IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

DESIGN SYNTHESIS AND BIOLOGICAL EVALUATION OF COUMARIN DERIVATIVES AS AN ANTIMICROBIAL AGENTS

[1]Mukesh Pawar, [2] Dr. Govind Nayak, [3] Dr. Parul Mehta, [4] Dr. Shivakant Shukla

[1]M. Pharmacy, [2nd] Professor, [3] Director and Professor [4]Associate Professor
[1]Lakshmi Narain College of Pharmacy, Bhopal, India.

ABSTRACT: New compounds were designed based on docking studies. GLIDE (Grid-based ligand docking with energetics, version autodock vina 1.5.7) was used to dock all the designed pyrazole containing coumarin derivatives within the binding site (PDB ID 4cv0). Docking based synthesis of coumarin contain pyrazoline derivatives, being used as potential medicinal agents, 3-Acetyl-2H-chromen-2one (I) was prepared by Knoevenagel condensation of salicylaldehyde with ethyl acetoacetate in presence of piperidine. A series of 3-[(2E)-3-substituted-prop-2-enoyl]-2H-chromen-2-one derivatives (II A-H) were prepared by Claisen-Schmidt condensation of 3-acetyl coumarin with aromatic aldehydes. Treatment of 3-substituted cinnamoyl coumarin with hydrazine hydrate in the presence of ethanol gave [5-substitutedphenyl]-4, 5-dihydro-1H pyrazol-3-yl]-2H-chromen-2-one (III A-H). Title compound were synthesized by conventional method. The structures of the newly synthesized compounds were confirmed by FT-IR and ^{1}H-NMR spectroscopy. All the synthesized compounds were tested for their antibacterial activities using filter paper disc method. The antibacterial activity screening revels that the compound 3A, 3B and 3C are promising against *S. aureus* and *E.coli.* show moderate activity as that of standard amikacin against gram positive and gram-negative bacteria.

IndexTerms **– Docking, 3-Acetyl coumarin, Pyrazoline, hydrazine hydrate, Antibacterial activity**

INTRODUCTION

Coumarins (2H1benzopyran2one) belong to the lactones sub group chemically.[1] The six basic classes of natural coumarins include simple coumarins, furanocoumarins, pyrano coumarins (linear and angular type), dihydrofurano coumarins, phenyl coumarins, and bicoumarins. In 1820, Vogel isolated the first parent coumarin from tonka bean (Dipteryx odorata)[2]. Coumarin is derived from the French word "Coumarou," which meaning "tonka bean." Coumarins are abundant in nature and can be found as secondary metabolites in a wide variety of plant roots, flowers, leaves, peels, seeds, and fruits.[3] Coumarins can be synthesized using several reactions, including the Perkin reaction, the Knoevenagel condensation, the Pechmann condensation, the Wittig reaction, the Baylis Hillman reaction, the Claisen rearrangement, and the Vilsmeier Haack and Suzuki cross coupling reactions. Many researchers have published studies on coumarins' medical biomolecules characteristics. Coumarins have antimicrobial, antibacterial, and antifungal properties. Researchers have been interested in studying the biological features of natural coumarins or synthesizing their analogues for medicinal applications for decades. Coumarin analogues (both synthetic and natural) are a necessary component in the cosmetics and perfume industries. The coumarin skeleton is found in biologically active natural products and is employed as an intermediary in the production of bioactive heterocyclic compounds.[4,5]

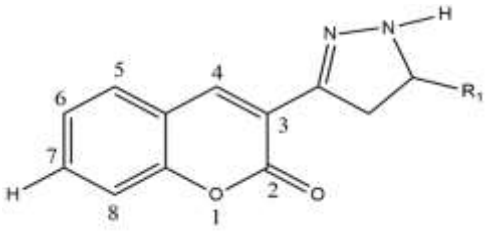
NEED OF THE STUDY

Numerous investigations suggest that both natural and synthesized coumarin derivatives have antibacterial properties.[6] There are several coumarin derivatives with anticoagulant, anti-analgesic,[7] anti-inflammatory,[8] anti-cancer, anti-proliferative, and antiviral properties have been reported. In the treatment of infectious diseases brought on by bacteria and fungi, drug resistance has emerged as a significant issue. The discovery and development of efficient antibacterial and antifungal medicines with novel mechanisms of action have thus become critical goals for infectious disease research programmers due to the major medical problem of bacterial and fungal resistance and the rapid rate at which it develops.[3,4]

RESEARCH ENVISAGED

The emergence of novel antimicrobial medications with greater therapeutic efficacy than existing ones has been made possible by the rise in microbial resistance to already available antimicrobial treatments.
Designing new molecules can be done in one of two ways:

- Through the utilization of verified targets to produce novel medications.
- Through locating fresh prospective targets.



In this SAR analysis of coumarin, the integument of the congener's active sites was briefly highlighted for its qualities of inhibitory effects due to the presence of electron withdrawing NO_2, halogen groups and electron donating -OCH_3, -CH_3 of phenyl rings, respectively. Due to the presence of withdrawing chloro substituents, which have a better zone of inhibition than methylation ligand, metal complexes including coumarinyl carbohydrazide with indole Schiff base derivatives have also demonstrated significant antibacterial activity. In fact, the increased antibacterial effectiveness is inversely correlated with the metal chelate ions' lipophilicity, which may favor permeability via the lipid layer of bacterial cell membranes.[9,10]

MOLECULAR DOCKING

Molecular docking is the process of arranging molecules in specific configurations to engage with a receptor. Docking is the study of protein and ligand interaction. Where, 2D structure ligand draw in ChemDraw Ultra 12.0 tools convert in 3D form with energy minimization process, protein was selected antibacterial literature study and protein downloaded in RCSB (PDB ID 4cv0). Protein was prepared autodock vina tools. Where water molecules are deleted and the protein grid was produced by configuring the grid box by selecting amino acids where the ligands expected to dock at the catalytic site, both the protein and ligand were prepared, running at autodock vina in command path, docking score result was generated, after docking of the proposed compounds was used to investigate the structure's binding posture with the protein, Compounds with the highest docking scores were compared. Then visualized in visualizing tool pymol. The data displayed in the table 01 revealed that all the developed compounds have significant anti-microbial action, although some compounds (3A-3B-3C-3D-3E-3F-3G-3H) demonstrated the best docking score.[11,12]

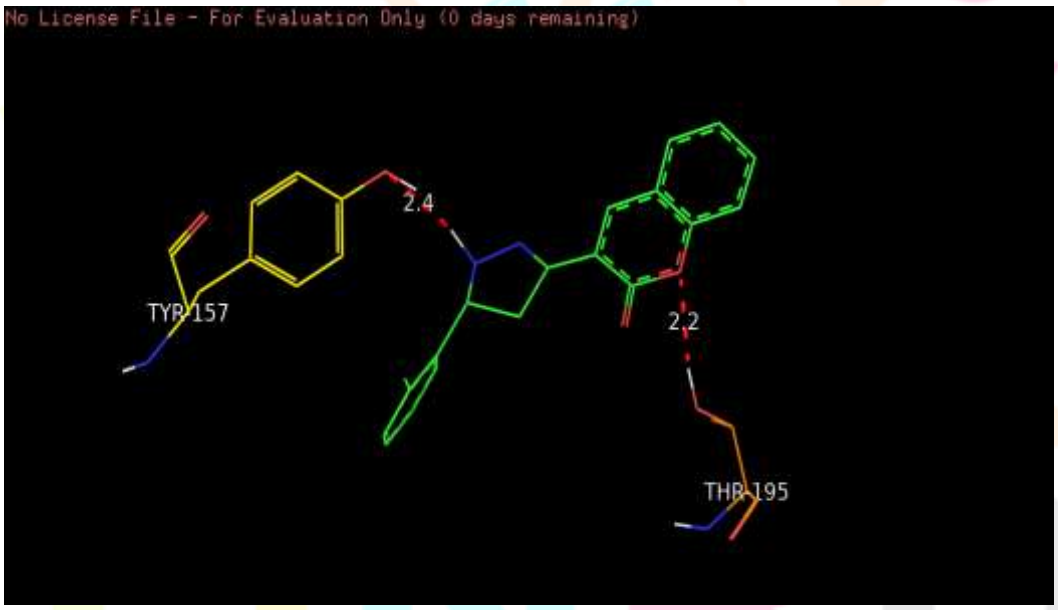


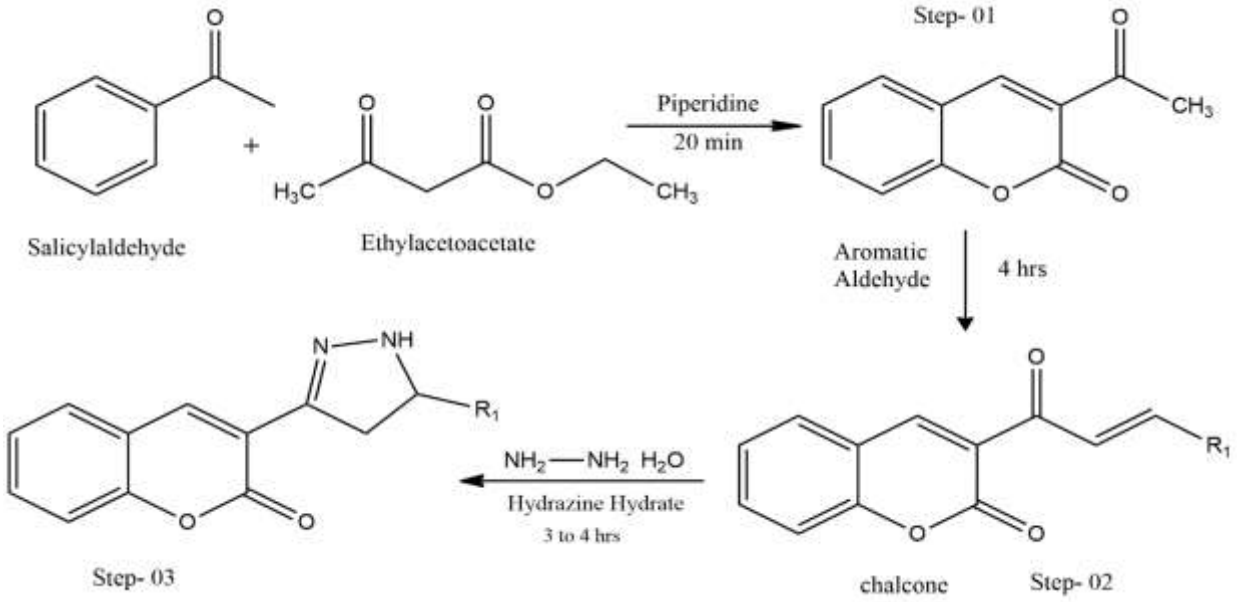
Figure 01. Drug binding with protein

S.NO	COMPOUND CODE	DOCKING SCORE
1	3A	− 10.1
2	3B	−10.3
3	3C	−10.2
4	3D	−8.6
5	3E	−10.2
6	3F	−10.3
7	3G	−7.6
8	3H	−9.9

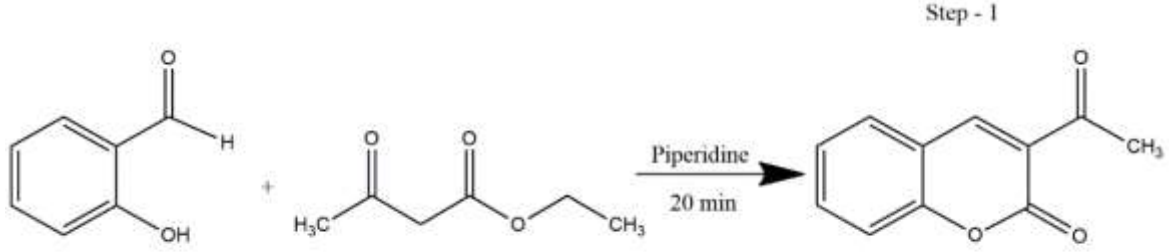
Table 01. Best docking Score

RESEARCH METHODOLOGY

Materials And Methods: Chemicals used in the synthesis of the title compounds described were purchased from chemdyes corporation, sunchem India, oxford laboratory and loba chemie pvt. ltd. The different aromatic aldehydes, ethyl acetoacetate, piperidine, mueller hinton agar, microorganism strain purchase for online Biomall.in. These chemicals were used as it is without further purification. All other LR grade reagents were used after purification using the literature methods. Melting point were determined with open capillary and are uncorrected. FT-IR spectra were recorded in Bruker – 300 MHz Spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker – 500 MHz spectrometer using CDCL3 as an internal standard. Progress of the reaction and purity of the products were ascertained by thin-layer chromatography (TLC) using silica gel G as stationary phase and various solvent combination as mobile phase; the spots were visualized by UV Chamber and Iodine vapours.

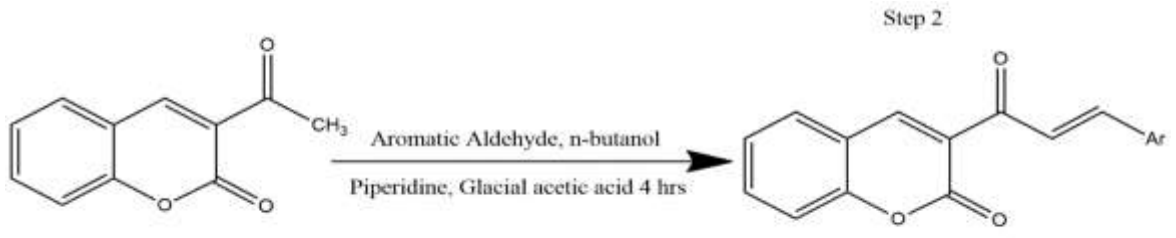


Step-01) Preparation of 3-Acetyl Coumarin (I): To a mixture of salicylaldehyde (1.8 g. 0.02 mol) and ethyl acetoacetate (2.5 g, 0.02 mol) was catalyzed with Piperidine (0.2 ml) added by rapid stirring and heated under reflux for a period of 20 min to produce a yellowish crude solid mass of 3-acetyl-coumarin separated was filtered off and washed with ethanol. It was recrystallized from ethanol, (TLC monitored) It melts at 120 ^{O}C (lit mp 120 – 122 ^{O}C) and yield was 82.40 %.



(I)

Step-02) General Procedure for the Synthesis of 3-[(2E)-3-substituted-prop-2-enoyl]-2H-chromen-2-one (II A-H): A combination amount of 3-Acetyl-2H-chromen-2-one (I) (0.01 mol) and (0.012 mol) of the corresponding aromatic aldehydes was dissolved in 10 ml of n-Butanol under heating; then subsequently, 0.3 ml of glacial acetic acid and addition of a catalytic amount of piperidine (5 drops) were added. The reaction mixture was refluxed for 4 hr and then the solvent was extracted under vacuum. The residue was triturated with 20 ml of ethanol until a precipitate formed, then separated by filtration and recrystallized with a suitable solvent methanol and Ethanol. Physical properties of the produced synthesis compounds determine (IIA-H).

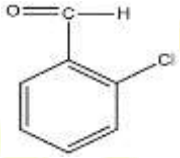
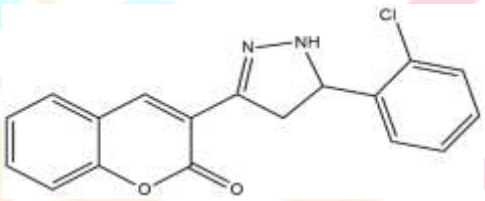
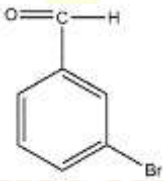
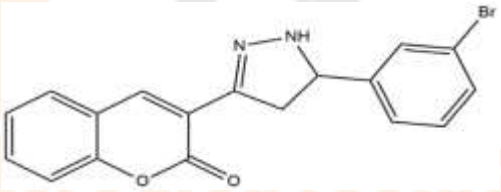
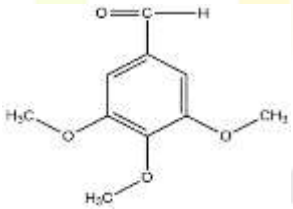
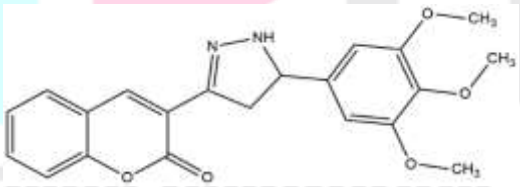
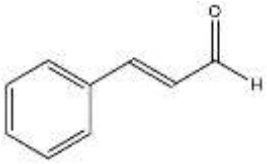
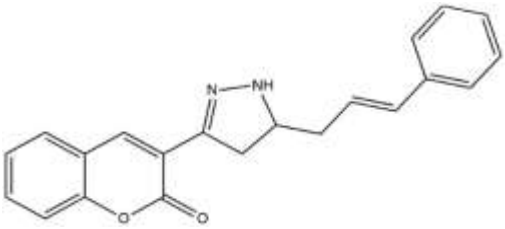


(2)

Step-03) General Procedure for the 3- [5-substituted phenyl]-4,5-dihydro-1H-pyrazol-3-yl]-2H - chromen-2-one (III A-H): Hydrazine hydrate (0.02 mol) and 10 ml of ethanolic solution (0.01 mol) chalcone were refluxed for 3 to 4 hours. The reaction mixture was poured over crushed ice and thoroughly mixed. The solids produced are filtered and washed with water before being crystallized from appropriate solvents, yielding the corresponding (III 3A-H). Data on the physicochemical and spectral properties of produced substances.[13],[14]

(3)

Table 02. Aromatic Aldehyde (R_1) Used in Synthesis of Coumarin Derivatives

S.no	Aromatic aldehyde (R_1)	Final Coumarin derivatives IUPAC name
1.	2- Chloro Benzaldehyde 	3-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one 
2	3- Bromo Benzaldehyde 	3-(5-(3-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one 
3	3,4,5 Trimethoxy Benzaldehyde 	3-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one 
4	Cinnamaldehyde 	(E)-3-(5-cinnamyl-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one 

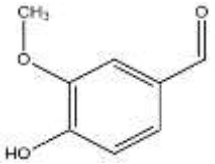
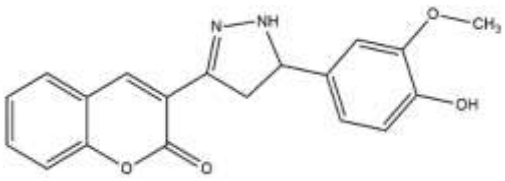
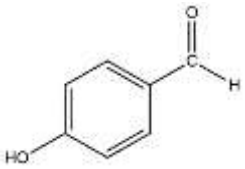
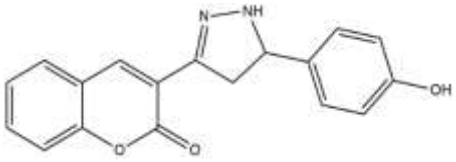
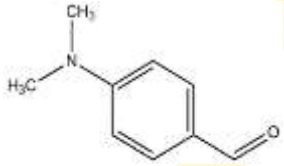
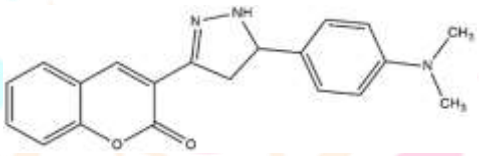
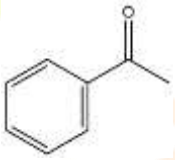
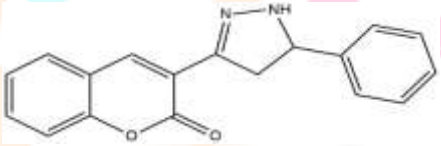
5	**4-Hydroxy-3-methoxy Benzaldehyde** 	**3-(5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one** 
6	**4-Hydroxy Benzaldehyde** 	**3-(5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one** 
7	**4-dimethyl Amino Benzaldehyde** 	**3-(5-(4-(dimethyl amino) phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one** 
8	**Benzaldehyde** 	**3-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one** 

Table 03: Physicochemical data of 3-[5-substitutedphenyl]-4, 5 - dihydro -1h-pyrazol-3-yl]- 2h-chromen-2-one (3 A-H)

Compound	R	Molecular Weight	Yield (%)	M.P. (^{O}C)	Rf Value
3A	2-Cl	324.76	78.56	260-262	0.63
3B	3-Br	369.21	64.21	120-122	0.64
3C	3,4,5- OCH_3	380.39	56.06	210-212	0.67
3D	5-cinnamyl	330.37	86.37	126-128	0.52
3E	4-OH, 3-OCH_3	336.34	67.43	170-172	0.71
3F	4- OH	306.31	77.62	232-234	0.63
3G	4-NCH_2	333.38	74.20	210-212	0.68
3H	5-Phenyl	290.31	71.55	120-122	0.72

3 Acetyl Coumarin

FT-IR (KBr cm^{-1}): Characteristics peak at 1741.40 (lactone of coumarin); 1647.46 (Ketone C=O)

^{1}H NMR (500 MHz, CDCl3) δ 2.70 (s, 3H, -CH_3); δ 7.28-7.52 (m, 4H, -ArH) and δ 8.52 (s, 1H, C_4 of Coumarin).

3A) 3-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one

FT-IR (KBr cm^{-1}): 3490.05 (NH), 2924.37 (CH_2), 1647.41 (lactone of Coumarin), 1531.52 (C=C), 780.72(Cl).

^{1}H NMR (500 MHz, CDCl3) δ 3.11 (dd, 1H, 4-H_t), δ 3.84 (dd, 1H, 4-H_c), δ 6.22 (dd, 1H, 5-H of pyrazoline), δ 7.26 -7.69 (m, 8H, Ar-H), δ 7.62 (s, 1H, 4-H of coumarin), δ 8.80(s, H, NH)

3B) 3-(5-(3-bromophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one

FT-IR (KBr cm^{-1}): 3491.37 (NH), 2922.25 (CH_2), 1646.40 (lactone of Coumarin), 1531.05 (C=C), 740.88(Br).

^{1}H NMR (500 MHz, CDCl3) δ 2.75 (dd, 1H, 4-H_t), δ 3.51 (dd, 1H, 4-H_c), δ 6.98 (dd, 1H, 5-H of pyrazoline), δ 7.28 -7.63 (m, 8H, Ar-H), δ 7.56 (s, 1H, 4-H of coumarin), 8.73(s, H, NH).

3C) 3-(5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one
FT-IR (KBr cm^{-1}): 3491.36 (NH), 2923.55 (CH_2), 1647.15 (lactone of Coumarin), 1531.17 (C=C), 1170.60(OCH_3).

^{1}H NMR (500 MHz, CDCl3) δ 2.65 (dd, 1H, 4-H_t), δ 3.95 (dd, 1H, 4-H_c), δ 3.95 (s, 3-H, OCH3), δ 6.97 (dd, 1H, 5-H of pyrazoline), δ 7.11 -7.63 (m, 8H, Ar-H), δ 8.02 (s, 1H, 4-H of coumarin), δ 8.79(s, H, NH).

3D) (E)-3-(5-cinnamyl-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one
FT-IR (KBr cm^{-1}): 3491.44 (NH), 2925.88 (CH_2), 1647.83 (lactone of Coumarin), 1531.27 (C=C)

^{1}H NMR (500 MHz, CDCl3) δ 1.28 (dd, 1H, 4-H_t), δ 2.25 (dd, 1H, 4-H_c), δ 3.01 (s, 3-H, OCH3), δ 7.21 (dd, 1H, 5-H of pyrazoline), δ 7.19 -7.38 (m, 8H, Ar-H), δ 7.65 (s, 1H, 4-H of coumarin), δ 8.15(s, H, NH).

3E) 3-(5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one
FT-IR (KBr cm^{-1}): 3491.25 (NH), 2925.65(CH_2), 1647.74 (lactone of Coumarin), 1531.27(C=C), 1170.75 (OCH_3), 1038.81(C-OH)

3F) 3-(5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one
FT-IR (KBr cm^{-1}): 3491.26 (NH), 2925.53(CH_2), 1647.55 (lactone of Coumarin), 1531.39 (C=C), 1038.35(C-OH).

3G) 3-(5-(4-(dimethyl amino) phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one
FT-IR (KBr cm^{-1}): 3491.37 (NH), 2926.38 (CH_2), 1647.52 (lactone of Coumarin), 1531.35 (C=C)

3H) 3-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one
FT-IR (KBr cm^{-1}): 3492.35 (NH), 2924.27 (CH_2), 1647.04 (lactone of Coumarin), 1531.27 (C=C)

The ADME Prediction was done in the Swiss ADME lab table 04.

S. No	Com - pounds	Lipinski Rule	Log P Lipophilicy	Log S Water Solubility	GI Absorption	BBB	Skin Permeation	Bio avail-ability
1	3A	Accepted	2.30	-4.85	High	Yes	-5.33 cm/s	0.55
2	3B	Accepted	2.56	-5.16	High	Yes	-5.56 cm/s	0.55
3	3C	Accepted	2.91	-4.46	High	No	-6.18 cm/s	0.55
4	3D	Accepted	2.59	-7.2	High	Yes	-5.27 cm/s	0.55
5	3E	Accepted	2.89	-4.18	High	No	-6.12 cm/s	0.55
6	3F	Accepted	1.84	-4.12	High	Yes	-5.92 cm/s	0.55
7	3G	Accepted	2.27	-4.48	High	Yes	-5.74 cm/s	0.55
8	3H	Accepted	2.22	-4.26	High	Yes	-5.57 cm/s	0.55

Biological Evaluation

Antibacterial activity: -

To melt the disinfected Muller Hinton Agar Media, it was heated in a water bath. The organisms were inoculated separately and poured aseptically into sterile petri dishes when the media was lukewarm. The standard drug disc Amikacin was placed on the medium and the whatmann no.2 filter disc (5mm diameter) was cut and poured into cotton-plugged vials. These vials were boiled in a hot air oven at 160^{o}C for 30 minutes. Then it was individually soaked in created compounds, evaporated to dryness, and then stored on the medium (5 mm height). As a control, another disc was dipped in Dimethyl formamide and left on the media. It was placed in the refrigerator for one hour to allow consistent drug diffusion before being placed in the incubator for 24 hours at 37^{o}C. The zone of inhibition around the synthesized compounds was observed and compared to that of conventional compounds. Antibacterial activity of the test compounds in DMF was determine by filter paper disc method at a concentration of 25, 50, 75 and

100 µg/ml. All the compounds showed comparable activity as that of the standard Amikacin (13 mm) against *staphylococcus aureus* & *Escherichia Coli* the compounds 3A, 3B and 3C are promising against *S. aureus* and *E.coli.*[15],[16]

Antimicrobial activity of compound 3A to 3H

(Diameter of the zone of inhibition in mm)

The solvent (control) is Dimethyl formamide

1. *Staphylococcus aureus* standard compounds amikacin
2. *Escherichia coli* standard compound amikacin

Antibacterial activity of all compounds against gram positive bacteria *Staphylococcus aureus*

Compound code	Zone of inhibition on different concentrations			
	25 µg/m	50 µg/ml	75 µg/ml	100 µg/ml
3A	0 mm	5.2 mm	8.5 mm	11.6 mm
3B	0 mm	4.0 mm	6.6 mm	9.2 mm
3C	0 mm	5.5 mm	8.9 mm	10.2 mm
3D	0 mm	4.2 mm	7.5 mm	8.5 mm
3E	0 mm	3.2 mm	5.6 mm	6.4 mm
3F	0 mm	3.4 mm	5.2 mm	6.1 mm
3G	0 mm	3.6 mm	6.7 mm	8.4 mm
3H	0 mm	3.3 mm	6.3 mm	7.6 mm
Amikacin	0 mm	5.8 mm	10.4 mm	13.0 mm

Table 05. Compound code, Diameter in mm Inhibition

Antibacterial activity of all compounds against gram negative bacteria *Escherichia coli*

Compound code	Zone of inhibition on different concentrations			
	25 µg/m	50 µg/ml	75 µg/ml	100 µg/ml
3A	0 mm	5.3 mm	9.6 mm	11.8 mm
3B	0 mm	4.0 mm	7.6 mm	9.8 mm
3C	0 mm	4.2 mm	8.1 mm	10.8 mm
3D	0 mm	3.6 mm	6.5 mm	8.4 mm
3E	0 mm	3.3 mm	5.8 mm	6.3 mm
3F	0 mm	3.2 mm	5.6 mm	7.2 mm
3G	0 mm	3.8 mm	7.2 mm	8.9 mm
3H	0 mm	3.4 mm	6.2 mm	8.2. mm
Amikacin	0 mm	5.5 mm	10.6 mm	13.0 mm

Table 06. Compound code, Diameter in mm Inhibition

IV. RESULTS AND DISCUSSION

This project was focused on the design and synthesis of new active molecule for microbial treatment. GLIDE (Grid-based ligand docking with energetics, version Autodock Vina 1.5.7) was used to dock all the designed pyrazole containing coumarin derivatives within the binding site (PDB ID 4cv0). New compounds were designed based on docking studies. Designed pyrazole containing coumarin derivatives show hydrogen bond interactions with catalytic dyad TYR 157 and THR 195 bond length of 2.4 and 2.2

respectively. 3-Acetyl coumarin was synthesized by Knoevenagel condensation of salicylaldehyde with ethyl acetoacetate. Claisen Schmidt condensation of 3-Acetyl coumarin with aromatic aldehydes gave 3-substituted cinnamoyl coumarin. Treatment of 3-substituted cinnamoyl coumarin with hydrazine hydrate in ethanol gave 3- (5-substitutedphenyl)-4, 5-dihydro-1H-pyrazol-3- yl]-2H-chromen-2-one. The purity of the synthesized compounds was analyzed by thin-layer chromatography (TLC) on a silica gel G. The Pyrazole containing coumarin derivatives were synthesized successfully and their structure was confirmed using FT-IR and ^{1}HNMR. Various physicochemical properties such as melting point, R_f value were determined experimentally. In FT-IR spectra of compounds the characteristic peak of (lactone of coumarin) and C=O of α-pyrone were observed at 1741.40 and 1647 respectively; and the title compounds (3A-H), NH, CH_2 and C=C of pyrazoline were observed at 3480-3500, 2920-2930 and 1530-1535 cm^{-1} respectively confirmed the structure of the title compounds. Further, the structure was ascertained by detailed ^{1}H-NMR study of the compounds. In ^{1}H-NMR spectra of compounds (3A-3H), the presence of three doublet-doublet between δ 3.01 to 4.28 of - CH2 and -CH of pyrazoline and multiple between δ 6.87 to 8.59 is characteristic peaks of aromatic protons in spectrum of 3-[5-substitutedphenyl]-4,5- dihydro-1H-pyrazol-3-yl]-2H-chromen-2-one reveals confirmation of structures. The confirmed pyrazole containing coumarin derivatives were evaluated against sensitive strain of *Staphylococcus aureus* and *Escherichia coli.* This study was performed at LNCP Laboratories, Bhopal. From the 8 compounds 3A, 3B and 3C have shown their antimicrobial activity less than 12 mm against microbial strain. The activity of compound 3A, 3B and 3C was found to be good activity.

CONCLUSION: As some docking of synthesized compounds showed antibacterial activity, further studies can be performed to evaluate the efficacy and safety of these derivatives.

ACKNOWLEDGMENT: The authors are thankful to Prof. Govind Nayak. Parul Mehta Director and Principal of Lakshmi Narain College of Pharmacy, Bhopal, India for providing research facilities and encouragement.

REFERENCES

[1] Saloni kakkar *et al* (2018) Design, synthesis, and biological evaluation of 3-(2-aminooxazol-5-yl)-2H-chromen-2-one derivatives Chemistry Central Journal, vol 12, 130.

[2] Melita Loncaric, Dajana Gaso sokac *et al* (2020). Recent Advances in the Synthesis of Coumarin Derivatives from Different Starting Materials 11 December 2019; Accepted: 14 January 2020; Published: 16 January 2020.

[3] R. Kenchappa and Yadav D. Bodke *et al* (2014) A Synthesis of coumarin derivatives containing pyrazole and indenone rings as potent antioxidant and antihyperglycemic agents published by Arabian Journal of Chemistry, vol 10, S3895 to S3906

[4] Watee Srikong and Pimonsri Mittraparp-arthorn *et al* (2015) Antimicrobial activity of seaweed extracts from Pattani, Southeast coast of Thailand Published by Food and Applied Bioscience Journal, 2015, 3 (1), 39–49.

[5] Yasameen K. and Al-Majedy *et al* (2017). A Coumarins: The Antimicrobial agents published by Systematic Reviews in Pharmacy, vol 8, Issue 1, 62-70.

[6] Prashant Aragade *et al* (2012) Synthesis and antibacterial activity of a new series of 3- [3-(substituted phenyl)-1-phenyl-1H-pyrazol-5-yl]-2Hchromen-2-one derivative, published by Journal of Enzyme Inhibition and Medicinal Chemistry, 27(6), 849–853.

[7] Suresh Khode and Veeresh Maddi *et al* (2009). Synthesis and pharmacological evaluation of a novel series of 5-(substituted) aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines as novel anti-inflammatory and analgesic agents, published by European Journal of Medicinal Chemistry 44 (2009), 1682–1638.

[8] Girish S. Boyapati and Veeresh S. Maddi *et al* (2008). Synthesis and Evaluation of Anti-inflammatory and Analgesic Activities of a Novel Series of Coumarin Mannich Bases, published by Arzneimittel-Forschung (Drug Research) 2008;58(10), 515–520.

[9] Chita Ranjan Sahoo and Jyotirmaya Sahoo *et al* (2020). A Coumarin derivatives as promising antibacterial agents, Published by Arabian Journal of Chemistry, vol 14, 102922.

[10] Pratik Swarup Das and Puja Saha *et al (*2017). A Review on Computer Aided Drug Design in Drug Discovery, Published By World Journal Of Pharmacy And Pharmaceutical Sciences vol 6, Issue 7, 2017.

[11] She-Feng Wang and Yong Yin *et al* (2014). Synthesis, molecular docking, and biological evaluation of coumarin derivatives containing piperazine skeleton as potential antibacterial agents.

[12] Leonardo G. Ferreira and Ricardo N. dos Santos *et al* (2015). A Molecular Docking and Structure-Based Drug Design Strategies, Published by Molecules 2015, 20, 13384-13421; doi:10.3390/molecules200713384.

[13] Olayinka O. Ajani *et al* (2019). Microwave assisted synthesis, characterization, and investigation of antibacterial activity of 3-(5-(substituted-phenyl)-4,5-dihydro-1Hpyrazol-3-yl)-2H-chromen-2-one derivative published Arab Journal of Basic and Applied Sciences 26:1, 362-375, DOI: 10.1080/25765299.2019.1632141.

[14] A. S. Lunkad and R. L. Sawant. (2019). A conventional and microwave assisted synthesis of some new derivatives of coumarin containing pyrazoline and investigation of their antibacterial and antifungal activities. Published in, IJPSR, 2018; vol. 9(7): 2852-2858.

[15] Daoudi Yacine and Daoudi Smail *et al* (2017) An Effect of culture media on the agar diffusion method for testing antibacterial activity against Escherichia coli published by ElWahat pour les Recherche's et les Etudes vol.10, 157-165.

[16] Margaret a. Kenny *et al* (1980) Cation Components of Mueller-Hinton Agar Affecting Testing of Pseudomonas aeruginosa Susceptibility to Gentamicin published by antimicrobial agents and chemotherapy, Jan. 1980. vol. 17, 55-62.