RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2015, **5**, 33562

# 3-(1-Phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)-methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones: one-pot three component condensation, *in vitro* antimicrobial, antioxidant and molecular docking studies†

Ramesh Gondru,[a] Janardhan Banothu,[a] Ranjith Kumar Thatipamula,[b] Althaf Hussain SK[c] and Rajitha Bavantula*[a]

Received 10th March 2015
Accepted 2nd April 2015

DOI: 10.1039/c5ra04196a

www.rsc.org/advances

In an attempt to find a new class of heterocyclic bio-active agents, a series of novel 3-(1-phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one derivatives (**5a–l**) have been synthesized efficiently both quantitatively and qualitatively *via* a three-component one-pot manner by Hantzsch condensation. Structures of all the newly synthesized compounds were established by their spectral data and elemental analyses, and they were evaluated for their *in vitro* antimicrobial and antioxidant activities. Among the tested compounds (**5a–l**), the derivatives **5k**, **5h** and **5a** have displayed broad spectrum antibacterial activity, whereas the compounds **5b** and **5f** were found to be potent antifungal agents. Antioxidant activity results revealed that compounds **5a**, **5b** and **5i** exhibited higher radical scavenging ability than the positive control drug Trolax. Further, molecular docking of synthesized compounds (**5a–l**) into the binding site of the crystal structure of *E. coli* MurB enzyme (PDB Id: 1MBT), a key enzyme in peptidoglycan biosynthesis, was performed to gain a comprehensive understanding of the plausible binding modes and also to compare the theoretical and experimental results of these compounds. Docking results revealed that the docking scores and H-bonding interactions of the ligands are in good agreement with the *in vitro* results and also indicated that compounds **5k**, **5h** and **5a** have considerable binding energies and greater affinity towards the active site of MurB enzyme. Thus, they can be further optimized and developed as lead compounds.

## 1. Introduction

During the past two decades, there has been an increase in the population severely suffering with infectious diseases due to multi-drug resistance, often resulting from the over-expression of multidrug efflux systems and their wide spread usage.[1] Microbial infections are the second most prominent death causing diseases after heart attack in the world, due to their ability to spread rapidly, combined with their toxicity and resistance towards existing antibiotic drugs. Hence there is an urgent need for the development of more potent, broad spectrum antimicrobial novel drugs with fewer side effects and improved efficacy to cure microbial infections. In this context, the microbial target based synthesis of novel antimicrobial agents has attracted considerable interest in drug discovery. In this regard, a well known key enzyme MurB, an NADPH dependent enolpyruvyl reductase,[2] which is essential for the growth and bio-synthesis of peptidoglycan polymeric layer of bacterial cell wall,[3] has emerged as an important and attractive target for the development of new antibiotic drugs.[4] MurB enzyme is unique to prokaryotic cells and has no counterpart in eukaryotes. In addition, the molecular docking technique has also emerged as an important tool in drug design and discovery of novel potential ligands. This computer aided drug designing suite is very useful in studying the mechanism involved in the non-covalent interactions between small molecule drug candidates and the binding site of a macromolecule and also to predict the accurate binding conformations of the ligands with the active pockets of pathogen macromolecules.[5]

Antioxidants play a vital role in the body defence mechanism by regulating the generation and elimination of reactive oxygen species (ROS) such as hydroxyl radicals, superoxide radicals, singlet oxygen and hydrogen peroxide radicals those generated from excessive oxidative stress and normal metabolic activities.

---

[a] *Department of Chemistry, National Institute of Technology, Warangal-506 004, Telangana, India. E-mail: rajitabhargavi@yahoo.com*

[b] *Department of Chemistry, Kakatiya University, Warangal-506 009, Telangana, India*

[c] *Department of Biotechnology, Kakatiya University, Warangal-506 009, Telangana, India*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ra04196a

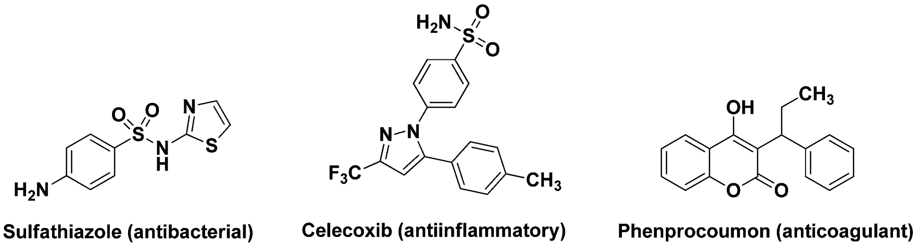


Fig. 1 Biologically active thiazole, pyrazole and coumarin derivatives.

The regulating mechanism includes detoxification of excess ROS, if not the high concentrations of free radicals damages the normal cell structures, embedded proteins, lipids, carbohydrates and also damages the nitrogen bases of nucleic-acids leading to mutations[6] and also causes cancer, aging and neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.[7,8] In addition to the body's defence mechanism includes superoxide dismutase (SOD), catalase and glutathione peroxidase, antioxidants also regulate the concentration of ROS by interacting with them and prevent their influence on other molecules. Thus, the discovery and development of novel synthetic radical scavengers attained great importance in organic chemistry.

Most of the literature studies revealed that, thiazoles and pyrazoles are the key moieties in heterocyclic chemistry and are important structural backbone of various natural and synthetic biologically active molecules. They are known to possess a wide range of pharmacological activities that includes, antimicrobial,[9] anticancer,[10] anti-inflammatory,[11] antitubercular,[12] antihypertensive,[13] antidepressant,[14] anti-HIV,[15] anti-parkinsonian,[16] antiviral,[17] antiallergenic,[18] anticonvulsant,[19] antipyretic[20] and fibrinogen receptor antagonists with antithrombic activity.[21] Among the pyrazoles, especially 4-functionalized pyrazoles have been known to exhibit better antimicrobial and anti-inflammatory activities.[22] Similarly, coumarin is a core structural motif present in numerous naturally occurring compounds,[23] and have been reported to possess anticancer, anticoagulant, anti-inflammatory, antimicrobial, antioxidant, antiviral and cardiovascular activities[24,25] (Fig. 1).

Further extension to our earlier works[26] and as a part of our endeavour towards the synthesis of biologically potent new heterocyclic scaffolds. Here in we report, the synthesis of novel heterocyclic scaffold bearing a coumarin nucleus with a pyrazole and 4-functionalized thiazole rings. This work is with an expectation to find a new and more potent antioxidant and antimicrobial agents which competitively inhibits the bacterial peptidoglycan bio-synthesis by restricting the vital MurB enzyme.

## 2. Results and discussion

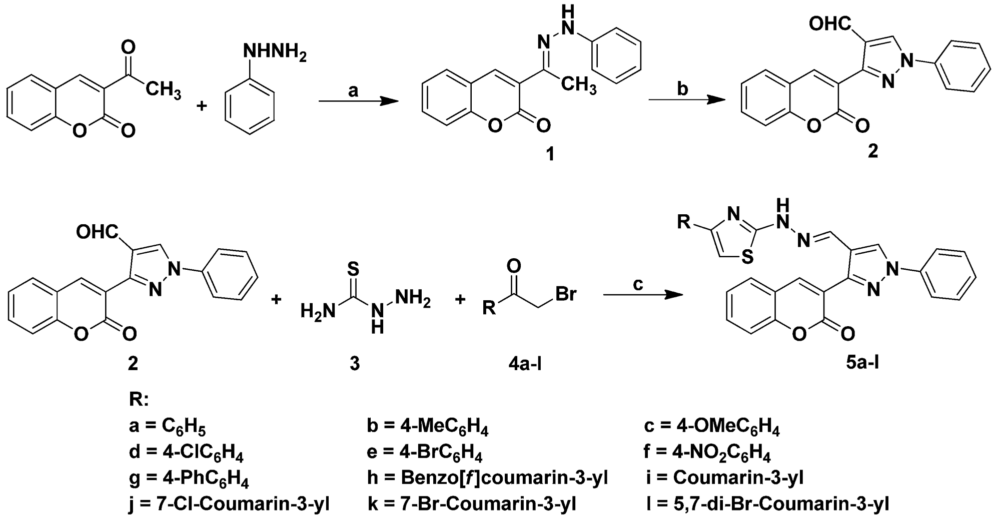
### 2.1. Chemistry

The synthetic protocol for the title compounds, 3-(1-phenyl-4-((2-(4-aryl/heteryl-thiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones (**5a–l**) has outlined in Scheme 1, and were synthesized by the one-pot three-component condensation reaction of 3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**2**), thiosemicarbazide (**3**) and phenacyl bromides (**4a–g**)/2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**4h**)/3-(2-bromoacetyl)-2*H*-chromen-2-ones (**4i–l**) in ethanol in the presence of catalytic amount of acetic acid under reflux conditions with good yields (85–92%) in shorter reaction times (30–50 min). The starting materials, 3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**2**), 2-(2-bromoacetyl)-3*H*-benzo[*f*]chromen-3-one (**4h**) and 3-(2-bromoacetyl)-2*H*-chromen-2-ones (**4i–l**) were synthesized by following the literature procedures.[26b,27] The physical data of the title compounds (**5a–l**) were presented in Table 1.

Structures of all the synthesized compounds (**5a–l**) were established with the aid of their spectral (IR, NMR and Mass) and (C, H and N) elemental analyses. Analytical and spectral data of all the synthesized compounds were in full agreement with the proposed structures and also discussed for a representative compound **5d**: From the IR spectrum, the appearance of a broad absorption band at 3414 $cm^{-1}$ and sharp bands at 1720 and 1628 $cm^{-1}$ are ascribed to –N–H, –C=O and –C=N stretching frequencies respectively, confirming the formation of the proposed compound. From the $^1H$ NMR spectrum, the appearance of singlets at 12.02 ppm (NH proton), 8.92 ppm (pyrazole 5th proton), 8.35 ppm (–CH=N proton), 8.06 ppm (coumarin 4th proton) and 7.20 ppm (thiazole 5th proton), and from the $^{13}C$ NMR the presence of signals at 168.1 ppm (thiazole –C=N carbon) & 158.8 ppm (lactone carbonyl carbon), and the molecular ion peak from the mass spectrum as well as elemental analyses data confirmed the formation of the product.

### 2.2. Biological studies

**2.2.1. *In vitro* antimicrobial activity.** All the synthesized compounds (**5a–l**) were screened for their *in vitro* antibacterial activity against four pathogenic microorganisms, including two Gram-positive bacteria, *Staphylococcus aureus* (MTCC 121) and *Bacillus subtilis* (MTCC 96), and two Gram-negative bacteria *Escherichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 2453). The standard pathogenic microbial cultures were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India and were incubated on sterile nutrient agar at room temperature and inoculated into the fresh nutrient broth of 10 mL, in order to yield bacterial suspension of about 10–100 colony forming units (CFU) per mL. The inoculums size of approximately $10^6$ CFU per plate was spread plated over the



Scheme 1 *Reagents and conditions*: (a) $H_2O$, AcONa, reflux, 1 h; (b) DMF, $POCl_3$, rt., 6 h; (c) EtOH, Cat. AcOH, reflux, 30-50 min. Synthesis of 3-(1-phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones.

Table 1 Physical data of the title compounds (**5a–l**)[a]

| Product | Time (min) | Yield[b] (%) | M.p. (°C) |
|---|---|---|---|
| **5a** | 35 | 89 | 175–177 |
| **5b** | 40 | 86 | 211–213 |
| **5c** | 30 | 88 | 195–197 |
| **5d** | 45 | 92 | 166–168 |
| **5e** | 45 | 90 | 189–191 |
| **5f** | 50 | 90 | 199–201 |
| **5g** | 40 | 88 | 210–212 |
| **5h** | 45 | 86 | 253–255 |
| **5i** | 35 | 89 | 257–259 |
| **5j** | 40 | 91 | 223–225 |
| **5k** | 45 | 86 | 233–235 |
| **5l** | 45 | 85 | 246–248 |

[a] Reaction conditions: Coumarin pyrazole aldehyde (**2**, 1 mmol), thiosemicarbazide (**3**, 1 mmol) and phenacyl bromides/3-(2-bromoacetyl) coumarins/2-(2-bromoacetyl)-3*H*-benzo[*f*] chromen-3-one (**4a–l**, 1 mmol), ethanol (5 mL), acetic acid (3 drops), reflux. [b] Isolated yields.

surface of the nutrient agar by diluting the initial microbial suspension 10 times with distilled water. 30 μL of Antibacterial suspension of 100 $\mu g\ mL^{-1}$ concentration was transferred into the 6 mm diameter well made by the sterile cork borer and incubated for about 24 h at 37 ± 1 °C. Antibacterial screenings were conducted in triplicates by well-plate method in Mueller-Hinton Agar[28] at 100 $\mu g\ mL^{-1}$ concentration for the synthesized compounds (**5a–l**) with respect to positive control streptomycin at 30 $\mu g\ mL^{-1}$. Zone of inhibition (ZOI) values were measured in mm and minimum inhibitory concentration (MIC) for the tested compounds, as well as standards was measured in $\mu g\ mL^{-1}$ by micro dilution method.[29] DMSO used as a solvent control and the results are depicted in Table 2.

All the compounds (**5a–l**) were also screened for their *in vitro* antifungal activity against *Candida albicans*, *Aspergillus niger*, *Candida glabrata* and *Aspergillus parasiticus* fungal strains using positive control clotrimazole.

Evaluation of antibacterial data (Table 2) revealed that, most of the tested compounds exhibited moderate to excellent antibacterial and good to moderate antifungal activity against all the tested microbial strains. Among them, the compound **5k** has exhibited excellent activity against *E. coli* (ZOI = 22 mm and MIC = 12.5 $\mu g\ mL^{-1}$), good activity against *S. aureus* (ZOI = 22 mm and MIC = 50 $\mu g\ mL^{-1}$) and moderate activity against *B. subtilis* (ZOI = 18 mm and MIC = 50 $\mu g\ mL^{-1}$), and *P. aeruginosa* (ZOI = 17 mm and MIC = 50 $\mu g\ mL^{-1}$). Similarly, the compound **5h** has shown good activity against *E. coli* (ZOI = 21 mm and MIC = 25 $\mu g\ mL^{-1}$) and *P. aeruginosa* (ZOI = 20 mm and MIC = 25 $\mu g\ mL^{-1}$), and moderate inhibiting activity against *S. aureus* (ZOI = 19 mm and MIC = 50 $\mu g\ mL^{-1}$). The compound **5a** has also exhibited good activity against *E. coli* (ZOI = 19 mm and MIC = 50 $\mu g\ mL^{-1}$) and moderate activity against *S. aureus* (ZOI = 17 mm and MIC = 50 $\mu g\ mL^{-1}$) and *P. aeruginosa* (ZOI = 17 mm and MIC = 50 $\mu g\ mL^{-1}$) with respect to the standard antibacterial drug streptomycin. From the antifungal results (Table 2) we have observed that, the compounds **5b** (ZOI = 20 mm) and **5f** (ZOI = 19 mm) have shown good inhibiting activity against *A. niger* on comparing with the positive control drug clotrimazole. Remaining all the compounds have shown moderate activity against all the tested microbial strains with ZOI ranging from 7–16 mm and MIC 50–200 $\mu g\ mL^{-1}$ for bacteria, and ZOI 8–18 mm for fungi.

Structure–activity relationship of the compounds (**5a–l**) revealed that, the 4th position of thiazole ring bearing 7-bromo coumarinyl (**5k**), benzo[*f*]coumarinyl (**5h**) and simple phenyl (**5a**) were found to be potent antibacterial agents and the compounds bearing 4-methyl phenyl and 4-fluoro phenyl were found to be good antifungal agents than the remaining compounds.

### 2.2.2. *In vitro* antioxidant activity.

In order to investigate the possible biological studies for the synthesized compounds (**5a–l**), also screened *in vitro* antioxidant activity in terms of hydrogen donating or radical scavenging ability by rapid and convenient technique *i.e.* 1,1-diphenyl-2-picrylhydrazyl (DPPH)

Table 2 *In vitro* antimicrobial activity of **5a–l**[a]

| | Antibacterial activity | | | | | | | | Antifungal activity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus* | | *B. subtilis* | | *E. coli* | | *P. aeruginosa* | | *C. albicans* | *A. niger* | *C. glabrata* | *A. parasiticus* |
| Product | ZOI | MIC | ZOI | MIC | ZOI | MIC | ZOI | MIC | ZOI | | | |
| **5a** | 17 | 50 | 16 | 50 | 19 | 50 | 17 | 50 | 10 | 14 | 8 | 8 |
| **5b** | 13 | 200 | 8 | 200 | 8 | 200 | 7 | 200 | 20 | 20 | 18 | 17 |
| **5c** | 16 | 100 | 7 | 200 | 8 | 200 | 8 | 200 | 10 | 8 | 10 | 10 |
| **5d** | 15 | 100 | 8 | 200 | 8 | 200 | 8 | 200 | 8 | 12 | 17 | 17 |
| **5e** | 12 | 200 | 8 | 200 | 8 | 200 | 8 | 200 | 8 | 8 | 12 | 10 |
| **5f** | 13 | 50 | 15 | 50 | 16 | 50 | 15 | 50 | 18 | 19 | 16 | 15 |
| **5g** | 9 | 200 | 8 | 200 | 8 | 200 | 13 | 100 | 8 | 10 | 10 | 8 |
| **5h** | 19 | 50 | 16 | 50 | 21 | 25 | 20 | 25 | 15 | 8 | 8 | 8 |
| **5i** | 8 | 200 | 8 | 200 | 8 | 200 | 8 | 200 | 10 | 8 | 10 | 12 |
| **5j** | 13 | 200 | 7 | 200 | 12 | 100 | 7 | 200 | 8 | 8 | 12 | 12 |
| **5k** | 22 | 50 | 18 | 50 | 22 | 12.5 | 17 | 50 | 12 | 12 | 10 | 12 |
| **5l** | 8 | 200 | 8 | 200 | 12 | 200 | 8 | 200 | 12 | 8 | 8 | 15 |
| Streptomycin | 22 | 25 | 21 | 12.5 | 20 | 12.5 | 20 | 12.5 | — | — | — | — |
| Clotrimazole | — | — | — | — | — | — | — | — | 24 | 20 | 22 | 20 |

[a] Zone of inhibition (ZOI) values (in mm) for analogs (**5a–l**) at 100 μg mL$^{-1}$ and positive control drugs streptomycin and clotrimazole at 30 μg mL$^{-1}$. MIC values were given in μg mL$^{-1}$. Bacterial strains: *S. aureus* – *Staphylococcus aureus*, *B. subtilis* – *Bacillus subtilis*, *E. coli* – *Escherichia coli* and *P. aeruginosa* – *Pseudomonas aeruginosa*; fungal strains: *C. albicans* – *Candida albicans*, *A. niger* – *Aspergillus niger*, *C. glabrata* – *Candida glabrata* and *A. parasiticus* – *Aspergillus parasiticus*. '—' – Not performed.

assay[30] using Trolax and Ascorbic acid as standard drugs. Methanol (95%), DPPH solution and standard drugs were used as blank, control and reference respectively. Absorbance was calculated at 517 nm (at absorption maximum of DPPH) after keeping the mixture of 100 μL of synthesized compounds of concentration 10 μg mL$^{-1}$ (dissolved in DMSO) and 900 μL of DPPH radical solution (0.004% w/v of DPPH in methanol) in a dark place for 30 min incubation period. Antioxidant activity was evaluated in IC$_{50}$ in μM (the effective concentration at which 50% of the radicals were scavenged) and depicted in Table 3.

Evaluation of antioxidant activity revealed that, most of the tested compounds exhibited moderate to strong DPPH radical scavenging ability compared with the positive controls Trolox and Ascorbic acid. Among them, the compounds **5a** bearing phenyl, **5b** bearing 4-methyl phenyl and **5i** having 2*H*-chromen-2-one were found to be more effective and potent DPPH radical scavenging ability with ~1.11, ~1.09, ~1.02 folds than positive control drug Trolax. Remaining all the compounds have shown good to moderate radical scavenging activity with IC$_{50}$ values in the range of 15.51–89.92 μM. It was noticed that, the compounds with electron donating groups on the phenyl ring were found to be potent radical scavenging ability.

Table 3 Antioxidant activity of **5a–l** by DPPH method

| Product | IC$_{50}$ in μM |
|---|---|
| **5a** | 12.79 |
| **5b** | 13.01 |
| **5c** | 16.80 |
| **5d** | 89.92 |
| **5e** | 67.85 |
| **5f** | 81.29 |
| **5g** | 15.51 |
| **5h** | 44.75 |
| **5i** | 13.89 |
| **5j** | 76.01 |
| **5k** | 74.56 |
| **5l** | 63.33 |
| Trolox | 14.22 |
| Ascorbic acid | 3.8 |

**2.2.3. Molecular modelling studies.** To explore and support the antibacterial mechanism, docking studies for the synthesized compounds (**5a–l**) was performed. This drug designing tool helpful to investigate and to gain a deep insight in to the mode of binding interactions of each of these ligands (**5a–l**) with the receptor sites of UDP-*N*-acetylenolpyruvoylglucosamine reductase, MurB and also to determine the best *in silico* conformation. Docking of the synthesized ligands was employed by using Lamarckian Genetic Algorithm (LGA),[31] inculcated in the docking program AutoDock 4.2. MurB is an essential enzyme that catalyzes the reduction of enolpyruvyl uridine diphosphate *N*-acetyl glucosamine (EP-UNAG), an intermediate in the assembly of the UNAM-pentapeptide (m-A2pm) portion to uridine diphosphate *N*-acetyl muramic acid (UNAM), of cell wall precursor. Mur proteins (Mur A-F, Y and G) catalyze more than 10 biosynthetic transformations involved in the formation of peptidoglycan layer of the cell walls of bacteria and they also conserved among several bacterial strains. Because of this, we selected the MurB enzyme as a target receptor.

The co-crystallized structure of target enzyme MurB (PDB id: 1MBT) was obtained from Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). To carry out *in silico* studies, the 2D structures of the synthesized ligands (**5a–l**) were drawn in ChemBioOffice 2010 and converted to energy minimized 3D structures in pdb file format using MarvinSketch (ChemAxon). The target protein file was prepared by removing the structural

Table 4 Autodock binding energies, no. of hydrogen bonds and residues involved in hydrogen bonding interaction of ligands for *E. coli* (PDB id: 1MBT)

| Product | Binding energy (kcal $mol^{-1}$) | Inhibition constant $K_i$ (nM) | Residues involved in hydrogen bonding interactions (no. of hydrogen bonds) |
|---|---|---|---|
| **5a** | −10.93 | 9.77 | SER229 (2), ARG214 (2), ARG159 (1) |
| **5b** | −10.44 | 22.39 | SER229 (1), ARG214 (3), ARG159 (1), SER50 (1) |
| **5c** | −10.07 | 41.70 | SER50 (2), SER229 (3), ARG214 (2) |
| **5d** | −10.34 | 26.44 | ARG214 (3), SER229 (1), ARG159 (2), GLY123 (1) |
| **5e** | −10.08 | 40.66 | ARG214 (3), SER229 (1), ARG159 (2), GLY123 (2) |
| **5f** | −10.85 | 11.21 | SER229 (1), ARG214 (1), ARG159 (1), SER50 (1), GLU48 (1), GLY49 (1), CYS113 (1) |
| **5g** | −10.44 | 22.40 | SER229 (4), ARG159 (1) |
| **5h** | −11.14 | 6.84 | ARG214 (3), ARG159 (2), SER50 (1) |
| **5i** | −9.83 | 62.09 | SER116 (2), GLU48 (1), SER50 (3), ARG159 (1), ARG214 (2), SER229 (1) |
| **5j** | −10.23 | 31.68 | SER116 (2), SER50 (1), CYS113 (1), ARG214 (1), SER229 (2) |
| **5k** | −11.15 | 6.71 | SER116 (2), CYS113 (1), SER50 (1), ARG159 (2), ARG214 (1), SER229 (1) |
| **5l** | −9.02 | 244.22 | SER229 (2), SER50 (2), SER116 (1) |

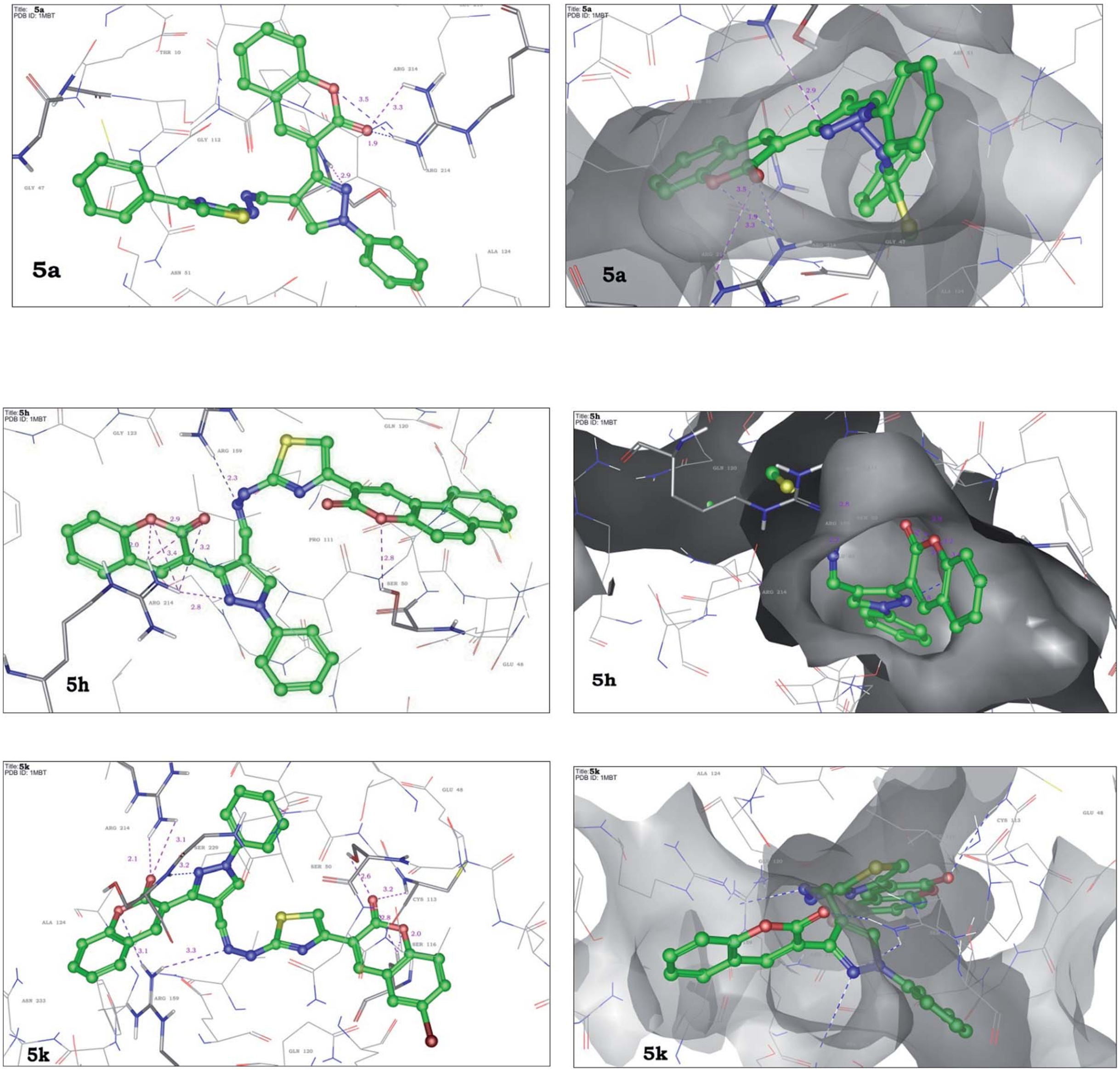


Fig. 2 Docking pose of **5a**, **5h** and **5k** (ball and stick) with UDP-*N*-acetylenolpyruvoylglucosamine reductase (MurB) (thin wire) with intermolecular H-bonding (pink and blue dotted lines) and 3D surface interaction (green) with the enzyme (represented in molecular cloud).

water molecule, hetero atoms and co-factors by leaving only the residues associated with protein by using Discovery Studio 4.0 Visualizer (DSV). AutoDock 1.5.6 (MGL tools-1.5.6) tool was used to prepare target protein file that involves, assigning AD4 type atoms, calculating Gasteiger charges for every atom of the macromolecule, addition of polar hydrogen's to the macromolecule, an essential step to correct the calculation of partial charge by keeping all other values as default. The binding site of protein identification was carried out using CastP (serversts-fw.bioengr.uic.edu/castp/calculation.php). Docking simulations for the compounds **5a–l** were performed against the active site of MurB enzyme. Then, finally docking results were visualized using Maestro elements tutorial 1.8.

All inhibitors were compared out of 100 docking runs. The docking studies revealed that, all the synthesized molecules exhibited excellent binding energies towards the receptor active pocket ranging from $-9.02$ to $-11.15$ kcal mol$^{-1}$ and summarized in Table 4. Among them, the conformations with lowest binding energies and those ligands exhibiting well established H-bonds with the closest range of 1.8–3.4 Å with one or more amino acids in the receptor active pocket were chosen as best docked ligand orientations (supporting file). Hence, the compounds **5a**, **5h** and **5k** were energetically favored for MurB active site and are exhibiting bonds with amino acids of active pocket of the receptor and considered as the best docking poses. The ligand **5a** exhibited H-bonding with SER229, ARG214, ARG159 amino acids, whereas **5h** with ARG214, ARG159, SER50 amino acids and **5k** with SER116, CYS113, SER50, ARG159, ARG214, SER229 amino acids. Best docked orientations of synthesized ligands were shown in Fig. 2. The binding energies, inhibition constants and hydrogen bond interactions of all the compounds were tabulated in Table 4. These results revealed a variety of binding modes that may provide a sufficient explanation and good compromise between docking scores and *in vitro* results of antibacterial activity.

## 3. Conclusion

In conclusion, a series of novel pyrazolyl coumarin bearing 2,4-disubstituted thiazole derivatives (**5a–l**) were reported in quantitative yields *via* MCR approach and evaluated for their *in vitro* antimicrobial and antioxidant studies. Among the series, compounds possessing 7-bromo coumarinyl (**5k**), benzo[*f*]coumarinyl (**5h**) and simple phenyl (**5a**) on thiazole ring were found to be potent and broad spectrum antibacterial agents with respect to standard drug streptomycin. The compounds possessing 4-methyl phenyl (**5b**) and 4-nitro phenyl (**5f**) on thiazole ring were found to be good antifungal agents. Antioxidant studies revealed that, the compounds **5a**, **5b** and **5i** have excellent radical scavenging ability than the positive control Trolax. In order to support the *in vitro* antibacterial results, the synthesized compounds were docked in to the plausible target UDP-*N*-acetylenolpyruvoylglucosamine reductase, MurB enzyme. The binding energies and H-bond interactions with amino acids in active site of target enzyme well supported the antibacterial inhibiting activity of **5k**, **5h** and **5a** and further helped to investigate the binding orientations of ligands with active pockets of an enzyme. All these results could be useful to evaluate novel antibacterial inhibitors and can be consider as a lead compounds for the development of antibacterial agents for the treatment of bacterial infection.

## 4. Experimental

### 4.1. General

All the solvents and the starting materials were purchased from commercial sources and used without further purification. Melting points were determined in open capillaries using Stuart SMP30 melting point apparatus and are uncorrected. The progress of the reactions as well as the purity of the compounds was checked with TLC plates (E. Merck, Mumbai, India) and the developed chromatogram was visualized under UV light and iodine vapors. IR spectra were recorded on Perkin-Elmer 100S spectrophotometer using KBr disk. NMR spectra were recorded on Bruker-400 MHz spectrometer using DMSO-$d_6$ as solvent and TMS as internal standard. Elemental analyses were performed on a Carlo-Erba model EA1108 analytical unit and the values are ±0.4% of theoretical values. Mass spectra were recorded on a Jeol JMSD-300 spectrometer.

### 4.2. General procedure for the synthesis of 3-(1-phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones (5a–l)

A mixture of 3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**2**, 1 mmol), thiosemicarbazide (**3**, 1 mmol) and phenacyl bromides/3-(2-bromoacetyl)-2*H*-chromen-2-ones/2-(2-bromoacetyl)-3*H*-benzo[*f*] chromen-3-one (**4a–l**, 1 mmol) were dissolved in 5 mL of ethanol in the presence of catalytic amount of acetic acid (3 drops) and refluxed for about 30-50 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid separated out was filtered, dried and washed with hot ethanol which afforded the analytically pure products (**5a–l**) in good yields.

**4.2.1. 3-(1-Phenyl-4-((2-(4-phenylthiazol-2-yl)hydrazono)-methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5a).** Yellow solid; IR (KBr, cm$^{-1}$) $\nu_{\max}$: 3442 (NH), 1722 (C=O), 1630 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 11.98$ (s, 1H), 8.92 (s, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 7.93 (d, $J = 8.0$ Hz, 2H), 7.88 (d, $J = 7.2$ Hz, 1H), 7.84 (d, $J = 8.0$ Hz, 2H), 7.68–7.78 (m, 2H), 7.50–7.58 (m, 3H), 7.35–7.45 (m, 4H), 7.27 (t, $J = 7.2$ Hz, 1H); MS (ESI) *m*/*z*: 490 [M + H]$^+$; anal. calcd for $C_{28}H_{19}N_5O_2S$: C, 68.70; H, 3.91; N, 14.31. Found: C, 68.49; H, 3.82; N, 14.58%.

**4.2.2. 3-(1-Phenyl-4-((2-(4-(*p*-tolyl)thiazol-2yl)hydrazono)-methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5b).** Yellow solid; IR (KBr, cm$^{-1}$) $\nu_{\max}$: 3447 (NH), 1721 (C=O), 1629 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 11.83$ (s, 1H), 8.91 (s, 1H), 8.34 (s, 1H), 8.05 (s, 1H), 7.93 (d, $J = 7.6$ Hz, 2H), 7.86 (d, $J = 6.4$ Hz, 1H), 7.67–7.70 (m, 3H), 7.50–7.58 (m, 3H), 7.36–7.45 (m, 2H), 7.17 (d, $J = 8.0$ Hz, 2H), 7.03 (s, 1H), 2.30 (s, 3H); MS (ESI) *m*/*z*: 504 [M + H]$^+$; anal. calcd for $C_{29}H_{21}N_5O_2S$: C, 69.17; H, 4.20; N, 13.91. Found: C, 69.36; H, 4.03; N, 13.74%.

**4.2.3. 3-(4-((2-(4-(4-Methoxyphenyl)thiazol-2-yl)hydrazono)-methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5c).** Pale yellow solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3448 (NH), 1724 (C=O), 1627 (C=N), 1217 (C–O–C); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.03$ (s, 1H), 8.91 (s, 1H), 8.34 (s, 1H), 8.05 (s, 1H), 7.85–7.94 (m, 3H), 7.71 (t, $J$ = 8.8 Hz, 3H), 7.50–7.58 (m, 3H), 7.38–7.45 (m, 2H), 6.93 (d, $J$ = 9.6 Hz, 3H), 3.76 (s, 3H); MS (ESI) $m/z$: 520 [M + H]$^+$; anal. calcd for $C_{29}H_{21}N_5O_3S$: C, 67.04; H, 4.07; N, 13.48. Found: C, 67.31; H, 4.26; N, 13.25%.

**4.2.4. 3-(4-((2-(4-(4-Chlorophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5d).** Yellow solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3414 (NH), 1720 (C=O), 1628 (C=N), 750 (C–Cl); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.02$ (s, 1H), 8.92 (s, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 7.93 (d, $J$ = 8.0 Hz, 2H), 7.80–7.87 (m, 3H), 7.68–7.72 (m, 1H), 7.38–7.58 (m, 7H), 7.20 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 168.0, 158.8, 153.5, 148.9, 145.3, 142.5, 138.9, 134.3, 133.3, 132.2, 131.8, 129.6, 128.8, 128.5, 128.1, 127.1, 126.9, 124.7, 121.7, 118.9, 118.7, 118.5, 116.0, 103.9; MS (ESI) $m/z$: 525 [M]$^+$; anal. calcd for $C_{28}H_{18}ClN_5O_2S$: C, 64.18; H, 3.46; N, 13.37. Found: C, 64.02; H, 3.63; N, 13.52%.

**4.2.5. 3-(4-((2-(4-(4-Bromophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5e).** Yellow solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3436 (NH), 1720 (C=O), 1630 (C=N), 684 (C–Br); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.00$ (s, 1H), 8.91 (s, 1H), 8.34 (s, 1H), 8.06 (s, 1H), 7.85–7.94 (m, 2H), 7.74 (d, $J$ = 8.8 Hz, 1H), 7.69 (d, $J$ = 7.6 Hz, 3H), 7.37–7.58 (m, 7H), 7.21 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 168.0, 158.8, 153.5, 149.0, 145.3, 142.5, 138.9, 134.3, 133.7, 132.2, 131.4, 129.6, 128.7, 128.1, 127.4, 126.9, 124.7, 121.7, 120.4, 118.9, 118.7, 118.5, 116.0, 103.9; MS (ESI) $m/z$: 568 [M]$^+$; anal. calcd for $C_{28}H_{18}BrN_5O_2S$: C, 59.16; H, 3.19; N, 12.32. Found: C, 59.33; H, 3.01; N, 12.57%.

**4.2.6. 3-(4-((2-(4-(4-Nitrophenyl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5f).** Brown solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3436 (NH), 1704 (C=O), 1632 (C=N), 1504, 1344 ($NO_2$); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.12$ (s, 1H), 8.93 (s, 1H), 8.35 (s, 1H), 7.86–8.25 (m, 8H), 7.69–7.73 (m, 1H), 7.37–7.58 (m, 6H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 168.3, 158.8, 153.5, 148.3, 146.1, 145.3, 142.5, 140.5, 138.9, 134.5, 132.2, 129.6, 128.8, 128.2, 126.9, 126.2, 124.7, 124.0, 121.6, 118.9, 118.5, 116.0, 107.9; MS (ESI) $m/z$: 535 [M + H]$^+$; anal. calcd for $C_{28}H_{18}N_6O_4S$: C, 62.91; H, 3.39; N, 15.72. Found: C, 63.12; H, 3.16; N, 15.54%.

**4.2.7. 3-(4-((2-(4-([1,1′-Biphenyl]-4-yl)thiazol-2-yl)hydrazono)methyl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (5g).** Pale brown solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3444 (NH), 1737 (C=O), 1627 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.00$ (s, 1H), 8.92 (s, 1H), 8.35 (s, 1H), 8.08 (s, 1H), 7.85–7.95 (m, 5H), 7.68–7.71 (m, 5H), 7.34–7.58 (m, 9H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 167.9, 158.8, 153.5, 149.7, 145.3, 142.5, 139.5, 138.9, 134.2, 133.5, 132.2, 129.6, 128.8, 128.7, 128.1, 127.3, 126.9, 126.7, 126.4, 126.0, 124.6, 121.7, 118.9, 118.7, 118.5, 116.0, 103.3; MS (ESI) $m/z$: 566 [M + H]$^+$; anal. calcd for $C_{34}H_{23}N_5O_2S$: C, 72.19; H, 4.10; N, 12.38. Found: C, 72.53; H, 4.37; N, 12.19%.

**4.2.8. 2-(2-(2-((3-(2-Oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-3*H*-benzo[*f*]chromen-3-one (5h).** Yellow solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3438 (NH), 1717 (C=O), 1638 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.17$ (s, 1H), 9.25 (s, 1H), 8.94 (s, 1H), 8.20–8.36 (m, 3H), 8.10 (d, $J$ = 7.6 Hz, 2H), 7.95 (d, $J$ = 7.6 Hz, 2H), 7.87 (d, $J$ = 6.4 Hz, 2H), 7.81 (s, 3H), 7.39–7.72 (m, 6H); MS (ESI) $m/z$: 608 [M + H]$^+$; anal. calcd for $C_{35}H_{21}N_5O_4S$: C, 69.18; H, 3.48; N, 11.53. Found: C, 69.39; H, 3.74; N, 11.36%.

**4.2.9. 3-(2-(2-((3-(2-Oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (5i).** Pale yellow solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3439 (NH), 1721 (C=O), 1633 (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.04$ (s, 1H), 8.92 (s, 1H), 8.50 (s, 1H), 8.35 (s, 1H), 8.09 (s, 1H), 7.69–7.95 (m, 4H), 7.64 (t, $J$ = 7.2 Hz, 1H), 7.36–7.60 (m, 9H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 167.4, 158.8, 158.6, 153.5, 152.2, 145.3, 143.8, 142.5, 138.9, 138.0, 134.6, 132.2, 131.6, 129.6, 128.7, 128.1, 126.9, 124.6, 121.5, 120.3, 119.1, 118.9, 118.5, 116.1, 115.8, 109.7; MS (ESI) $m/z$: 558 [M + H]$^+$; anal. calcd for $C_{31}H_{19}N_5O_4S$: C, 66.78; H, 3.43; N, 12.56. Found: C, 66.98; H, 3.22; N, 12.84%.

**4.2.10. 7-Chloro-3-(2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (5j).** Pale brown solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3434 (NH), 1723 (C=O), 1607 (C=N), 751 (C–Cl); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.06$ (s, 1H), 8.92 (s, 1H), 8.44 (s, 1H), 8.35 (s, 1H), 8.10 (s, 1H), 7.91–8.00 (m, 3H), 7.86 (d, $J$ = 7.6 Hz, 1H), 7.69 (t, $J$ = 7.2 Hz, 1H), 7.62 (t, $J$ = 6.4 Hz, 1H), 7.39–7.58 (m, 7H); MS (ESI) $m/z$: 593 [M]$^+$; anal. calcd for $C_{31}H_{18}ClN_5O_4S$: C, 62.89; H, 3.06; N, 11.83. Found: C, 62.72; H, 3.26; N, 11.69%.

**4.2.11. 7-Bromo-3-(2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (5k).** Brown solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3414 (NH), 1723 (C=O), 1600 (C=N), 685 (C–Br); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.06$ (s, 1H), 8.92 (s, 1H), 8.42 (s, 1H), 8.35 (s, 1H), 8.11 (t, $J$ = 6.8 Hz, 2H), 7.93 (t, $J$ = 7.6 Hz, 1H), 7.85 (s, 2H), 7.68–7.73 (m, 1H), 7.51–7.56 (m, 4H), 7.40 (d, $J$ = 8.8 Hz, 4H); MS (ESI) $m/z$: 636 [M]$^+$; anal. calcd for $C_{31}H_{18}BrN_5O_4S$: C, 58.50; H, 2.85; N, 11.00. Found: C, 58.63; H, 2.98; N, 11.25%.

**4.2.12. 5,7-Dibromo-3-(2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-phenyl-1*H*-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)-2*H*-chromen-2-one (5l).** Yellow solid; IR (KBr, cm$^{-1}$) $\nu_{max}$: 3437 (NH), 1702 (C=O), 1597 (C=N), 684 (C–Br); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta = 12.07$ (s, 1H), 8.92 (s, 1H), 8.39 (s, 2H), 8.35 (s, 1H), 8.14 (t, $J$ = 8 Hz, 2H), 7.94 (d, $J$ = 8.4 Hz, 2H), 7.86 (d, $J$ = 7.6 Hz, 1H), 7.71 (t, $J$ = 8.4 Hz, 1H), 7.46–7.58 (m, 4H), 7.37–7.44 (m, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ = 167.4, 158.8, 153.5, 145.3, 143.3, 142.6, 138.8, 136.3, 134.8, 132.3, 130.3, 129.6, 128.7, 128.1, 126.9, 124.7, 121.9, 121.5, 118.9, 118.5, 116.2, 116.1, 111.2, 109.7; MS (ESI) $m/z$: 715 [M]$^+$; anal. calcd for $C_{31}H_{17}Br_2N_5O_4S$: C, 52.05; H, 2.40; N, 9.79. Found: C, 52.22; H, 2.18; N, 9.96%.

## Acknowledgements

The authors thank the Director, National Institute of Technology, Warangal for providing research facilities. One of the authors RG thanks the MHRD for research fellowship.

## References and notes

1 (*a*) H. Okusu, D. Ma and H. Nikaido, *J. Bacteriol.*, 1996, **178**, 306; (*b*) R. E. Isturiz, *Int. J. Antimicrob. Agents*, 2010, **36**, S19.

2 (*a*) R. W. Sarver, J. M. Rogers and D. E. Epps, *J. Biomol. Screening*, 2002, **7**, 21; (*b*) M. S. Harris, J. T. Hergerg, J. I. Cial-della, J. P. Martin Jr, T. E. Benson, G. H. Choi and E. T. Baldwin, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2001, **D57**, 1032.

3 (*a*) T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K. A. Datsenko, M. Tomita, B. L. Wanner and H. Mori, *Mol. Syst. Biol.*, 2006, **2**, 2006.008; (*b*) T. E. Benson, C. T. Walsh and V. Massey, *Biochemistry*, 1997, **36**, 796.

4 Y. Shen, J. Liu, G. Estiu, B. Isin, Y. Y. Ahn, D. S. Lee, A. L. Barabási, V. Kapatral, O. Wiest and Z. N. Oltvai, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 1082.

5 (*a*) A. A. M. Abdel-Aziz, K. E. H. ElTahir and Y. A. Asiri, *Eur. J. Med. Chem.*, 2011, **46**, 1648; (*b*) M. A. El-Sayed, N. I. Abdel-Aziz, A. A. Abdel-Aziz, A. S. El-Azab, Y. A. Asiri and K. E. El-Tahir, *Bioorg. Med. Chem.*, 2011, **19**, 3416; (*c*) G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, *J. Comput. Chem.*, 2009, **30**, 2785; (*d*) N. K. Sharma, K. K. Jha and Priyanka, *J. Adv. Sci. Res.*, 2010, **1**, 67.

6 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, 3rd edn, 1999.

7 T. J. Montine, K. S. Montine, W. McMahan, W. R. Markesbery, J. F. Quinn and J. D. Morrow, *Antioxid. Redox Signaling*, 2005, **7**, 269.

8 J. A. Berliner and J. W. Heinecke, *Free Radical Biol. Med.*, 1996, **20**, 707.

9 (*a*) K. Tsuji and H. Ishikawa, *Bioorg. Med. Chem. Lett.*, 1994, **4**, 1601; (*b*) C. H. Oh, H. W. Cho, D. Baek and J. H. Cho, *Eur. J. Med. Chem.*, 2002, **37**, 743; (*c*) A. M. Isloor, B. Kalluraya and P. Shetty, *Eur. J. Med. Chem.*, 2009, **44**, 3784.

10 (*a*) B. Jiang and X. H. Gu, *Bioorg. Med. Chem.*, 2000, **8**, 363; (*b*) I. V. Magedov, M. Manpadi, S. V. Slambrouck, W. F. Steelant, E. Rozhkova, N. M. Przheval'skii, S. Rogelj and A. Kornienko, *J. Med. Chem.*, 2007, **50**, 5183.

11 (*a*) B. S. Holla, K. V. Malini, B. S. Rao, B. K. Sarojini and N. S. Kumari, *Eur. J. Med. Chem.*, 2003, **38**, 313; (*b*) G. Szabo, J. Fischer, A. Kis-Varga and K. Gyires, *J. Med. Chem.*, 2008, **51**, 142.

12 A. Andreani, M. Granaiola, A. Leoni, A. Locatelli, R. Morigi and M. Rambaldi, *Eur. J. Med. Chem.*, 2001, **36**, 743.

13 (*a*) S. Bondock, W. Khalifa and A. A. Fadda, *Eur. J. Med. Chem.*, 2007, **42**, 948; (*b*) B. A. Steinbaugh, B. L. Batley, C. A. Painchaud, S. T. Rapundalo, B. M. Michniewicz and S. C. J. Olson, *J. Med. Chem.*, 1992, **35**, 2562.

14 P. Y. Rajendra, R. A. Lakshmana, L. Prasoona, K. Murali and K. P. Ravi, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5030.

15 F. W. Bell, A. S. Cantrell, M. Hogberg, S. R. Jaskunas, N. G. Johansson, C. L. Jordon, M. D. Kinnick, P. Lind, J. M. Morin Jr, R. Noreen, B. Oberg, J. A. Palkowitz, C. A. Parrish, P. Pranc, C. Sahlberg, R. J. Ternansky, R. T. Vasileff, L. Vrang, S. J. West, H. Zhang and X. X. Zhou, *J. Med. Chem.*, 1995, **38**, 4929.

16 F. Azam, B. A. El-Gnidi, I. A. Alkskas and M. A. Ahmed, *J. Enzyme Inhib. Med. Chem.*, 2010, **25**, 818.

17 O. I. El-Sabbagh, M. M. Baraka, S. M. Ibrahim, C. Pannecouque, G. Andrei, R. Snoeck, J. Balzarini and A. A. Rashad, *Eur. J. Med. Chem.*, 2009, **44**, 3746.

18 K. D. Hargrave, F. K. Hess and J. T. Oliver, *J. Med. Chem.*, 1983, **26**, 1158.

19 Z. Ozdemir, B. Kandilici, B. Gumusel, U. Calis and A. Bilgin, *Eur. J. Med. Chem.*, 2007, **42**, 373.

20 A. Sener, M. K. Şener, I. Bildmci, R. Kasımogullari and Y. Akcamur, *J. Heterocycl. Chem.*, 2002, **39**, 869.

21 A. Badorc, M. F. Bordes, P. De Cointet, P. Savi, A. Bernat, A. Lale, M. Petitou, J. P. Maffrand and J. M. Herbert, *J. Med. Chem.*, 1997, **40**, 3393.

22 (*a*) R. Sridhra, P. T. Perumal, S. Etti, G. Shanmugam, M. N. Ponnuswamy, V. R. Prabavathy and N. Mathivanan, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 6035; (*b*) P. K. Sharma, S. Kumar, P. Kumar, P. Kaushik, D. Kaushik, Y. Dhingra and K. R. Aneja, *Eur. J. Med. Chem.*, 2010, **45**, 2650.

23 (*a*) H. Raistrick, C. E. Stilkings and R. Thomas, *Biochemistry*, 1953, **55**, 421; (*b*) J. Abaul, E. Philogene and P. Bourgeois, *J. Nat. Prod.*, 1994, **57**, 846.

24 F. Borges, F. Roleira, N. Milhazes, L. Santana and E. Uriarte, *Curr. Med. Chem.*, 2005, **12**, 887.

25 (*a*) R. O. Kennedy and R. D. Thornes, *Coumarins: biology, applications and mode of action*, Wiley & Sons, Chichester, UK, 1997; (*b*) M. Zahradnik, *The production and applications of fluorescent brightening agent*, Wiley & Sons, Chichester, UK, 1992.

26 (*a*) G. Rajitha, B. Janardhan and B. Rajitha, *J. Heterocycl. Chem.*, 2014, DOI: 10.1002/jhet.2116; (*b*) S. Vijaya Laxmi, B. Suresh Kuarm and B. Rajitha, *Med. Chem. Res.*, 2013, **22**, 768; (*c*) B. Janardhan, K. Manjulatha, B. Srinivas, B. Rajitha, N. Muralikrishna and A. Sadanandam, *RSC Adv.*, 2014, **4**, 22866; (*d*) G. Rajitha, B. Janardhan, P. Mahendar, V. Ravibabu, B. Rajitha and A. Sadanandam, *RSC Adv.*, 2014, **4**, 53812.

27 S. K. Sushanta, M. Arnaresh and M. B. K. Rajani, *Indian J. Heterocycl. Chem.*, 1996, **6**, 91.

28 B. A. Arthington-Skaggs, M. Motley, D. W. Warnock and C. J. Morrison, *J. Clin. Microbiol.*, 2000, **38**, 2254.

29 P. A. Wayne, *Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing*, 18th informational supplement, M100-S18, 2008.

30 Z. G. Mir, A. Farhan, K. K. Anand, A. Q. Insaf and A. G. Irfan, *BMC Complementary Altern. Med.*, 2013, **13**, 53.

31 G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *J. Comput. Chem.*, 1998, **19**, 1639.