Microwave-Assisted Rapid and Efficient Synthesis of New Series of Chromene-Based 1,2,4-Oxadiazole Derivatives and Evaluation of Anti-bacterial Activity with Molecular Docking Investigation

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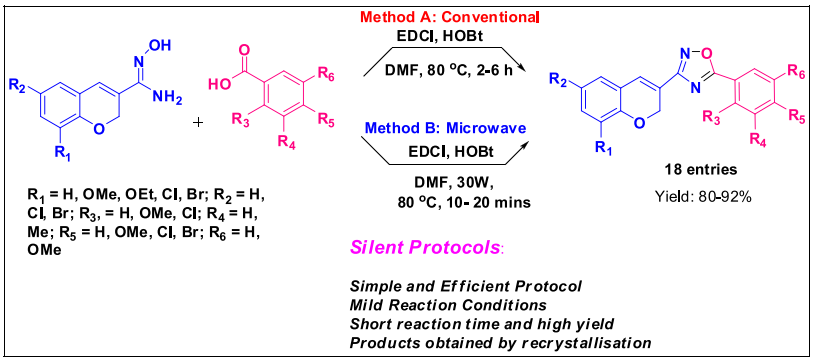


A new series of novel chromene-based oxadiazole derivatives were synthesized from a variety of chromene-based amidoximes with readily available carboxylic acids under conventional oil bath heating as well as under microwave irradiation. The use of commercially available EDCI and HOBt as coupling reagents in DMF combined with microwave heating resulted in high yields and purities of the product 1,2,4-oxadiazoles in an expeditious manner. This methodology is successfully applied to synthesize 18 numbers of new 2*H*-chromene-substituted 1,2,4-oxadiazole derivatives in good to high yields. The structure of the product was ascertained by X-ray crystallographic analysis. All the synthesized compounds were evaluated for their *in vitro* antibacterial activity against two different pathogenic bacterial strains, that is, *Escherichia coli* (MTCC614) and *Klebsiella pneumoniae* (MTCC4031). The obtained results from *in vitro* antimicrobial assays indicated that **6g** and **6h** exhibited good antibacterial activity nearer to the standard drug, gentamicin. The molecular docking studies showed that compounds **6g** and **6h** show hydrogen bonding interaction with the bacterial target DNA gyrase of *E. coli*.

INTRODUCTION

In recent years, the occurrence of bacterial infections has reached alarming levels around the world. The widespread use of antibacterial agents considered as the leading weapons used for the treatment of infectious diseases resulted in resistance to drug therapy against bacterial infections, which led to serious health hazards [1,2]. Among several bacterial infections, the urinary tract infections (UTIs) are common infectious diseases in clinical practice. An estimated 150 million people worldwide are diagnosed with a UTI in each year [3], and 40–50% of women experiences at least one UTI during their lifetime [4,5]. Most cases of UTI are caused by Gram-negative bacilli, with *Escherichia coli* accounting for over 90% of uncomplicated UTIs [6]. Other pathogens included such as *Klebsiella* species, *Proteus* species, *Pseudomonas aeruginosa*, and *Enterococcus* species [7–9]. Uncomplicated infections can be treated with short courses of antibiotics, while complicated UTIs require longer and more intensive courses of antibiotics. Effective therapy is based on antibiotics, but bacterial resistance is an ongoing issue for the management of UTI. In an era of increasing bacterial resistance to classical antibacterial agents, the design and synthesis of new antibacterial agents become an imperative need to support for the battle against pathogenic bacteria [10]. The development of novel structure leads remains a key challenge for medicinal chemists to design new, effective, and broad spectrum antimicrobial drug targets *via* genomic improving or overcoming the phenomenon of multiple drug resistance strains of bacteria in existing antibiotics and most importantly by identifying new antibacterial agents with novel structures and mode of action with enhanced activity profile and high potency without or with at least reduced systemic adverse effects. A wide variety of approaches are being used in the search for

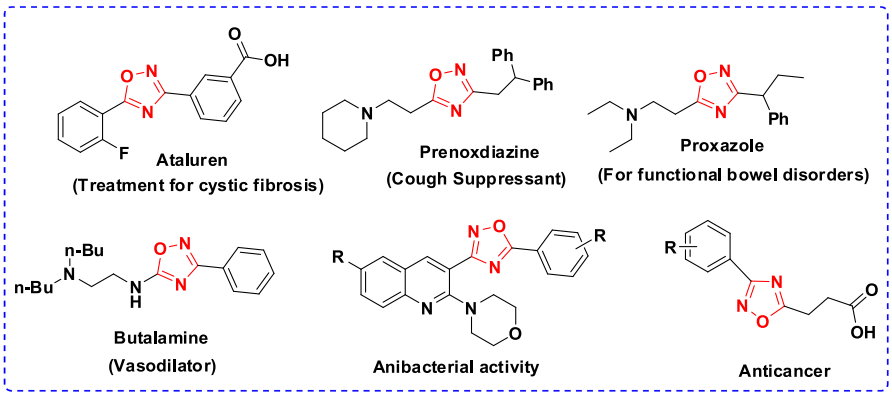


Figure 1. Examples of biologically active 1,2,4-oxadiazole derivatives. [Color figure can be viewed at wileyonlinelibrary.com]

novel antibacterial agents [11]. For a long period of time, natural products are still one of the major sources of new antibacterial agents [12]. Following in this vein, it was apparent that heterocycles containing nitrogen(s) and an oxygen atom particularly oxadiazoles have received considerable attention in the field of medicinal chemistry due to their interesting and diverse clinical applications such as antibacterial [13], antifungal [14], anthelmintic [15], antitubercular [16], anticancer [17], anti-HIV [18], antioxidant [19], anti-inflammatory [20], and anticonvulsant [21] activities (Fig. 1). Due to their huge biomedical importance, several synthetic methods for preparing the oxadiazole ring systems have been reported [22]. Literature survey reveals the synthesis of 1,3,4-oxadiazole derivatives as antibacterial agents [2,13–22]. However, there are very few reports on the antibacterial activity of 1,2,4-oxadiazole derivatives, which encouraged us to move further [11,23,24]. Various methods reported for the preparation of 1,2,4-oxadiazoles such as cyclization of *O*-acylamidoximes obtained from acylation of amidoximes with acyl halides, esters, or anhydrides [25]. Katritzky and co-workers have used *N*-acyl benzotriazoles for the synthesis of 1,2,4-oxadiazoles [26]. A strong recent trend toward the use of a carboxylic acid that is activated *in situ* and then reacted with an amidoxime has emerged. Several acid activators have been reported for this purpose including BOP-Cl, TBTU, 1,1′-carbonyldiimidazole, CDMT, and propylphosphonicanhydride (T3P) [27]. Microwave-assisted synthesis of 1,2,4-oxadiazole derivatives was reported by Santagada *et al.* [27] in 2004 and Wang *et al.* [27] in 2005 by using polymer supported reagents as well as using suitable coupling reagents. Despite the wide generality and the high efficiency of the aforementioned methodologies with their own merits, some limitations still remain, that is, most of the reaction conditions such as high temperature, long duration of reaction time, and chromatographic separation of the products do not satisfy each and every aspect of ideal and green synthesis. Therefore, in view of environmental concerns, there is still scope for the development of better alternative protocols for promoting this reaction.

On the other hand, chromenes are key structural units of a variety of biologically important compounds, many of which are pharmaceutically significant. In recent times, there are several drug moieties that are in use, bearing chromene entity in the treatment of various diseases like antiallergic, antitumor, antiviral, antioxidant, anti-inflammatory, antibacterial, and anticancer [28–35]. A key feature is that the lipophilic nature of the benzopyran derivatives helps to cross the bacterial cell membrane easily [36], which encouraged the chemist to develop novel methodologies for the synthesis of various 2*H*-chromene derivatives.

Nowadays, the synthesis of hybrid compound drawn much interest because hybrid compounds are more potent than their individual counterpart [37–41]. Hybrid compounds that possess important skeletons present in drugs/clinical agents have become an important research area for medicinal chemists. This can be an effective approach to possibly avoid the

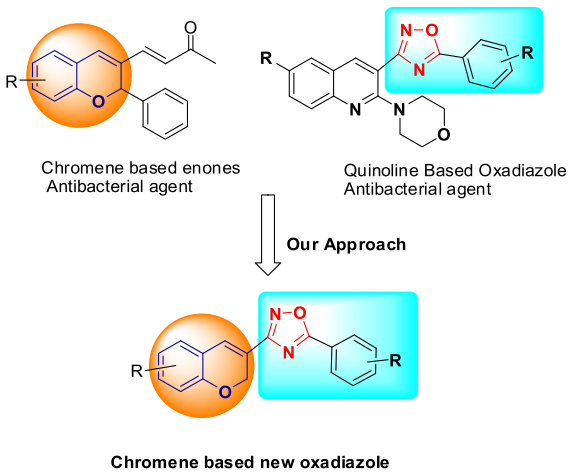


Figure 2. Designed new chromene-based oxadiazole derivative. [Color figure can be viewed at wileyonlinelibrary.com]

physicochemical/pharmacokinetic/toxicity problems that appear in the later stages of development. Literature report reveals that chromene-based enones [34] and quinoline-based oxadiazoles [11] show potent antibacterial activity. All these observations and our interest in chromene and oxadiazole derivatives prompted us to explore a series of new hybrid compounds (Fig. 2).

The activities of chromene, as well as oxadiazoles, lead us to embark both the pharmacophores in a single framework and to synthesize a set of chromene-substituted oxadiazole derivatives and to study its antibacterial activity. To the best of our knowledge, these are the newly synthesized chromene-fused 1,2,4-oxadiazole derivatives and have not been reported before.

Herein, we have designed the molecules based on structure–activity relationship studies and explored their antibacterial activity against two different pathogenic bacteria, that is, *E. coli* (MTCC614) and *Klebsiella pneumoniae* (MTCC4031). Among all the tested compounds, **6g** and **6h** exhibited good antibacterial activity nearer to the standard drug, gentamicin. Bacterial DNA gyrase is playing a vital role for catalyzing negative super coiling of DNA and involves with binding and cleaving the protein of the DNA. The literature reported that novobiocin has inhibited DNA gyrase, which is a chromene derivative. Thus, the selected antibacterial DNA gyrase has been performed for molecular docking [42]. The molecular docking studies showed that the compounds **6g** and **6h** have hydrogen bonding interaction with the bacterial target DNA gyrase of *E. coli*.

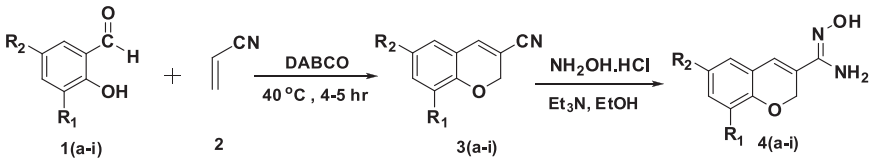
RESULTS AND DISCUSSION

In continuation of our enormous research interest in the development of efficient and environmentally friendly procedures for the synthesis of biologically important heterocycles, we devised a convenient and eco-friendly method for the synthesis of chromene-fused oxadiazole derivatives to provide privileged scaffolds for the generation of target compounds for drug discovery. In our preliminary studies, in this protocol, chromene-fused oxadiazole derivatives were synthesized starting from chromene-3-carbonitrile **3**(**a–i**). Initially, the starting chromene nitriles were prepared by following a green and efficient one-pot two component approach using salicylaldehyde **1**(**a–i**) and acrylonitrile **2** in the presence of DABCO under solvent-free conditions in good to high yield following Morita–Bayllis–Hillman reaction [43]. All the synthesized chromene-3-carbonitriles were successfully characterized by ^{1}H-NMR, ^{13}C-NMR, IR, and mass spectroscopy. In the subsequent step, compound **3**(**a–i**) was treated with hydroxylamine hydrochloride using Et_3N in ethanol for 2 h to obtain *N*′-hydroxy-2*H*-chromene-3-carboximidamide **4**(**a–i**) in 87–93% yield (Scheme 1). All the synthesized molecules were successfully characterized by ^{1}H-NMR and ^{13}C-NMR.

We mainly focused on developing a facile procedure to generate 2*H*-chromene-substituted 1,2,4-oxadiazole library with a variety of substitutions. It requires investigating the scope of the reaction by screening with various functionalities. In the process of evaluating best methodology, for the synthesis of 1,2,4-oxadiazole derivative, we have tried with various reagents such as EDCI, EDCI/HOBt, and DCC in different solvents such as DMF, CH_3CN, toluene, and THF (Scheme 2). As shown in Table 1, EDCI/HOBt in DMF under heating at 80°C provided higher yield of 75% (Table 1, entry 5). The yields were not improved further even on raising the temperature and elongating the time period. Therefore, EDCI/HOBt in DMF under reflux condition was chosen as optimal conditions for all further reactions.

In order to reduce the reaction time as well as to enhance the yield of chromene-substituted oxadiazole derivatives, the reaction was subsequently investigated under microwave irradiation. This technique is a valuable

Scheme 1. Synthesis of chromene-3-carbontrile **3**(**a–i**) and *N*′-hydroxy-2*H*-chromene-3-carboximidamide **4**(**a–i**).



Scheme 2. Methodology screening.

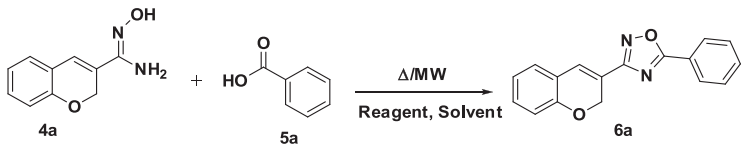


Table 1

Reaction system screening results.

Entry	Coupling reagent	Solvent	Temp. (°C)	Time (h)	Yield (%)[a]	MW (W)	Temp. (°C)	Time (min)	Yield (%)[a]
1	DCC	DMF	80	12	50	30	50	10	30
2	DCC	DMF				30	60	10	40
3	DCC	DMF				30	70	10	50
4	DCC	DMF				30	80	10	60
5	**EDCI/HOBt**	**DMF**	**80**	**6**	**75**	30	50	10	70
6	EDCI/HOBt	DMF				30	70	10	85
7	**EDCI/HOBt**	**DMF**				**30**	**80**	**10**	**92**
8	EDCI/HOBt	CH_3CN	80	12	n.r	30	80	10	n.r
9	EDCI/HOBt	Toluene	80	12	30	30	80	10	52
10	EDCI/HOBt	THF	80	12	35	30	80	10	48

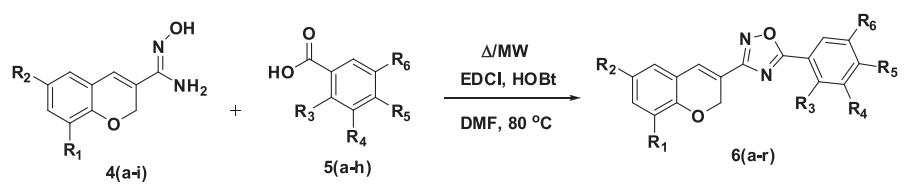
Bold signifies for best result.
n.r = No Reaction.
[a]Isolated yield.

alternative to conventional heating, introduces energetic radiations into the reaction, and thereby, enhances the rate and yields of the reaction and simultaneously decreases the time. Similar to conventional heating, the coupling reagents, as well as the solvents, were again screened under microwave irradiation. It has been observed that among all the reagents and solvents used, EDCI/HOBt in DMF at 80°C for 10 min provided 92% yield (Table 1, entry 7) of chromene-substituted oxadiazole derivative without any unwanted side products by simple recrystallization. To our delight in microwave irradiation excellent yield of the product, the formation was observed, and the reaction time period reduced from hours to minutes, which encouraged us to move further.

Having these preliminary observations in hand, we wished to broaden the scope of this protocol for the synthesis of a new class of 2*H*-chromene-substituted 1,2,4-oxadiazoles **6**(**a**–**r**) (Scheme 3). The reaction of 2*H*-chromene-3-carboximidamide **4**(**a**–**i**) with a variety of carboxylic acid derivatives **5**(**a**–**h**) in the presence of EDCI/HOBt in anhydrous DMF was carried out. All of them underwent the reaction smoothly affording the corresponding chromene-substituted oxadiazole derivatives. The results obtained for both conventional and microwave irradiation methods are summarized in Table 2, which clearly shows that microwave irradiation led to an enhancement in the rate as well as yield of the products (80–92%) over the conventional method (65–78%).

As shown in Table 2, a variety of aromatic carboxylic acid derivatives bearing functional groups such as halo, methoxy, and alkyl participated effectively with chromene-3-carboximidamide and resulted in chromene-substituted 1,2,4-oxadiazoles with good to excellent yields (Table 2). To our delight, a wide range of chromene-3-carboximidamide containing an electron-donating as well as electron-withdrawing groups was well tolerated. However, from Table 2, it was noticed that the product yield is affected by the substituents and their positions at the aromatic ring of the carboxylic acid. Without substitution in the carboxylic acid functionalities, compounds (**6a**, **6b**, **6d**, **6f**, **6g**, **6h**, **6i**, and **6k)** provided an excellent yield (82–92%). Presence of electron-donating groups (—OMe) at 2,4,5-position of the aromatic ring of carboxylic acid (**6c**, **6e**, **6j**, and **6l)** leads to decrease in the yield (80–82%). However, when the electron-donating group is changed from —OMe to $-CH_3$ in the carboxylic acid, good yield was observed (**6q**, 90%). Excitingly halogenated carboxylic acid derivatives such as —Cl and —Br were tolerated in entries **6m**, **6n**, and **6p** and provided better yield. Also, presence of single —OMe group at the carboxylic acid ring (**6o** and **6r)** decreases the yield (80–85%). The structures of the products **6**(**a**–**r**) were confirmed by ^{1}H-

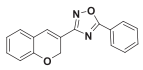
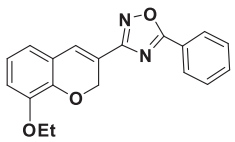
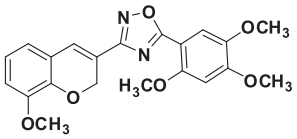
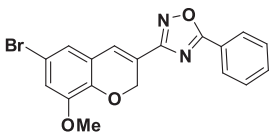
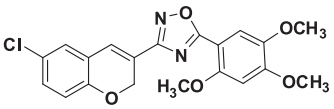
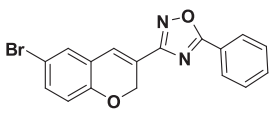
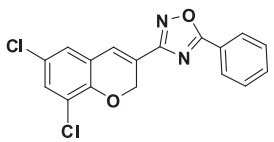
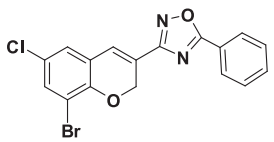
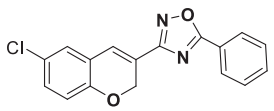
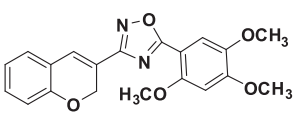
Scheme 3. Synthesis of chromene-fused oxadiazole derivatives **6**(**a**–**r**).



R_1 = H, OMe, OEt, Cl, Br; R_2 = H, Cl, Br; R_3 = H, OMe, Cl; R_4 = H, Me; R_5 = H, OMe, Cl, Br; R_6 = H, OMe

Table 2

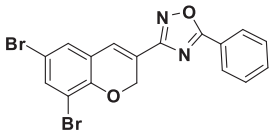
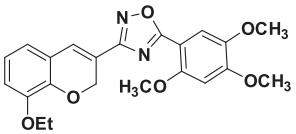
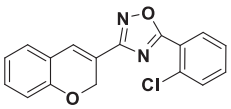
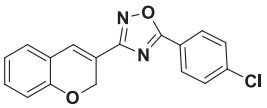
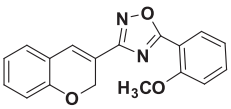
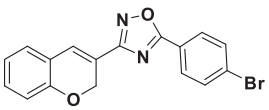
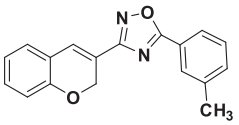
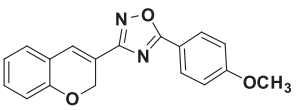
Substrate scope for the synthesis of chromene-fused 1,2,4-oxadiazole derivatives **6**(**a**–**r**).

		Conventional method: A (DMF, 80°C)		Microwave method: B (DMF, 80°C, 30 W)	
Entry	Product	Time (h)	Yield (%)[a]	Time (min)	Yield (%)[a]
6a		6	71	10	92
6b		6	68	10	88
6c		6	65	15	81
6d		4	72	15	88
6e		6	65	20	80
6f		2	75	10	86
6g		2.5	72	10	89
6h		5	67	15	82
6i		3	78	12	92
6j		6	65	20	82

(*Continues*)

Table 2

(*Continued*)

		Conventional method: A (DMF, 80°C)		Microwave method: B (DMF, 80°C, 30 W)	
Entry	Product	Time (h)	Yield (%)[a]	Time (min)	Yield (%)[a]
6k		5	65	15	82
6l		6	65	20	80
6m		6	75	20	83
6n		5	71	15	82
6o		4	66	12	80
6p		2	72	10	90
6q		3	70	10	90
6r		5	67	15	85

[a]Isolated yield.

NMR, ^{13}C-NMR, and HRMS. Also, the structure of the compound was unambiguously confirmed by single-crystal X-ray analysis of compound **6m** (Figs 3 and 4) [44].

Antibacterial activities. After successful synthesis of chromene-fused 1,2,4-oxadiazole derivatives **6(a–r)**, we further studied the antibacterial activity of the newly synthesized molecules against two gram-negative pathogenic bacteria *E. coli* (MTCC614) and *K. pneumoniae* (MTCC4031). The minimum inhibitory concentration values were determined by comparison with standard drug gentamicin (Table 3). The antibacterial activity studies indicated that all the synthesized compounds showed moderate to excellent activity against the tested gram-negative bacterial

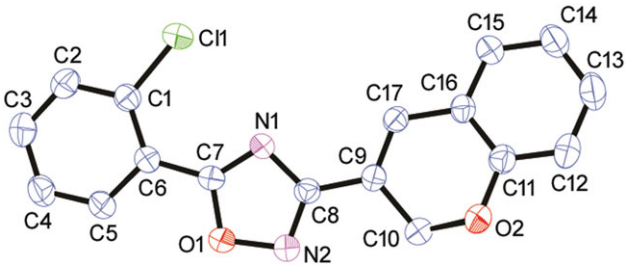


Figure 3. Single crystal analysis data of compound **6m**. [Color figure can be viewed at wileyonlinelibrary.com]

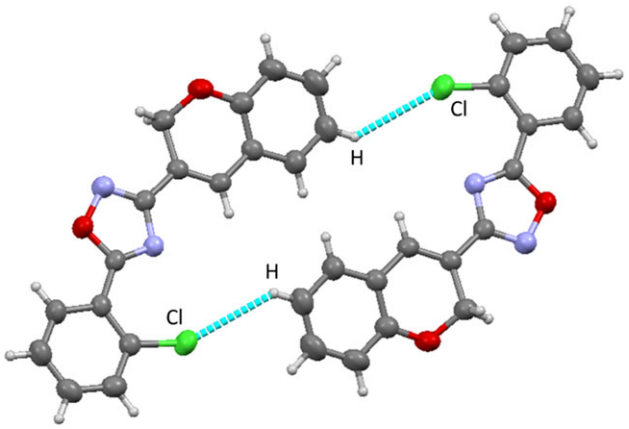


Figure 4. View of the dimeric unit formed from the C—H···Cl hydrogen bonding (cyan-colored dotted line) of 5-(2-chlorophenyl)-3-(2*H*-chromen-3-yl)-1,2,4-oxadiazole **6m**. [Color figure can be viewed at wileyonlinelibrary.com]

pathogens. From the antibacterial activity data, it was observed that compounds **6g** and **6h** are the most active among all the tested compound, while compounds **6a**, **6b**, **6d**, and **6p** showed less antibacterial activity against *E. coli*. Similarly, compounds **6a**, **6d**, and **6r** exhibit less antibacterial activity against *K. pneumoniae* in comparison with gentamicin. Rest of the compounds of the series showed moderate to good antibacterial activity against both the bacteria.

Structure–activity relationship study. The structure–activity relationship indicates that the potency of antibacterial activity exhibited by compounds is preferably due to the presence of halogen substituents at the structures because the introduction of halogen atoms into the chromene scaffold can be beneficial for antimicrobial activity so far as this improves the lipid solubility of the active ingredients. Most potent activity was observed when chromene moiety was substituted with electron-withdrawing halogen atom chlorine at C-6 position and chlorine/bromine at C-8 positions (**6g–h**). Also, chromene-fused 1,2,4-oxadiazole derivatives with electron-donating group methoxy at C-2, C-4, and C-5 positions of aromatic ring of carboxylic acid and methoxy, ethoxy, —Cl, and —H at C-8 position of chromene ring resulted in better antibacterial activity **(6c**, **6e**, **6l**, and **6j**). Introduction of halogen atoms (**6f**, **6i**, **6k**, **6m**, and **6n)** and methoxy group at C-2 position of carboxylic ring (**6o)** also led to an increase in the microbial activity. Inhibition was decreased when there is

Table 3

Antibacterial activity as size of zone of inhibition and MIC of synthesized compounds against two pathogenic bacteria.

Serial no.	Tested compound code	Diameter of inhibition zone in three different concentration (mm in µg/disc)						MIC (µg/mL)	
		Escherichia coli (MTCC614)			*Klebsiella pneumoniae* (MTCC4031)			*E. coli*	*K. pneumoniae*
1	**6a**	9	10	11	7	9	8	80	80
2	**6b**	8	10	10	7	8	8	80	60
3	**6c**	10	10	11	10	12	13	60	60
4	**6d**	8	9	12	7	8	8	80	80
5	**6e**	11	12	14	11	12	13	60	60
6	**6f**	10	12	12	11	12	13	60	60
7	**6g**	**14**	**17**	**20**	**12**	**14**	**19**	**40**	**40**
8	**6h**	**12**	**13**	**16**	**11**	**14**	**17**	**40**	**40**
9	**6i**	10	11	11	8	10	13	60	60
10	**6j**	10	11	11	10	12	13	40	60
11	**6k**	12	13	14	11	13	16	60	60
12	**6l**	10	12	11	10	12	13	60	60
13	**6m**	11	12	12	9	10	11	60	60
14	**6n**	11	12	12	10	12	14	60	60
15	**6o**	9	9	13	8	11	12	60	60
16	**6p**	7	8	9	8	8	10	80	60
17	**6q**	10	10	12	9	10	15	60	60
18	**6r**	8	9	13	7	10	11	60	80
Gen[a]	—	15	16	18	14	15	17	60	60

Bold signifies for best result.
MIC, minimum inhibitory concentration.
[a]Gentamicin used as reference antibiotic as positive control.

Table 4

Docking energy of two effective compounds against two bacterial targets.

Chemical	Docking scores (kcal/mol)	
	PDB ID: 1KZN	PDB ID: 4G89
6g	−8.57	−6.76
6h	−8.85	−6.93
Gentamicin	−6.71	−6.56

PDB, protein data band.

no substitution in the aromatic ring (**6a**). Also, from the result, it was observed that methoxy substitution at C-4 position of carboxylic acid (**6r**) and introduction of bromo group at C-6 position and methoxy at C-8 position of (**6d**) of chromene ring decrease the activity.

Molecular modeling. Molecular docking study was performed parallel to the synthesis and *in vitro* evaluation of chromene-based 1,2,4-oxadiazole to gain a more exhaustive perception of the interactions and binding ability of the molecule to the active sites of bacterial DNA gyrase. In order to find out the molecular binding interaction of compounds with bacterial DNA strain, compounds **6g** and **6h** were subjected to molecular docking study. The docking study was performed by Discovery Studio 4.0 program. Molecular docking was achieved by employing two targets *E. coli* (PDB ID: 1KZN) and *K. pneumoniae* (PDB ID: 4G89). The standard ligands were (**6g–h**, gentamicin). The docking energy gained by two effective compounds **6g** and **6h** against two bacterial targets was depicted in Table 4. It is stimulating to note that both compounds **6(g–h**) exhibited binding energy in the range between −6.76 and −8.57 kcal/mol. The 2D diagram illustrated that compounds **6(g–h)** and the standard drugs have interrelated to the active binding sites through various bindings. Through molecular docking study from Figures 5, 6, and 7, it was noticed that the most potent compounds **6g** and **6h** selectively bind to the DNA gyrase of the target bacteria *E. coli* with respect to confirmation of hydrogen bonding, alkyl, π–π interaction, π–donor hydrogen bonding, and π–σ interactions. A greater number of interactions found in compounds **6g** and **6h** that exactly fit into the DNA gyrase in the model resulted in greater interaction, which explains their potent antibacterial activity as compared with standard drug, gentamicin.

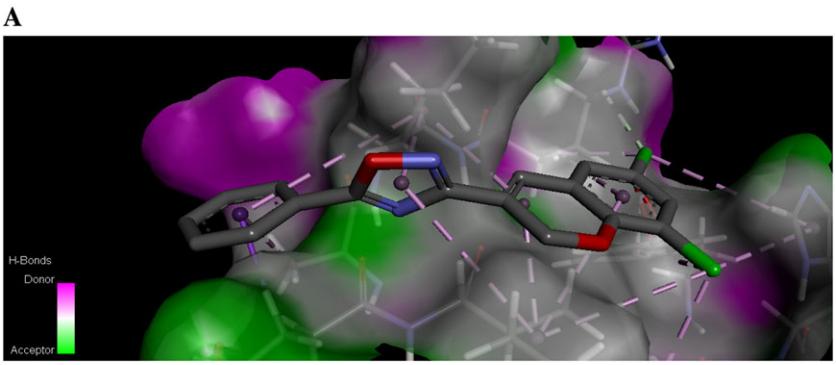


Figure 5. Interactions of chemical **6g**, with the bacterial target DNA gyrase of *Escherichia coli* visualization during molecular docking. [Color figure can be viewed at wileyonlinelibrary.com]

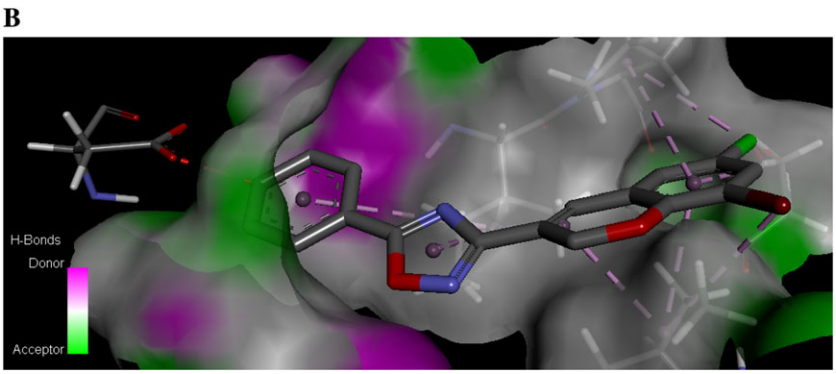


Figure 6. Interactions of chemical **6h**, with the bacterial target DNA gyrase of *Escherichia coli* visualization during molecular docking study. [Color figure can be viewed at wileyonlinelibrary.com]

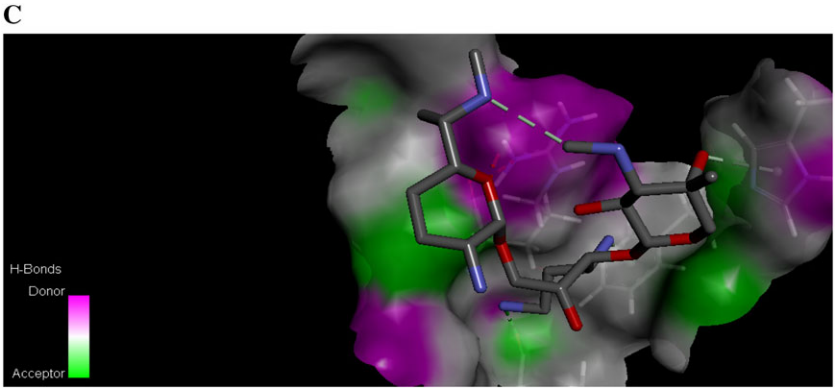
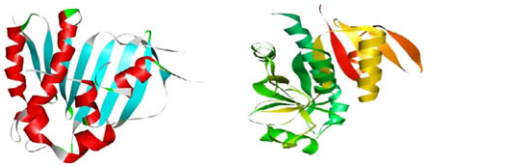


Figure 7. Interactions of antibiotic gentamicin with the bacterial target DNA gyrase of *Escherichia coli* visualization during molecular docking study. [Color figure can be viewed at wileyonlinelibrary.com]



Escherichia coli target (PDB ID:1KZN) *Klebsiella. Pneumoniae* target (PDB ID:4G89)

CONCLUSIONS

In summary, a series of new chromene-fused 1,2,4-oxadiazole derivatives were synthesized and evaluated for their *in vitro* antibacterial activity against two pathogenic bacteria, which are causing UTI: *E. coli* (MTCC614) and *K. pneumoniae* (MTCC4031). Among all the synthesized molecules, in particular, compounds **6g** and **6h** were identified as the most potent antibacterial candidates against *E. coli*. Through molecular docking study, it was noticed that the most potent compounds **6g** and **6h** selectively bind to the DNA gyrase of the target bacteria. Together, given their potent antibacterial activities, these newly synthesized hybrid molecules can be potentially be developed into useful antimicrobial agents that can prompt future researcher to synthesize a series of oxadiazole derivatives containing wide varieties of substituents with the aim of obtaining some novel heterocyclic candidates for the potential treatment of bacterial infection.

EXPERIMENTAL

Melting points were recorded on SMP10 digital melting point apparatus using open capillary tubes and uncorrected. IR spectra were recorded on a Nicolet FT-IR500 spectrophotometer using KBr. ^{1}H-NMR spectra were recorded on 400 MHz (100 MHz for ^{13}C-NMR) JEOL NMR spectrometer with $CDCl_3$ as solvent and tetramethylsilane as an internal standard. Chemical shifts were reported in parts per million (ppm, δ scale) downfield from tetramethylsilane at 0.00 ppm and referenced to the $CDCl_3$ at 7.26 ppm (for ^{1}H-NMR) or 77.00 ppm (for ^{13}C-NMR). HRMS analysis was conducted on a Brucker micro-TOF-Q-MS analyzer. All reagents and solvents used in this study were commercially available (from Sigma-Aldrich) and were used without further purification.

Synthesis of chromene-3-carbonitrile 3(a–i). A neat reaction was carried out by taking substituted salicylaldehydes **1(a–i)** (1.0 mmol), DABCO (0.2 mmol), and acrylonitrile **2** (1.0 mmol) in one pot and stirred at 40°C for 4–5 h. The progress of the reaction was monitored by TLC. After completion, reaction mixture was diluted with water and extracted with EtOAc. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by general silica gel (100–200 mesh) column chromatography using ethyl acetate/hexane to furnish the pure compound **3(a–i)** in 82–93% yield. All compounds were characterized through ^{1}H-NMR, ^{13}C-NMR, IR, and mass data.

2H-chromene-3-carbonitrile (3a). White solid. (93%). m.p.: 53–55°C; IR (KBr): *ν* 3436, 2215, 1606, 1484, 1459, 1211, 1150, 1037, 757 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.28–7.23 (m, 1H, Ar—H), 7.13 (s, 1H, CH), 7.12–7.09 (dd, J = 4.0 Hz, 8.0 Hz, 1H, Ar—H), 6.98–6.94 (m, 1H, Ar—H), 6.87 (d, J = 8.0 Hz, 1H, Ar—H), 4.79 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 154.4, 138.9, 132.8, 128.6, 122.5, 120.1, 116.7, 116.6, 103.4, 64.4. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{10}H_7NO$ $[M + H]^+$ 157.05276; found: 157.05265.

8-Ethoxy-2H-chromene-3-carbonitrile (3b). Off white solid. (92%). m.p.: 92–95°C; IR (KBr): *ν* 2931, 2209, 1626, 1578, 1475, 1398, 1335, 1282, 1212, 1113, 1087,

1015, 910, 894, 775, 728 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.50 (s, 1H, CH), 7.25–7.22 (m, 2H, Ar—H), 7.17–7.14 (m, 1H, Ar—H), 5.01 (s, 2H, CH_2), 4.12 (q, J = 8.0 Hz, 2H, CH_2), 1.79 (t, J = 8.0 Hz, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 147.5, 143.7, 139.1, 122.2, 120.9, 120.4, 116.7, 116.5, 103.4, 64.7, 64.6, 14.9. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{12}H_{11}NO_2$ $[M + H]^+$ 201.07898; found: 201.07811.

8-Methoxy-2H-chromene-3-carbonitrile (3c). Off white solid. (90%). m.p.: 101–103°C; IR (KBr): *v* 3429, 2924, 2205, 1606, 1577, 1483, 1464, 1275, 1223, 1174, 1098, 1021, 974, 775, 729 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.13 (s, 1H, CH), 6.90–6.87 (m, 2H, Ar—H), 6.70–6.68 (m, 1H, Ar—H), 4.83 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 148.1, 143.3, 139.0, 122.3, 120.8, 120.4, 116.5, 115.3, 103.5, 64.6, 56.2. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{11}H_9NO_2$ $[M + H]^+$ 187.06333; found: 187.06333.

6-Bromo-8-methoxy-2H-chromene-3-carbonitrile (3d). Yellow solid. (87%). m.p.: 97–101°C; IR (KBr): ν 3433, 3082, 2924, 2853, 2211, 1624, 1469, 1283, 1215, 1114, 851, 735 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.10 (s, 1H, CH), 7.02 (s, 1H, Ar—H), 6.90 (d, J = 4.0 Hz, 1H, Ar—H), 4.88 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 148.9, 142.2, 137.7, 122.5, 121.8, 118.2, 116.0, 114.2, 104.8, 64.7, 56.5. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{11}H_8BrNO_2$ $[M + H]^+$ 264.97384; found: 264.97370.

6-Chloro-2H-chromene-3-carbonitrile (3e). Faint yellow solid. (82%). m.p.: 132–134°C; IR (KBr, ν_{max}/cm^{-1}): 3444, 3066, 2923, 2213, 1629, 1480, 1239, 1213, 1113, 1021, 914, 815, 675, 628; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.19 (d, J = 8.0 Hz, 1H, CH), 7.09–7.06 (m, 2H, Ar—H), 6.80 (d, J = 8.0 Hz, 1H, Ar—H), 4.80 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 152.8, 137.7, 132.4, 127.8, 127.4, 121.2, 118.1, 116.0, 104.9, 64.5. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{10}H_6ClNO$ $[M + H]^+$ 191.01379; found: 191.01365.

6-Bromo-2H-chromene-3-carbonitrile (3f). Yellow solid. (82%). m.p.: 105–107°C; IR (KBr): *v* 3064, 2919, 2857, 2211, 1672, 1476, 1413, 1212, 1112, 1021, 917, 815, 757, 662 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.37 (d, J = 8.0 Hz, 1H, CH), 7.25–7.23 (m, 1H, Ar—H), 7.13–7.09 (m, 1H, Ar—H), 6.78 (d, J = 8.0 Hz, 1H, Ar—H), 4.80 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 153.2, 137.5, 135.3, 130.7, 121.7, 118.5, 116.0, 114.6, 104.8, 64.5. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{10}H_6BrNO$ $[M + H]^+$ 234.96328; found: 234.96344.

6,8-Dichloro-2H-chromene-3-carbonitrile (3g). Yellow solid. (86%). m.p.: 94–96°C; IR (KBr, ν_{max}/cm^{-1}): 3446, 2924, 2854, 2227, 1634, 1467, 1216, 762; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.32 (s, 1H, CH), 7.10 (s, 1H, Ar—H), 7.0 (s, 1H, Ar—H), 4.92 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 148.7, 137.0, 132.5, 127.3, 126.4, 122.8, 121.9, 115.5, 105.8, 65.1. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{10}H_5Cl_2NO$ $[M + H]^+$ 224.97482; found: 224.97470.

8-Bromo-6-chloro-2H-chromene-3-carbonitrile (3h). Yellow solid. (88%). m.p.: 143–145°C; IR (KBr, ν_{max}/cm^{-1}): 2925, 2854, 2209, 1732, 1465, 1252, 1222, 1193, 1021, 916, 864, 817, 729, 694; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.46 (s, 1H, CH), 7.06 (d, J = 8.0 Hz, 2H, Ar—H), 4.91 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.7, 136.9, 135.2, 127.6, 126.9, 121.6, 115.4, 111.1, 105.7, 65.1. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{10}H_5BrClNO$ $[M + H]^+$ 268.92430; found: 268.92420.

6,8-Dibromo-2H-chromene-3-carbonitrile (3i). Yellow solid. (83%). m.p.: 161–163°C; IR (KBr, ν_{max}/cm^{-1}): 2924, 2853, 2210, 1732, 1630, 1465, 1252, 1224, 1192, 1017, 914, 726, 661; ^{1}H-NMR (400 MHz,$CDCl_3$): δ 7.62 (s, 1H, CH), 7.19 (s, 1H, Ar—H), 7.09 (s, 1H, Ar—H), 4.94 (s, 2H, CH_2); ^{13}C-NMR (100 MHz,$CDCl_3$): δ 150.1, 137.9, 136.8, 129.8, 122.2, 115.4, 114.4, 111.5, 105.6, 65.1. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{10}H_5Br_2NO$ $[M + H]^+$ 312.87379; found: 312.8770.

Synthesis of (*Z*)-*N*′-hydroxy-2*H*-chromene-3-carboximidamide 4(a–i). In a reaction bottle, hydroxylamine hydrochloride ($NH_2OH.HCl$) (1.5 eqv.), Et_3N (1.5 eqv.), and ethanol were taken and stirred at room temperature for half an hour. Then chromene-3-carbonitrile (1 eqv.) was added to it, and the reaction mixture was stirred at room temperature. The progress of the reaction was monitored by TLC. The reaction was found completed after 2 h. After completion, reaction mixture was diluted with water and extracted with EtOAc. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to get the pure product **4(a–i)** in 87–93% yield. All compounds were characterized through ^{1}H-NMR and ^{13}C-NMR.

(Z)-N′-hydroxy-2H-chromene-3-carboximidamide (4a). White solid. (93%). m.p.: 200–202°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.18–7.14 (td, J = 7.7 Hz, 1.7 Hz, 1H, Ar—H), 7.08–7.06 (dd, J = 7.6 Hz, 1.6 Hz, 1H, Ar—H), 6.92–6.88 (m, 1H, Ar—H), 6.84 (d, J = 8.0 Hz, 1H, Ar—H), 6.69 (s, 1H, CH), 4.97 (s, 2H, CH_2), 4.64 (s, 2H, NH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 154.5, 145.0, 130.2, 127.5, 124.5, 122.0, 121.7, 121.5, 116.0, 64.3.

(Z)-8-ethoxy-N′-hydroxy-2H-chromene-3-carboximidamide (4b). White solid. (91%). m.p.: 112–115°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.86–6.78 (m, 2H, Ar—H, CH), 6.73–6.65 (m, 2H, Ar—H), 5.03 (s, 2H, CH_2), 4.68 (s, 2H, NH_2), 4.10 (q, J = 7.0 Hz, 2H, CH_2), 1.45 (t, J = 7.2 Hz, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.7, 147.0, 143.6, 124.4, 122.3, 122.1, 121.2, 119.7, 114.5, 64.6, 64.4, 14.8.

(Z)-N′-hydroxy-8-methoxy-2H-chromene-3-carboximidamide (4c). White solid. (90%). m.p.: 150–152°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.87–6.78 (m,

2H, Ar—H, CH), 6.71–6.63 (m, 2H, Ar—H), 5.01 (s, 2H, CH_2), 4.68 (s, 2H, NH_2), 3.86 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.5, 147.8, 143.3, 124.5, 122.2, 121.9, 121.2, 119.7, 112.9, 64.5, 56.0.

(Z)-6-bromo-N′-hydroxy-8-methoxy-2H-chromene-3-carboximidamide (4d). Yellow solid. (87%). m.p.: 158–160°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.88–6.81 (m, 2H, Ar—H), 6.57 (s, 1H, CH), 4.98 (s, 2H, CH_2), 4.65 (s, 2H, NH_2), 3.85 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.2, 148.6, 142.5, 125.7, 123.4, 122.0, 120.6, 115.9, 113.1, 64.5, 56.2.

(Z)-6-chloro-N′-hydroxy-2H-chromene-3-carboximidamide (4e). White solid. (89%). m.p.: 215–217°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.99 (s, 1H, OH), 7.15–7.12 (m, 1H, Ar—H), 7.10–7.09 (m, 1H, Ar—H), 6.93 (s, 1H, Ar—H), 6.79 (d, J = 8.0 Hz, 1H, CH), 5.62 (s, 2H, NH_2), 4.85 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 152.9, 149.1, 129.3, 127.2, 126.8, 125.6, 124.3, 119.9, 117.6, 64.5.

(Z)-6-bromo-N′-hydroxy-2H-chromene-3-carboximidamide (4f). Yellow solid. (91%). m.p.: 210–212°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.00 (s, 1H, OH), 7.27–7.22 (m, 2H, Ar—H), 6.93 (s, 1H, Ar—H), 6.74 (d, J = 8.0 Hz, 1H, CH), 5.62 (s, 2H, NH_2), 4.86 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 153.4, 149.1, 132.2, 129.7, 127.1, 124.8, 119.8, 118.1, 113.2, 64.5.

(Z)-6,8-dichloro-N′-hydroxy-2H-chromene-3-carboximidamide (4g). White solid. (87%). m.p.: 202–204°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.18–7.06 (m, 1H, Ar—H), 6.92–6.83 (m, 1H, Ar—H), 6.69 (s, 1H, CH), 4.97 (s, 2H, CH_2), 4.65 (s, 2H, NH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 154.4, 149.9, 130.2, 127.4, 124.4, 121.9, 121.6, 121.5, 115.9, 64.2.

(Z)-8-bromo-6-chloro-N′-hydroxy-2H-chromene-3-carboximidamide (4h). White solid. (87%). m.p.: 194–197°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1H, OH), 7.47 (s, 1H, Ar—H), 7.12 (s, 1H, Ar—H), 6.93 (s, 1H, CH), 5.66 (s, 2H, NH_2), 4.97 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 149.8, 148.8, 131.6, 127.9, 126.4, 126.0, 125.2, 119.4, 110.0, 65.4.

(Z)-6,8-dibromo-N′-hydroxy-2H-chromene-3-carboximidamide (4i). White solid. (87%). m.p.: 194–196°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.49 (d, J = 2.4 Hz, 1H, Ar—H), 7.12 (d, J = 2.4 Hz, 1H, Ar—H), 6.57 (s, 1H, CH), 5.08 (s, 2H, CH_2), 4.62 (s, 2H, NH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 149.1, 135.2, 134.2, 128.9, 126.4, 124.0, 120.0, 113.4, 110.7, 65.1.

Synthesis of chromene-based oxadiazole 6(a–r).

Procedure. Method A. EDCI/HOBt (1.5 mmol) and benzoic acid (1 mmol) were added to a solution of hydroxy-chromene-carboximidamide (1 mmol) in DMF (2 mL), and the mixture was refluxed for 2–6 h in a sealed tube at 80°C. The progress of the reaction was monitored by TLC. On completion of the reaction, the reaction mixture was cooled to room temperature and was diluted with water and extracted with EtOAc. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The pure product was obtained by recrystallization from isopropyl alcohol to provide compound **6(a–r)** in 65–78% yield.

Method B. EDCI/HOBt (1.5 mmol) and benzoic acid (1 mmol) was added to a solution of hydroxy-chromene-carboximidamide (1 mmol) in DMF (2 mL), and the mixture was irradiated under microwave irradiation (30W power) at 80°C for 10–20 min. The progress of the reaction was monitored by TLC. On completion of the reaction, the reaction mixture was cooled to room temperature and was diluted with water and extracted with EtOAc. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The pure product was obtained by recrystallization from isopropyl alcohol to provide compound **6(a–r)** in 80–92% yield.

3-(2H-chromen-3-yl)-5-phenyl-1,2,4-oxadiazole (6a). Light yellow solid. (92%). m.p.: 118–120°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.20 (d, J = 8.0 Hz, 2H, Ar—H), 7.65–7.55 (m, 4H, Ar—H), 7.27–7.20 (m, 2H, Ar—H), 6.97 (t, J = 8.0 Hz, 1H, Ar—H), 6.91 (d, J = 8.0 Hz, 1H, Ar—H), 5.27 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.2, 166.2, 154.6, 132.9, 131.0, 129.1, 128.2, 124.0, 121.8, 121.4, 119.3, 116.0, 64.5. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{12}N_2O_2$ $[M + H]^+$ 277.0899; found: 277.0998.

3-(8-Ethoxy-2H-chromen-3-yl)-5-phenyl-1,2,4-oxadiazole (6b). White solid. (88%). m.p.: 180–182°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.19 (d, J = 8.0 Hz, 2H, Ar—H), 7.64–7.60 (m, 1H, Ar—H, CH), 7.58–7.54 (m, 3H, Ar—H, CH), 6.90–6.83 (m, 3H, Ar—H), 5.33 (s, 2H, CH_2), 4.14 (q, J = 8.0 Hz, 2H, CH_2), 1.49 (t, J = 8.0 Hz, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.8, 165.9, 146.8, 143.5, 132.5, 128.8, 127.8, 123.6, 121.8, 121.1, 120.1, 119.0, 114.8, 64.4, 64.2, 14.5. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{19}H_{16}N_2O_3$ $[M + H]^+$ 321.1161; found: 321.1248.

3-(8-Methoxy-2H-chromen-3-yl)-5-(2,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (6c). Yellow solid. (81%). m.p.: 203–205°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.58(s, 1H, CH), 7.55 (s, 1H, Ar—H), 6.91–6.87 (m, 2H, Ar—H), 6.83 (dd, J = 4.0 Hz, 8.0 Hz, 1H, Ar—H), 6.60 (s, 1H, Ar—H), 5.31 (s, 2H, CH_2), 3.98 (s, 6H, OCH_3), 3.94 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.4, 165.4, 154.5, 153.9, 147.8 143.4, 143.2, 127.7, 122.1, 121.4, 120.3, 119.7, 113.4, 112.9, 104.2, 64.9, 56.9, 56.5, 56.1, 56.0. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{21}H_{20}N_2O_6$ $[M + H]^+$ 397.13214; found: 397.13979.

3-(6-Bromo-8-methoxy-2H-chromen-3-yl)-5-phenyl-1,2,4-oxadiazole (6d). Off white solid. (88%). m.p.: 200–202°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.12 (d,

J = 8.0 Hz, 2H, Ar—H), 7.63–7.46 (m, 5H, Ar—H), 6.97 (s, 1H, Ar—H), 5.31 (s, 2H, CH_2), 3.89 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.4, 165.9, 148.6, 133.8, 133.0, 130.2, 129.3, 129.1, 128.5, 128.2, 126.8, 122.6, 116.6, 113.3, 64.8, 56.3. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{18}H_{13}BrN_2O_3$ $[M + H]^+$ 385.0110; found: 385.0119.

3-(6-Chloro-2H-chromen-3-yl)-5-(2,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (6e). Yellow solid. (80%). m.p.: 158–160°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.56 (s, 1H, CH), 7.46–7.44 (m, 1H, Ar—H), 7.36–7.33 (m, 1H, Ar—H), 6.91 (d, J = 8.0 Hz, 1H, Ar—H), 6.79 (d, J = 8.0 Hz, 1H, Ar—H), 6.60 (s, 1H, Ar—H), 5.22 (s, 2H, CH_2), 3.97 (s, 6H, OCH_3), 3.93 (s, 3H, OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.5, 165.2, 154.6, 154.2, 153.0, 143.2, 130.3, 127.5, 126.8, 126.5, 125.7, 123.4, 117.9, 117.3, 113.0, 103.9, 64.7, 56.7, 56.5, 56.2. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{20}H_{17}ClN_2O_5$ $[M + H]^+$ 401.0826; found: 401.0922.

3-(6-Bromo-2H-chromen-3-yl)-5-phenyl-1,2,4-oxadiazole (6f). Light brown solid. (86%). m.p.: 190–193°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.18 (d, J = 8.0 Hz, 2H, Ar—H), 7.67–7.53 (m, 3H, Ar—H), 7.48 (s, 1H, Ar—H), 7.41–7.30 (m, 2H, Ar—H), 6.78–6.76 (m,1H, Ar—H), 5.26 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.3, 165.9, 153.6, 133.4, 132.9, 130.4, 129.1, 128.2, 126.8, 123.9, 123.1, 120.6, 117.8, 113.7, 64.6. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{11}BrN_2O_2$ $[M + Na]^+$ 377.0004; found: 377.2188.

3-(6,8-Dichloro-2H-chromen-3-yl)-5-phenyl-1,2,4-oxadiazole (6g). Yellow solid. (89%). m.p.: 112–114°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.18 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 8.0 Hz, 1H), 7.56 (m, 2H), 7.48 (s, 1H), 7.28 (s, 1H), 7.085 (d, J = 2.0 Hz, 1H), 5.37 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.5, 165.7, 148.9, 133.1, 130.6, 129.2, 128.2, 126.4, 126.3, 126.1, 123.8, 123.4, 121.9, 121.4, 65.3. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{10}Cl_2N_2O_2$ $[M + H]^+$ 345.0119; found: 345.0167.

3-(8-Bromo-6-chloro-2H-chromen-3-yl)-5-phenyl-1,2,4-oxadiazole (6h). White solid. (82%). m.p.: 185–187°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.17 (d, J = 8.0 Hz, 2H), 7.65–7.61 (m, 1H, Ar—H), 7.57–7.54 (m, 2H, Ar—H), 7.46 (s, 1H, Ar—H), 7.44 (d, J = 2.0 Hz, 1H), 7.11 (d, J = 2.4 Hz, 1H), 5.37 (s, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.5, 165.6, 150.0, 139.0, 133.4, 133.1, 130.7, 129.2, 128.2, 126.8, 126.3, 123.3, 121.4, 110.5, 65.4. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{10}BrClN_2O_2$ $[M + H]^+$ 388.9614; found: 388.9583.

3-(6-Chloro-2H-chromen-3-yl)-5-phenyl-1,2,4-oxadiazole (6i). White solid. (92%). m.p.: 104–106°C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.12 (d, J = 8.0 Hz, 2H), 7.69–7.50 (m, 5H), 7.26 (dd, J = 8.8, 2.4 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 5.18 (s, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 175.5, 166.1, 153.3, 134.1, 131.1, 130.2, 128.5, 128.4, 127.2, 126.0, 123.6, 123.2, 120.8, 118.0, 64.6. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{11}ClN_2O_2$ $[M]^+$ 310.0509; found: 310.0673.

3-(2H-chromen-3-yl)-5-(2,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (6j). Dirty yellow solid. (82%). m.p.: 200–202°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.58 (s, 1H), 7.21–719 (m, 2H), 6.95 (td, J = 7.4, 1.1 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 6.62 (s, 1H), 5.27 (s, 2H), 4.00 (s, 6H, OCH_3), 3.97 (s, 3H, OCH_3).^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.7, 165.8, 154.9, 154.8, 154.3, 143.5, 131.1, 128.4, 128.0, 122.0, 121.8, 119.9, 116.3, 113.2, 104.5, 64.9, 57.2, 56.8, 56.4. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{20}H_{18}N_2O_5$ $[M + H]^+$ 367.1216; found: 367.1329.

3-(6,8-Dibromo-2H-chromen-3-yl)-5-phenyl-1,2,4-oxadiazole (6k). Brown solid. (82%). m.p.: 220–222°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.19 (d, J = 8.0 Hz, 2H), 7.65–7.62 (m, 1H, Ar—H), 7.59–7.57 (m, 2H, Ar—H), 7.52–7.48 (m, 2H, Ar—H), 7.17 (d, J = 4.0 Hz, 1H, Ar—H), 5.39 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.0, 165.8, 149.2, 136.0, 133.1, 129.8, 129.7, 129.5, 129.2, 128.6, 128.2, 126.2, 123.8, 121.3, 113.4, 108.4, 65.4. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{10}Br_2N_2O_2$ $[M + H]^+$ 434.9089; found: 434.1509.

3-(8-Ethoxy-2H-chromen-3-yl)-5-(2,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (6l). Brown solid. (80%). m.p.: 183–185°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.61 (s, 1H, Ar—H), 7.57 (s, 1H, Ar—H), 6.89–6.82 (m, 3H, Ar—H), 6.62 (s, 1H, Ar—H), 5.33 (s, 2H, CH_2), 4.14 (q, J = 8.0 Hz, 2H, CH_2), 4.0 (s, 6H, OCH_3), 3.96 (s, 3H, OCH_3), 1.48 (t, J = 8.0 Hz, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.4, 165.5, 154.5, 153.9, 147.1, 143.8, 143.2, 127.8, 125.2, 122.3, 121.3, 120.3, 119.6, 115.0, 112.9, 104.3, 64.8, 64.6, 56.9, 56.5, 56.1, 14.8. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{22}H_{22}N_2O_6$ $[M + K]^+$ 449.1478; found: 449.9642.

5-(2-Chlorophenyl)-3-(2H-chromen-3-yl)-1,2,4-oxadiazole (6m). Light yellow solid. (83%). m.p.: 109–110°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.11 (dd, J_1 = 7.8, J_2 = 1.4 Hz, 1H, Ar—H), 7.39–7.35 (m, 4H, Ar—H), 7.26–7.20 (m, 2H, Ar—H), 6.98–6.93 (m, 1H, Ar—H), 6.91 (d, J = 8.0 Hz, 1H, Ar—H), 5.29 (s, 2H, CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.1, 166.3, 154.9, 135.0, 133.9, 133.6, 132.8, 132.2, 131.8, 131.3, 128.8, 128.7, 127.4, 127.0, 122.1, 116.4, 64.7. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{11}ClN_2O_2$ $[M - H]^+$ 309.05091; found: 309.04273.

5-(4-Chlorophenyl)-3-(2H-chromen-3-yl)-1,2,4-oxadiazole (6n). Yellow solid. (82%). m.p.: 114–116°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.13 (d, J = 8.0 Hz, 2H), 7.56–7.53 (m, 3H), 7.23–7.19 (m, 2H), 6.96 (td, J = 7.4, 1.1 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 5.25 (s, 2H) .^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.4, 166.4, 154.7, 139.5, 131.2, 129.7, 129.6, 128.5, 128.4, 122.5, 121.9, 121.4, 119.2, 116.2, 64.5. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{11}ClN_2O_2$ $[M - H]^+$ 309.05091; found: 309.04283.

3-(2H-chromen-3-yl)-5-(2-methoxyphenyl)-1,2,4-oxadiazole (6o). Brown solid. (80%). m.p.: 124–126°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.10 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.58–7.55 (m, 2H), 7.23–7.17 (m, 2H), 7.12–7.06 (m, 2H), 6.96 (td, *J* = 7.6, 0.9 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 5.27 (s, 2H), 4.0 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.6, 165.7, 158.5, 154.6, 134.2, 131.6, 130.8, 128.2, 127.9, 121.7, 121.4, 120.8, 119.5, 116.0, 113.6, 112.1, 64.6, 56.0. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{18}H_{14}N_2O_3$ $[M + H]^+$ 307.10044; found: 307.10751.

5-(4-Bromophenyl)-3-(2H-chromen-3-yl)-1,2,4-oxadiazole (6p). Yellow solid. (90%). m.p.: 198–200°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.05 (d, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.56 (s, 1H), 7.27–7.19 (m, 2H), 6.98–6.96 (m, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 5.25 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.0, 166.0, 154.3, 132.2, 130.7, 129.2, 128.1, 127.9, 127.6, 122.5, 121.5, 120.9, 118.8, 115.7, 64.1. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{17}H_{11}BrN_2O_2$ $[M + H]^+$ 355.0004; found: 354.9960.

3-(2H-chromen-3-yl)-5-(m-tolyl)-1,2,4-oxadiazole (6q). Brown solid. (90%). m.p.: 162–164°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.99–7.96 (m, 2H), 7.86–7.83 (m, 1H), 7.56 (s, 1H), 7.19 (td, *J* = 7.9, 1.6 Hz, 2H, Ar—H), 6.94 (td, *J* = 7.4, 1.1 Hz, 1H), 6.91–6.85 (m, 2H), 5.25 (s, 2H), 2.45 (s, 3H, OCH3). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 175.7, 166.5, 154.9, 139.4, 134.0, 131.2,129.3, 129.0, 128.5, 128.4, 126.8, 125.6, 122.1, 116.3, 64.8, 21.6. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{18}H_{14}N_2O_2$ $[M + H]^+$ 291.1055; found: 291.1142.

3-(2H-chromen-3-yl)-5-(4-methoxyphenyl)-1,2,4-oxadiazole (6r). White solid. (85%). m.p.: 128–130°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.13 (d, *J* = 12 Hz, 2H, Ar—H), 7.55 (s, 1H, Ar—H), 7.27–7.18 (m, 2H), 7.05–7.03 (m, 2H), 6.96 (td, *J* = 7.6, 0.9 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 5.25 (s, 2H), 3.90 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.7, 165.8, 162.9, 154.2, 130.5, 129.7, 127.9, 127.6, 121.4, 121.1, 119.2, 116.6, 115.7, 114.2, 64.2, 55.2. ESI-HRMS (*m/z*): *Anal*. Calcd for $C_{18}H_{14}N_2O_3$ $[M + H]^+$ 307.1004; found: 307.1093.

Biological assay. An *in vitro* activity of a series of compounds was evaluated by agar-well diffusion method against two pathogenic bacterial strains, viz. *E. coli* (MTCC614) and *K. pneumoniae* (MTCC4031) using Muller–Hinton agar medium. The antibacterial agar diffusion test was carried out using a bacterial cell suspension of about 1.5 × 106 CFU/mL employing a McFarland turbidity standard no. 0.5. The different concentrations for respective compounds were the plates loaded into wells and incubated 37°C for 24 h. Antibacterial activity of the congener was evaluated by measuring the diameter of the zone of inhibition (millimeter). An experiment of each candidate was conducted thrice, and the average inhibitory zone data were in the tabular form [45]. The reference drug gentamicin were aliquot in 100 micro leter at concentration 10 microgram per mL for both, gram -ve bacteria. The zone of inhibition rate 20 mm and/or more than consider as highest active and consequently, less than 20 mm as measured moderate active. Gentamicin 10 μg/mL with an average size of the zone of inhibition of 20 mm and no zone of inhibition by DMSO 10% solution was taken [46].

Determination of minimum inhibitory concentration. The six different concentrations (μg/mL) of an individual synthesized test sample and along with standard antibiotic gentamicin were prepared in DMSO by serial dilution technique; the values of concentration of tested samples were 80, 60, and 40 μg/mL, respectively. The calculated value of test sample was added to 96-well plates along aliquots 20 μL inoculated around 10^7 CFU/mL. Five microliter of an aliquot with 0.5% 2,3,5-triphenyltetrazolinium chloride was added in each well of 96-well plates. The bacterial growth was observed by pink coloration. After incubation of the well plates in 37°C for 24 h, the minimum inhibitory concentration value of tested samples was determined by observing the absence of bacterial growth in well plate.

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SUPPORTING INFORMATION

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