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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

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Abstract

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 in both quantitatively and qualitatively *via* 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 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, the compounds **5a**, **5b** and **5i** have exhibited high radical scavenging ability than the positive control drug Trolax. Further, molecular docking of synthesized compounds (**5a-l**) in to binding site of crystal structure of *E.coli* MurB enzyme (PDB Id: 1MBT), a key enzyme in the peptidoglycan biosynthesis, was performed to gain a comprehensive understanding into 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 the 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.

Key words: Antimicrobial activity; Antioxidant activity; Molecular docking study; Pyrazolyl coumarin; Thiazole.

1. Introduction

During the past two decades the world population suffering severely with the infectious diseases due to multi-drug resistance often results from the over-expression of a multidrug

efflux system and their wide spread usage.[1] Among them, microbial infections are the second most leading death causing diseases after heart attack in the world, due to their rapid spread, toxicity and resistance towards the 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 the drug discovery. In this regard, a well known key enzyme MurB, an NADPH dependant enolpyruvyl reductase,[2] which is essential for the growth and bio-synthesis of peptidoglycan polymeric layer of bacterial cell wall,[3] was emerged as an important and attractive target for the development of new antibiotic drugs.[4] MurB enzyme is unique to the prokaryotic cells and has no counter parts in eukaryotes. In addition, molecular docking technique was also emerged as an important tool in drug designing and discovery of novel potential ligands. This computer aided drug designing suite is very much useful in studying the mechanism involved in the non-covalent interactions between the 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 macromolecules of pathogens.[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. 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).

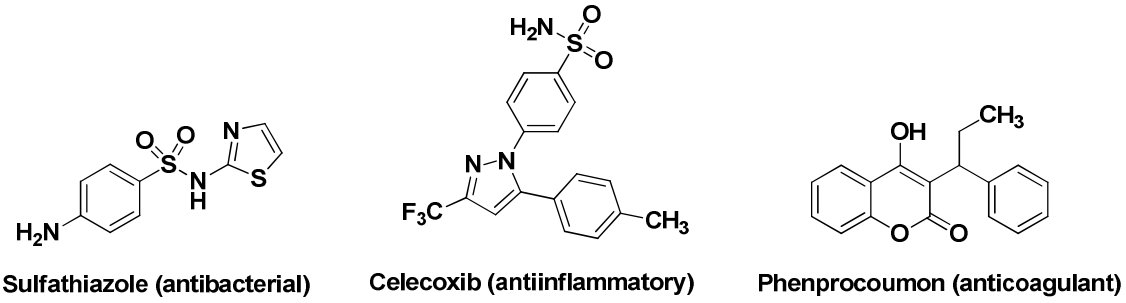


Figure 1. Biologically active thiazole, pyrazole and coumarin derivatives.

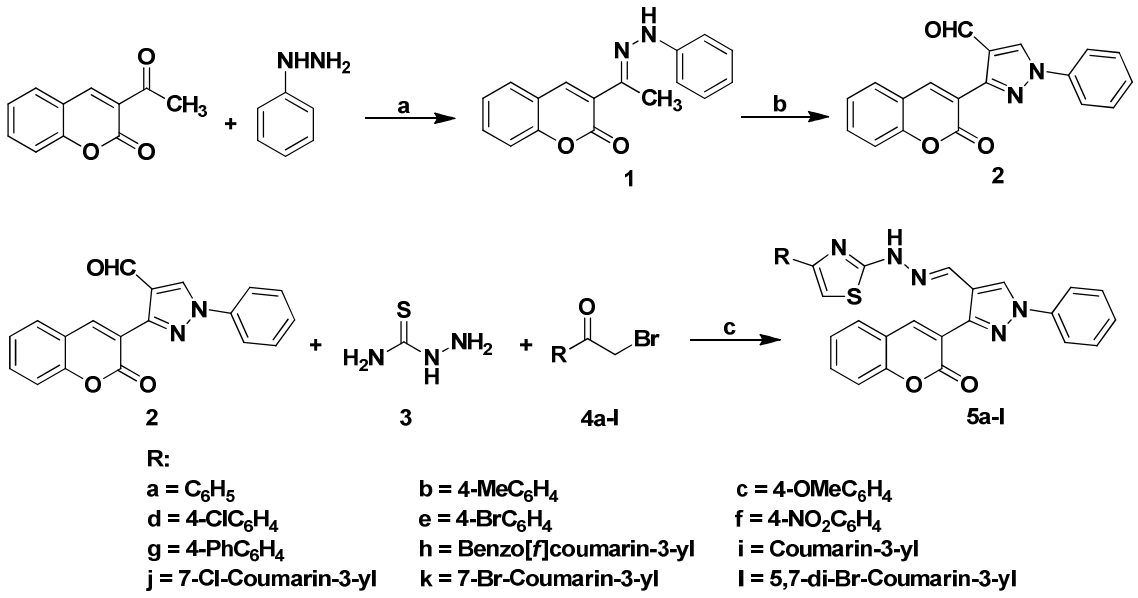
Further extension to our earlier works[26] and as a part of our endeavour towards the synthesis of biologically potent new heterocyclic scaffolds. Here in we report, the synthesis of novel heterocyclic scaffold bearing a coumarin nucleus with a pyrazole and 4-functionalized thiazole rings. This work 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.



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.

Scheme 1. Synthesis of 3-(1-phenyl-4-((2-(4-arylthiazol-2-yl)hydrazono)methyl)-1*H*-pyrazol-3-yl)-2*H*-chromen-2-ones.

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.

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.

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/plate was spread plated over the surface of the nutrient agar by diluting the initial microbial suspension 10 times with distilled water. 30 µL of Antibacterial suspension of 100 µg/mL 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 µg/mL

concentration for the synthesized compounds (**5a-l**) with respect to positive control Streptomycin at 30 µg/mL. 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 µg/mL 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.

Table 2. *In vitro* antimicrobial activity of **5a-l**.

Product	Antibacterial activity								Antifungal activity			
	S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*		*C.albicans*	*A.niger*	*C. glabrata*	*A. parasiticus*
	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

Zone of inhibition (ZOI) values (in mm) for analogs (**5a-l**) at 100 µg/mL and positive control drugs Streptomycin and Clotrimazole at 30 µg/mL. MIC values were given in µg/mL.

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.

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 µg/mL), good activity against *S. aureus* (ZOI = 22 mm and MIC = 50 µg/mL) and moderate activity against *B. subtilis* (ZOI = 18 mm and MIC = 50 µg/mL), and *P. aeruginosa* (ZOI = 17 mm and MIC = 50 µg/mL). Similarly, the compound **5h** has shown good activity against *E. coli* (ZOI = 21 mm and MIC = 25 µg/mL) and *P. aeruginosa* (ZOI = 20 mm and MIC = 25 µg/mL), and moderate inhibiting activity against *S. aureus* (ZOI = 19 mm and MIC = 50 µg/mL). The compound **5a** has also exhibited good activity against *E. coli* (ZOI = 19 mm and MIC = 50 µg/mL) and moderate activity against *S. aureus* (ZOI = 17 mm and MIC = 50 µg/mL) and *P. aeruginosa* (ZOI = 17 mm and MIC = 50 µg/mL) 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 µg/mL 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-pic-ryl-hydrazyl (DPPH) 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 (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.

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

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.

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.

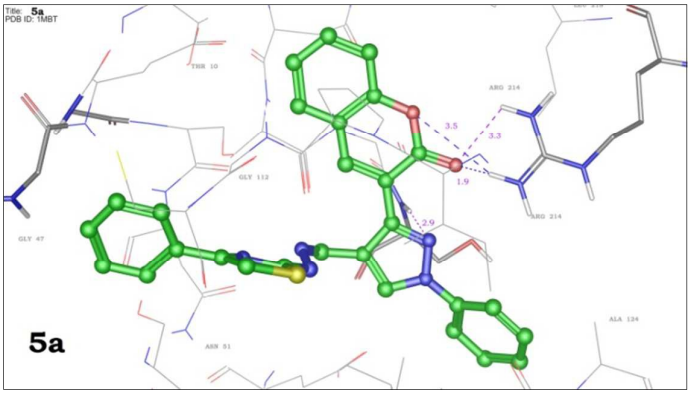
The co-crystallized structure of target enzyme MurB (PDB id: 1MBT) was obtained from Protein Data Bank (RCSB) (http://www.rcsb.org/pdb). To carry out in silico studies, the 2D structures of the synthesized ligands (**5a-l**) were drawn in ChemBioOffice 2010 and converted to energy minimized 3D structures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

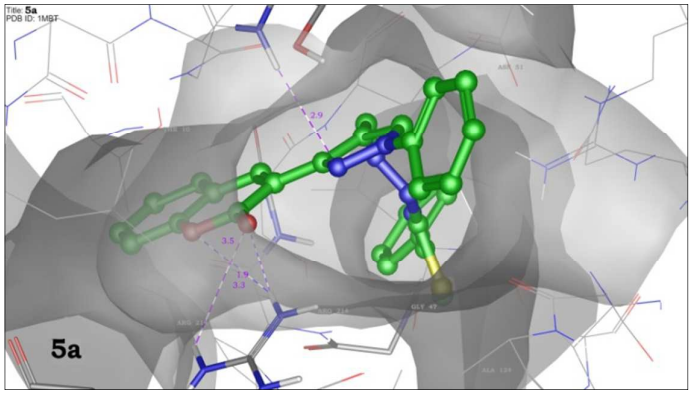
All inhibitors were compared out of 100 docking runs. The docking studies revealed that, all the synthesized molecules exhibited excellent binding energies towards the receptor active pocket ranging from -9.02 to -11.15 kcal/mol and summarized in Table 4. Among them, the conformations with lowest binding 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.

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)	Inhibition Constant Ki (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)





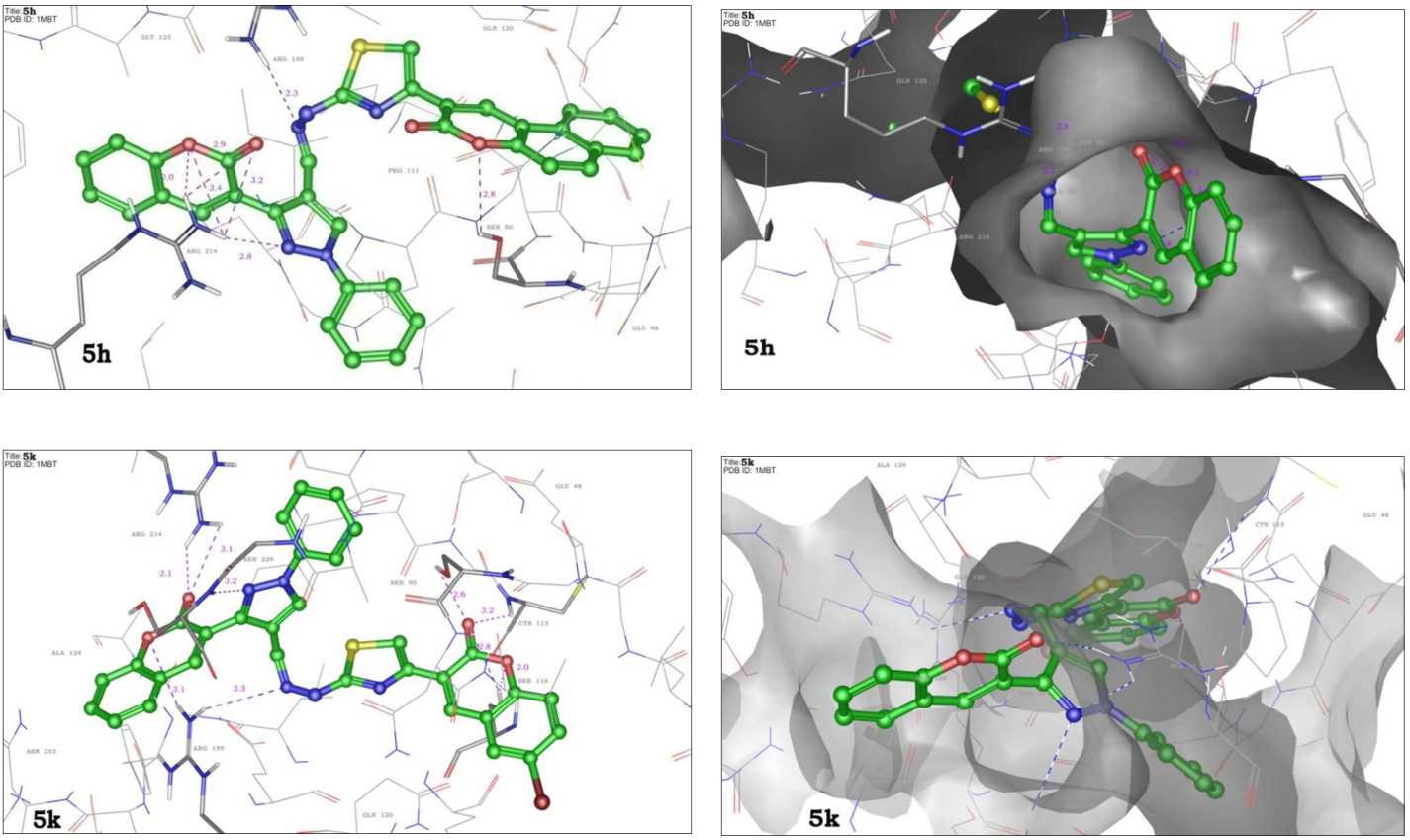


Fig. 2. Docking pose of **5a**, **5h** and **5k** (ball and stick) with UDP-*N*-acetylenolpyruvoylglucosamine reductase (MurB) (thin wire) with intermolecular H-bonding (pink and blue dotted lines) and 3D surface interaction (green) with the enzyme (represented in molecular cloud).

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)-1H-pyrazol-3-yl)-2H-chromen-2-one (5a)

Yellow solid; IR (KBr, cm^{-1}) υ_{max}: 3442 (NH), 1722 (C=O), 1630 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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)-1H-pyrazol-3-yl)-2H-chromen-2-one (5b)

Yellow solid; IR (KBr, cm^{-1}) υ_{max}: 3447 (NH), 1721 (C=O), 1629 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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-1H-pyrazol-3-yl)-2H-chromen-2-one (5c)

Pale yellow solid; IR (KBr, cm^{-1}) υ_{max}: 3448 (NH), 1724 (C=O), 1627 (C=N), 1217 (C-O-C); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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-1H-pyrazol-3-yl)-2H-chromen-2-one (5d)

Yellow solid; IR (KBr, cm^{-1}) υ_{max}: 3414 (NH), 1720 (C=O), 1628 (C=N), 750 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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): δ = 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-1H-pyrazol-3-yl)-2H-chromen-2-one (5e)

Yellow solid; IR (KBr, cm^{-1}) υ_{max}: 3436 (NH), 1720 (C=O), 1630 (C=N), 684 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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): δ = 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-1H-pyrazol-3-yl)-2H-chromen-2-one (5f)

Brown solid; IR (KBr, cm^{-1}) υ_{max}: 3436 (NH), 1704 (C=O), 1632 (C=N), 1504, 1344 (NO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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): δ = 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-1H-pyrazol-3-yl)-2H-chromen-2-one (5g)

Pale brown solid; IR (KBr, cm^{-1}) υ_{max}: 3444 (NH), 1737 (C=O), 1627 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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): δ = 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-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)hydrazinyl) thiazol-4-yl)-3H-benzo[f]chromen-3-one (5h)

Yellow solid; IR (KBr, cm^{-1}) υ_{max}: 3438 (NH), 1717 (C=O), 1638 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)hydrazinyl) thiazol-4-yl)-2H-chromen-2-one (5i)

Pale yellow solid; IR (KBr, cm^{-1}) υ_{max}: 3439 (NH), 1721 (C=O), 1633 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 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): δ = 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-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (5j)

Pale brown solid; IR (KBr, cm^{-1}) υ_{max}: 3434 (NH), 1723 (C=O), 1607 (C=N), 751 (C-Cl); ^1H NMR (400 MHz, DMSO-d_6): δ = 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-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (5k)

Brown solid; IR (KBr, cm^{-1}) υ_{max}: 3414 (NH), 1723 (C=O), 1600 (C=N), 685 (C-Br); ^1H NMR (400 MHz, DMSO-d_6): δ = 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-2H-chromen-3-yl)-1-phenyl-1H-pyrazol-4-yl)methylene) hydrazinyl)thiazol-4-yl)-2H-chromen-2-one (5l)

Yellow solid; IR (KBr, cm^{-1}) υ_{max}: 3437 (NH), 1702 (C=O), 1597 (C=N), 684 (C-Br); ^1H NMR (400 MHz, DMSO-d_6): δ = 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): δ = 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.

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Supplementary data

Supplementary data related to this article can be found in the online version at.....

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