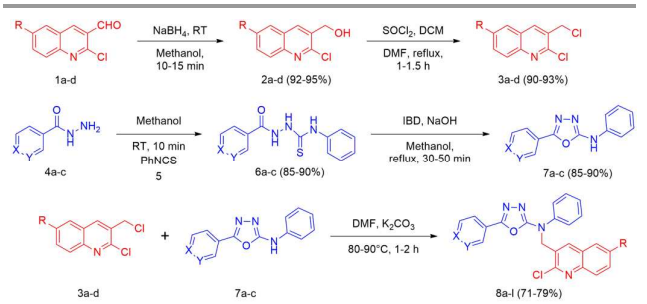
Based on the observations, a challenge to develop novel series of bioactive molecules by coupling 1,3,4-oxadiazole with substituted quinoline moiety using molecular hybridization approach has been proposed. We have synthesized quinoline based 1,3,4-oxadiazoles scaffolds and evaluated them for their antibacterial, antifungal, antitubercular and antimalarial activities. Further we also evaluated effect of different substitution on biological activities.

2. Chemistry

The synthetic approach adopted to obtain the targeted quinoline nucleus incorporated 1,3,4-oxadiazole derivatives are depicted in **Scheme 1**. The starting material 6-(un)substituted-2-chloro-3-(chloromethyl)quinoline **3a-d** were prepared by reduction followed by chlorination of 6-(un)substituted-2-chloroquinoline-3-carbaldehyde **1a-d**[36] and another starting material *N*-phenyl-5-(substituted)-1,3,4-oxadiazol-2-amine **7a-c** were prepared by refluxing different hydrazide **4a-c** and phenylisothiocyanide **5** using different cyclizing reagent like mercuric acetate, lead dioxide, chloramine-T, iodobenzenediacetate and aqueous sodium hydroxide with iodine in aqueous potassium iodide.



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 the *N*-((2-chloro-6-(un)substituted-quinolin-3-yl)methyl)-*N*-phenyl-5-(substituted)-1,3,4-oxadiazol-2-amine **8a-l.**

For screening of different cyclizing reagents we select a model reaction between isonicotinohydrazide and phenylisothiocyanide (**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-amine **8a-l** were synthesized using chloro-amine coupling reaction between **3a-d** and **7a-c**. The chloro-amine coupling reaction of **3a** and **7a** was used as a model reaction to optimize reaction condition (**Scheme 3**), and the results are summarized in **Table 2**

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) were carried out by broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS)[37-39]. 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, *Vibrio cholera* MTCC 3906) bacteria using ampicillin, ciprofloxacin, norfloxacin and chloramphenicol as the reference antibacterial drugs. 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 were gathering from the Institute of Microbial Technology, Chandigarh (MTCC-Micro Type Culture Collection). Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. The results of antimicrobial screening data are shown in **Table 3**.

3.2. *In vitro* antituberculosis activity

The encouraging results from the antibacterial activity provoked us to decide on preliminary screening of the title compounds for their *in vitro* antituberculosis activity. Primary screening of targeted compounds **8a-l** were performed at 250 µg/mL against *Mycobacterium tuberculosis* H37Rv strain using Lowenstein-Jensen medium (conventional method) as described by Rattan[38-40]. The acquire results are presented in **Table 4** in the form of % inhibition. Rifampicin and Isoniazid were used as the reference drugs. From, the result four compounds those exhibited highest % inhibition, were again screened to get their MIC values (**Table 5**).

3.3. *In vitro* antimalarial activity

All the targeted compounds **8a-l** was screened for their *in vitro* antimalarial activity against *P. falciparum* strain using chloroquine and quinine as the reference compounds. The consequences 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**.

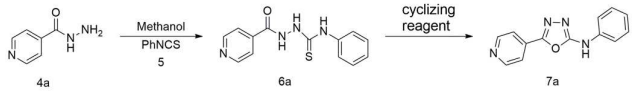
3.4. Cytotoxicity (brine shrimp lethality bioassay)

The *in vitro* 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 exhibited highest % inhibition, were again screened for their cytotoxicity by using the protocol of Meyer *et al*[41]. The corresponding LC_{50} values of all 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 condition for substituted 1,3,4-oxadiazole 7a-c



Scheme 2. Optimize of the reaction condition for substituted 1,3,4-oxadiazole **7a**.

We began our investigation with thiosemicarbazide **6a**, which was synthesized *via* 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, which often lead to inconvenient handling and undesirable by-product. Different coupling reagents are used for the final cyclization including DIC, DCC as carbodiimides, TBTU as 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) works in stoichiometric quantities, and (c) solubility in the currently used solvents. Considering these characteristics, IBD is one of the common coupling reagents and additives used in cyclization chemistry[47-50].

For optimized of reaction condition initially, the reaction in acetic acid in presence of concentrated H_2SO_4 as catalyst could afford the desired product **7a** in 40% yield (entry 1). But there is a lower yield than reported in literature. Than we have tried to chloramine-T as cyclizing reagent for the 1,3,4-oxadiazole synthesis from literature[51], in this case also we obtained lower yield (entry 2). Further investigation of various catalysts, IBD was found to be more effective than other catalysts like chloramine-T, lead dioxide and mercuric acetate, and the reaction yield increased up to 85% when IBD was employed (entry 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 reaction media as compared to other bases (entry 5). In addition, lower yields were observed if reaction perform without base or weak bases (entry 3,4,6,7).

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

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

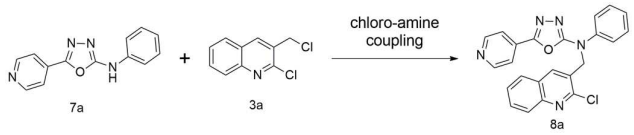
[a] Reaction of isonicotinohydrazide **4a** (1 mmol) with phenylisothiocyanate **5** (1 mmol) in the presence of 1 mmol of catalyst

[b] Reaction progress monitored by TLC

[c] Isolated yield

According to these results, IBD was selected as coupling reagent for the synthesis of 1,3,4- oxadiazole because 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 base and IBD (iodobenzenediacetate) as coupling reagents producing the desired 2-amino-1,3,4-oxadiazole **7a-c**. The results are summarized in **Table 1**.

4.1.2. Optimization of reaction condition for quinoline incorporated 1,3,4-oxadiazole 8a-l



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

The reaction of N-phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine **7a** with 2-chloro-3-(chloromethyl)quinoline **3a** was a selected model reaction to optimize reaction conditions (**Scheme 2**), and the results are summarized in **Table 2**. Subsequently, with the intention of finding a suitable condition for the reaction, we tested different catalysts and solvents for the synthesis of desired product **8a-l**. For initial feasibility studies, **7a** was subjected to reaction with **3a** in THF using NaH as catalyst at room temperature which resulted **8a** in trace amount (entry 1). Further, we tested the chloro-amine coupling reaction with NaH in DCM as a solvent which gave the required product in 20% yield after 12 h (entry 2). Next the same reaction was tested in DCM using catalyst TEA, in this case we got 30% yield (entry 3). Also, we used TEA with ethanol and n-butanol as solvent which proceeded reaction in forward direction; in this case yield was not much higher (entry 7 and 9). These observations enforced us to screen different catalyst. In our attempt, we have studied K_2CO_3 as catalyst with different solvent which are summarized in **Table 2** (entry 4-6).

Table 2. The influence of different reaction condition for chloro-amine coupling reaction.

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

After the extensive screening of different catalysts, we concluded that K_2CO_3 was the most appropriate catalyst with DMF as a solvent for chloro-amine coupling reaction and hence resulted in high yield of the product within a shorter reaction time (entry 6). Consequently, the present protocol can keep away from the use of expensive metallic catalyst and harsh reaction condition.

4.2. Analytical results

The structures of all the synthesized compounds were confirmed by ^{1}H NMR, FT-IR, ^{13}C NMR, mass and elemental analysis. The ^{1}H NMR spectra of compound **8a-l** shows signals of N-CH_2 (methylene protons at quinoline ring) proton as a sharp singlet around δ 5.39-5.48. The aromatic protons resonate as multiplets at around δ 7.23-8.99 ppm. The IR spectrum of compounds **8a-l** exhibited characteristic absorption band around 1605-1659 cm^{-1} which was attributed to the presence C=N stretching of 1,3,4-oxadiazole ring. The 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 1033-1096 cm^{-1} may be attributed to symmetric stretching of nitrogen-nitrogen in 1,3,4-oxadiazole ring. In ^{13}C NMR spectra, C-2 carbon of 1,3,4-oxadiazole ring was displayed very down field at δ 161.8-163.5 ppm for the reason that was in between one oxygen atom and two nitrogen atom. The signals at around δ 52.4-53.2 ppm was assigned to methylene carbon of quinoline at 3-position attached to nitrogen of 1,3,4-oxadiazole at 2-position. The mass spectrum of all the compounds showed molecular ion peak at M^+ corresponding to their molecular weights, which confirmed the respective chemical structures.

4.3. Biological section

4.3.1. Antibacterial activity

The analysis of antibacterial screening data (**Table 3**) revealed that all the compounds **8a-l** demonstrates moderate to very good inhibitory activity. The compound **8b** (0.151 mM) and **8k** (0.139 mM) showed maximum activity adjacent to *B. subtilis*. Majority of the compounds display excellent activity towards gram positive bacteria *B. subtilis* and *C. tetani* as compared to

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

Entry	Gram-positive bacteria			Gram-negative bacteria			Fungi	
	S.P.	C.T.	B.S.	S.T.	V.C.	E.C.	C.A.	A.F.
	MTCC 1936	MTCC 449	MTCC 441	MTCC 98	MTCC 3906	MTCC 443	MTCC 227	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.

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ampicillin, while compounds **8a** and **8j** showed similar potency to that of the standard drugs against *C. tetani* and *B. subtilis*. The compounds **8a**, **8b** and **8l** displayed comparatively good activities against *S. pneumonia*. Compounds **8d**, **8h**, **8k** and **8l** showed similar potency as that of standard drugs close to *S. typhi*. While compounds **8a**, **8b, 8c**, **8e** and **8l** showed comparable potency as that of standard drugs against *V. cholera*. The compounds **8d** (0.143 mM) and **8h** (0.140 mM) illustrated highest activity in inhibiting gram negative bacteria to *E. coli*. Remaining other compounds are moderate or less active against all gram positive and gram negative bacteria.

4.3.2. Antifungal activity

The result of antifungal study (**Table 3**) of the synthesized quinoline based 1,3,4-oxadiazole derivatives revealed that all the compounds have poor activity to *A. fumigatus*. While in comparison with standard fungicidal griseofulvin, compounds **8d** and **8e** contributed excellent antifungal activity against *C. albicans.* Although compounds **8a** and **8h** illustrated similar potency towards *C. albicans*. Remaining compounds showed weak antifungal potency than nystatin and griseofulvin.

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

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 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

4.3.3. Antituberculosis activity

Some excellent results from the antimicrobial studies encouraged us to go for the 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 is summarized in **Table 4**. The outcome of the result 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 respectively) against *M. tuberculosis* H37Rv. The compound **8a** shows moderate potency against *M. tuberculosis* i.e. MIC = 0.060 mM as compared to rifampicin i.e. MIC = 0.048 mM at 98% inhibition. Compound **8e** MIC = 0.146 mM and **8j** MIC = 0.111 mM at 96% inhibition and compounds **8k** MIC = 0.223 mM at 95% inhibition displayed less activity towards *M. tuberculosis* (**Table 5**). Compound **8a** was emerged out as the most potent member of the series and opens up a new door to optimize this series for new class of antituberculer agent.

4.3.4. Antimalarial activity

All the synthesized compounds **8a-l** was evaluated for their antimalarial activity against chloroquine and quinine sensitive strain of *P. falciparum*. All experiments were performed in duplicate and a mean value of IC_{50} is mentioned in **Table 6**. As shown in **Table 6**, the compounds **8b**, **8e**, **8h**, **8j** and **8k** were found to have IC_{50} in the range of 0.089 to 0.467 µM against *P. falciparum* strain. These compounds displayed excellent activity against *P. falciparum* strain as compared to quinine IC_{50} = 0.826 µM. Furthermore compounds **8j** was found to possess moderate activity i.e. IC_{50} = 0.089 µM as compared to chloroquine. Remaining all other compounds was found to be less active against chloroquine sensitive strain of *P. falciparum*.

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

4.3.5. Cytotoxicity (brine shrimp lethality bioassay)

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

5. Conclusion

Some magnificent results have been obtained with the quinoline-oxadiazole hybridized scaffold. Furthermore, this work contributes to validate the choice of the quinoline based

1,3,4-oxadiazole scaffold, as a template, useful to design new antimicrobial, antitubercular and antimalarial compounds. This synthetic approach allows the inclusion of potent bioactive nuclei in a single scaffold through an easy way. The compounds **8a**, **8e**, **8j** and **8k** exhibited good antituberculosis activities. Majority of the compounds showed excellent activity against *P. Falciparum* strains as compared to quinine. Compound **8k** emerged as the promising antimicrobial member within varies it shows better antitubercular activity, antimalarial activity and lower toxicity. Consequently, such type of compound would represent a fertile matrix for further development of more biologically potent agents and that deserve further investigation and derivatization in order to discover the scope and limitation of its biological activities.

Table 7 Effect of compounds on brine shrimp lethality bioassay.

Entry	Concentration (µg/mL)	Log (Conc.)	No. of nauplii taken	No. of nauplii dead	% of Mortality	LC_{50} (µg/mL)	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

6. Experimental section

6.1. Chemistry

All reactions were performed with commercially available reagents. They 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}, 0.25 mm thickness (Merck). Detection of the components was made by exposure to iodine vapours or UV light. Melting points were taken in melting point apparatus µThermoCal$_{10}$ (Analab Scientific Pvt. Ltd, India) and are uncorrected. Mass spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under PURSE program of DST at Sardar Patel University, Vallabh Vidyanagar, India. The IR spectra were recorded on a FTIR MB 3000 spectrophotometer (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 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using 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 doublet and m, multiplet. The elemental analysis was carried out by using 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-sustituted-1,3,4-oxadiazol-2-amine (7a-c)

Substituted hydrazides **4a-c** (10 mmol), Phenylisothiocyanide **5** (10 mmol) and methanol 5 mL were charged in a 50mL round bottom flask with mechanical stirrer and condenser. The reaction mixture was at ambient temperature stirred for 10 min. After add the IBD (10 mmol) to reaction mixture and reaction mixture was reflux for 30-50 min. After the completion of reaction checked by TLC, the reaction mixture cool to room temperature to obtained solid. The solid were filtered and wash with cold methanol and recrystallized from methanol to obtain substituted 1,3,4-oxadiazole **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-amine (8a-l)

2-chloro-3-(chloromethyl)-6-(un)substitutedquinoline **3a-d** (1 mmol), *N*-phenyl-5-sustituted-1,3,4-oxadiazol-2-amines **7a-c** (1 mmol) and anhydrous potassium carbonate (2 mmol) in dimethylformamide (5 mL) were charged in a 50 mL round bottom flask with mechanical stirrer and 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 the completion of 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 until pH 7. The separated precipitates of *N*-((2-chloro-6-(un)substitutedquinolin-3-yl)methyl)-*N*-phenyl-5-substituted-1,3,4-oxadiazol-2-amine **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.); ^{1}H 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.); ^{1}H 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.); ^{1}H 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.); ^{1}H 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.); ^{1}H 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.); ^{1}H 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.); ^{1}H 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.); ^{1}H 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

Broth micro dilution method was used for screening of *in vitro* antimicrobial activity of all synthesized compounds. For desire concentration of compounds, DMSO was used as diluents to test upon standard bacterial strains. Mueller-Hinton broth and Sabouraud Dextrose broth were used as nutrient medium to grow and dilute the compound suspension for the test bacteria and fungus respectively. Inoculum size for test strain was adjusted to 10^8 $CFUmL^{-1}$ (Colony Forming Unit per millilitre) by comparing the turbidity (turbidimetric method). Serial dilutions were prepared in primary and secondary screening. Each synthesized compounds and the standard drugs were diluted obtaining 2000 µg/mL concentration as a stock solution. The drugs which were found to be active in primary screening (i.e. 500, 250 and 200 µg/mL concentrations) were further screened in their second set of dilution at 100, 62.5, 50, 25 and 12.5 µg/mL concentration against all microorganisms. 10 micro litre suspensions were further inoculated on appropriate media and growth was noted after 24 and 48 h. The control tube containing no antibiotic was instantaneously 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 put for incubation at 37°C overnight. The highest dilution preventing appearance of turbidity after spot subculture was considered as minimal inhibitory concentration (MIC, µg/mL). All the tubes showing no visible growth (same as control tube) were subcultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation was compared. In this study Ampicillin, Norfloxacin and Chloramphenicol were used as the standard antibacterial drugs. Nystatin and Griseofulvin were used as standard antifungal drugs. The results are summarized in Table 3.

6.2.2. *In vitro* antituberculosis assay

Drug susceptibility and determination of antituberculosis activity of the test compounds against *M. tuberculosis* H37Rv were performed by Lowensteine-Jensen method[38-40] with slight modification where 250 mg/mL dilution of each test compound were added liquid Lowenstein-Jensen Medium and then media were sterilized by inspissations method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein-Jensen medium was collected in 0.85% saline in bijou bottles. All test compound make solution of 250 mg/mL concentration of compounds was 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. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which no development of colonies occurred or < 20 colonies was taken as MIC concentration of test compound. The screening results are summarized as % inhibition (**Table 4**) relative to standard drugs isoniazid and rifampicin. MIC values of four compounds with highest % inhibition i.e. **8a**, **8e**, **8j** and **8k** are shown in **Table 5**. The standard strain *M. tuberculosis* H37Rv was tested with standard drugs isoniazid and rifampicin for comparison purpose.

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* strains were cultivated by a modified method described by Trager and Jensen[52]. 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 in 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. A positive control with reference to 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 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

Brine shrimp lethality bioassay technique was applied for the determination of general toxic property of compounds. The in vitro 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 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 were sprinkled into the large compartment, which was darkened while the minor compartment was opened to ordinary light. After two days 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 concentration 5, 10, 20, 30, 40, 50 mg/mL (dilutions were used in triplicate for each test sample and LC_{50} is the mean of three values) and three vial was kept as control having of DMSO only. After two days, when the of nauplii were ready, 1 mL of seawater and 10 of 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. Data were analysed by simple logit method to determine the LC_{50} values, in which log of dose concentration of samples were plotted against percent of mortality of nauplii.[38, 39, 55]

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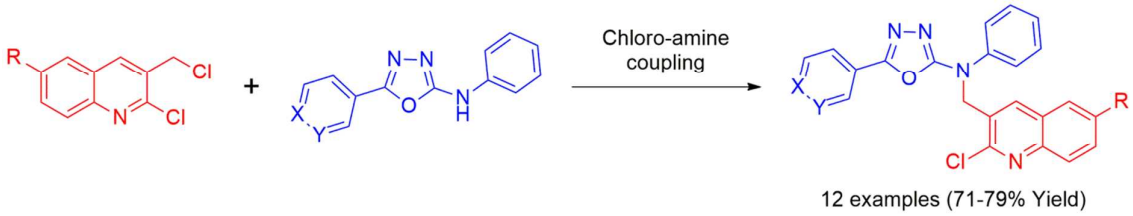

12 examples (71-79% Yield)