ORIGINAL RESEARCH ARTICLES

DESIGN, SYNTHESIS AND EVALUATION OF *N*-(BENZO[D]THIAZOL-2-YL)-2-OXO-2*H*-CHROMENE-3-CARBOXAMIDE DERIVATIVES AS POTENTIAL ANTIOXIDANT AND ANTIBACTERIAL AGENTS

Ranjit V. Gadhave[a]*, Sachin S. Khade[a], Yogita S. Ozarde[a], Somdatta Y. Chaudhari[b], Arti G. Swami[a] and Mukesh K. Meena[c]

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ABSTRACT

This research is focused on designing, synthesis and biological evaluation of a series of coumarin based benzothiazole derivatives. The ligands were identified by docking study for antioxidant and antibacterial potential using target proteins PDB:4H1J and PDB:3G75, respectively. The target molecules were synthesized as a series of substituted *N*-(benzothiazol-2-yl)-2-oxo-chromene-3-carboxamides (7a–h) by condensation of substituted benzo[d]thiazol-2-amines with *in situ* synthesized substituted 2-oxo-2*H*-chromene-3-carbonyl chlorides. Infrared spectroscopy and ^{1}H- nuclear magnetic resonance spectra were used to characterize the synthesized molecules. *In vitro* antioxidant activity of compounds was evaluated by DPPH and H_2O_2 radical scavenging assays. Antibacterial potential of compounds was evaluated using well diffusion method against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Among synthesized derivatives, 7a showed good antioxidant potential whereas 7f showed antibacterial activity, which might be employed as lead molecules for future investigation for respective activities.

Keywords: Antibacterial, antioxidant, benzothiazole, coumarin, docking, synthesis

INTRODUCTION

Heterocyclic compounds play a significant role in drug design & discovery. Their derivatization leads to formation of various category drugs, therefore they received considerable attention of many researchers. The incorporation of heterocyclic nucleus can make the molecule more versatile for biological activity and can be converted to drug by lead modification. In the family of heterocyclic compounds, coumarin and benzothiazole played significant role because their derivatives are pharmacologically active and are found in many different categories of drugs. Oxygen-rich heterocyclic coumarin (2*H*-chromen-2-one) derivatives possess many biological activities like antimalarial[1], anticancer[1], monoamino oxidase inhibitor[2], anti-inflammatory[3], antioxidant[3,4], antibacterial[4], antiviral[5], analgesic[6] and antimicrobial[7]. Sulfur-rich heterocyclic benzothiazole (1,3-thiazole) derivatives have been reported to exhibit biological activities like antifungal[8], antimicrobial[9], antibacterial[10], antiviral[11], anticancer[11], antimalarial[12], anti-inflammatory[13] and antioxidant[14]. Condensed heterocyclic derivatives showed antioxidant[15], antimicrobial[16] and anti-inflammatory[17] activities.

Cellular damage is caused by reactive oxygen species produced as a result of severe oxidative stress or inadequate antioxidant scavenging activity. Antioxidants slow down the progress of oxidative damage associated with chronic diseases such as cancer, alzheimer, parkinson, inflammation, depression and cataracts, therefore it is essential to design novel antioxidants. The antioxidant potential of heterocyclic compounds can be determined *In vitro* using DPPH and H_2O_2 scavenging methods. Similarly, increased bacterial

[a] School of Pharmacy, Dr. Vishwanath Karad MIT World Peace University, Pune-411 038, Maharashtra, India
[b] Department of Pharmaceutical Chemistry, PES's Modern College of Pharmacy, Nigdi, Pune-411 044, Maharashtra, India
[c] Department of Pharmaceutical Sciences, Mohanlal Sukhadia University, Udaipur-313 001, Rajasthan, India
*For Correspondence: E-mail: ranjitgadhave@gmail.com

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resistance is caused by mutation due to the frequent use of antibacterial drugs and less patient compliance. The emergence of more resistant and pathogenic bacterial strains necessitates the designing of novel antibacterial drugs effective against mutant and pathogenic strains of microorganisms. The therapeutic effect of antibacterial medicines can be evaluated by inhibiting bacterial growth under standardized parameters.

The current research builds on previous attempts to design and synthesize benzothiazole-based coumarin compounds as possible antioxidant and antibacterial leads.

MATERIALS AND METHODS

S. D. Fine / E. Merck / Loba / Qualigens laboratory grade solvents and chemicals were used for synthesis of designed molecules. Solvents were purified before use by the established purification methods. TLC and melting point were used to monitor reactions progress. TLC plates (Type 60 GF254, Merck) were activated at 110 °C temperature for 30 min., developed in twin trough chamber and visualization was done by iodine vapours and UV-Lamp. Though different systems were employed, R*f* values are reported for better comparable solvent systems. MP was determined by VEEGO (Model: VMP-D) or with open capillary using Thiele's tube and temperatures are expressed in °C and are uncorrected. All the intermediates and final products have been dried in vacuum desiccators and recrystallized. The percentage yields are based upon the products obtained after purification by recrystallization. All computational studies carried out with ligands built in Chem Bio Draw version 13.0.0.3015 (PerkinElmer Software) and Discovery studio visualizer v.16.1.0.15350 program used for geometry optimization. IR study was performed by Jasco FT/IR-4600, MS by Impact HD QTOF Mass Spectrometer (Bruker-GmbH) and ^{1}H-NMR by Bruker Advance III HD 500 MHz.

Molecular docking study[18,19]

The Autodock tool was used to conduct a molecular docking study of designed molecules in order to find ligand affinity with target enzyme. The antioxidant and antibacterial docking studies were performed using enzymes PDB Code: 4H1J and 3G75 (retrieved from protein data bank) with ascorbic acid (AA) and ciprofloxacin (CPF) as reference standards, respectively. Target enzymes were identified and used for docking. X-ray crystallographic structures of target proteins are validated as per Ramchandran plot. PDB files were corrected in terms of missing atoms using PDB Fixer. The proteins converted to PDBQT file format using in-house script by adding Gasteiger partial charges. All the proposed compounds were drawn and optimized in ChemBioDraw Ultrav. 20 software (Cambridge Software) using MMFF94 force field. Structures were converted to PDBQT by MGL tools 1.5.6. The docking simulations were carried out using an in-house batch script for automatic running of AutoDock Vina. In all experiments, genetic algorithm search method was applied to determine the best pose of each ligand in the active site of the target enzymes. Docking was done by setting up parameters like no. of GA runs were 50, center coordinates X-; Y-; & Z-; size was x-; y-; z-, no. of evaluations were 2500000 and numbers of generations were 27000. Conformations generated for the ligand for the receptor active site to obtain desired result. All proposed compounds were docked and few benzothiazole based coumarin derivatives were selected for synthesis considering the intermolecular binding interactions and binding energies of ligands with target enzyme cavity. The model skeleton of the lead molecules selected for structural modification is as depicted in Fig.1.

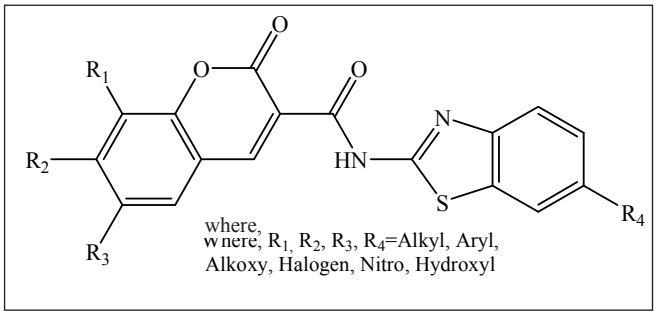

Fig. 1: Model skeleton of lead molecule

Synthesis of designed target molecules

Synthesis scheme was designed for target molecules (Fig. 2), where substituted methyl 2-oxo-chromene-3-carboxylates (**3a-e**) were synthesized using substituted salisaldehyde (**1a-e**) and dimethyl malonate (**2**). Compounds **3a-e** on alkaline hydrolysis gives corresponding carboxylic acids (**4a-e**), which when treated with $SOCl_2$ gives reactive 2-oxo-chromene-3-carbonyl chloride derivatives (**5a-e**). Compounds **5a-e** were treated with substituted 2-aminobenzothiazoles (**6a-b**) to obtained desired title compounds *N*-benzothiazolyl-2-oxo-chromene-3-carboxamide (**7a-h**).

Individual reactions were optimized for solvents, stoichiometric ratios of reactants, purification methods, temperature and heating time required. The procedures were optimized to get high yield with pure product with mild reaction conditions with less complicated reaction

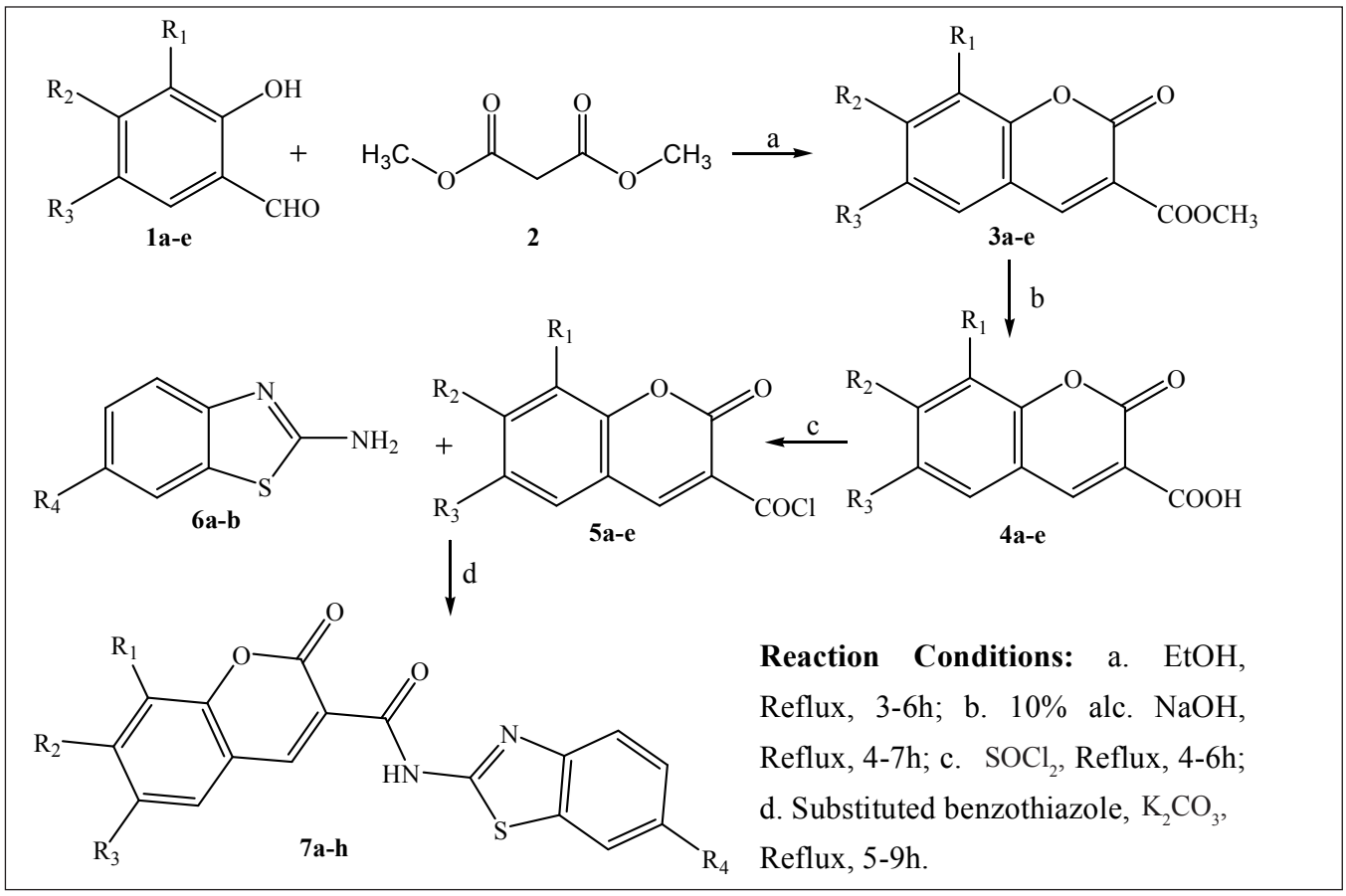

Fig. 2: Synthesis scheme for *N*-(benzo[d]thiazol-2-yl)-2-oxo-2*H*-chromene -3-carboxamides

steps and using economical and less hazardous chemicals.

The synthesis of selected molecules from designed library of compounds was carried out by developing suitable optimized synthesis schemes. Preliminary characterization of synthesized compounds was carried out by determination of physical constant; purified by recrystallization or column chromatography and purity was confirmed by TLC.

METHOD OF SYNTHESIS

Step-I: Synthesis of substituted methyl 2-oxo-chromene-3-carboxylate (3a-e) (Table I)

Placed mixture of substituted Salisaldehyde **1a-e** (0.033 mol) and dimethyl malonate **2** (0.1 mol) in alcohol, refluxed for 3-6 h using piperidine as catalyst. Cooled reaction mixture and extracted (ethyl acetate). Excess solvent was distilled off and residues were obtained as title compounds.

Step-II: Synthesis of substituted 2-oxo-chromene-3-carboxylic acid derivatives (4a-e) (Table II)

Placed compound **3a-e** (0.03 mol) in 10 % w/V alcoholic sodium hydroxide solution (0.034 mol) and refluxed for 4-7 h. Reaction mixture was cooled and acidified to get precipitate of title compounds.

Step-III: Synthesis of 2-oxo-chromene-3-carbonyl chloride derivatives (5a-e) (Table III)

Compound **4a-e** (0.01 mol) was placed in thionyl chloride (0.06 mol) and refluxed for 4-6 h. Excess thionyl chloride was removed by vacuum distillation to get title compounds.

Step-IV: Synthesis of substituted *N*-benzothiazolyl-2-oxo-chromene-3-carboxamide derivatives (7a-h) (Table IV)

Mixture of compound **5a-e** (0.024 mol), substituted 2-aminobenzothiazole **6a-b** (0.03 mol) and potassium carbonate (0.036 mol) was placed in dioxane. Reaction

Table I: Physical characterization of compounds (3a-e)

Code	R_1	R_2	R_3	Molecular formula	Mol. Wt.	% Yield	m.p. (°C)	R_f Value*
3a	H	H	H	$C_{11}H_8O_4$	204.18	64	124-126	0.50
3b	CH_3	H	H	$C_{12}H_{10}O_4$	218.21	69	143-146	0.42
3c	OCH_3	H	Cl	$C_{12}H_9ClO_5$	268.65	51	110-112	0.72
3d	CH_3	CH_3	H	$C_{13}H_{12}O_4$	232.23	68	158-162	0.55
3e	H	OC_2H_5	H	$C_{13}H_{12}O_5$	248.23	54	210-212	0.65

**Mobile phase: Ethyl acetate*

Table II: Physical characterization of compounds (4a-e)

Code	R_1	R_2	R_3	Molecular formula	Mol. Wt.	% Yield	m.p. (°C)	R_f Value*
4a	H	H	H	$C_{10}H_6O_4$	190.15	80	228-230	0.64
4b	CH_3	H	H	$C_{11}H_8O_4$	204.18	72	>300	0.58
4c	OCH_3	H	Cl	$C_{11}H_7ClO_5$	254.62	66	212-214	0.76
4d	CH_3	CH_3	H	$C_{12}H_{10}O_4$	218.21	45	164-166	0.54
4e	H	OC_2H_5	H	$C_{12}H_{10}O_5$	234.20	54	188-192	0.48

**Mobile phase: Ethyl acetate*

Table III: Physical characterization of compounds (5a-e)

Code	R_1	R_2	R_3	Molecular Formula	Mol. Wt.	% Yield	m.p. (°C)	R_f Value*
5a	H	H	H	$C_{10}H_5ClO_3$	208.60	48	86-88	0.60
5b	CH_3	H	H	$C_{11}H_7ClO_3$	222.62	61	132-134	0.46
5c	OCH_3	H	Cl	$C_{11}H_6Cl_2O_4$	273.07	42	126-128	0.64
5d	CH_3	CH_3	H	$C_{12}H_9ClO_3$	236.65	60	118-120	0.54
5e	H	OC_2H_5	H	$C_{12}H_9ClO_4$	252.65	53	174-176	0.70

**Mobile phase: Ethyl acetate*

Table IV: Physical characterization of compounds (7a-h)

Code	R_1	R_2	R_3	R_4	Molecular Formula	Mol. Wt.	% Yield	m.p. (°C)	R_f Value*
7a	H	H	H	OCH_3	$C_{18}H_{12}N_2O_4S$	352.36	64	154-156	0.62
7b	CH_3	H	H	OCH_3	$C_{19}H_{14}N_2O_4S$	366.39	55	148-150	0.55
7c	OCH_3	H	Cl	OCH_3	$C_{19}H_{13}ClN_2O_5S$	416.83	68	166-168	0.68
7d	CH_3	CH_3	H	OCH_3	$C_{20}H_{16}N_2O_4S$	380.42	55	200-204	0.42
7e	H	OC_2H_5	H	OCH_3	$C_{20}H_{16}N_2O_5S$	396.42	58	188-190	0.50

7f	CH_3	H	H	NO_2	$C_{18}H_{11}N_3O_5S$	381.36	46	154-156	0.42
7g	OCH_3	H	Cl	NO_2	$C_{18}H_{10}ClN_3O_6S$	431.81	55	142-144	0.58
7h	CH_3	CH_3	H	NO_2	$C_{19}H_{13}N_3O_5S$	395.39	52	164-166	0.44

**Mobile phase: Ethyl acetate: Toluene (4:1)*

Table V: Binding energy of ligand-target complex (Code: 4H1J and 3G75)

Code	PDB	7a	7b	7c	7d	7e	7f	7g	7h	Standard drug
Binding Energy (Kcal $mole^{-1}$)	Code: 4H1J	-6.286	-3.658	-5.495	-4.381	-5.224	-5.296	-5.13	-5.782	-7.288 AA
	Code: 3G75	-3.413	-3.452	-2.841	-3.054	-3. 616	-4.225	-2.143	-3.229	-4.969 CPF

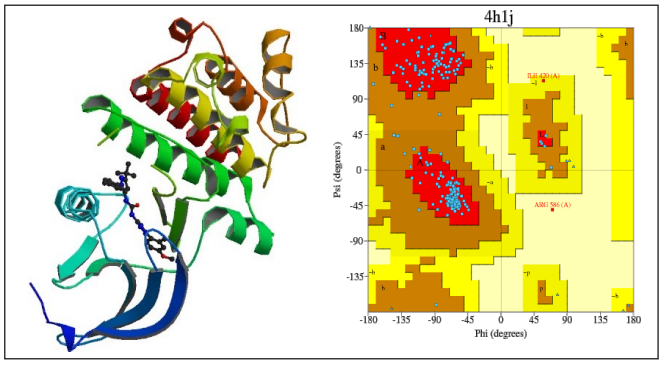

Fig. 3: X-ray crystallographic structure of protein (PDB: 4H1J) and its Ramachandran Plot

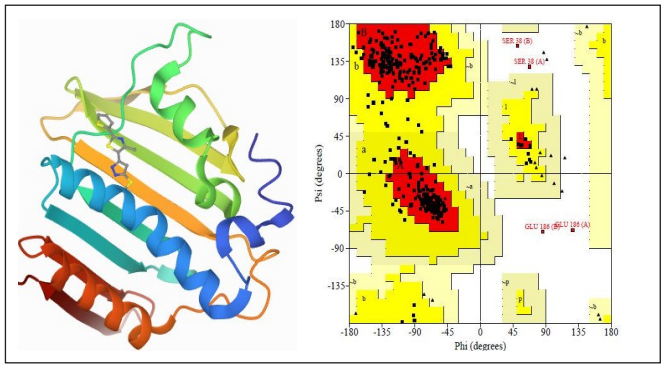

Fig. 4: X-ray crystallographic structure of protein (PDB: 3G75)and its Ramachandran Plot

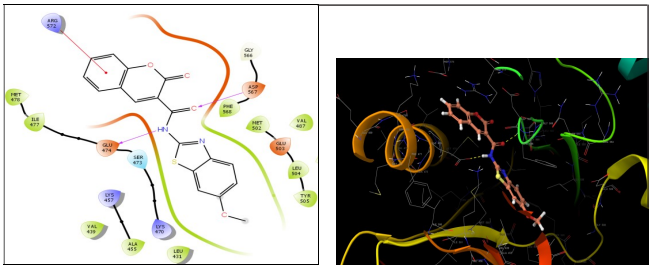

Fig. 5: Molecular docking 2D and 3D pose of 7a interacting at the binding cleft of PDB: 4H1J

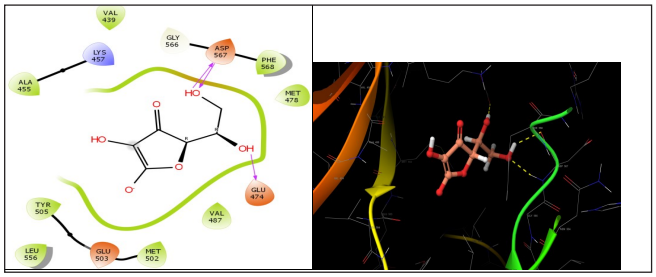

Fig. 6: Molecular docking 2D and 3D pose of AA interacting at the binding cleft of PDB: 4H1J

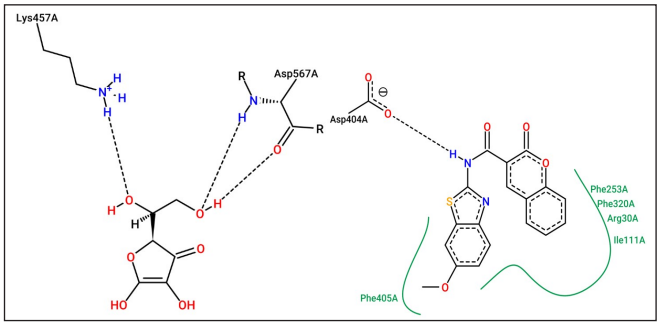

Fig. 7: Poseview image showing ligand-target interaction (AA and 7a with PDB: 4H1J)

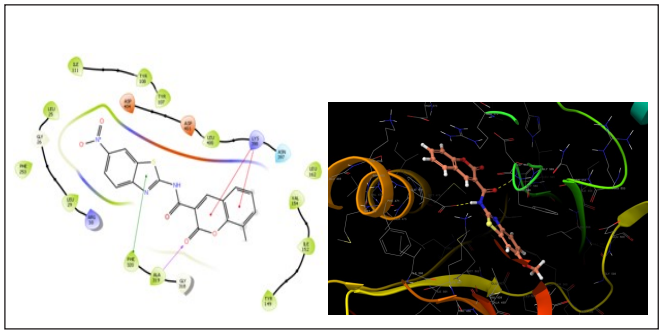

Fig. 8: Molecular docking 2D and 3D pose of compound 7f interacting at the binding cleft at PDB: 3G75

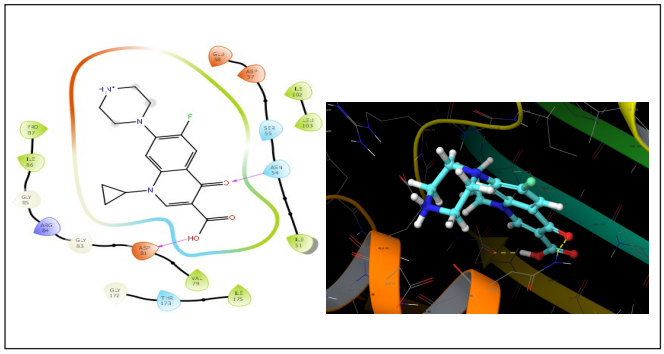

Fig. 9: Molecular docking 2D and 3D pose of CPF interacting at the binding cleft at PDB: 3G75

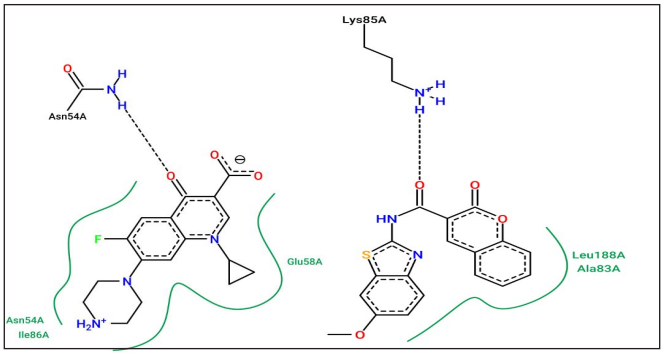

Fig. 10: Poseview image showing ligand-target interaction (CPF and 7a with PDB: 3G75)

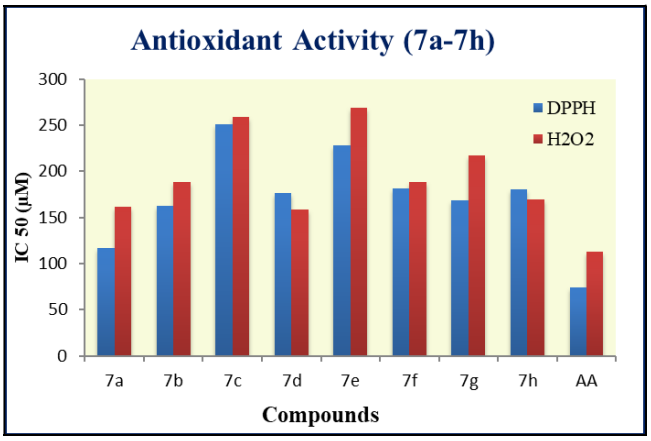

Fig. 11: *In vitro* antioxidant activity of compounds 7a-h and AA

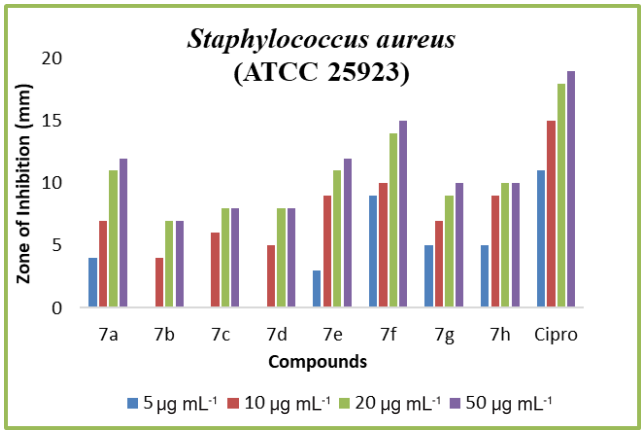

Fig. 12: Antibacterial activity: Zone of inhibition against *Staphylococcus aureus* (ATCC 25923)

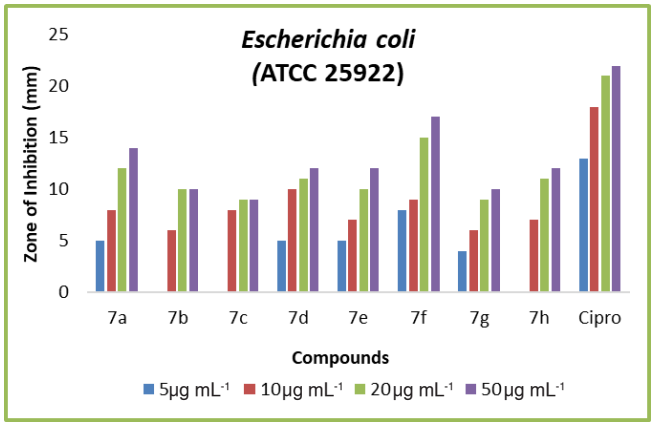

Fig. 13: Antibacterial activity: Zone of Inhibition against *Escherichia coli* (ATCC 25922)

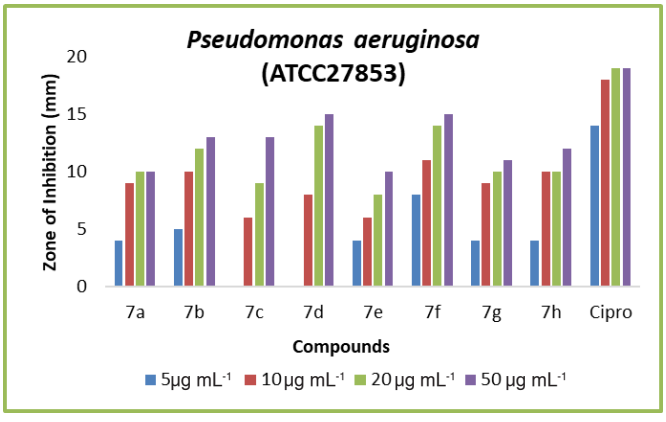

Fig. 14: Antibacterial activity: Zone of inhibition against *Pseudomonas aeruginosa* (ATCC 27853)

was refluxed for 5-9 h and cooled to RT, which was then poured to ice and slight yellow to green precipitate was filtered and dried to obtain title compounds.

Biological evaluation

Antioxidant activity[20,21,22]

Synthesized heterocyclic compounds were screened by following *in vitro* methods for their antioxidant potential.

Diphenyl picryl hydrazyl (DPPH) method

Material

1. DPPH solution: The working solution of (400 µM) DPPH in methanol.
2. Standard solution: Standard solution of AA was prepared in methanol (Standard free radical scavenger).
3. Test solutions: Test samples of 50-500 µM concentrations were prepared in methanol.
4. UV-VIS Spectrophotometer: Varian Carry100.
5. Method: Change in the UV absorbance was measured as measure of scavenging activity.

Test procedure [23,24]

The antioxidant activity was performed using method described by Venkatachalam H. et al. and Stefania F. B. et al. with few modifications. DPPH solution (2 mL, 400 µM) was mixed with test compound solutions 50, 100, 150, 200, 250 and 500 µM in methanol. The samples were then incubated for 20 minute and measured absorbance (517 nm) in UV spectrophotometer. Reference standard was AA.

$$\text{DPPH Scavenging effect (\%)} = \frac{(\text{Abs of Blank} - \text{Abs of Test}) * 100}{\text{Abs of Blank}}$$

The antioxidant activity was expressed as IC_{50} value. IC_{50} calculated from a plot of concentration v/s % scavenging using Microsoft office excel software.

Hydrogen peroxide (H_2O_2) scavenging method

Material

1. H_2O_2 Solution: 40 mM H_2O_2 in phosphate buffer (pH 7.4).
2. Standard solution: Standard solution of AA in phosphate buffer (Standard free radical scavenger).
3. Test solutions: Test solution of 50-500 µM concentrations were prepared in phosphate buffer.
4. Method: Change in the UV absorbance was measured as measure of scavenging activity.

Test procedure[25]

Method reported by Munir O. et al. was used with minor modifications. 3.4 mL (50, 100, 150, 200, 250 and 500 µM) of test compounds mixed with H_2O_2 solution (0.6 mL). Samples were incubated for 10 minute and absorbance taken in UV spectrophotometer at 230 nm. AA was standard used for comparison.

$$H_2O_2 \text{ scavenging effect (\%)} = \frac{(\text{Abs of Blank} - \text{Abs of Test}) * 100}{\text{Abs of Blank}}$$

The antioxidant activity was expressed as IC_{50} value, calculated using Microsoft office excel software.

Antibacterial activity[21, 22]

Synthesized heterocyclic compounds were screened by well diffusion method for their antibacterial potential.

Well diffusion method

Antibacterial screening conducted against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC27853). 5, 10, 25 and 50 µg mL^{-1} test samples were prepared in dimethyl sulphoxide (DMSO). The wells in the petriplates containing culture media were inoculated with bacterial culture and then they were filled with test samples of different strength. Zone of inhibition in each plate was measured after incubation at 37 °C for 24 h. DMSO (control) and CPF (reference standard) were used for analysing results.

RESULTS AND DISCUSSION

Molecular docking

Validation of target protein PDB:4H1J and PDB:3G75

- Ramachandran plot (PDB ID: 4H1J) showed resolution 2.00 Å and 99.6 % amino acid residues fall in allowed region. Therefore, protein structure is considered as validated for docking study (Fig. 3).
- Ramachandran plot (PDB: 3G75) showed resolution 1.96 Å and 98.8 % amino acid residues fall in allowed region. Therefore, protein structure is considered as validated for docking study (Fig. 4).

Target enzymes (PDB: 4H1J and 3G75) were used for molecular docking studies. Amino acid residues Glu503A, Arg572A, Phe253A, Phe320A, Arg30A, Ile111A, Phe405A, Asp404A, Asp567A, Lys457A and Glu474A of target PDB: 4H1J and Phe320A, Lys398A, Ala319A,

Asp81A, Asn54A, Glu58A, Ile86A, Lys85A, Leu188A and Ala83A residues of target PDB: 3G75 were bound with respective ligands (Figs. 5 -10).

Docking results showed that designed benzothiazole based coumarin derivatives gives good docking scores for antioxidant and antibacterial activity using target proteins PDB: 4H1J and PDB: 3G75, respectively (Table V). Molecules from logically designed library were selected for synthesis based on the docking score (antioxidant activity-7a and antibacterial activity-7f).

Antioxidant target (PDB: 4H1J) residues Asp567A, Glu474A, Asp404A showed hydrogen bond interactions with C=O and N-H group respectively from amide bridge between coumarin and benzothiazole. Amino acid residue Arg572A showed Pi-cation interactions with coumarin aromatic ring. Phe253A, Phe320A, Arg30A, Arg30A, Ile111A, Phe405A residues interacts with coumarin and benzothiazole rings by hydrophobic interactions.

Antibacterial target (PDB: 3G75) residue Ala319A showed hydrogen bond interaction with C=O of coumarin ring. Amino acid residue Lys398A showed Pi-cation interactions with coumarin aromatic rings. Phe320A residue showed Pi-Pi stacking interaction with thiazole ring in benzothiazole. Leu188A and Ala83A residues interact with coumarin and benzothiazole by hydrophobic interactions.

Characterization of synthesized compounds

The synthesized compounds were characterized by melting point, TLC, Infra-red spectroscopy, Mass spectroscopy and ^{1}H-NMR spectroscopy.

Physical, analytical and spectral data of synthesized compounds (7a-h)[26,27]

***N*-(6-methoxybenzo[d]thiazol-2-yl)-2-oxo-2*H*-chromene-3-carboxamide (7a):**Yield: 64 %, m.p.: 154-156 °C and Rf: 0.62 (ethyl acetate : toluene, 4:1 V/V); IR: 3342 (N-H, amide), 2925 (C-H, Ar), 1642 (C=O, amide), 1650 (C=N, Ar), 1212 (C-O-C, ether);MS: $[M]^+$352, $[M+1]^+$353; fragments:179, 173, 145, 164, 133;^{1}H NMR: 7.37-7.51 (m, 4H, coumarin), 7.44-8.16 (m, 3H, benzothiazole), 8.37 (s, 1H, amide), 8.79 (s, 1H, coumarin), 2.88 (s, 3H, Methoxy); elemental analysis for $C_{18}H_{12}N_2O_4S$: C, 61.16; H, 3.48; N, 7.56; S, 9.22.

***N*-(6-methoxybenzo[d]thiazol-2-yl)-8-methyl-2-oxo-2*H*-chromene-3-carboxamide(7b):** Yield: 55 %, m.p.: 148-150 °C and Rf: 0.55 (ethyl acetate : toluene, 4:1 V/V); IR: 3356 (N-H, amide), 2942 (C-H, Ar), 1651 (C=O, amide), 1644 (C=N, Ar), 1238 (C-O-C, ether); MS: $[M]^+$366, $[M+1]^+$367; fragments: 179, 164, 133, 187, 159; ^{1}H NMR: 6.87-7.24 (m, 3H, coumarin), 7.36-7.96 (m, 3H, benzothiazole), 9.04 (s, 1H, amide), 8.20 (s, 1H, coumarin), 2.12 (s, 3H, methyl), 3.06 (s, 3H, methoxy); elemental analysis for $C_{19}H_{14}N_2O_4S$: C, 62.25; H, 3.68; N, 7.33; S, 8.21.

6-chloro-8-methoxy-*N*-(6-methoxybenzo[d]thiazol-2-yl)-2-oxo-2*H*-chromene-3-carboxamide (7c):Yield: 68 %, m.p.: 166-168 °C and Rf: 0.68 (ethyl acetate : toluene, 4:1 V/V); IR: 3362 (N-H, amide), 2913 (C-H, Ar), 1633 (C=O, amide), 1652 (C=N, Ar), 1269 (C-O-C, ether); MS: $[M]^+$416, $[M+1]^+$417; fragments: 179, 164, 133, 237, 209, 177; ^{1}H NMR: 6.68-6.89 (m, 2H, coumarin), 7.05-7.56 (m, 3H, benzothiazole), 8.60 (s, 1H, amide), 8.37 (s, 1H, coumarin), 3.31 (s, 6H, methoxy);elemental analysis for $C_{19}H_{13}ClN_2O_5S$: C, 54.49; H, 3.12; N, 7.10; S, 7.33.

***N*-(6-methoxybenzo[d]thiazol-2-yl)-7,8-dimethyl-2-oxo-2*H*-chromene-3-carboxamide (7d):**Yield: 55 %, m.p.: 200-204 °C and Rf: 0.42 (ethyl acetate : toluene, 4:1 V/V); IR: 3327 (N-H, amide), 2967 (C-H, Ar), 1638 (C=O, amide), 1640 (C=N, Ar), 1217 (C-O-C, ether); MS: $[M]^+$380, $[M+1]^+$381; fragments: 179, 164, 133, 201, 173; ^{1}H NMR: 6.68-6.96 (m, 2H, coumarin), 7.18-8.03 (m, 3H, benzothiazole), 8.81 (s, 1H, amide), 8.01 (s, 1H, coumarin), 3.11 (s, 3H, methoxy), 2.31 (s, 6H, methyl); elemental analysis for $C_{20}H_{16}N_2O_4S$: C, 63.58; H, 4.21; N, 7.62; S, 8.46.

7-ethoxy-*N*-(6-methoxybenzo[d]thiazol-2-yl)-2-oxo-2*H*-chromene-3-carboxamide (7e):Yield: 58 %, m.p.: 188-190 °C and Rf: 0.50(ethyl acetate : toluene, 4:1 V/V); IR: 3386 (N-H, amide), 2953 (C-H, Ar), 1649 (C=O, amide), 1638 (C=N, Ar), 1243 (C-O-C, ether); MS: $[M]^+$396, $[M+1]^+$397; fragments: 179, 164, 133,217,189, 172; ^{1}H NMR: 6.48-6.85 (m, 3H, coumarin), 7.04-8.36 (m, 3H, benzothiazole), 8.11 (s, 1H, amide), 8.27 (s, 1H, coumarin), 3.25 (s, 3H, methoxy), 1.76-3.84 (s, 5H, ethoxy); elemental analysis for $C_{20}H_{16}N_2O_5S$: C, 60.10; H, 3.98; N, 7.12; S, 8.17.

8-methyl-*N*-(6-nitrobenzo[d]thiazol-2-yl)-2-oxo-2*H*-chromene-3-carboxamide (7f):Yield: 46 %, m.p.: 154-156 °C and Rf: 0.42(ethyl acetate : toluene, 4:1 V/V); IR: 3330 (N-H, amide), 2968 (C-H, Ar), 1647 (C=O, amide), 1676 (C=N, Ar), 1185 (C-O-C, ether); MS: $[M]^+$381, $[M+1]^+$382; fragments: 194, 179, 133, 187, 159; ^{1}H NMR: 6.70-7.16 (m, 3H, coumarin), 8.21-9.64 (m, 3H, benzothiazole), 9.12 (s, 1H, amide), 8.41 (s, 1H, coumarin), 2.33 (s, 3H, methyl); elemental analysis for $C_{18}H_{11}N_3O_5S$: C, 56.71; H, 3.01; N, 11.11; S, 8.35.

6-chloro-8-methoxy-*N*-(6-nitrobenzo[d]thiazol-2-yl)-2-oxo-2*H*-chromene-3-carboxamide(7g): Yield 55 %, m.p.:142-144 °C and Rf: 0.58 (ethyl acetate : toluene, 4:1 V/V); IR: 3308 (N-H, amide), 2975 (C-H, Ar), 1645 (C=O, amide), 1650 (C=N, Ar), 1220 (C-O-C, ether); MS: $[M]^+$432, $[M+1]^+$433; fragments: 194, 179, 133, 238, 210; ^{1}H NMR: 6.55-6.83 (m, 2H, coumarin), 8.16-9.42 (m, 3H, benzothiazole), 9.06 (s, 1H, amide), 8.01 (s, 1H, coumarin), 3.63 (s, 6H, methoxy); elemental analysis for $C_{18}H_{10}ClN_3O_6S$: C, 50.05; H, 2.21; N, 9.66; S, 7.47.

7,8-dimethyl-*N*-(6-nitrobenzo[d]thiazol-2-yl)-2-oxo-2*H*-chromene-3-carboxamide (7h): Yield 52 %, m.p.: 168-170 °C and Rf: 0.54(ethyl acetate : toluene, 4:1 V/V); IR: 3388 (N-H, amide), 2984 (C-H, Ar), 1660 (C=O, amide), 1635 (C=N, Ar), 1246 (C-O-C, ether); MS: $[M]^+$395, $[M+1]^+$396; fragments: 194, 179, 133, 201, 173; ^{1}H NMR: 6.70-6.92 (m, 2H, coumarin), 8.41-9.25 (m, 3H, benzothiazole), 8.43 (s, 1H, amide), 8.32 (s, 1H, coumarin), 2.24 (s, 6H, methyl); elemental analysis for $C_{19}H_{13}N_3O_5S$: C, 57.65; H, 3.24; N, 10.76; S, 8.07.

IR spectra peaks at 1633-1651 cm^{-1} (C=O, stretching, 2° amide) and 3308-3388 cm^{-1} (N-H, stretching, 2° amide) confirmed the presence of -CONH- bridge connecting benzothiazole and coumarin. MS peak $[M]^+$ and $[M+1]^+$ were characteristic for synthesized compounds fragments. ^{1}H NMR spectra showed peaks at 6.48-8.79 ppm for aromatic protons of coumarin, 7.36-9.64 ppm for aromatic protons of benzothiazole, 8.11-9.12 ppm for N-H bridge, alkoxy proton at 2.88-3.63 and alkyl proton peak at 2.12-2.33 ppm. So, all the values of spectral analysis were in compliance with the literature and correspond to structures of synthesized compounds.

Antioxidant activity

Antioxidant activity was performed using *in vitro* methods like DPPH and H_2O_2 free radical scavenging methods. Antioxidant activity results are expressed as IC_{50} value in µM (Fig. 11).

Results of DPPH method showed IC_{50} value of **7a** as 116.39 µM, however H_2O_2 method showed 161.90, which shows compound **7a** was most active compound in the series, when evaluated using AA as reference standard. The less binding energy, H-bonding and other interactions with target may be responsible for its better antioxidant activity.

Antibacterial activity

Antibacterial activity was performed using well diffusion method and results are expressed as zone of inhibition in mm by test compounds and standard drug (Figs. 12 - 14).

Antibacterial study revealed that compounds **7f** was most active and **7a** and **7e** were moderately active against *S. aureus* (ATCC 25923), compounds **7f** was most active and **7a** was moderately active against *E. coli* (ATCC 25922), whereas compounds **7f** and **7d** were most active and **7b** and **7c** were moderately active against *P. aeruginosa* (ATCC 27853), when evaluated using CPF as reference standard. Other compounds in the series showed less antibacterial activity. The binding affinity of most active ligands with target may be responsible for the better antibacterial activity. Compound **7d** showed antibacterial activity (In contrast to docking results) against *P. aeruginosa* (ATCC 27853). The reason behind more activity of compound **7d** needs to be further explore by continuing the study by structural modifications.

Further, different models for antioxidant activity and evaluation of antibacterial activity against different mutant pathogenic strains should be taken into consideration for further studies. The most active benzothiazole based 2*H*-chromen-2-one derivative could serve as lead compounds for designing and developing novel, potent and safe antioxidant and antibacterial agents.

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