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ORIGINAL PAPER

Design, synthesis, antibacterial and quorum quenching studies of 1,2,5-trisubstituted 1,2,4-triazoles

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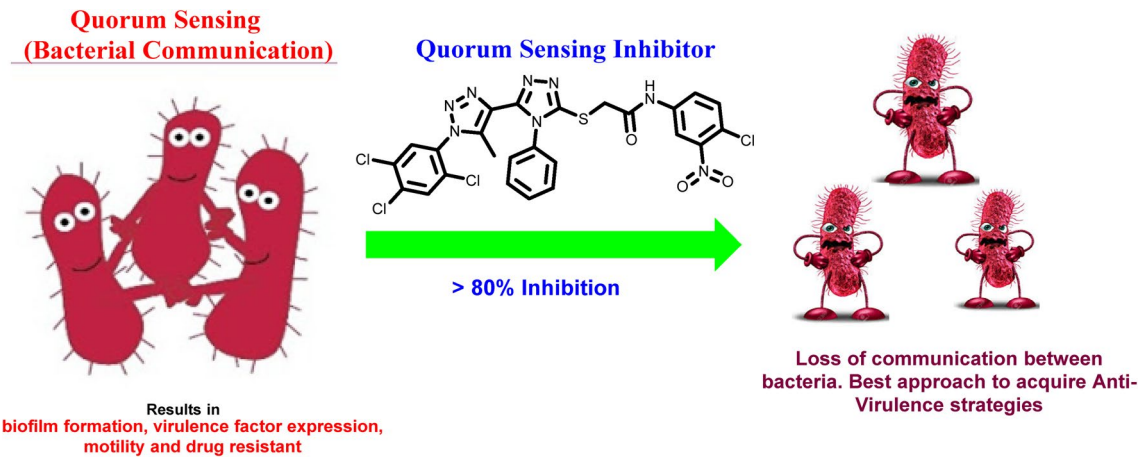
Abstract

In view of discovering novel bioactive molecules, 1-phenyl-1*H*-2-(1-aryl-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-(*N*-aryl-carbamoylmethylthio)-1,2,4-triazoles (**8a–n**) were designed and synthesized in good yield. Preliminary antibacterial activity was tested against *Chromobacterium violaceum* and *Xanthomonas campestris* pv. *Campestris* (*Xcc*). Out of 14 derivatives, compound **8g** selectively possessed antibacterial activity against *C. violaceum*. Further derivatives that possessed an electron-withdrawing group and halogen atoms in *N*-phenylacetamide moiety were moderately active against *Xcc* (plant pathogen). After observing the reduction of violacein production through plate assay, compounds **8a**, **8c**, **8h**, **8i** and **8m** were subjected to quantification of quorum sensing inhibition. Compounds with the electron-withdrawing group in *N*-phenylacetamide moiety showed admirable activity with > 80% inhibition of violacein. Mainly compound **8c** which was inactive against the growth of bacteria were identified as excellent QSI which could be a lead compound for further development.

Graphic abstract

One of the best approaches to acquire anti-virulence strategies and new direction for the discovery of antibacterial drugs



Keywords 1,2,3-Triazole · 1,2,4-Triazole · Quorum quenching · Molecular docking · ADME · *Xcc*

Introduction

Antibiotic resistance is a common problem worldwide due to the selection pressure from human applications of antibiotics. The pathogenic bacteria use various mechanisms to get shelter against antimicrobials. Due to this, bacteria

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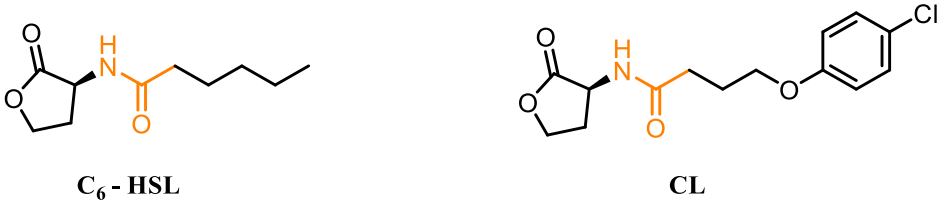
attain genotypic and phenotypic resistance to antibiotics and emerge as superbugs [1]. As a result, antibiotic treatments remain ineffective to the patients [2]. Hence, it's the foremost duty of the researchers to discover novel targets with better mechanisms of action [3, 4]. Among several mechanisms, quorum sensing (QS) inhibition shows to be an encouraging approach, as these molecules will not prompt resistance [5]. These pathogenic bacteria communicate by producing low molecular weight chemical signalling molecules called autoinducers (AIs) by the process called quorum sensing [6]. Bacteria use QS signalling pathway to govern virulence factor and biofilm formation which help in antibiotic resistance. Hence, researchers believe that directing the QS as an important pathway to acquire anti-virulence with minimal risk of resistance development. The QS targeted antimicrobial drugs enact differently from traditional antibiotics; hence this kind of drug is considered as ideal antibacterial drugs in the near future [7, 8]. The bacterial cell density is proportional to the concentration of the signalling molecules in a given environment [9]. QS mechanism is one of the reasons for pathogenicity and survival of bacteria [10] which should be under consideration for the development of novel anti-quorum drugs. The inhibition of QS results in control over biofilm formation, virulence factor expression, bioluminescence, motility and drug-resistant [11]. Henceforth, the novel research strategy includes the development of novel synthetic ligands that behave as antagonists of autoinducer. Numerous works have been carried on synthetic QS modulators [12–14]. Most of the QS modulators imitates natural products produced by natural antifoulants and certain algae [15, 16].

Chromobacterium violaceum is a gram-negative, aquatic bacterium that rarely behaves as a pathogen causing skin lesions, extreme virulence, fatal septicaemia [17]. The gram-negative bacteria communicate by producing violet pigment called violacein with the help of autoinducer like *N*-acyl homoserine lactone (C_6-HSL) to maintain threshold cell density [18]. The best chemical approach to obstruct QS is to block signal receptor binding via non-native small molecules [19]. The quorum sensing inhibitors (QSIs) will not affect the bacterial growth but stop the bacterial signalling [20]. A prodigious number of derivatives of *N*-acyl-homoserine lactone (AHL) has been discovered based on their inhibition of violacein production. The most effective quorum sensing inhibitors reported to date are chloro lactone (CL) (Fig. 1) [21]. As AHLs posses hydrophilic homoserine lactone ring and hydrophobic aliphatic amide chain, it can freely acess cell membrane and associate with receptor protein [22]. Recently Srinivasarao, S et al. [23] worked on QSI and stated that the presence of at least one methylene functionality between triazole nucleus and *N*-acyl group enhances the activity and moieties with no methylene group showed poor QSIs. Figure 3 depicts few synthetic QSI (**1–5**) [19, 21–24] possessing 1,2,3-triazole, amide and methylene functionalities.

Xanthomonas campestris pv. *campestris* (*Xcc*) is a gram-negative plant pathogen which causes black rot. This is considered as one of the trivial and most damaging disease of cruciferous crops which is present globally. One of the methods to increase agricultural crop is through usage of organic fertilizers, agrochemicals and biological controls [25]. Some of the commercially available agrofungicides are diniconazole, triadimenol, flusilazole, triadimefon, difenoconazole, oxathiapiprolin and acetazoiamide which has 1,2,4-triazole as a core moiety, amide linkage and electron-withdrawing atoms like chlorine and fluorine (Fig. 2).

Few researchers discovered drug against phytopathogens and explained the importance of electron-withdrawing groups attached to the core moiety like 1,2,4-triazole and quinoline to enhance the activity [26–30]. From these findings it can be assumed that the moieties responsible for potent antibacterial activity is 1,2,3-triazole, 1,2,4-triazole and electron-withdrawing groups like chlorine. Moreover, most of the commercially available agrofungicides (**6**, **7**) and synthesized drug (**8**, **9**) [28, 29] against phytopathogenic bacteria contain 1,2,4-triazole moieties along with amide functionality (Fig. 3).

Considering all above facts we are herewith reporting novel 1,2,4-triazole analogues to evaluate for their QS against *C. violaceum*, antibacterial activity against *C. violaceum* and *Xanthomonas campestris* pv. *campestris* (*Xcc*). Drug-receptor interaction can be understood by one of the routinely used computational studies (molecular docking). It provides binding orientations of ligands to their targeted proteins. To understand the efficiency of targeted molecules as anti-quorum inhibitors, it was docked against CviR protien (PDB ID: 3QP5).



C_6 - HSL **CL**

Fig. 1 Structure of autoinducer (C_6-HSL) and QSI (CL)

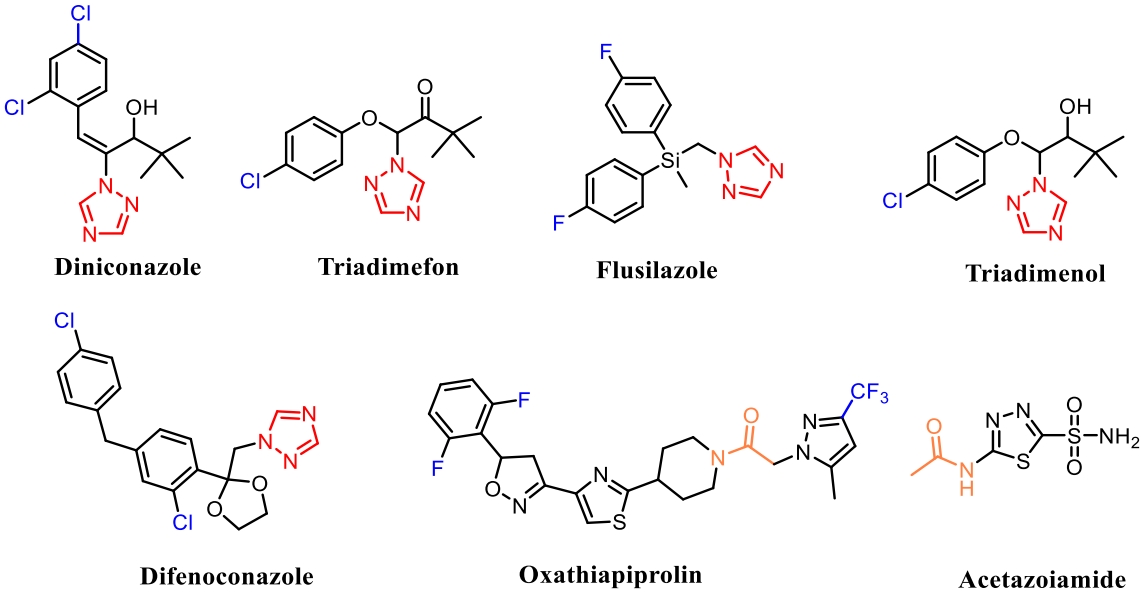


Fig. 2 Commercially available agrofungicides

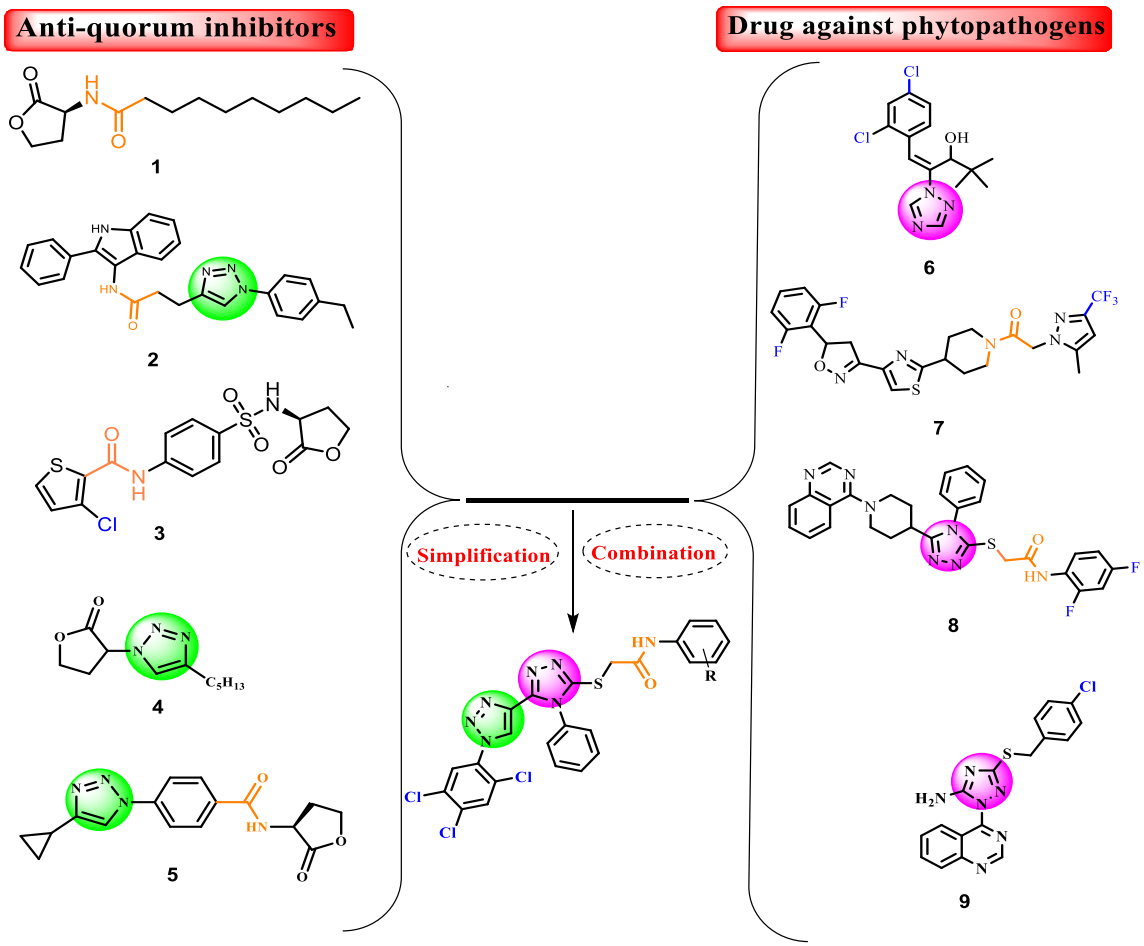


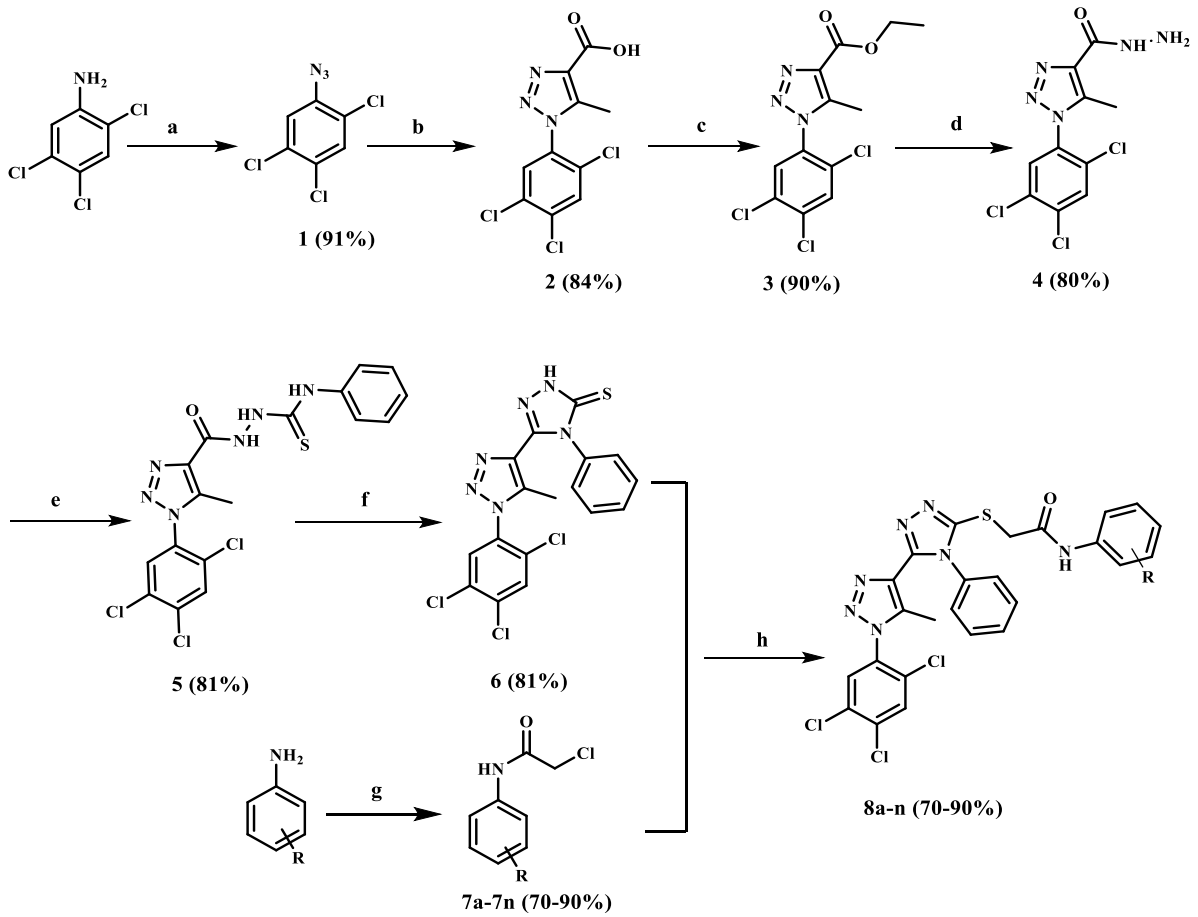
Fig. 3 Synthetic strategy for designing target drug

Results and discussion

Chemistry and spectral analysis

A new series of 1,2,4-triazole conjugated with 1,2,3-triazole **8a–n** was synthesized in good yield following the synthetic pathway depicted in Scheme 1. At first trisubstituted aniline was converted to azide (**1**) by treating with sodium nitrite and sodium azide in 1:1 mixture of Conc. HCl and water. The intermediate 5-methyl-1-(2,4,5-trichlorophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (**4**) was obtained by converting azides (**1**) to acid derivative of *N*-aryl-1,2,3-triazole (**2**), followed by esterification (**3**) and converting to corresponding carbohydrazide (**4**). Further intermediate **4** upon treating with phenyl isocyanate yielded carbothioamide (**5**), which underwent cyclization in basic condition to obtain pre-final 5-(5-methyl-1-(2,4,5-trichlorophenyl)-1*H*-1,2,3-triazol-4-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**6**). Later on intermediate **6** was treated with different *N*-(substituted phenyl)chloroacetamide to yield the target compounds **8a–n**. Physical properties and structural features of target compounds are descriped in Table S1 (ESI).

The structures of intermediate and target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, FT-IR, mass spectrum (MS) and elemental analyses. The final compound taking **8e** into the consideration, the IR spectrum showed absorption band at 3240, 1683 and 1176 cm^{-1} which were assigned to NH, amide C=O and C–F, respectively. The 400 MHz NMR spectrum showed the characteristic sharp singlet at δ 3.95 ppm for SCH_2 proton thus confirming the attachment



8a : R = 4-Cl-3-NO_2; **8b :** R = 4 -F; **8c :** R = 3-NO_2; **8d :** R = 3-F-2-CH_3 ; **8e :** R = 3-Cl-4-F ;
8f : R = 4-CH_3 ; **8g :** R = 4-OCH_3; **8h :** R = 4-Br-3-CH_3; **8i :** R = 2-F; **8j :** R = 3-Cl-2-CH_3 ;
8k : R = 3,4-$(CH_3)_2$; **8l :** R = 2-C_2H_5-6-CH_3; **8m :** R = 2-Br; **8n :** R = 4-Br

Scheme 1 The synthesis of target compounds **8a–n**. Reagents and conditions: **a** $NaNO_2/H_2O/HCl$, NaN_3, 0 °C to R.T. 16 h; **b** Ethylacetoacetate/EtOH, refluxed, 4 h; **c** EtOH/HCl, 90 °C, 16 h; **d** NH_2NH_2/EtOH, 90 °C, 16 h; **e** PhNCS/EtOH, 90 °C, 8h; **f** 5% NaOH, 100 °C, 3 h; **g** $ClCH_2COCl/Et_3N$/EtOH, 90 °C, 3 h; **h** K_2CO_3/CH_3COCH_3, R.T. 10 h

of substituted phenyl acetamide to C=S group, singlet at δ 10.55 ppm was attributed for NH proton and signal at δ 2.53 ppm was assigned for $–CH_3$ group. The chemical shift values for the aromatic region are in agreement with the structure. The structure was also supported by ^{13}C NMR, the desheilded signal resonance at 166.8 ppm confirmed the presence of amide C=O group. The signal at 36.1 ppm showed the presence of CH_3 carbon. Its mass spectrum displayed $[M+NH_4]^+$ at m/z 638.00 along with isotopic peaks.

Pharmacological studies

Antibacterial activity and quorum sensing inhibition against *C. violaceum*

The quorum sensing inhibition (QSI) is directed to inhibition of bacterial quorum sensing signal/receptor and not on the growth of bacteria, therefore, we subjected the synthesized moieties **8a–n** initially for antibacterial activity over gram-negative bacteria *C. violaceum.* The antibacterial activity was evaluated in terms of zone of inhibition (ZOI) against bacterial strain by disc diffusion method, and results are reported in Table 1. Only compounds **8b, 8f**, **8g**, **8n** showed antibacterial activity against gram-negative *C. violaceum* bacteria. The SAR trends revealed that monosubstituted *N*-phenylacetamide with electron-donating groups and halogen derivative in the para position showed better activity when compared to halogen in ortho position and disubstituted *N*-phenylacetamide moiety. Ciprofloxacin was used for comparison.

The synthesized derivatives **8a–n** were subjected to quorum sensing inhibition (QSI). The autoinducer acyl HSLs control the production of violet pigment (violacein) which is used as a reporter system in detecting QS communication between bacteria. Henceforth, hindrance of QS efficacy in *C. violaceum* will forbid the production of violacein. Here, we observed for reduction of violacein production upon QSI activity for the compounds **8a**, **8c, 8h**, **8i** and **8m** (Table 2) and violacein production as well as growth was quantified (Fig. 4). The SAR trends revealed that among 14 derivatives, **8a**, **8c**, **8h**, **8i**, **8m** which possessed electron-withdrawing group on *N*-phenylacetamide moiety showed excellent quorum sensing inhibition activity (> 60% inhibition) at 300 µg/mL. These moieties which displayed QSI were antibacterial inactive. Among the five derivatives which showed QSI, **8c** and **8a** exhibited outstanding activity with > 80% QSI which possessed strong electron-withdrawing nitro and 4-Cl-3-NO_2 groups, respectively, attached to *N*-phenyl acetamide moiety (Fig. 4) when compared to standard quercetin. Exchanging 4-Cl-3-NO_2 in **8a** with a combination of weak electron-withdrawing group bromo and donating group methyl led to compound **8h** which showed 70% inhibition,

Table 1 Zone of inhibition (mm) of **8a–n** against tested microorganism at 300 µg/ml

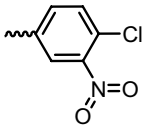
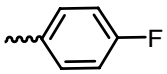
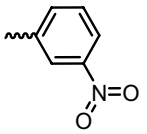
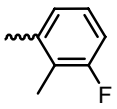
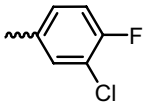
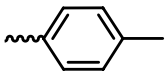
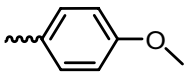
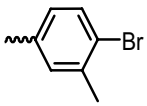
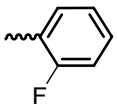
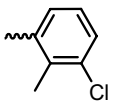
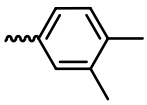
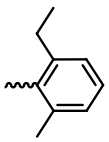
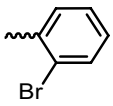
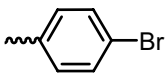
Compound	*R*	Zone of inhibition (mm)**	
		C. violaceum	*Xcc*
8a		NA	7 ± 0.2
8b		21	14 ± 0.5
8c		NA	13 ± 0.6
8d		NA	8 ± 0.2
8e		NA	7 ± 0.4
8f		20	8 ± 0.2
8g		**27**	9 ± 0.5
8h		NA	12 ± 0.2
8i		NA	7 ± 0.3
8j		NA	11 ± 0.5
8k		NA	8 ± 0.2
8l		NA	8 ± 0.3
8m		NA	10 ± 0.2
8n		16	10 ± 0.2
Ciprofloxacin (30 µg/disc)		27 ± 0.5	18 ± 0.5

Table 1 (continued)

NA no activity

**The zone of inhibition was expressed as an average from three experiments ± standard deviation

Table 2 Anti-QS activity against tested *C. violaceum* biosensor (ATCC 12427)

Compound	Inhibition of quorum sensing in mm (300 µg/disc)*
8a	12 ± 0.2
8b	–
8c	12 ± 0.4
8d	–
8e	–
8f	–
8g	–
8h	10 ± 0.2
8i	8 ± 0.1
8j	–
8k	–
8l	–
8m	8 ± 0.4
8n	–
Quercetin	10 ± 0.2

*The zone of violacein inhibition was expressed as an average from three experiments ± standard deviation

whereas monosubstituted halogen derivatives (**8m** and **8i**) showed < 70% inhibition. The above experiment revealed that target compounds **8a**, **8c**, **8h**, **8i**, **8m** had no effect on the normal growth of *C. violaceum* but inhibited bacterial signalling.

In silico studies

Target protein LuxR-type protein CviR

The molecules which play a role as an antagonist of LuxR-type proteins are likely to be a lead molecule in the evolution of novel antibacterial therapeutics. The gram-negative bacteria frequently use acyl-homoserine lactone molecules (AHLs) as autoinducers. The stable LuxR:AHL complexes bind DNA to initiate transcription of quorum sensing in a broad range of pathogens.

C. violaceum strain produces an autoinducer *N*-hexanoyl homoserine lactone (C_6-HSL), which is detected via the LuxR-type protein CviR [31]. The autoinducer C_6-HSL could bind to the CviR protein from *C. violaceum* to regulate the production of violacein. Chlorolactone (CL) compound is one of the potent quorum sensing antagonists which is successful in competing against C_6-HSL for binding to the autoinducer binding site resulting in closed conformation incapable to bind DNA [32].

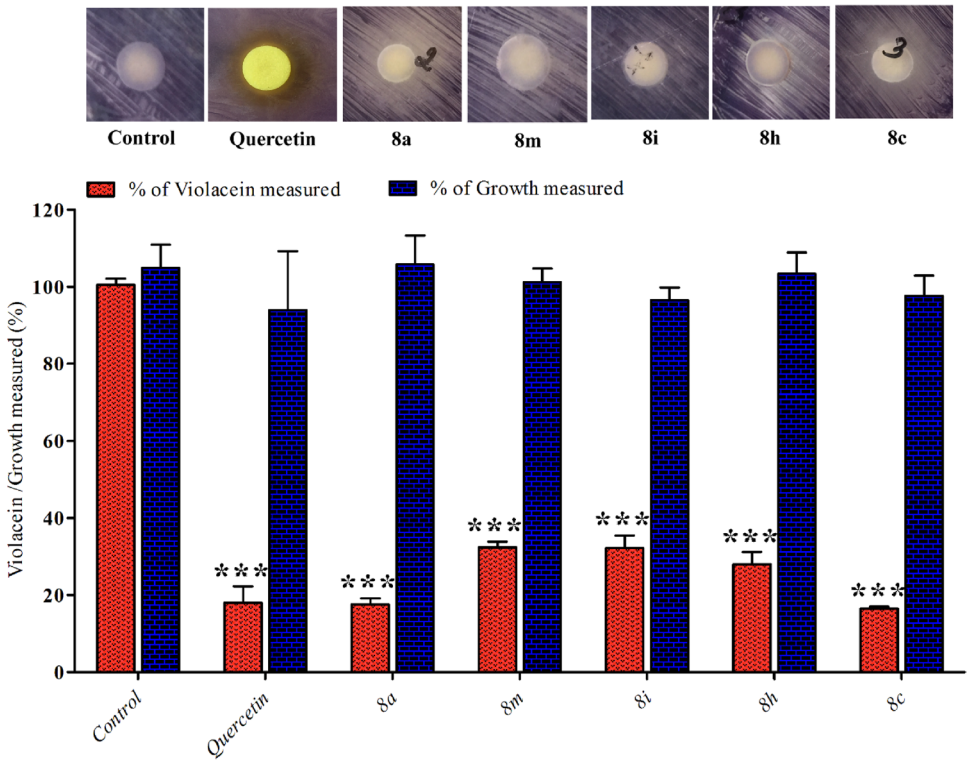


Fig. 4 Anti-quorum sensing assay (plate assay-insert) and quantification of violacein inhibition with measurement of growth under assay condition by microtiter plate method. Quantification of violacein produced was expressed in percentage, more than 60% inhibition of violacein was observed by respective compounds ($p < 0.0001$), and the absence of growth inhibition. The error bar represents standard deviations from reproduced results in three repeated experiments

Molecular docking

To obtain a possible correlation of in vitro quorum sensing inhibition, all the newly synthesized molecules were subjected to in silico docking studies. Larger the negative value, greater is the binding efficiency of molecules with CviR protein (PDB ID: 3QP5). The activity trend was replicated in the docking results. The docking results obtained for binding interaction of the molecules (**8a, 8c, 8h, 8i** and **8m**) with protein are shown in Fig. 5. The docking score of

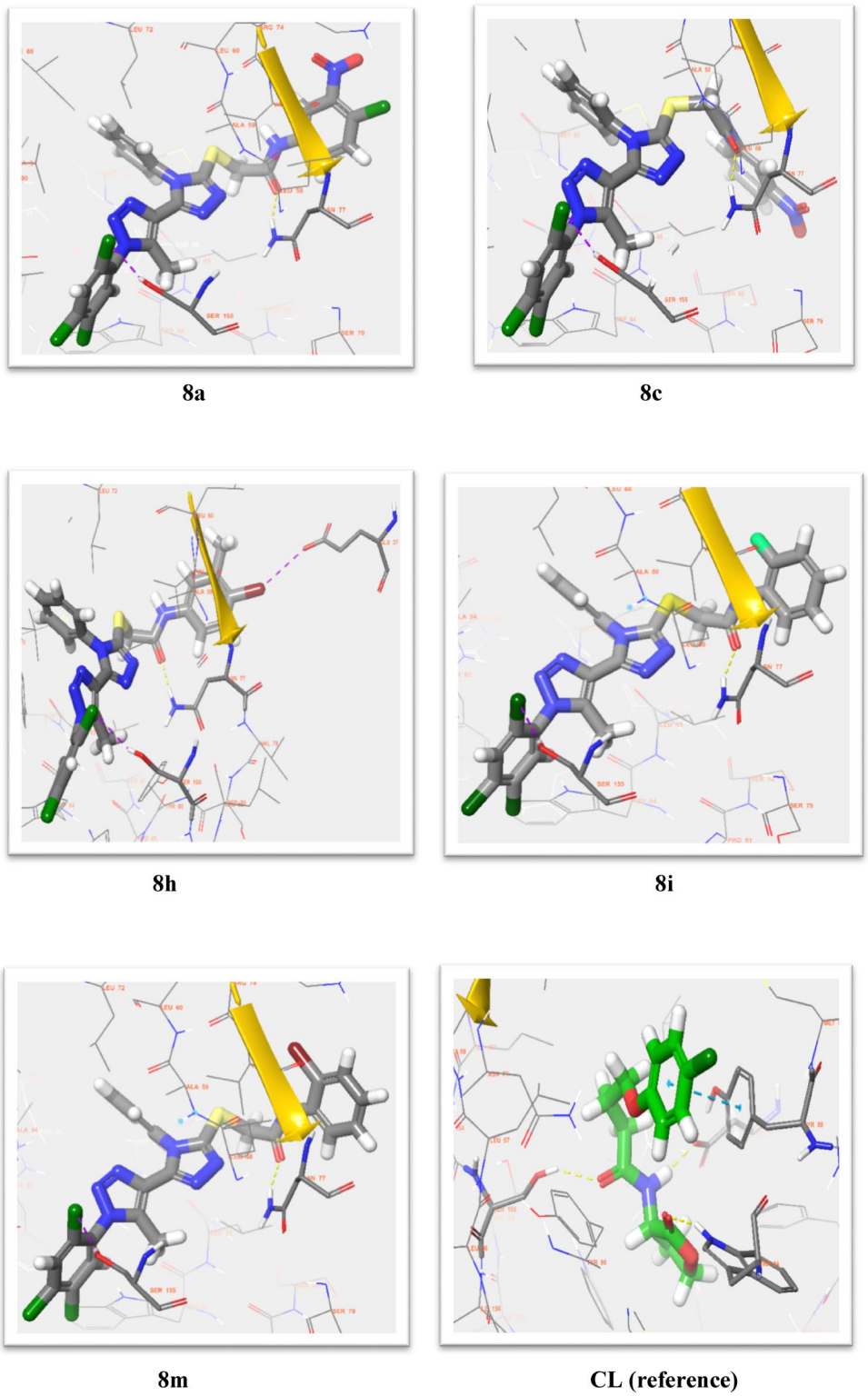


Fig. 5 The docking poses of the compounds with CviR protein (PDB ID: 3QP5). Lines indicate the type of interactions: purple—halogen bond, yellow—hydrogen bond

Table 3 Docking score and interacting amino acid residues for binding of the compounds with CviR protien

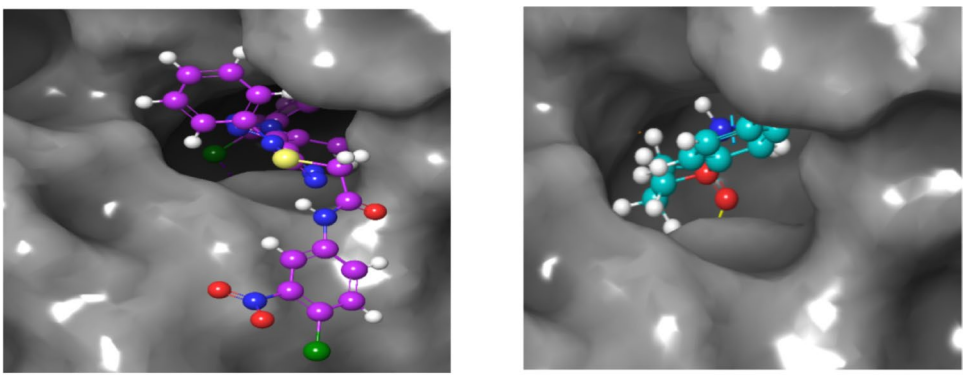
Compound	Docking score	Halogen bond	Hydrogen bond
8a	− 8.69	SER155	ASN77
8c	− 8.17	SER155	ASN77
8h	− 8.11	SER155 GLU37	ASN77
8i	− 8.06	SER155	ASN77
8m	− 8.20	SER155	ASN77
CL (reference)	− 8.46	–	ASP97 SER155 TRP84

the molecules varied from − 8.69 to − 8.06 kJ/mol (Table 3). Compared to the chlorolactone (CL) which was taken as a reference (− 8.46 kJ/mol), the best docking score was obtained to compound **8a** which showed − 8.69 kJ/mol. And from Fig. 6, It is evident that the ligand **8a** is bound to the same pocket of CviR protein as that of the reference. This represents that the docking procedure reproduced the receptor-bound conformation with the ligand molecules.

All the molecules exhibited halogen bonding to SER155 via C_2 chlorine atom of the 2,4,5-trichlorophenyl ring, whereas hydrogen bonding to ASN77 through an oxygen atom of the amide group. Additionally, the compound **8h** exhibited halogen bonding to GLU37 by bromo atom of *N*-(4-bromo-3-methylphenyl)acetamide group. These moieties which showed good activity also showed excellent docking score.

Physiochemical properties

The important tool for the determination of drug-likeness property of a bioactive molecule is the Lipinski rule of five. Drug-likeness is nothing, but to know whether the synthesized novel bioactive molecule is alike to marketed drugs. For any molecule to be an effective drug candidate it should obey Lipinski's rule of five [33] which in turn determined by ADME properties, i.e. absorption, distribution, metabolism and excretion. According to this, a chemical species should possess a molecular weight ≤ 500, partition coefficient (log P) ≤ 5, the number of hydrogen bond donor and acceptor ≤ 5 and ≤ 10, respectively. In addition to this, the drug should have less than or equal to ten rotatable bonds and polar surface area ≤ 140Å which determine oral bioavailability [34]. Other parameters such as #stars (0–5), percentage human oral absorption (> 80% high, < 25% low), CNS (− 2: inactive; +2: active), QlogKp (− 8.0–− 10.0), QPlogKhsa (− 1.5–1.5), #metab (1–8), QPPMDCK (< 25 poor > 500 Great) and QPHERG (> − 5) were also determined.



(a) (b)

Fig. 6 Surface representation of LuxR-type protein CviR with ligand **8a** (**a**) and **CL** (**b**)

Table 4 Predicted Lipinski's parameter for **8a, 8c, 8h, 8i** and **8m**

Compounds	Molecular weight	Donor HB	Acceptor HB	logPo/w[a]	#rotor[b]	Rule of 5
Acceptable range	≤ 500	≤ 5	≤ 10	(− 2.0 to 6.5)	≤ 10	< 5
8a	647.98	1	7.5	6.24	5	3
8c	614.02	1	7.5	6.23	5	3
8h	660.96	1	6.5	7.86	4	2
8i	587.03	1	6.5	6.59	4	2
8m	646.95	1	6.5	7.05	4	2

[a]Predicted octanol/water partition coefficient logp

[b]Predicted rotatable bonds

Table 5 Predicted physiochemical properties of synthesized compounds **8a, 8c, 8h, 8i** and **8m**

Compounds	#stars[a]	% Human oral absorption[b]	QP logBB[c]	CNS[d]	QlogKp[e]	QPlogKhsa[f]	#metab[g]	QPPMDCK[h]	QPHERG[i]	PSA[j]
Acceptable range	(0–5)	> 80% High, <25% low	(− 3 to 1.2)	(− 2 inactive; +2 active	(− 8.0–− 10.0)	(− 1.5–1.5)	(1–8)	<25 poor >500 Great	> − 5	≤140Å
8a	3	60	− 1.40	− 2	− 3.89	1.30	3	1305.41	− 6.57	137–82
8c	4	60	− 1.87	0	− 3.4	− 1.34	4	534.42	− 7.9	138–78
8h	5	100	− 0.44	0	− 1.85	1.53	3	100.00	− 7.79	93.91
8i	3	91	− 0.34	0	− 2.04	1.28	3	6190.22	− 6.51	92.28
8m	4	94	− 0.39	0	− 1.86	1.44	3	7591.58	− 7.11	94.55

[a]Predicted #stars

[b]Predicted percentage human absorption

[c]Predicted brain/blood partition coefficient

[d]Predicted central nervous system

[e]Predicted skin permeability

[f]Prediction of binding to human serum albumin

[g]Predicted metabolic reactions

[h]Predicted apparent MDCK cell permeability

[i]Predicted IC_{50} values to block HERG K + channels

[j]Predicted polar surface area

In this study, the physiochemical properties of **8a, 8c, 8h, 8i** and **8m** were determined and summarised in Tables 4 and 5. For a drug to be effective orally it should not violate more than one Lipinski's rule of five. It's worth noting that though all the compounds violate Lipinski's rule, it showed an excellent percentage of human oral absorption. Absorption is the main criteria for the bioavailability of the drug sequentially absorption depends on permeability and solubility of the drug [35]. All the compounds obey most of the parameters expect molecular weight and log Po/w. Though **8a** and **8c** violate 3 rules it has a good percentage of human oral absorption=60%. As these compounds possess good oral absorption (60–100%) violation in any rule will not affect the bioavailability [36].

Antibacterial activity against *Xcc*

Furthermore, the newly synthesized compounds (**8a–n**) were also screened for their in vitro growth inhibitory activity against phytopathogenic bacteria *Xanthomonas campestris pv. campestris*. The preliminary screening was carried out through the disc diffusion method and MIC by broth microdilution method. The results of the preliminary antimicrobial testing of compounds **8a–n** (300 µg/mL) and standard antibacterial drug ciprofloxacin are shown in Table 1. As shown in Table 1, in terms of ZOI, most of the compounds showed promising antibacterial activity against *Xanthomonas campestris* pv. *campestris* at 300 µg/mL. Among all the derivatives, the strong antibacterial activity was observed in **8b** which has fluoro substitution on *N*-phenylacetamide moiety, produced growth inhibition zone of 14 mm. The

bromo derivatives exhibited optimal activity (**8h**, **8m**, **8n**). Except for the **8b**, other fluoro derivatives exhibited weak activity (**8d**, **8e**, **8i**). The moiety with the strong electron-withdrawing nitro group at meta position (**8c**) showed excellent activity, whereas **8a** with 4-Cl-3-NO_2 comparatively showed weak activity. However, *N*-phenylacetamide moiety with the electron-donating group did not have noticeable antibacterial activity (**8f**, **8g**, **8k**, **8e**). The minimum concentration of antibacterial agent required to inhibit bacterial growth (MIC µg/mL) was determined for entire derivatives. Out of 14 derivatives, compounds **8b**, **8c**, **8h** and **8j** showed MIC value at < 200 µg/ml (Table 6).

Table 6 Antibacterial activity against *Xanthomonas campestris* pv. *campestris* compounds

Compound	MIC (µg/mL)
8a	300
8b	< 200
8c	< 200
8d	200
8e	400
8f	300
8g	200
8h	< 200
8i	200
8j	< 200
8k	200
8l	300
8m	200
8n	200
Ciprofloxacin	< 32

Conclusions

In this study, novel 1-phenyl-1*H*-2-(1-aryl-5-methyl-1*H*-1,2,3-triazol-4-yl)-3-(*N*-aryl-carbamoylmethylthio)-1,2,4-triazoles derivatives (**8a–n**) were designed, synthesized in good yield and screened for in vitro antibacterial activity and anti-QS activity. The results revealed that moiety with monosubstitution bearing electron-donating on *N*-phenylacetamide was selectively active against bacteria *C. violaceum*, whereas five molecules bearing electron-withdrawing on *N*-phenylacetamide moiety exhibited fabulous anti-QS inhibition. Hence, the statement "The QSIs will not affect the bacterial evolution but stop the bacterial signalling" is proved by this experiment as these moieties (**8a**, **8c**, **8h**, **8i**, **8m**) were antibacterial inactive. The compounds showing antibacterial activity are not suitable for the anti-QS activity. Henceforth, the design was successful in discovering novel anti-QS inhibitors. Also, the moieties with the electron-withdrawing group exhibited moderate activity against *Xcc*.

Experimental section

Materials and methods

The reagents and chemicals used in this series were bought from Sigma-Aldrich, S.D. Fine and Merck company. All reagents were analytical grade and were used directly. The open capillary tube method was used to determine the melting point of the target compounds. The characterization of desired compounds was done using ^{1}H NMR (Bruker Avance III, 400 MHz NMR spectrometer), ^{13}C NMR (Bruker Avance III, 100 MHz) and IR (ATR). Mass spectra were recorded on Waters, synapt G2 high detection mass spectrometry. All the reactions were monitored by TLC, performed on silica plate and observed under UV light. Tetramethylsilane was used as a standard in ^{1}H NMR, chemical shift values (δ) were denoted in ppm and coupling constants in Hz. Spin multiplicities were stated singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). Elemental analysis was conducted in CHNS ElementarVarioEL III.

Synthesis of 1-azido-2,4,5-trichlorobenzene (1)

To a stirred solution of 2,4,5-trichloroaniline (5.0 g, 25.6 mmol) in water (25 mL), added 15% HCl solution (25 mL). The reaction mixture was then cooled to 0 °C followed by dropwise addition of 30% $NaNO_2$ solution (7 mL) at the same temperature and allowed to stir for 15 min. To the above reaction mixture, NaN_3 (3.3 g, 51.3 mmol) was added a lot wise at the same temperature and was stirred overnight at room temperature. The precipitate formed was filtered, washed thoroughly with water, dried and recrystallized from ethanol to give intermediate **1** (5.2 g) as off-white solid. Yield: 91%; Mp. 48–50 °C.

Procedure for synthesis of 5-methyl-1-(2,4,5-trichlorophenyl)-1*H*-1,2,3-triazole-4-carboxylic acid (2)

Intermediate **1** (5.2 g, 23.3 mmol) in ethanol (50 mL) was taken in a round bottom flask fixed with a guard tube, added ethyl acetoacetate (3.0 g, 23.3 mmol). The reaction mixture was cooled to 0 °C, followed by the lot wise addition of NaOMe (2.5 g, 46.7 mmol) and refluxed for 4 h. The completion of the reaction was monitored by thin-layer chromatography. After completion of the reaction, the reaction mass was added to ice-cold water and neutralized with acetic acid. The precipitate formed was filtered, dried and recrystallized from EtOAc to yield intermediate **2** (6.0 g) as white solid.

Yield: 84%; Mp. 146–148 °C; Off-white solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3442 (OH), 1685 (C=O), 1577 (C=C), 775 (C–Cl) cm^{-1}; ESI–MS (*m/z*): 305.95 $[M+H]^+$; Anal. calcd. for $C_{10}H_6N_3Cl_3O_2$: C, 39.18; H, 1.97; N, 13.71%. Found: C, 39.15; H, 1.95; N, 13.69%.

Procedure for synthesis of ethyl 5-methyl-1-(2,4,5-trichlorophenyl)-1*H*-1,2,3-triazole-4-carboxylate (3)

To a solution of intermediate **2** (6.0 g, 19.5 mmol) in ethanol, a catalytic amount of Conc. H_2SO_4 was added and heated under reflux for 16 h. After completion of the reaction, the excess solvent was removed under vacuum. The leftover residue was poured into ice-cold water. The precipitate formed was filtered and dried to obtain intermediate **3** as white solid (5.9 g).

Yield: 90%; Mp. 128–130 °C; Off-white solid; FT-IR (ATR, υ_{max}, cm^{-1}): 1732 (C=O), 1577 (N=N), 1448 (C–H), 773 (C–Cl); ESI–MS (*m/z*): 334.00 $[M+H]^+$; Anal. calcd. for $C_{12}H_{10}N_3Cl_3O_2$: C, 43.08; H, 3.01; N, 12.56%. Found: C, 43.05; H, 3.02; N, 12.54%.

Procedure for synthesis of 5-methyl-1-(2,4,5-trichlorophenyl)-1*H*-1,2,3-triazole-4-carbohydrazide (4)

Hydrazine hydrate 98% (20 mL) was added to a solution of intermediate **3** (5.9 g, 17.6 mmol) in ethanol (50 mL) and heated under reflux for 16 h. After the completion of the reaction, the reaction mass was added to ice-cold water. The precipitate formed was filtered, dried and recrystallized from ethanol to yield intermediate **4** (4.5 g) as off-white solid.

Yield: 80%; Mp. 184–186 °C; Off-white solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3309 (N–H), 2966 (C–H), 1625 (C=O), 1585 (C=C), 854 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.28 (s, 1H, NH), 7.69 (s, 1H, Ar–H), 7.50 (s, 1H, Ar–H), 4.00 (bs, 2H, NH_2), 2.48 (s, 3H, CH_3); ESI–MS (*m/z*): 319.95 $[M+H]^+$; Anal. calcd. for $C_{10}H_8N_5Cl_3O$: C, 37.47; H, 2.52; N, 21.85%. Found: C, 37.45; H, 2.50; N, 21.83%.

Procedure for synthesis of 2-(5-methyl-1-(2,4,5-trichlorophenyl)-1*H*-1,2,3-triazole-4-carbonyl)-*N*-phenylhydrazine-1-carbothioamide (5)

To a solution of intermediate **4** (4.5 g, 17.1 mmol) in EtOH (50 mL), added phenyl isothiocyanate (1.7 mL, 17.1 mmol) and heated to reflux for 8 h. The progress of the reaction was monitored by thin-layer chromatography. Excess solvent was removed under high vacuum. Solid formed was recrystallized from ethanol to yield intermediate **5** (5.0 g) as white solid.

Yield: 81%; Mp. low melting; Off-white solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3219 (N–H), 2972 (C–H), 1680 (C=O), 1593 (C=C), 1145 (C=S), 742 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.77 (bs, 1H, NH), 8.70 (bs, 1H, NH), 8.47 (s, 1H, NH), 7.25–7.74 (m, 7H, Ar–H), 2.47 (s, 3H, $-H_3$); ESI–MS (*m/z*): 454.99 $[M+H]^+$; Anal. calcd. for $C_{17}H_{13}Cl_3N_6OS$: C, 44.80; H, 2.88; N, 18.44%. Found: C, 44.78; H, 2.87; N, 18.42%.

Procedure for synthesis of intermediate 5-(5-methyl-1-(2,4,5-trichlorophenyl)-1*H*-1,2,3-triazol-4-yl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6)

30% NaOH (50 mL) solution was added to intermediate **5** (5.0 g, 11.3 mmol) and heated to reflux for 3 h. After the completion of the reaction, the reaction mass was cooled and neutralized to pH = 7 using dil. HCl. The precipitate formed was filtered, dried and recrystallized from ethanol to yield intermediate **6** (4.0 g) as off-white solid. Yield: 81%.

Yield: 81%; Mp. 270–272 °C; Off-white solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3255 (N–H), 2908 (C–H), 1591 (C=C), 1143 (C=S), 763 (C–Cl); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 14.27 (s, 1H, NH), 8.20–8.24 (m, 1H, Ar–H), 7.35–7.48 (m, 6H, Ar–H), 2.21 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 164.4, 139.3, 133.4, 131.0, 130.3, 128.0, 127.6, 127.4, 127.2, 126.9, 126.2, 125.0, 124.8, 124.4, 4.3; ESI–MS (*m/z*): 437.00 $[M+H]^+$; Anal.

calcd. for $C_{17}H_{11}N_6Cl_3S$: C, 46.65; H, 2.53; N, 19.20%. Found: C, 46.63; H, 2.50; N, 19.15%.

Procedure for synthesis of intermediates 7a–n

To a stirred solution of substituted aniline (8.2 mmol) in ethanol (10 mL), added dry triethylamine (8.2 mmol). The reaction mixture was cooled to 0 °C, chloroacetyl chloride (8.2 mmol) was added dropwise and heated under reflux for 3 h. The reaction mass was quenched with ice-cold water. The precipitate formed was filtered, dried and washed with hexane to yield intermediate **7a–n** in good yield. Yield: 70–90%.

Procedure for synthesis of target compound 8a–n

To a mixture of triazole (0.3 g, 0.7 mmol) and substituted amides (0.7 mmol) in acetone, added K_2CO_3 (0.7 mmol) and stirred at room temperature for 5 h. The completion of the reaction was monitored by thin-layer chromatography. The reaction mass was filtered, and the filtrate was evaporated under a high vacuum to yield the desired product which was recrystallized using ethanol. Yield 70–95%.

N-(4-Chloro-3-nitrophenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (8a)

Yield: 72%; Mp. 200–202 °C; Brown solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3132 (NH), 2939 (C–H), 1683 (C=O), 1608 (C=N), 1585 (C=C), 1510, 1327 (C–NO_2) 765, 819 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.95 (s, 1H, NH), 8.24 (d, J = 4.0 Hz, 1H, Ar–H), 7.71–7.78 (m, 1H, Ar–H), 7.51–7.56 (m, 3H, Ar–H), 7.37–7.44 (m, 3H, Ar–H), 7.16 (s, 2H, Ar–H), 4.02 (s, 2H, –CH_2–), 2.49 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.0, 148.4, 147.9, 137.9, 137.0, 136.4, 133.0, 132.6, 132.5, 132.2, 132.1, 131.8, 131.7, 130.5, 130.5, 130.4, 129.8, 128.8, 128.1, 127.3, 123.9, 121.0, 116.3, 36.3, 9.4; ESI–MS (*m/z*): 648.98 $[M+H]^+$; Anal. calcd. for $C_{25}H_{16}N_8Cl_4O_3S$: C, 46.17; H, 2.48; N, 17.23%. Found: C, 46.10; H, 2.45; N, 17.20%.

N-(4-Fluorophenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (8b)

Yield: 77%; Mp. 202–203 °C; Creamish solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3120 (N–H), 2940 (C–H), 1682 (C=O), 1607 (C=N), 1583 (C=C), 1021 (C-F), 766, 820 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.23 (s, 1H, N–H), 7.73 (s, 1H, Ar–H), 7.73–7.74 (m, 2H, Ar–H), 7.44–7.55 (m, 6H, Ar–H), 7.01–7.02 (m, 2H, Ar–H), 3.97 (s, 2H, –CH_2–), 2.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.2, 160.5 (J = 210.1 Hz), 144.7, 144.0, 141.9, 137.5, 137.4, 137.3, 130.5, 129.8, 129.3, 129.2, 129.0, 124.7, 122.1, 118.7 (J = 8.0 Hz), 117.5 (J = 25.2 Hz), 117.1, 32.1, 9.6; ESI–MS (*m/z*): 588.03 $[M+H]^+$; Anal. calcd. for $C_{25}H_{17}N_7C_{l3}FOS$: C, 50.99; H, 2.91; N, 16.65%. Found: C, 50.95; H, 2.92; N, 16.63%.

2-((5-(5-Methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-(3-nitrophenyl)acetamide (8c)

Yield: 75%; Mp. 196–197 °C; Off-white solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3201 (NH), 2985 (C–H), 1684 (C=O), 1613 (C=N), 1580 (C=C), 1327, 1514 (C–NO_2), 764, 815 (C–Cl), ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.98 (s, 1H, NH), 7.89 (s, 1H, Ar–H), 7.40–7.75 (m, 9H, Ar–H), 3.97 (s, 2H, –CH_2–), 2.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.2, 144.7, 144.0, 141.9 140.5, 137.6, 137.5, 137.4, 137.1, 130.5, 129.8, 129.4, 129.3, 129.2, 129.0, 124.7, 122.1, 119.1, 117.3, 117.1, 103.4, 32.1, 9.6; ESI–MS (*m/z*): 614.02 $[M+H]^+$; Anal. calcd. for $C_{25}H_{17}N_8Cl_3O_3S$: C, 48.75; H, 2.78; N, 18.19%. Found: C, 48.70; H, 2.75; N, 18.15%.

N-(3-Fluoro-2-methylphenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (8d)

Yield: 75%; Mp. 160–162 °C; Brown solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3109 (NH), 2940 (C–H), 1682 (C=O), 1607 (C=N), 1583 (C=C), 766, 820 (C–Cl), 1023 (C-F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.92 (s, 1H, NH), 7.73–7.79 (m, 2H, Ar–H), 7.53–7.54 (m, 4H, Ar–H), 7.36–7.38 (m, 2H, Ar–H), 7.12–7.18 (m, 1H, Ar–H), 6.81–6.86 (m, 1H, Ar–H), 4.04 (s, 2H, –CH_2–), 2.50 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.0, 161.0 (J = 240.2 Hz), 154.0, 148.4, 137.7 (J = 6.4 Hz), 136.8, 136.3, 133.1, 132.4, 132.2, 131.7, 130.4, 130.3, 129.7, 127.2, 126.8, 126.7, 118.0, 116.9, 116.7, 111.4 (J = 22.5 Hz), 35.8, 9.4; ESI–MS (*m/z*): 602.04 $[M+H]^+$; Anal. calcd. for $C_{26}H_{19}N_7Cl_3FOS$: C, 51.80; H, 3.18; N, 16.26%. Found: C, 51.78; H, 3.15; N, 16.25%.

N-(3-Chloro-4-fluorophenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (8e)

Yield: 77%; Mp. 209–210 °C; Light cream solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3240 (NH), 2989 (C–H), 1683 (C=O), 1612 (C=N), 1583 (C=C), 1176 (C-F), 763, 823 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.55 (s, 1H, NH), 7.85 (d, J = 4.0 Hz, 1H, Ar–H), 7.73 (s, 1H, Ar–H), 7.51–7.56 (m, 4H, Ar–H), 7.45–7.48 (dd, 1H, Ar–H), 7.34–7.38 (m,

3H, Ar–H), 3.95 (s, 2H, –CH_2–), 2.53 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 166.8, 154.2, 148.4, 137.7, 136.9, 133.1, 132.6, 132.5, 132.3, 131.7, 130.5, 130.4, 129.8, 127.3, 121.4, 119.1, 36.1, 9.5; ESI–MS (*m/z*): 638.00 $[M + NH_4]^+$; Anal. calcd. for $C_{25}H_{16}N_7Cl_4FOS$: C, 48.17; H, 2.59; N, 15.73%. Found: C, 48.15; H, 2.55; N, 15.70%.

2-((5-(5-Methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-(4-tolyl) acetamide (8f)

Yield: 88%; Mp. 120–122 °C; Light cream solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3086 (NH), 2989 (C–H), 1687 (C=O), 1612 (C=N), 1583 (C=C), 767, 817 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.02 (s, 1H, NH), 7.72 (s, 1H, Ar–H), 7.49–7.54 (m, 6H, Ar–H), 7.36–7.38 (m, 2H, Ar–H), 7.11 (d, 2H, $J = 8.3$ Hz, Ar–H), 3.97 (s, 2H, –CH_2–), 2.52 (s, 3H, CH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 166.4, 154.1, 148.3, 136.8, 136.3, 135.6, 133.8, 133.2, 132.9, 132.5, 132.3, 131.7, 130.5, 130.4, 129.7, 129.3, 127.3, 119.9, 36.1, 20.8, 9.5; ESI–MS (*m/z*): 584.03 $[M + H]^+$; Anal. calcd. for $C_{26}H_{20}N_7Cl_3OS$: C, 53.39; H, 3.45; N, 16.76%. Found: C, 53.30, H, 3.44; N, 16.70%.

N-(4-Methoxyphenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (8g)

Yield: 75%; Mp. 188–190 °C; Light grey solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3041 (N–H), 2940 (C–H), 1676 (C=O), 1606 (C=N), 1552 (C=C), 1082 (C–O), 767, 829 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.98 (s, 1H, NH), 7.72 (s, 1H, Ar–H), 7.50–7.54 (m, 6H, Ar–H), 7.36–7.38 (m, 2H, Ar–H), 6.83–6.86 (m, 2H, Ar–H), 3.97 (s, 2H, –CH_2–), 3.77 (s, 3H, –OCH_3–), 2.51 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 166.2, 156.3, 154.1, 148.2, 136.8, 136.3, 133.2, 132.9, 132.5, 132.3, 131.7, 131.4, 130.5, 130.4, 129.7, 127.3, 121.5, 114.0, 55.4, 30.9, 9.5; ESI–MS (*m/z*): 600.05 $[M + H]^+$; Anal. calcd. for $C_{26}H_{20}N_7Cl_3O_2S$: C, 51.97; H, 3.35; N, 16.32%. Found: C, 51.95; H, 3.30; N, 16.30%.

N-(4-Bromo-3-methylphenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (8h)

Yield: 72%; Mp. 194–195 °C; Creamish solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3251 (NH), 2939 (C–H), 1683 (C=O), 1608 (C=N), 1585 (C=C), 765, 819 (C–Cl), 650 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.23 (s. 1H, NH), 7.73 (s, 1H, Ar–H), 7.50–7.55 (m, 5H, Ar–H), 7.43 (d, 1H, $J = 8.0$ Hz, Ar–H), 7.32–7.38 (m, 3H, Ar–H), 3.96 (s, 2H, CH_2), 2.52 (s, 3H, CH_3), 2.37 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 166.6, 154.1, 148.3, 138.4, 137.4, 136.8, 136.3, 133.1, 132.8, 131.7, 130.5, 130.5, 134.4, 129.7, 122.0, 119.3, 118.9, 36.2, 23.0, 9.5; ESI–MS (*m/z*): 660.96 $[M + H]^+$; Anal. calcd. for $C_{26}H_{19}N_7BrCl_3OS$: C, 47.04; H, 2.98; N, 14.77%. Found: C, 47.03; H, 2.95; N, 14.72%.

N-(2-Fluorophenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio) acetamide (8i)

Yield: 65%; Mp. 176–178 °C; Off-white solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3121 (NH), 2940 (C–H), 1682 (C=O), 1607 (C=N), 1583 (C=C), 766, 820 (C–Cl), 1021 (C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.17 (s, 1H, NH), 8.27–8.30 (m, 1H, Ar–H), 7.72 (s, 1H, Ar–H), 7.51–7.55 (m, 4H, Ar–H), 7.37–7.39 (m, 2H, Ar–H), 7.03–7.13 (m, 3H, Ar–H), 4.07 (s, 2H, –CH_2–), 2.16 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 166.8, 153.6, 152.9 ($J = 200.0$ Hz), 148.3, 136.9, 136.2, 133.1 ($J = 27.6$ Hz), 132.4 ($J = 3.3$ Hz), 131.7, 130.5, 130.4, 130.3, 129.7, 127.3, 126.6, 126.5, 124.6, 124.5, 124.36, 124.39, 122.0, 114.9 ($J = 15.6$ Hz), 36.0, 9.5; ESI–MS (*m/z*): 588.02 $[M + H]^+$; Anal. calcd. for $C_{25}H_{17}N_7Cl_3FOS$: C, 50.99; H, 2.91; N, 16.65%. Found: C, 50.95; H, 2.92; N, 16.63%.

N-(3-Chloro-2-methylphenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (8j)

Yield: 65%; Mp. 180–182 °C; Light brown solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3112 (NH), 2985 (C–H), 1684 (C=O), 1613 (C=N), 1580 (C=C), 764, 815 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.23 (s, 1H, NH), 7.89 (s, 1H, Ar–H), 7.46–7.55 (m, 6H, Ar–H), 6.64–7.29 (m, 3H, Ar–H), 3.68 (s, 2H, –CH_2–), 2.56 (s, 3H, CH_3), 2.24 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.2, 144.7, 144.0, 141.9, 141.1, 137.5, 137.4, 137.1, 134.4, 131.7, 129.9, 129.8, 129.3, 129.2, 129.0, 124.8, 124.7, 122.1, 117.7, 117.1, 32.1, 14.6, 9.6; ESI–MS (*m/z*): 618.01 $[M + H]^+$; Anal. calcd. for $C_{26}H_{19}N_7Cl_4OS$: C, 50.42; H, 3.09; N, 15.83%. Found: C, 50.41; H, 3.08; N, 15.85%.

N-(3,4-Dimethylphenyl)-2-((5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (8k)

Yield: 83%; Mp. 132–134 °C; Brown solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3098 (NH), 2940(C–H), 1681 (C=O), 1609 (C=N), 1583 (C=C), 766, 817 (C–Cl), ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.93 (s, 1H, NH), 7.73 (s, 1H, Ar–H), 7.52–7.55 (m, 4H, Ar–H), 7.26–7.39 (m, 4H, Ar–H), 7.05–7.07 (d, 1H, Ar–H), 3.97 (s, 2H, –CH_2–), 2.52 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); ^{13}C NMR

(100 MHz, $CDCl_3$, δ ppm): 167.2, 144.7, 144.0, 141.9, 139.5, 137.5, 137.4, 137.1, 135.8, 132.6, 130.5, 129.9, 129.8, 129.3, 129.2, 129.0, 124.7, 122.1, 120.8, 116.9, 32.1, 19.7, 19.4, 9.6; ESI–MS (*m/z*): 598.07 $[M+H]^+$; Anal. calcd. for $C_{27}H_{22}N_7Cl_3OS$: C, 54.14; H, 3.70; N, 16.37%. Found: C, 54.12; H, 3.69, N, 16.35%.

N-(2-Ethyl-6-methylphenyl)-2-(5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio)acetamide (8l)

Yield: 60%; Mp. 118–120 °C; Light brown solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3211 (NH), 2989 (C–H), 1687 (C=O), 1612 (C=N), 1583 (C=C), 767, 817 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.23 (s, 1H, NH), 7.89 (s, 1H, Ar–H), 7.50–7.58 (m, 5H, Ar–H), 7.43 (s, 1H, Ar–H), 6.85–7.00 (m, 3H, Ar–H), 3.74 (s, 2H, $-CH_2-$), 2.65–2.71 (q, 2H, $-CH_2-$), 2.56 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 1.09–1.06 (t, 3H, $J=12.0$ Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.2, 144.7, 144.0, 141.9, 140.9, 137.5, 137.4, 137.1, 136.5, 130.5, 129.8, 129.7, 129.5, 129.3, 129.3, 129.2, 129.0, 128.5, 124.7, 122.6, 122.1, 117.1, 32.1, 24.4, 18.2, 14.0, 9.6; ESI–MS (*m/z*): 612.08 $[M+H]^+$; Anal. calcd. for $C_{28}H_{24}N_7Cl_3OS$: C, 54.86; H, 3.95; N, 16.00%. Found: C, 54.85; H, 3.93; N, 16.02%.

N-(2-Bromophenyl)-2-(5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio) acetamide (8m)

Yield: 60%; Mp. 126–128 °C; Creamish solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3099 (NH), 2939 (C–H), 1683 (C=O), 1608 (C=N), 1585 (C=C), 765, 819 (C–Cl), 650 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.23 (s, 1H, NH), 7.89 (s, 1H, Ar–H), 7.74–7.76 (m, 1H, Ar–H), 7.50–7.55 (m, 5H, Ar–H), 6.90–7.48 (m, 4H, Ar–H), 3.97 (s, 2H, $-CH_2-$), 2.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.2, 144.7, 144.0, 141.9, 137.5, 137.4, 137.1, 134.9, 132.4, 130.5, 129.8, 129.3, 129.3, 129.0, 128.4, 126.7, 124.7, 122.1, 121.9, 117.1, 113.5, 32.1, 9.6; ESI–MS (*m/z*): 647.95 $[M+H]^+$; Anal. calcd. for $C_{25}H_{17}N_7BrCl_3OS$: C, 46.1; H, 2.64; N, 15.09%. Found: C, 46.3; H, 2.66; N, 15.02%.

N-(4-Bromophenyl)-2-(5-(5-methyl-1-(2,4,5-trichlorophenyl)-1H-1,2,3-triazol-4-yl)-4-phenyl-4H-1,2,4-triazol-3-ylthio) acetamide (8n)

Yield: 70%; Mp. 212–214 °C; Off-white solid; FT-IR (ATR, υ_{max}, cm^{-1}): 3044 (N–H), 2939 (C–H), 1683 (C=O), 1608 (C=N), 1585 (C=C), 765, 819 (C–Cl), 650 (C–Br); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.41 (s, 1H, N–H), 7.73 (s, 1H, Ar–H), 7.43–7.53 (6H, m, Ar–H), 7.36–7.41 (m, 4H, Ar–H), 3.95 (s, 2H, $-CH_2-$), 2.52 (s, 3H, CH_3), ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 167.2, 144.7, 144.0, 1419, 137.5, 137.4, 137.1, 132.0, 130.5, 129.8, 129.3, 129.2, 124.7, 122.1, 121.6, 117.9, 117.1, 32.1, 9.6; ESI–MS (*m/z*): 647.95 $[M+H]^+$; Anal. calcd. for $C_{25}H_{17}N_7BrCl_3OS$: C, 46.1; H, 2.64; N, 15.09%. Found: C, 46.3; H, 2.66; N, 15.02%.

Biological screening and computational studies

Bacterial strains

The bacterial cultures used in the study *Xanthomonas campestris* pv. *campestris* (*Xcc*) (5028) were procured National Collection of Industrial Microorganisms Pune, India. Stock cultures of bacteria were maintained on nutrient agar (Himedia, Mumbai) slants at 4 °C with periodical subculturing. The wild-type strain *C. violaceum* ATCC 12427 (provided by Robert McLean) used as a biosensor for anti-quorum sensing studies. *C. violaceum* was maintained in Luria–Bertani (LB) broth at 30 °C in a rotary shaker incubator for 24 h.

Antibacterial activity

The antibacterial activity of the compounds was screened preliminarily by the disc diffusion method [37]. Briefly, the synthetic compounds were loaded into the sterile paper discs and these were placed on the pre-seeded Mueller–Hinton (Himedia, Mumbai) agar plates. Overnight culture of test microorganisms in broth (turbidity adjusted to 0.5 McFarland unit) was used for the seeding. Ciprofloxacin discs (6 mm, 30 µg/disc) were used as a positive control for bacteria. Then, the bacterial plates were incubated at 37 °C for 24 h and fungal plates for three days at 30 °C temperature. The diameters of the inhibition zones (in mm) were measured after the incubation.

Determination of minimum inhibitory concentration (MIC)

MIC of the synthetic compounds against *Xanthomonas campestris* pv. *Campestris* was performed as per the prevoius method [37]. A serial two-fold dilution of the synthetic compounds was prepared in a 96-well microtiter plate (Tarsons, U bottom) over the range 1.25 mg/ml–0.019 mg/ml. Each of the wells was inoculated with $1X10^8$ of the inoculum. The plates were incubated at 37 °C for 24 h. Appropriate growth controls and sterility controls were maintained. The bacterial growth was assessed by measuring the turbidity after the incubation.

Anti-quorum sensing activity

The anti-quorum sensing assay was performed as per the previously reported method with some modification [38]. Briefly, the synthetic compounds were loaded into the sterile

paper discs and placed into LB agar plates were pre-seeded with an overnight culture of *C. violaceum.* The plates were incubated at 30 °C for 24 h. Quercetin (Sigma Aldrich, USA) was used as a standard QSI molecule [39]. After the incubation time, the plates were observed for the presence of clear zone (inhibition of violacein production) around the discs suggesting the inhibition of quorum sensing.

Quantification of anti-quorum sensing activity

The quorum sensing inhibition by synthetic compounds was quantified as per the reported method [40] with few modifications. Briefly, the overnight culture of *C. violaceum* inoculated to LB medium along with synthetic compounds in a 10 ml culture tube. The culture tubes were incubated at 30 °C for 24 h. Then, 1 ml of culture from each tube was transferred to a microcentrifuge tube and centrifuged at 10000 rpm for 5. Supernatant was separated, cell pellet was used to extract violacein with an equal volume of DMSO (Dimethyl sulphoxide). It was vortexed for 1 to 2 min and centrifuged at 10,000 rpm for 5 min to remove the cells. Two hundred microlitres of violacein containing supernatants were transferred to 96-well microplates, and the absorbance was read with a microplate reader at a wavelength of 585 nm. The harvested bacterial cells from individual treatment were resuspended in 1 ml sterile water to check the cell growth by measuring the optical density at 600 nm. The results were compared with respective assay control (without synthetic compound) and standard (quercetin).

In silico molecular docking studies

The binding interactions and orientations of targeted compounds with CviR protein were understood with the aid of molecular docking studies. The molecular docking studies reported were performed on a Schrodinger software suite, LLC, NY, USA, 2015. The crystallographic structure of CviR protein was collected with PDB ID: 3QP5 from the RSCB protein data bank (www.rscb.org). Firstly, ligands were drawn in 2D sketchers and prepared with the help of LigPrep tool. Using the Protein Preparation Wizard tool, the protein was pre-processed for docking by retrieving chain-A and deleting remaining chains, heteroatoms and water molecules that were far away from the ligand. The extra precision mode of docking using glide application of the Schrodinger suite was used to identify the best fit of molecules in the active pockets of the docked protein.

Physicochemical properties

The physiochemical properties have been calculated using the QikProp programme (Schrödinger) running in fast mode.

Statistical analysis

Statistical significance of variance for collected data was determined by ANOVA. The quantification of QSI activity by microtiter plate assay was analysed by one-way ANOVA followed by Tukeys test. Statistical analyses were performed using Graphpad Prism 5.03 software.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

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