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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antimicrobial evaluation of 1,3,4-oxadiazole-based chalcone derivatives

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Abstract A series of chalcones-bearing 1,3,4-oxadiazole derivatives was synthesized as novel bio-active antimicrobial agents against multidrug-resistant bacteria and fungi. The lead compounds (*Z*)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-(3-(aryl)acryloyl)phenyl)acetamides **5a–n** were synthesized via acid-catalyzed aldol condensation ($SOCl_2$) by reacting *N*-(4-acetylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide (**4**) with differently substituted aldehydes. Compound (**4**) was obtained by reacting 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol (**2**) with *N*-(4-acetylphenyl)-2-chloroacetamide (**3**) in the presence of K_2CO_3. The intermediates (**2**) and (**3**) were synthesized simultaneously from 3-nitrobenzohydrazide (**1**) and 4-aminoacetophenone, respectively. The formation of intermediates and targeted compounds were confirmed for their structure by means of various spectral–analytical techniques like IR, ^{1}H NMR, ^{13}C NMR, elemental analysis, and mass spectra. Antimicrobial properties of all the synthesized compounds have been evaluated against broad panel of bacteria and fungi.

Keywords Chalcones · 1,3,4-Oxadiazole · Antifungal activity · Antibacterial activity · MIC

Introduction

The high resistance acquired by microbes against the antimicrobial drugs existing in the market is of a great challenge to the scientific fraternity, involved in the development of novel and effective drugs against human diseases. It is, therefore, necessary to develop therapeutic agents with improved potential for treating broad-spectrum microbial infections. In continuation with our earlier contribution of presenting clubbed molecules (Parikh and Joshi, 2013), we report here a series of hybrid heterocyclic scaffolds by clubbing chalcone with 1,3,4-oxadiazole in the present study.

The versatility of chalcone and its wide range of applicability in medicinal chemistry has attracted scientists all over the globe to concentrate their research around it. Chalcones have always been promising antifungal (Sarma *et al*., 2007; Parikh and Joshi, 2013) and antibacterial (Parikh and Joshi, 2013) entities. They are found to be prominent as antioxidants (Padhyee *et al*., 2009) and anti-inflammatory agents (Iwalewa *et al*., 2008). Some of the substituted chalcones have exhibited excellent anticancer (De Meyer *et al*., 1991), antimitotic (Ducki *et al*., 1998), antiplasmodial (Mei-Lin *et al*., 2004), and antiprotozoal activities (Azam *et al*., 2011). Chalcones have also displayed a variety of important biological properties such as anti-tubercular (Linn *et al*., 2002) and analgesics (Iwalewa *et al*., 2008) and a few of them have exhibited anti-hyperglycemic activity (Satyanarayana *et al*., 2004).

The small nitrogen and oxygen containing molecules have been under investigation since long because of their important medicinal properties. The chemistry and pharmacology of 1,3,4-oxadiazole ring system is of a considerable interest as the core structure remains the same as that of the various bio-active drugs possessing nitrogen and oxygen atoms. It can be clearly noticed from the literature survey that many of the lead molecules utilized for developing potent bio-active agents possess 1,3,4-oxadiazole as a nucleus. The presence of 1,3,4-oxadiazole as an important entity in the synthesis of several therapeutic

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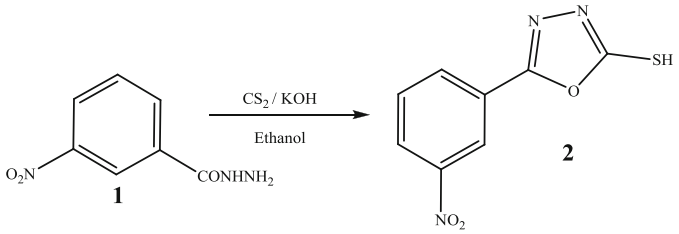
Step-1: Synthesis of 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol (2)



5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol

Reagents utilized in-

Step-1
CS_2 : Carbondisulphide
KOH : Pottasiumhydroxide

Step-2
CAC : Chloroacetylchloride
TEA : Triethylamine

Step-3
K_2CO_3 : Pottasiumcarbonate

Step-4
$SOCl_2$: Thionylchloride

Solvents used in-

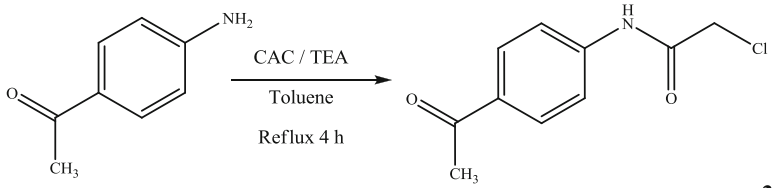
Step-1
Ethanol

Step-2
Toluene

Step-3
Acetone

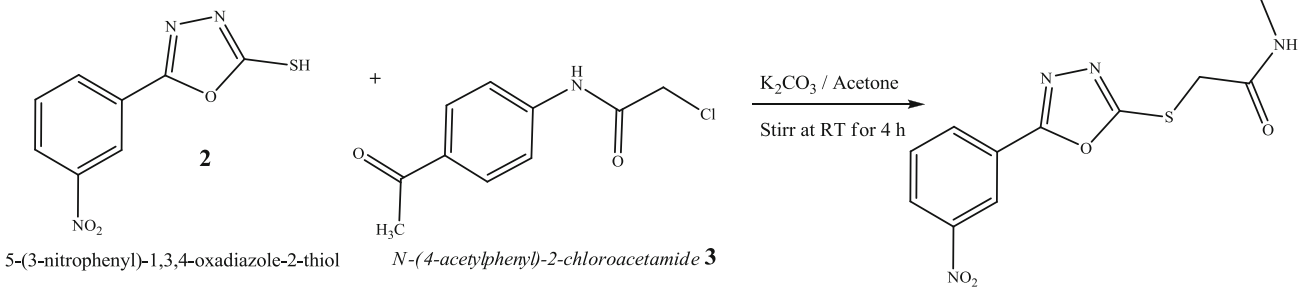
Step-4
Ethanol

Step-2: 4-aminoacetophenone to N-(4-acetylphenyl)-2-chloroacetamide (3)



4-aminoacetophenone *N-(4-acetylphenyl)-2-chloroacetamide* **3**

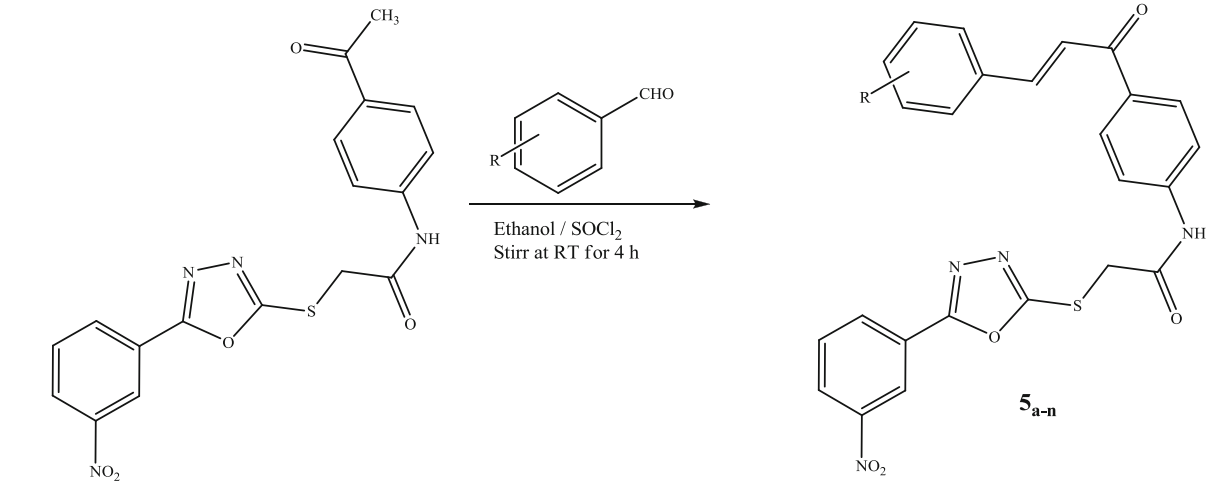
Step-3: Synthesis of N-(4-acetylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide (4)



5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol *N-(4-acetylphenyl)-2-chloroacetamide* **3**

N-(4-acetylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide
4

Step-4: Synthesis of Titled compounds (5_{a-n})



N-(4-acetylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide
4

Scheme 1 Synthesis of Compounds **5a–n**

Table 1 Results for antibacterial and antifungal screening of compounds **5a–n**

–R (Derivatives)	Minimum inhibitory concentration (MIC) in μg/ml					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
-4-OH-3-OCH_3	62.5	62.5	31.25	62.5	250	250
-4-NO_2	62.5	62.5	31.25	62.5	250	250
-4-Br	62.5	62.5	62.5	62.5	250	250
-3,4-diOCH_3	62.5	31.25	62.5	62.5	500	500
-2-NO_2	125	125	62.5	62.5	250	250
-C_6H_5	31.25	31.25	31.25	62.5	250	125
-3,4,5-triOCH_3	62.5	62.5	62.5	62.5	250	125
N,*N*-diCH_3	31.25	125	31.25	62.5	62.5	125
-2-OH	62.5	125	62.5	62.5	125	125
-4-OCH_3	62.5	125	62.5	62.5	125	125
-2,5-diOCH_3	62.5	125	62.5	62.5	125	125
-2-Hydroxy-1-napthaldehyde	62.5	62.5	62.5	62.5	62.5	62.5
-4-Cl	62.5	62.5	62.5	31.25	62.5	62.5
-4-OH	125	125	31.25	31.25	62.5	62.5
Fluconazole	–	–	–	–	125	62.5
Ciprofloxacin	62.5	125	125	125	–	–

MIC, minimum inhibitory concentration; *Std.*, standard drug Fluconazole for antifungal and Ciprofloxacin for antibacterial tests

agents is because of its several biological properties viz. antimicrobial (Palaska *et al*., 2002; Ahsan *et al*., 2011), antimitotic (Lokanatha Rai and Linganna, 2000), and antitubercular (Joshi *et al*., 2008). The mechanism of action and chemistry of various 1,3,4-oxadiazoles are of great interest; as they possess various biological activities such as antioxidant (Fadda *et al*., 2011; Kotaiah *et al*., 2012), anti-inflammatory (Maddi *et al*., 2012), and antitumor (Bondock *et al*., 2012). A variety of substituted 1,3,4-oxadiazole derivatives are utilized as benzodiazepine receptor agonist (Zarghi *et al*., 2005). Several other biological activities associated with 1,3,4-oxadiazole nucleus include antimicrobial (Krishnamurthy *et al*., 2012) and anticancer property (Maity *et al*., 2011).

Presently, in search for clubbed heterocyclic agents with potent antimicrobial activity and improved pharmacological properties, our group is pursuing investigations on designing various biologically active molecules by clubbing chalcones with different heterocyclic scaffolds. The research work conducted by Patil *et al.* supports that several heterocyclic scaffolds like benzofuran when incorporated with chalcones have resulted in the formation of bioactive scaffolds (Patil *et al*., 2010).

Result and discussion

Chemistry

The synthetic methods adopted for the formation of the targeted compounds (**5a–n**) are depicted in Scheme 1. 5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (**2**) was obtained by reacting 3-nitrobenzohydrazide with carbon disulfide and KOH in the presence of ethanol. Another reaction was undertaken to produce *N*-(4-acetylphenyl)-2-chloroacetamide (**3**) from 4-aminoacetophenone using chloroacetylchloride as a reactant and triethylamine as a catalyst. Thus obtained two intermediates: 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol (**2**) and *N*-(4-acetylphenyl)-2-chloroacetamide (**3**) were reacted in the presence of K_2CO_3 and acetone leading to the formation of *N*-(4-acetylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide (**4**). Various substituted aldehydes were made to react with *N*-(4-acetylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio) acetamide (**4**) via acid-catalyzed ($SOCl_2$) aldol condensation (Petrov *et al*., 2008) resulting in the syntheses of the desired compounds **5a–n**. The MIC values of the synthesized molecules are presented in Table 1.

Characterization

IR spectra

IR spectra of the compound **5g** ((*Z*)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)-*N*-(4-(3-(3,4,5-trimethoxyphenyl) acryloyl)phenyl)acetamide) have given a sharp absorption peak at 3260 cm^{-1} pointing out the presence of the secondary amine group. The stretching vibrations for the aromatic –C–H were observed at a frequency of 2981 cm^{-1}. The carbonyl functional group present in vicinity to the chalcone (–CH=HC–) has shown a sharp and intense absorption peak at 1663 cm^{-1}. Another stretching vibration at 1592 cm^{-1} in

the spectra of compound **5g** confirmed the presence of –CH=CH– (chalcone formation) near the carbonyl group. The presence of –C=C– in the aromatic ring was proved by the presence of an absorption peak at 1415 cm^{-1}. The carbon–nitrogen (–C–N–) linkage of the secondary amine group was further confirmed by an intensified absorption band at 1347 cm^{-1}. The formation of oxadiazole nucleus was confirmed by the presence of two absorption bands at 1035 and 1243 cm^{-1} indicating the –C–O–C– linkage in the structure. Thus the IR spectral data support the formation of the desired motifs **5a**–**n**.

^{1}H NMR

Let us consider compound **5g** and try to evaluate the proton NMR spectral data. Two broad but singlet peaks examined at $\delta = 3.41$ and $\delta = 3.78$ indicated the presence of methoxy group in the final structure. Of the three methoxy groups present, the protons of the two methoxy groups at C-22 and C-24 exhibited a single peak whereas the third present at C-23 (between C-22 and C-24) was observed at a higher δ (3.78) value than the other two. The singlet peak obtained at $\delta = 4.36$ helped to confirm the presence of the methylene group in the final structure. The presence of the protons intact with the two carbon atoms each of the chalcone group was confirmed by the presence of two doublets at $\delta = 7.71$ and $\delta = 7.89$, respectively. Also, the geometry of the compound **5g** was found to be of *cis* (*Z*) type, as the coupling constant values for the two protons were 8.8 Hz. The protons belonging to the aromatic rings of the final molecule were found to correspond between the δ values 7.01–8.72. A singlet peak observed at δ value of 10.69 proved the presence of proton on the secondary amine group.

^{13}C NMR

The conformation regarding the formation of the final compound **5g** was executed by the ^{13}C NMR data varying between $\delta = 38.8$ and $\delta = 190.5$. The carbon atoms of the two carbonyl groups present at C-10 and C-17 appeared more downfield at 168.3 and 190.5 ppm. The carbonyl carbon atom present in between the aromatic ring and the –CH=HC– appeared more downfield ($\delta = 190.5$) than the other obtained at $\delta = 168.3$ (C-10). The two carbon of the chalcone linkage corresponded to the absorption peaks at $\delta = 145.5$ (C-19) and $\delta = 121.3$ (C-18). The methylene group present in between the sulfur and the carbonyl (–C=O) functional group was observed to exhibit an absorption peak at $\delta = 38.5$. The two carbon C-7 and C-8 of the –C–O–C– linkage in oxadiazole nucleus exhibited absorption peaks at $\delta = 164.7$ and $\delta = 170.8$, respectively. The carbon atom

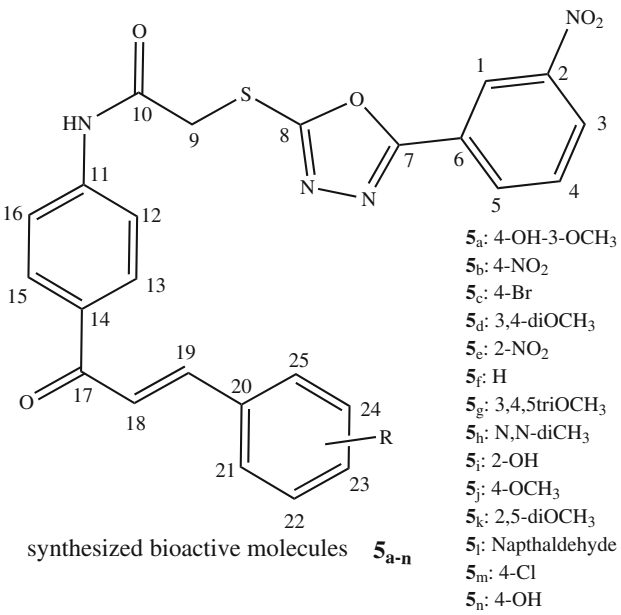


Fig. 1 Carbon numbering of the synthesized compounds **5a**–**n**

C-2 possessing the $-NO_2$ functional group was confirmed by an absorption peak obtained at a more downfield value of $\delta = 148.4$ compared to the other carbon atoms of the phenyl ring. The carbon numbering for the general structure is given in Fig. 1.

Antimicrobial activity

A broad panel of microbes was used for testing the antibacterial and antifungal properties of the molecules synthesized. The results obtained are reproduced in Table 1 as MIC values. The antimicrobial values showed that most of the synthesized derivatives exhibited excellent activities against different strains of bacteria and moderate activity against fungi. Ciprofloxacin and fluconazole were used as the standard drugs for antimicrobial and antifungal testing, respectively. When ciprofloxacin was used as a standard drug for testing the MIC values against the Gram-positive bacterial strains, it exhibited MIC value of 62.5 and 125 μg/ml against *S. aureus* and *E. faecalis*, respectively. The MIC values were found to be 125 μg/ml against both the Gram-negative bacteria (*E. coli* and *P. aeruginosa*) when tested for the standard drug ciprofloxacin. The standard drug fluconazole used against fungal strains *C. albicans* and *A. niger* showed MIC values of 125 and 62.5 μg/ml, respectively.

Antibacterial activity

The newly synthesized molecules **5a**–**n** were tested by micro dilution/broth titer method, keeping in consideration

the standard protocols put forward. All the compounds were screened against Gram-positive bacteria *S. aureus* (ATCC no. 25923) and *E. faecalis* (ATCC no. 29212) and Gram-negative bacteria *E. coli* (ATCC no. 25922) and *P. aeruginosa* (ATCC no. 27853) by preparing the serial dilutions of the samples. Compounds **5f** and **5h** exhibited excellent activity (31.25 μg/ml) against the Gram-positive bacterial strain *S. aureus*. Compounds **5a**, **5b**, **5c**, **5d**, **5g**, **5i**, **5j**, **5k**, **5l**, and **5m** showed MIC value (62.5 μg/ml) equivalent to that reported by the standard ciprofloxacin. Only two derivatives **5e** and **5n** were found to exhibit low activity (125 μg/ml) as compared to the standard, whereas most of the other compounds exhibited good activity against the Gram-positive bacteria *S. aureus*. When *E. faecalis* was introduced as another Gram-positive strain for conducting the antibacterial test, it was found that not a single derivative from the series exhibited poor activity as compared to the standard. The derivatives **5d** and **5f** showed excellent activity (31.25 μg/ml) against *E. faecalis*. It was also observed that the compounds **5a**, **5b**, **5c**, **5g**, **5l**, and **5m** exhibited even better MIC value (62.5 μg/ml) than the standard ciprofloxacin. MIC values equal to that of the standard drug was shown by the remaining derivatives **5e**, **5h**, **5i**, **5j**, **5k**, and **5n**. The complete series of derivatives synthesized was tested for their antibacterial property against two different strains of Gram-negative bacteria *E. coli* and *P. aeruginosa*. When the compounds were exposed against Gram-negative bacteria *E. coli*, it was observed that the compounds **5a**, **5b**, **5f**, **5m**, and **5n** exhibited excellent activity (31.25 μg/ml) as compared to the standard drug. Other derivatives **5c**, **5d**, **5e**, **5g**, **5h**, **5i**, **5j**, **5k**, and **5l** have also showed much better activity (62.5 μg/ml) than the standard drug ciprofloxacin. Overall, all the compounds **5a**–**n** have exhibited very good activity, even better than the standard drug against Gram-negative strain *E. coli*. Even the MIC values recorded for the derivatives against *P. aeruginosa* were found to be exhibiting much better activity than that of the standard used. The compounds **5m** and **5n** showed excellent activity (31.25 μg/ml) as compared to the standard drug ciprofloxacin. All the remaining derivatives **5a**–**l** showed very good activity (62.5 μg/ml) as compared to the standard. It was observed that very few derivatives were found to exhibit less MIC value than the standard ciprofloxacin, when tested against the bacterial strains. Other than that, all the compounds have shown excellent activity against the broad panel of Gram-positive and Gram-negative bacterial strains.

Antifungal activity

The synthesized motifs were tested against *C. albicans* (ATCC no. 10231) and *A. niger* (ATCC no. 1015) for their antifungal activity (MIC). It was found that as compared to the antibacterial activity, very few derivatives exhibited good activity. When the molecules **5a**–**n** were tested against *C. albicans,* derivatives **5h**, **5l**, **5m**, and **5n** showed excellent activity (62.5 μg/ml) as compared to the standard drug fluconazole. A few molecules **5i**, **5j**, and **5k** exhibited MIC value (125 μg/ml) equivalent to that of the standard drug fluconazole. Other chemical motifs **5a**, **5b**, **5c**, **5e**, **5f**, and **5g** showed less activity (250 μg/ml) as compared to the standard drug. The compound **5d** showed much poor MIC value (500 μg/ml). *A. niger* was also used as a fungal strain for checking the antifungal potency of the derived bio-active compounds. It was observed that very few compounds viz. **5l**, **5m**, and **5n** showed antifungal activity equivalent to the standard drug fluconazole (62.5 μg/ml). All the other derivatives were found to exhibit poor activity. The compound **5d** was found to be least active against the fungal strains.

SAR study

Structure–activity relationship (SAR) study helped to reveal the effect of different substituents on the microbial strains depending upon the different electronic environments developed on the aromatic ring by substituting both electron-withdrawing and electron-donating groups. It was observed that compounds **5h** (3,4-dimethoxy), **5i** (2-OH), **5j** (4-OCH_3), **5k** (2,5-diOCH_3), **5l** (2-OH-1-napthaldehyde), and **5n** (4-OH) with electron-donating functional groups executed good antifungal results, displaying much lesser MIC value than the standard drug fluconazole against *C. albicans* and *A. niger*. It was not very clear to justify what kind of substituted derivatives were more potent antibacterial agents, as most of them were having good MIC results as compared to the standard drug ciprofloxacin. On comparing the MIC values of all the synthesized bio-active molecules (**5a**–**n**) mutually, it was found that the derivatives with electron-donating substituents like –OH and –OCH_3 exhibited much promising results; leading to a conclusion that the derivatives having capacity of increasing the electron density (electron-withdrawing groups) can lead to generation of more potent bio-molecules and can be more effective toward the broad panel of microorganism.

Experimental

Methods, materials, and physical measurements

All the chemicals and solvents required for the synthesis were purchased from Merck ltd., sdfine chemicals, LOBA Chemie, and HIMEDIA. Open-end capillary method was used to

determine the melting points of the synthesized derivatives and the results were reported uncorrected. The completion of reaction was monitored on TLC plates purchased from Merck (TLC Silica gel 60 F_{254}) and appropriate solvents were used as mobile phase. Bruker FT-IR alpha-t (ATR) was used to determine the IR spectral data for the conformation of functional groups present in the synthesized derivatives. The ^{1}H NMR and ^{13}C NMR spectral data were obtained using Bruker Spectrophotometer-400 and -100 MHz, respectively, where DMSO-d_6 was used as solvent and TMS was used as reference. Schimadzu mass spectrophotometer was used for the Mass spectral analysis. Perkin-Elmer 2400 CHN was utilized for the elemental analysis.

Synthesis and physical data

*Step-1: synthesis of 5-(3-nitrophenyl)-1,3,4-oxadiazole-2-thiol (**2**)*

3-Nitrobenzohydrazide (0.01 mol, 181 g/mol, 1.81 g) was taken in a round bottom flask containing ethanol (40 ml) and KOH (0.01 mol, 56.11 g/mol, 0.56 g in 2 ml H_2O). Carbon disulfide (0.02 mol, 76.14 g/mol, 1.2 ml) was added to the well-stirred solution and refluxed for 12–15 h. The ethanol was then distilled off and cooled to room temperature. The content was poured into ice-cold water and acidified with diluted HCl till the precipitates were obtained. The separated solid was washed with cold water and dried to get the desired product. The formation of titled intermediate was confirmed by observing the TLC using ethyl acetate:benzene (6:4) as a mobile phase.

Compound **2**: Solid light-green crystals; Yield: 66 %; m.p.: 155 °C; IR (ATR, cm^{-1}): 1035, 1248 (–C–O–C– str.), 1419 (–C=C– str. aromatic ring), 2571 (–S–H str.), 2988 (–C–H str. aromatic ring); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.60–8.35 (4H, m, Ar–H), 11.72 (1H, s, –SH); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 122.8 (C_1), 123.9 (C_3), 127.4 (C_6), 130.4 (C_4), 133.6 (C_5), 148.4 (C_2), 164.5 (C_7), 170.8 (C_8); MS (*m/z*): 224 (M^+); Anal. calcd. for $C_8H_5ClN_3O_3$ S: C-43.05, H-2.26, N-18.83, S-14.37 Found: C-43.08, H-2.30, N-18.86, S-14.40 %.

*Step-2: synthesis of N-(4-acetylphenyl)-2-chloroacetamide (**3**)*

The titled compound (**3**) was obtained by reacting 4-aminoacetophenone (0.01 mol, 135 g/mol, 1.35 g) with chloroacetylchloride (0.015 mol, 113 g/mol, 1.19 ml) and Triethylamine (3–4 drops) in toluene (25 ml). The reaction mixture was refluxed for 4 h. The completion of reaction was monitored using TLC with mobile phase toluene:acetone (7:3). The obtained intermediate was buff in color and solid by state. Crystallization was carried in toluene.

Compound **3**: Solid light-brown crystals; Yield: 87 %; m.p.: 154 °C; IR (ATR, cm^{-1}): 740 (C–Cl str.), 1413 (C=C str. aromatic ring), 1640 (C=O str.), 3017 (–C–H str. aromatic ring), 3262 (NH str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.56 (3H, s, –CH_3), 4.89 (2H, s, –CH_2), 7.77 – 8.10 (4H, d, Ar–H), 9.97 (1H, s, –NH); ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 27.2 (C_{18}), 43.2 (C_9), 121.0 (C_{12}), 121.0 (C_{16}), 129.2 (C_{13}), 129.2 (C_{15}), 137.2 (C_{14}), 142.2 (C_{11}), 166.0 (C_{10}), 198.2 (C_{17}); (MS (*m/z*): 212 (M^+); Anal. calcd. for $C_{10}H_{10}ClNO_2$: C-56.75, H-4.76, N-6.62 Found: C-56.79, H-4.81, N-6.68 %.

*Step-3: synthesis of N-(4-acetylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide (**4**)*

The compounds (**2**) and (**3**) obtained above were used as precursors for the synthesis of titled compound (**4**). 5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (**2**) (0.01 mol, 223 g/mol, 2.23 g) was made soluble in acetone and *N*-(4-acetylphenyl)-2-chloroacetamide (**3**) (0.01 mol, 211 g/mol, 2.11 g) was added to it with stirring. K_2CO_3 (0.02 mol, 138 g/mol, 2.76 g) was added to it and the solution was stirred at room temperature for 4 h. The completion of reaction was monitored by using TLC using toluene:acetone (7:3) as mobile phase. The solution was poured in ice-cold water and stirred for 30 min. The separated product was filtered, dried, and recrystallized from methanol.

Compound **4**: Light yellow; Yield: 79 %; IR (ATR, cm^{-1}): 1036, 1244 (–C–O–C– str.), 1351 (–C–N– str. sec. amine), 1417 (–C=C– str. aromatic ring), 1593 (C=C str. conjugated to carbonyl group), 1643 (C=O str.), 2981 (C–H str. aromatic ring), 3266 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.37 (3H, s, –CH_3), 4.36 (2H, s, –CH_2), 7.01–8.62 (8H, m, Ar–H), 10.71 (1H, s, –NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 27.4 (C_{18}), 38.8 (C_9), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.8 (C_3), 127.4 (C_6), 130.3 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 136.8 (C_{14}), 133.7 (C_5), 144.6 (C_{11}), 148.4 (C_2), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 196.6 (C_{17}); LCMS (*m/z*): 399 (M^+); Anal. calcd. for $C_{18}H_{14}N_4O_5S$: C, 54.27 %; H, 3.54 %; N, 14.06 %; S, 8.05 %. Found: C, 54.32 %; H, 3.59 %; N, 14.10 %; S, 8.08 %.

*Step-4: general procedure for the synthesis of final compounds **5a–n***

The formation of final compounds was undertaken by utilizing popular acid-catalyzed aldol condensation. $SOCl_2$ was used as an acid catalyst in the reaction, where *N*-(4-acetylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio) acetamide **4** (398 g/mol, 0.01 mol, 3.98 g) was made

soluble in an appropriate amount of ethanol. To this well-stirred solution, various substituted aldehydes (0.01 mol) were added and stirred at room temperature for 4 h. The obtained precipitates were poured onto crushed ice and stirred for 1 h. The products were washed with water periodically and then dried. All the obtained derivatives were recrystallized from alcohol and their melting points were recorded.

(Z)-N-(4-(3-(4-Hydroxy-3-methoxyphenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5a*** Chrome yellow; Yield: 68 %; m.p.: 162 °C; IR (ATR, cm^{-1}): 1039, 1244 (–C–O–C– str.), 1350 (–C–N– Str. sec. amine), 1421 (–C=C– str. aromatic ring), 1595 (C=C str. conjugated to carbonyl group), 1668 (C=O str. α,β-unsaturation), 2981 (C–H str. aromatic ring), 3262 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.46 (3H, s, –OCH_3), 4.16 (2H, s, –$C\underline{H}_2$), 7.71 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.8 Hz) 7.79 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.8 Hz), 7.09–8.52 (11H, m, Ar–$\underline{H}$), 10.68 (1H, s, –$\underline{N}H$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 111.7 (C_{21}), 116.2 (C_{24}), 121.5 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.0 (C_{25}), 123.8 (C_3), 127.3 (C_{20}), 127.4 (C_6), 130.3 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 144.6 (C_{11}), 145.5 (C_{19}), 147.5 (C_{23}), 149.7 (C_{22}), 148.4 (C_2), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 533 (M^+); Anal. calcd. for $C_{26}H_{20}N_4O_7S$: C, 58.64 %; H, 3.79 %; N, 10.52 %; S, 6.02 %. Found: C, 58.68 %; H, 3.81 %; N, 10.56 %; S, 6.06 %.

(Z)-2-(5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-ylthio)-N-(4-(3-(4-nitrophenyl)acryloyl)phenyl)acetamide ***5b*** Dark brown; Yield: 74 %; m.p.: 156 °C; IR (ATR, cm^{-1}): 1038, 1240 (–C–O–C– str.), 1361 (–C–N– Str. sec. amine), 1417 (–C=C– str. aromatic ring), 1584 (C=C str. conjugated to carbonyl group), 1660 (C=O str. α,β-unsaturation), 2992 (C–H str. aromatic ring), 3254 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.22 (2H, s, –$C\underline{H}_2$), 7.76 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.8 Hz) 7.83 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.8 Hz), 7.12–8.59 (12H, m, Ar–$\underline{H}$), 10.52 (1H, s, –$\underline{N}H$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 121.5 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.8 (C_3), 123.8 (C_{24}), 123.8 (C_{22}), 129.4 (C_{21}), 129.4 (C_{25}), 127.4 (C_6), 130.3 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 141.3 (C_{20}), 144.6 (C_{11}), 145.5 (C_{19}), 147.7 (C_{23}), 148.4 (C_2), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 532 (M^+); Anal. calcd. for $C_{25}H_{17}N_5O_7S$: C, 56.49 %; H, 3.22 %; N, 13.18 %; S, 6.03 %. Found: C, 56.54 %; H, 3.26 %; N, 13.22 %; S, 6.06 %.

(Z)-N-(4-(3-(4-Bromophenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5c*** Brown; Yield: 65 %; m.p.: 158 °C; IR (ATR, cm^{-1}): 1041, 1242 (–C–O–C– str.), 1356 (–C–N– Str. sec. amine), 1427 (–C=C– str. aromatic ring), 1591 (C=C str. conjugated to carbonyl group), 1672 (C=O str. α,β-unsaturation), 2984 (C–H str. aromatic ring), 3263 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.30 (2H, s, –$C\underline{H}_2$), 7.70 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.7 Hz) 7.85 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.7 Hz), 7.03–8.56 (12H, m, Ar–$\underline{H}$), 10.63 (1H, s, –$\underline{N}H$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 121.5 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.5 (C_{23}), 122.8 (C_1), 123.8 (C_3), 127.4 (C_6), 128.9 (C_{21}), 128.9 (C_{25}), 130.3 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 131.8 (C_{24}), 131.8 (C_{22}), 133.4 (C_{14}), 133.7 (C_5), 134.2 (C_{20}), 144.6 (C_{11}), 145.5 (C_{19}), 148.4 (C_2), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 566 (M^{+2}); Anal. calcd. for $C_{25}H_{17}BrN_4O_5S$: C, 53.11 %; H, 3.03 %; N, 9.91 %; S, 5.67 %. Found: C, 53.15 %; H, 3.07 %; N, 9.95 %; S, 5.71 %.

(Z)-N-(4-(3-(3,4-Dimethoxyphenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5d*** Dark orange; Yield: 73 %; m.p.: 160 °C; IR (ATR, cm^{-1}): 1040, 1254 (–C–O–C– str.), 1363 (–C–N– Str. sec. amine), 1424 (–C=C– str. aromatic ring), 1598 (C=C str. conjugated to carbonyl group), 1671 (C=O str. α,β-unsaturation), 2992 (C–H str. aromatic ring), 3268 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.18 (2H, s, –$C\underline{H}_2$), 7.71 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.8 Hz) 7.88 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.8 Hz), 7.16–8.54 (11H, m, Ar–$\underline{H}$), 10.52 (1H, s, –$\underline{N}H$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 121.5 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.8 (C_3), 127.4 (C_6), 149.8 (C_{23}), 122.8 (C_{25}), 149.8 (C_{22}), 111.9 (C_{24}), 130.3 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 127.3 (C_{20}), 144.6 (C_{11}), 145.5 (C_{19}), 111.4 (C_{21}), 148.4 (C_2), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 547 (M^+); Anal. calcd. for $C_{27}H_{22}N_4O_7S$: C, 59.33 %; H, 4.06 %; N, 10.25 %; S, 5.87 %. Found: C, 59.38 %; H, 4.10 %; N, 10.28 %; S, 5.92 %.

(Z)-2-(5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-ylthio)-N-(4-(3-(2-nitrophenyl)acryloyl)phenyl)acetamide ***5e*** Brown; Yield: 71 %; m.p.: 148 °C; IR (ATR, cm^{-1}): 1035, 1249 (–C–O–C– str.), 1359 (–C–N– Str. sec. amine), 1414 (–C=C– str. aromatic ring), 1587 (C=C str. conjugated to carbonyl group), 1662 (C=O str. α,β-unsaturation), 2987 (C–H str. aromatic ring), 3271 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.09 (2H, s, –$C\underline{H}_2$), 7.78 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.7 Hz) 7.89 (1H, d, –$\underline{H}C$=C$\underline{H}$–, J = 8.7 Hz), 7.04–8.62 (12H, m, Ar–$\underline{H}$), 10.58 (1H, s, –$\underline{N}H$); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 121.5 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.8 (C_3), 127.4 (C_6), 127.9 (C_{23}), 128.5 (C_{25}), 128.8 (C_{22}), 128.8 (C_{24}), 130.3 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 127.4 (C_{20}), 144.6 (C_{11}), 145.5 (C_{19}), 147.8 (C_{21}), 148.4

(C_2), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 532 (M^+); Anal. calcd. for $C_{25}H_{17}N_5O_7S$: C, 56.49 %; H, 3.22 %; N, 13.18 %; S, 6.03 %. Found: C, 56.54 %; H, 3.25 %; N, 13.22 %; S, 6.07 %.

(Z)-N-(4-Cinnamoylphenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5f*** Black; Yield: 62 %; m.p.: 205 °C; IR (ATR, cm^{-1}): 1037, 1246 (–C–O–C– str.), 1351 (–C–N– Str. sec. amine), 1419 (–C=C– str. aromatic ring), 1599 (C=C str. conjugated to carbonyl group), 1654 (C=O str. α,β-unsaturation), 2991 (C–H str. aromatic ring), 3263 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.27 (2H, s, –C<u>H</u>$_2$), 7.74 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.7 Hz) 7.81 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.8 Hz), 7.16–8.57 (13H, m, Ar–<u>H</u>), 10.73 (1H, s, –<u>N</u>H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 121.5 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.8 (C_3), 127.4 (C_6), 127.9 (C_{23}), 128.5 (C_{21}), 128.5 (C_{25}), 128.8 (C_{22}), 128.8 (C_{24}), 130.3 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 135.2 (C_{20}), 144.6 (C_{11}), 145.5 (C_{19}), 148.4 (C_2), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 487.5 (M^+); Anal. calcd. for $C_{25}H_{18}N_4O_5S$: C, 61.72 %; H, 3.73 %; N, 11.52 %; S, 6.59 %. Found: C, 61.77 %; H, 3.76 %; N, 11.56 %; S, 6.63 %.

(Z)-2-(5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-ylthio)-N-(4-(3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide ***5g*** Light brown; Yield: 67 %; m.p.: 142 °C; IR (ATR, cm^{-1}): 1035, 1243 (–C–O–C– str.), 1347 (–C–N– str. sec. amine), 1415 (–C=C– str. aromatic ring), 1592 (C=C str. conjugated to carbonyl group), 1663 (C=O str. α,β-unsaturation), 2981 (C–H str. aromatic ring), 3260 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.41 (6H, s, –OC<u>H</u>$_3$), 3.78 (3H, s, –OCH$_3$), 4.36 (2H, s, –C<u>H</u>$_2$), 7.71 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.8 Hz), 7.89 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.8 Hz) 7.01–8.72 (10H, m, Ar–<u>H</u>), 10.69 (1H, s, –<u>N</u>H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 103.6 (C_{21}), 103.6 (C_{25}), 121.3 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.9 (C_3), 126.6 (C_{20}), 127.4 (C_6), 130.4 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 138.7 (C_{23}), 144.6 (C_{11}), 145.5 (C_{19}), 148.4 (C_2), 153.5 (C_{22}), 153.5 (C_{24}), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 577 (M^+); Anal. calcd. for $C_{28}H_{24}N_4O_8S$: C, 58.33 %; H, 4.20 %; N, 9.72 %; S, 5.56 %. Found: C, 58.37 %; H, 4.23 %; N, 9.75 %; S, 5.60 %.

(Z)-N-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5h*** Light yellow; Yield: 60 %; m.p.:170 °C; IR (ATR, cm^{-1}): 1042, 1256 (–C–O–C– str.), 1353 (–C–N– str. sec. amine), 1427 (–C=C– str. aromatic ring), 1594 (C=C str. conjugated to carbonyl group), 1669 (C=O str. α,β-unsaturation), 2995 (C–H str. aromatic ring), 3267 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.29 (2H, s, –C<u>H</u>$_2$), 7.71 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.8 Hz), 7.83 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.8 Hz) 7.10–8.48 (12H, m, Ar–<u>H</u>), 10.52 (1H, s, –<u>N</u>H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 111.9 (C_{22}), 111.9 (C_{24}), 121.3 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.9 (C_3), 124.9 (C_{20}), 127.4 (C_6), 129.6 (C_{21}), 129.6 (C_{25}), 130.4 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 144.6 (C_{11}), 145.5 (C_{19}), 148.4 (C_2), 150.8 (C_{23}), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 530 (M^+); Anal. calcd. for $C_{27}H_{23}N_5O_5S$: C, 61.24 %; H, 4.38 %; N, 13.22 %; S, 6.05 %. Found: C, 61.29 %; H, 4.41 %; N, 13.25 %; S, 6.11 %.

(Z)-N-(4-(3-(2-Hydroxyphenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5i*** Dark violet; Yield: 78 %; m.p.: 180 °C; IR (ATR, cm^{-1}): 1038, 1247 (–C–O–C– str.), 1356 (–C–N– str. sec. amine), 1424 (–C=C– str. aromatic ring), 1587 (C=C str. conjugated to carbonyl group), 1654 (C=O str. α,β-unsaturation), 2984 (C–H str. aromatic ring), 3271 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.38 (2H, s, –C<u>H</u>$_2$), 7.75 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.7 Hz), 7.88 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.7 Hz) 7.19–8.58 (12H, m, Ar–<u>H</u>), 10.34 (1H, s, –<u>N</u>H);^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 117.8 (C_{22}), 121.3 (C_{18}), 121.6 (C_{24}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 122.9 (C_{20}), 123.9 (C_3), 127.4 (C_6), 129.3 (C_{25}), 129.7 (C_{23}), 130.4 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 144.6 (C_{11}), 145.5 (C_{19}), 148.4 (C_2), 157.6 (C_{21}), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 503 (M^+); Anal. calcd. for $C_{25}H_{18}N_4O_6S$: C, 59.75 %; H, 3.61 %; N, 11.15 %; S, 6.38 %. Found: C, 59.80 %; H, 3.66 %; N, 11.18 %; S, 6.44 %.

(Z)-N-(4-(3-(4-Methoxyphenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5j*** Yellow; Yield: 72 %; m.p.: 170 °C; IR (ATR, cm^{-1}): 1029, 1239 (–C–O–C– str.), 1350 (–C–N– str. sec. amine), 1419 (–C=C– str. aromatic ring), 1594 (C=C str. conjugated to carbonyl group), 1663 (C=O str. α,β-unsaturation), 2993 (C–H str. aromatic ring), 3269 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.27 (2H, s, –C<u>H</u>$_2$), 7.72 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.8 Hz), 7.83 (1H, d, –<u>H</u>C=C<u>H</u>–, J = 8.8 Hz) 7.03–8.61 (12H, m, Ar–<u>H</u>), 10.51 (1H, s, –<u>N</u>H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 114.8 (C_{22}), 114.8 (C_{24}), 121.3 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.9 (C_3), 127.8 (C_{20}), 127.4 (C_6), 130.6 (C_{25}), 130.4 (C_4), 130.6 (C_{21}), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 144.6 (C_{11}), 145.5 (C_{19}), 148.4 (C_2), 160.2 (C_{23}), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 517 (M^+); Anal. calcd. for $C_{26}H_{20}N_4O_6S$: C, 60.46 %; H, 3.90 %; N, 10.58 %; S, 6.21 %. Found: C, 60.52 %; H, 3.95 %; N, 10.62 %; S, 6.25 %.

(Z)-N-(4-(3-(2,5-Dimethoxyphenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5k*** Orange; Yield: 61 %; m.p.: 160 °C; IR (ATR, cm^{-1}): 1032, 1246 (–C–O–C– str.), 1354 (–C–N– str. sec. amine), 1417 (–C=C– str. aromatic ring), 1597 (C=C str. conjugated to carbonyl group), 1657 (C=O str. α,β-unsaturation), 2979 (C–H str. aromatic ring), 3274 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.34 (2H, s, –C$\underline{H}_2$), 7.79 (1H, d, –$\underline{H}$C=C$\underline{H}$–, $J = 8.8$ Hz), 7.87 (1H, d, –$\underline{H}$C=C$\underline{H}$–, $J = 8.8$ Hz) 7.02–8.52 (11H, m, Ar–$\underline{H}$), 10.73 (1H, s, –$\underline{N}$H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 115.4 (C_{22}), 152.8 (C_{24}), 121.3 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.9 (C_3), 115.8 (C_{20}), 127.4 (C_6), 111.7 (C_{25}), 130.4 (C_4), 151.6 (C_{21}), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 144.6 (C_{11}), 145.5 (C_{19}), 148.4 (C_2), 114.6 (C_{23}), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 547 (M^+); Anal. calcd. for $C_{27}H_{22}N_4O_7S$: C, 59.33 %; H, 4.06 %; N, 10.25 %; S, 5.87 %. Found: C, 59.38 %; H, 4.10 %; N, 10.29 %; S, 5.90 %.

(Z)-N-(4-(3-(3-Hydroxynaphthalen-2-yl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5l*** Dark brown; Yield: 58 %; m.p.: 220 °C; IR (ATR, cm^{-1}): 1041, 1250 (–C–O–C– str.), 1352 (–C–N– str. sec. amine), 1422 (–C=C– str. aromatic ring), 1589 (C=C str. conjugated to carbonyl group), 1664 (C=O str. α,β-unsaturation), 2985 (C–H str. aromatic ring), 3259 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.28 (2H, s, –C$\underline{H}_2$), 7.72 (1H, d, –$\underline{H}$C=C$\underline{H}$–, $J = 8.7$ Hz), 7.82 (1H, d, –$\underline{H}$C=C$\underline{H}$–, $J = 8.7$ Hz) 6.94–8.61 (14H, m, Ar–$\underline{H}$), 10.49 (1H, s, –$\underline{N}$H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 111.8 (C_{22}), 121.3 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.9 (C_3), 126.7 (C_{20}), 127.0 (C_{25}), 127.4 (C_6), 129.3 (C_{24}), 130.4 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 137.9 (C_{23}), 141.3 (C_{19}), 144.6 (C_{11}), 148.4 (C_2), 154.9 (C_{21}), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 553 (M^+); Anal. calcd. for $C_{29}H_{20}N_4O_6S$: C, 63.04 %; H, 3.65 %; N, 10.14 %; S, 5.80 %. Found: C, 63.08 %; H, 3.68 %; N, 10.18 %; S, 5.84 %.

(Z)-N-(4-(3-(4-Chlorophenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5m*** Dark brown; Yield: 65 %; m.p.: 195 °C; IR (ATR, cm^{-1}): 1038, 1246 (–C–O–C– str.), 1359 (–C–N– Str. sec. amine), 1428 (–C=C– str. aromatic ring), 1592 (C=C str. conjugated to carbonyl group), 1661 (C=O str. α,β-unsaturation), 2982 (C–H str. aromatic ring), 3273 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.21 (2H, s, –C$\underline{H}_2$), 7.70 (1H, d, –$\underline{H}$C=C$\underline{H}$–, $J = 8.7$ Hz), 7.79 (1H, d, –$\underline{H}$C=C$\underline{H}$–, $J = 8.7$ Hz) 7.12–8.54 (12H, m, Ar–$\underline{H}$), 10.53 (1H, s, –$\underline{N}$H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 121.3 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.9 (C_3), 127.4 (C_6), 129.0 (C_{24}), 129.0 (C_{22}), 129.2 (C_{25}), 129.2 (C_{21}), 130.4 (C_4), 131.6 (C_{13}), 131.6 (C_{15}), 133.3 (C_{20}), 133.4 (C_{14}), 133.7 (C_5), 133.8 (C_{23}), 141.3 (C_{19}), 144.6 (C_{11}), 148.4 (C_2), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 522 (M^{+2}); Anal. calcd. for $C_{25}H_{17}ClN_4O_5S$: C, 57.64 %; H, 3.29 %; N, 10.75 %; S, 6.16 %. Found: C, 57.68 %; H, 3.32 %; N, 10.78 %; S, 6.20 %.

(Z)-N-(4-(3-(4-Hydroxyphenyl)acryloyl)phenyl)-2-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-ylthio)acetamide ***5n*** Yellow; Yield: 63 %; m.p.: 208 °C; IR (ATR, cm^{-1}): 1041, 1248 (–C–O–C– str.), 1360 (–C–N– str. sec. amine), 1421 (–C=C– str. aromatic ring), 1597 (C=C str. conjugated to carbonyl group), 1664 (C=O str. α,β-unsaturation), 2990 (C–H str. aromatic ring), 3258 (NH str. sec. amine); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 4.25 (2H, s, –C$\underline{H}_2$), 7.68 (1H, d, –$\underline{H}$C=C$\underline{H}$–, $J = 8.7$ Hz), 7.82 (1H, d, –$\underline{H}$C=C$\underline{H}$–, $J = 8.8$ Hz) 7.03–7.64 (12H, m, Ar–$\underline{H}$), 10.59 (1H, s, –$\underline{N}$H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.8 (C_9), 116.4 (C_{24}), 116.4 (C_{22}), 121.3 (C_{18}), 122.5 (C_{12}), 122.5 (C_{16}), 122.8 (C_1), 123.9 (C_3), 127.4 (C_6), 127.9 (C_{20}), 130.4 (C_4), 130.8 (C_{25}), 130.8 (C_{21}), 131.6 (C_{13}), 131.6 (C_{15}), 133.4 (C_{14}), 133.7 (C_5), 141.3 (C_{19}), 144.6 (C_{11}), 148.4 (C_2), 157.9 (C_{23}), 164.7 (C_7), 168.3 (C_{10}), 170.8 (C_8), 190.5 (C_{17}); LCMS (*m/z*): 503 (M^{+2}); Anal. calcd. for $C_{25}H_{18}N_4O_6S$: C, 59.75 %; H, 3.61 %; N, 11.15 %; S, 6.38 %. Found: C, 59.81 %; H, 3.65 %; N, 11.17 %; S, 6.42 %.

Conclusion

The synthesized 1,3,4-oxadiazoles-clubbed chalcone derivatives (**5a**–**n**) have shown significant antifungal and antibacterial property. More than half of the derivatives have exhibited MIC value even better than that of the standards used. These bio-active molecules can be further optimized as potent lead molecules by introducing more electron-donating groups in the basic structure as stated in the SAR study. It can be concluded that, the presence of electron-donating groups hydroxy (–OH) and methoxy (–OCH_3) in the titled derivatives having the property of generating optimum electron density has helped to achieve better heterocyclic scaffolds possessing potent antibacterial and antifungal properties. Furthermore, there is an ample scope in developing these derivatives as potent lead molecules, which can be used as antimicrobial therapeutics.

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References

Ahsan MJ, Samy JG, Khalilullah H, Nomani MS, Saraswat P, Gaur R, Singh A (2011) Molecular properties prediction and synthesis of novel 1,3,4-oxadiazole analogues as potent antimicrobial and antitubercular agents. Bioorg Med Chem Lett 21:7246–7250. doi:10.1016/j.bmcl.2011.10.057

Azam A, Hayat F, Mosely E, Attar S, Vanzyl R (2011) Antiprotozoal activity of chloroquinoline based chalcones. Eur J Med Chem 46:1897–1905. doi:10.1016/j.ejmech.2011.02.004

Bondock S, Adel S, Etman HA, Badria FA (2012) Synthesis and antitumor evaluation of some new 1,3,4-oxadiazole-based heterocycles. Eur J Med Chem 48:192–199. doi:10.1016/j.ejmech.2011.12.013

De Meyer N, Haemers A, Mishra L, Pandey HK, Peters LA, Vanden Berghe DA, Vlietinck AJ (1991) 4′-Hydroxy-3-methoxyflavones with potent antipicornavirus activity. J Med Chem 34:736–746. doi:10.1021/jm00106a039

Ducki S, Forrest R, Hadfield J, Kendall A, Lawerence NJ, McGrown AT, Rennison D (1998) Potent antimitotic and cell growth inhibitory properties of substituted chalcones. Bioorg Med Chem Lett 8:1051–1056. doi:10.1016/S0960-894X(98)00162-0

Fadda AA, Abdel-Rahman AAH, El-Sayed WA, Zidan TA, Badria FA (2011) Synthesis of novel 1,3,4-oxadiazole derivatives and their nucleoside analogs with antioxidant and antitumor activities. Chem Heterocycl Compd 47:856–864. doi:10.1007/s10593-011-0847-4

Iwalewa EO, Oyedapo OA, Adewunmi CO, Makunja VO (2008) Analgesic, antioxidant and anti-inflammatory related activities of 2^1-hydroxy-2,4^1-dimethoxychalcone and 4-hydroxychalcone in mice. J Biol Sci 8:131–136. doi:10.3923/jbs.2008.131.136

Joshi SD, Vagdevi HM, Vaidya VP, Gadaginamath GS (2008) Synthesis of new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: a novel class of potential antibacterial and antitubercular agents. Eur J Med Chem 43:1989–1996. doi:10.1016/j.ejmech.2007.11.016

Kotaiah Y, Harikrishna N, Nagaraju K, Venkata Rao C (2012) Synthesis and antioxidant activity of 1,3,4-oxadiazole tagged thieno[2,3-*d*]pyrimidine derivatives. Eur J Med Chem 58:340–345. doi:10.1016/j.ejmech.2012.10.007

Krishnamurthy G, Patil BS, Lokesh MR, Shashikumar ND, Bhojya Naik HS, Latthe PR, Ghate M (2012) Synthesis of some novel 1,2,4-triazole and 1,3,4-oxadiazole derivatives of biological interest. Med Chem Res 22:3341–3349. doi:10.1007/s00044-012-0332-3

Linn L-M, Zhou Y, Flavin MT, Zhou LM, Nie W, Chen F-C (2002) Chalcones and flavonoids as anti-tuberculosis agents. Bioorg Med Chem 10:2795–2802. doi:10.1016/S0968-0896(02)00094-9

Lokanatha Rai KM, Linganna N (2000) Synthesis and evaluation of antimitotic activity of alkylated 2-amino-1,3,4-oxadiazole derivatives. Il Farmaco 55:389–392. doi:10.1016/S0014-827X(00)00056-2

Maddi V, Ingale N, Palkar M, Ronad P, Mamledesai S, Vishwanathswamy AHM, Satyanarayana D (2012) Synthesis and evaluation of anti-inflammatory and analgesic activity of 3-[(5-substituted-1,3,4-oxadiazol-2-yl-thio)acetyl]-2*H*-chromen-2-ones. Med Chem Res 21:16–26. doi:10.1007/s00044-010-9494-z

Maity TK, Dash S, Ashok B, Singh J, Maiti BC (2011) Synthesis of some novel 3,5-disubstituted 1,3,4-oxadiazole derivatives and anticancer activity on EAC model. Med Chem Res 20:1206–1213. doi:10.1007/s00044-010-9455-6

Mei-Lin Go, Liu Mei, Wilairat P, Rosenthal PJ, Saliba KJ, Kirk K (2004) Antiplasmodial chalcones inhibit sorbitol-induced hemolysis of *plasmodium falciparum*-infected erythrocytes. Antimicrob Agents Chemother 48:3241–3245. doi:10.1128/AAC.48.9.3241-3245

Padhyee S, Ahmad A, Oswal N, Sarkar FH (2009) Emerging role of Garcinol, the antioxidant chalcone from *Garcinia indica* Choisy and its synthetic analogs. J Hematol Oncol 2:38. doi:10.1186/1756-8722-2-38

Palaska E, Şahin G, Ekizoğlu M, Özalp M (2002) Synthesis and antimicrobial activity of some 1,3,4-oxadiazole derivatives. Il Farmaco 57:539–542. doi:10.1016/S0014-827X(02)01245-4

Parikh KS, Joshi DS (2013) Antibacterial and antifungal screening of newly synthesized benzimidazole-clubbed chalcone derivatives. Med Chem Res 22:3688–3697. doi:10.1007/s00044-012-0369-3

Patil SL, Bhalgat C, Burli S, Chithale S (2010) Synthesis, antibacterial and antioxidant properties of newer 3-(1-benzofuran-2-yl)-5-substituted aryl-1,2-oxazole. Int J Chem Sci Appl 1(1):42–49

Petrov O, Ivanova Y, Gerova M (2008) $SOCl_2$/EtOH: catalytic system for synthesis of chalcones. Catal Commun 9:315–316. doi:10.1016/j.catcom.2007.06.013

Sarma BK, Mishra PK, Singhai PK, Singh UP (2007) Antifungal activity of 2-hydroxy 4,4′,6′trimethoxy chalcone. Mycobiology 35:72–75. doi:10.4489/MYCO.2007.35.2.072

Satyanarayana M, Tiwari P, Tripathi BK, Srivastava AK, Pratap R (2004) Synthesis and antihyperglycemic activity of chalcone based aryloxypropanolamines. Bioorg Med Chem 12:883–889. doi:10.1016/j.bmc.2003.12.026

Zarghi A, Faizi M, Shafaghi B, Ahadian A, Khojastehpoor HR, Zanganeh V, Tabatabai SA, Shafiee A (2005) Design and synthesis of new 2-substituted-5-(2-benzylthiophenyl)-1,3,4-oxadiazoles as benzodiazepine receptor agonists. Bioorg Med Chem Lett 15:3126–3129. doi:10.1016/j.bmcl.2005.04.018