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Graphical abstract

Synthesis, characterization and molecular docking studies of substituted 4-coumarinylpyrano[2,3-c]pyrazole derivatives as potent antibacterial and anti-inflammatory agents

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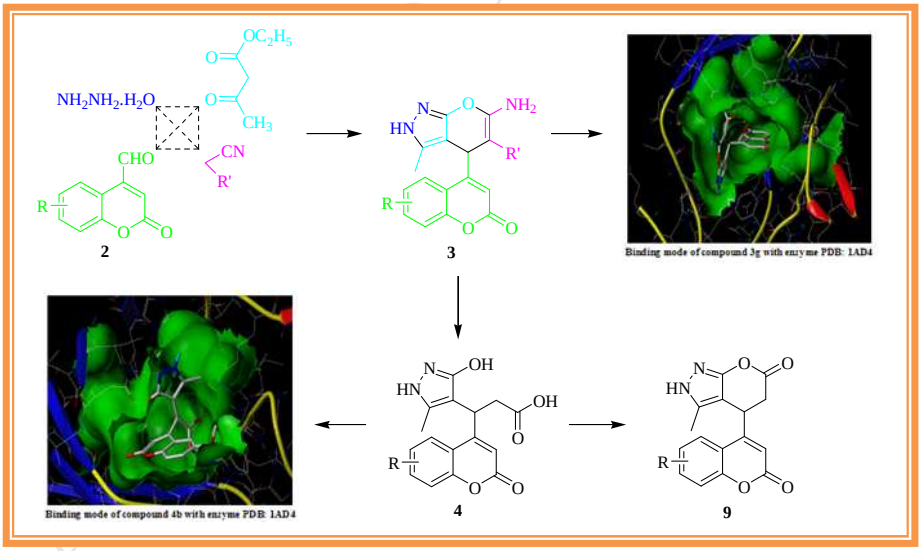

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Abstract:

A green, eco-friendly and efficient protocol has been developed and synthesized a series of coumarin based pyrano[2,3-c]pyrazole derivatives (**3**) by multi-component reaction (MCR). Unexpected 3-coumarinyl-3-pyrazolylpropanoic acids (**4**) have been isolated by the reaction of compound (**3**) in acidic conditions. Further, intramolecular cyclization of compounds (**4**) leads to C_4-C_4 chromons (**9**) and these compounds were screened for their biological activities using array of techniques. Most of the compounds exhibited promising antibacterial activity, in particular Gram-positive bacteria. The anti-inflammatory assay was evaluated against protein denaturation as well as HRBC membrane stabilization methods and compounds exhibit excellent anti-inflammatory activity in both methods. Molecular docking study has been performed for all the synthesized compounds with *S. aureus* dihydropteroate synthetase (DHPS) and results obtained are quite promising.

Key words: DMAP, Pyrano[2,3-c]pyrazole, Pyranopyrazolo-pyrimidine, 3-Coumarinyl-3-pyrazolylpropanoic acids, C_4-C_4 pyranone, Antibacterial, Anti-inflammatory, Molecular docking.

1. Introduction

Coumarin analogues are a group of bioactive molecules, found substantially in nature with a wide range of structural modifications [1]. Coumarins are a privileged oxygen heterocycles widely distributed in various species of plants as well as animals and microbial metabolite, playing an important role in the agricultural and pharmaceutical industries [2]. They exhibit antiviral [3], anti-cancer [4], anti-fungal [5], anti-inflammatory [6], anti-HIV [7] properties. They have been known to be particularly effective against Gram-positive bacterial species [8]. Incorporation of bio-compatible fragments like vanillin and paracetamol onto coumarin nucleus has resulted 4-aryloxymethyl coumarins which are exhibiting anti-inflammatory activity [9]. Coumarin based anti-biotics viz. novobiocin and clorobiocin affect the functioning of DNA gyrase, which is the basis for their broad spectrum of antibacterial activity [10]. The naturally occurring bromotyrosine derivatives have exhibited anti-microbial effect on the methicillin resistant *S. aureus* (MRSA) [11].

Pyrazolones fused pyran rings compose a very important class of compounds in the heterocyclic area [12], and are extensively used as important precursors in the field of medicinal chemistry due to their potential biological and pharmacological properties such as antimicrobial [13], anti-inflammatory [14], anticancer [15] insecticidal [16], and inhibitors of human Chk1 kinase [17]. Medicinally important pyrano[2,3-c]pyrazole derivatives have been synthesized via two component [18], three component [19], and more importantly four component reactions by using different homogeneous and heterogeneous catalysts [20].

Computational biology and bioinformatics play a major role in drug designing and accelerating the drug discovery process. Molecular docking of the drug molecule with the receptor (target) provides important information about drug-receptor interactions and is commonly used to identify the binding orientation of drug candidates to their protein targets in order to predict the affinity and activity [21]. DNA gyrase is an enzyme that relieves strain, while double-strand DNA is being unwound by helicase, this causes negative supercoiling of the DNA. DNA gyrase is the target of many antibiotics, including nalidixic acid, novobiocin and ciprofloxacin. The antibiotics such as ciprofloxacin prevents the two enzymatic steps from replicating of DNA [22].

Knowing the therapeutic importance of coumarin, pyrazole and pyran scaffolds, the present investigation pertains to the hybridization of these three pharmacophoric motifs in a

single molecule. We hereby report the synthesis, characterization and biological studies of some novel coumarin based pyrano[2,3-c]pyrazoles and pyrazolylpropanoic acid using conventional method. The newly synthesized compounds were screened for their antibacterial and anti-inflammatory activities, further molecular docking studies were carried out to understand the drug–receptor interactions.

2. Chemistry

Base catalyzed one-pot four component approach has been used for the synthesis of substituted 4-coumarinyl-6-amino-1,4-dihydro-3-methylpyrano[2,3-c]pyrazole-5-carbonitrile **3** under optimized conditions (Scheme 1).

Insert Scheme 1

Encouraged by gaining acceptable results in our first attempt, initial optimization studies were performed by employing 6-methyl-4-formylcoumarin **2** [23] as a precursor. Table 1 shows the optimizing effect for the synthesis of pyrano[2,3-c]pyrazoles **3** using different ratios of water and ethanol (0 : 100, 50 : 50, 80 : 20 and 100 : 0). The best results were obtained in terms of yield (> 90%) by using DMAP in water-ethanol medium (Table 1, entry 8) at ambient temperature.

Insert Table 1

The mechanism of reaction is believed to involve the Knoevenagel condensation [24] with compound **2** and malononitrile to form an alkene intermediate followed by Michael type addition with pyrazolin-5-one (formed from ethylacetoacetate and hydrazine hydrate) and intra-molecular cyclization to give polyfunctionalized pyrano[2,3-c]pyrazoles (Scheme 2). The structures for the newly synthesized pyrano[2,3-c]pyrazoles were assigned on the basis of their spectral data and X-ray single crystal studies.

Insert Scheme 2

Insert Table 2

The literature revels that pyrimidinone derivatives have wide range of applications in medicinal and pharmaceutical area; therefore we thought of synthesizing coumarin based pyranopyrazole pyrimidines **5** by utilizing polyfunctional compound **3**. The sequence of reaction illustrates (Scheme 3) the construction of desired compound **5** starting from compound **3** with

urea and thiourea in presence of acetic acid and HCl (3:1) by using literature methods [25]. Unfortunately, we noticed that the isolated expected product **5** was not supporting with spectral data, whereas spectral data agrees well with the unexpected compound **4**. Further, this observation inspired us to study the earlier reported reaction for the synthesis of various substituted pyranopyrazolo-pyrimidine derivatives using variety of reagents at different experimental conditions [26]. Most of the reported methods afforded desired product pyrimidine skeleton starting from 6-amino-1,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile [26]. The model reaction was initiated to construct pyrimidine skeleton starting from compound **3** with various reagents such as acetic anhydride in acetic acid, alone acetic anhydride, formic acid in polar solvents, alone formic acid, water-HCl with acetamide and formamide in polar solvents etc. Interestingly, we observed the formation of compound **4** almost >80% instead of pyrimidine structures **5, 6, 7** and **8** (Scheme 3).

Insert Scheme 3

There are very few references in the literature for the conversion of compound **3** into 3-aryl-3-(5-hydroxy-3-methyl-1H-pyrazol-4-yl)propanoic acid derivatives **4** using formic acid [27]. Finally, we noticed that reaction of compound **3** with various reagents in acidic condition resulted in the formation of compound **4** in high yield (Table 3). Mechanism of the reaction involves opening of the pyran ring, under acidic condition with subsequent hydrolysis of nitrile or ester group, accompanied by decarboxylation of one of the carboxylic group giving the compound **4**, the general plausible mechanism is presented in Scheme 4.

Insert Scheme 4

Insert Table 3

In order to confirm the formation of compound **4**, the study was extended to intramolecular cyclization to generate new class of compounds i.e. tail-tail pyranone (pyranone-4-pyranone or C_4-C_4 pyranone **9**) structure starting from compound **4** with thionyl chloride (Scheme 5). Successfully we achieved the synthesis of desired product 4,5-dihydropyrano[2,3-c]pyrazol-6(1H)-one **9** from compound **4** with excellent yield and purity (Table 4).

Insert Scheme 5

Insert Table 4

3. Results and Discussion

All new synthesized compounds were characterized and confirmed by spectral analysis. Formation of product **3** was supported by spectral data. In case of compound **3a**, IR spectrum shows intense bands at 1707 cm^{-1} and 2201 cm^{-1} due to lactone carbonyl and nitrile groups respectively. Whereas, pyrazole and pyran rings NH and NH_2 stretching bands were observed at 3339 cm^{-1} and 3311 cm^{-1}. It was further confirmed by ^{1}H-NMR, wherein the NH proton of pyrazole ring appeared as a singlet at 12.25 ppm. The C_5H of coumarin was resonated as singlet at 7.55 ppm and coumarin C_8H was observed as a doublet at 7.41 ppm (J = 8.8 Hz). The C_7H of coumarin was appeared as a doublet at 7.32 ppm (J = 8.8 Hz). The NH_2 proton of pyran ring was observed as a singlet at 7.15 ppm. Two singlet at 6.34 ppm and 5.13 ppm due to C_3H of coumarin and C_4H of pyran respectively. Whereas, singlet at 2.28 ppm due to C_6-CH_3 of coumarin and one more singlet at 1.87 ppm due to C_3-CH_3 of pyrazole. The protons correlations have been confirmed by using 2D NMR (Fig. 1).

Insert Figure 1

Formation of product **4** is well supported by spectral data. In case of compound **4a**, IR spectrum shows two intense bands at 1712 cm^{-1} and 1727 cm^{-1}, due to carboxylic acid and lactone carbonyl. The band at 3327 cm^{-1} is due to NH and OH groups. It was further confirmed by ^{1}H-NMR, wherein the NH of pyrazole ring and OH of propanoic acid appeared as a broad singlet at 11.26 ppm. The C_5H of coumarin was resonated as a doublet at 7.69 ppm (J = 1.2 Hz). The C_8H of coumarin was observed as a doublet at 7.38 ppm (J = 8.4 Hz) and C_7H of coumarin appeared as a doublet at 7.27 ppm (J = 8.4 Hz). The C_3H of coumarin was observed as a singlet at 6.41 ppm and C_3H of propanoic acid was observed as a triplet at 4.57 ppm (J= 7.6 Hz) and phenolic OH of pyrazole ring has appeared as broad singlet at 3.3 ppm. The two protons on C_2 of propanoic acid was resonated as two doublets of doublet at 3.08 ppm (J = 16.4 Hz & 8.0 Hz) and 2.89 ppm (J = 16.0 Hz & 7.2 Hz). The methyl groups on C_6 of coumarin and C_3 of pyrazole moiety were observed as two singlets at 2.34 ppm and 2.07 ppm respectively. Further this was confirmed by DEPT-135, in this two methyl group carbon containing odd number of protons were observed at 10.26 ppm and 20.22 ppm with +ve mode. The C_3 of propanoic acid containing odd number of proton has appeared at 30.66 ppm with +ve mode and remaining aromatic carbons containing odd number of protons was resonated in expected region with +ve mode.

Whereas, C_2 carbon of propanoic acid containing even number of proton were observed at 36.46 ppm with –ve mode. Further, proton correlations have been confirmed by 2D NMR (Fig.2).

Insert Figure 2

In case of compound **9a**, IR spectrum shows two intense bands at 1722 cm^{-1} and 1748 cm^{-1}, due to lactone carbonyls of coumarin and pyran nucleus and band at 3327 cm^{-1} due to -NH of pyrazole ring. It was further confirmed by ^{1}H-NMR, wherein the NH proton of pyrazole ring appeared as a singlet at 11.23 ppm. The C_5H of coumarin was resonated as singlet at 7.69 ppm and C_8H of coumarin was observed as a doublet at 7.40 ppm (J = 8.4 Hz). The C_7H of coumarin was appeared as a doublet at 7.28 ppm (J = 8.4 Hz) and C_3H of coumarin was observed as a singlet at 6.43 ppm. The two protons on C_3 of pyran ring have appeared as two doublets of doublet at 3.28 ppm (J = 14.8 Hz and 6.8 Hz) and 2.88 ppm (14.2 Hz and 6.7 Hz) respectively. Whereas, C_4H of pyran was resonated as a triplet at 4.60 ppm (J= 7.6 Hz). The C_3 and C_6 methyl groups of pyrazole and coumarin moiety were resonated as two singlets at 2.06 ppm and 2.48 ppm respectively. Further, the protons correlations have been confirmed by 2D NMR (Fig. 3).

Insert Figure 3

4. X-Ray Diffraction Studies

The single crystals of compound **3b** were developed by slow evaporation of methanol as a solvent. Suitable crystal was selected using polarizing microscope, then mounted on a Mitegen Micromount and refined using the Bruker SAINT Software package using a narrow-frame algorithm. The structure was solved and refined using Bruker SHELXTL Software Package. Crystal data was refined [28,29] and their properties were studied. Crystal information files (CIF) have been deposited at Cambridge Crystallographic Data Centre, the CCDC number for **3b** is **1463750**. The crystal and structure refinement data of compound **3b** is given in Table 5.

X-ray studies and molecular structure of pyranopyrazoles revealed that it exists in the crystal phase as 2H instead of 1H tautomer as shown in Fig. 4, which is in congruence with the literature reports of pyrano[2,3-c]pyrazole [30]. In contrast to this, N1-substituted pyrano[2,3-c]pyrazole derivatives exist as ‘immobilized’ 1H-tautomers [31]. The crystal structure of compound **3b** is non-planar in nature with a significant dihedral angle 88.18° with coumarin and pyranopyrazole moiety (Fig. 5 and 6).

Insert Figure 4

Insert Figure 5

Insert Figure 6

Insert Table 5

5. Pharmacological Screening

5.1. Antibacterial Studies

All the synthesized compounds **3a-j**, **4a-e** and **9a-e** were evaluated for their antibacterial activity against two Gram-positive (*S. aureus* and *E. faecalis*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains with ciprofloxacin as a standard by using Broth microdilution method [32]. The minimum inhibitory concentration (MIC) of the synthesized compounds were compared with ciprofloxacin, it revealed that almost all the newly synthesized compounds showed excellent antibacterial activity against Gram-positive *S. aureus* and *E. faecalis* bacterial strain. But in case of Gram-negative *E. coli* and *P. aeruginosa* bacterial strains some of the synthesized compounds have showed selective antibacterial activity.

The antibacterial screening of compounds **3a-j** reveals that almost all the coumarin substituted pyrano[2,3-c]pyrazoles are highly active against *S. aureus* at concentration of 1.56 to 6.25 µg/mL. Compounds **3b** (OCH_3 substitution at C_6 of coumarin and CN, NH_2 substations on pyran), **3c** (Cl substitution at C_6 of coumarin and CN, NH_2 substations on pyran), **3f** (CH_3 substitution at C_6 of coumarin and COOEt, NH_2 substations on pyran), **3g** (OCH_3 substitution at C_6 of coumarin and COOEt, NH_2 substations on pyran), **3h** (Cl substitution at C_6 of coumarin and COOEt, NH_2 substations on pyran), **3i** (CH_3 substitution at C_7 of coumarin and COOEt, NH_2 substations on pyran) and **3j** (benzo substitution at C_7-C_8 of coumarin and COOEt, NH_2 substations on pyran) have shown significant activity at lower concentration than standard drug, but compounds **3a** (CH_3 substitution at C_6 of coumarin and CN, NH_2 substations on pyran), **3d** (CH_3 substitution at C_7 of coumarin and CN, NH_2 substations on pyran) and **3e** (benzo substitution at C_7-C_8 of coumarin and CN, NH_2 substations on pyran) have exhibited good activity at 6.25µg/mL, which is same as standard drug concentration used (Table 6). Among **3a-j,** compound **3g** is more effective against *S. aureus*, showing the least MIC value of 1.56 µg/mL. The efficiency of the compounds (**3a-j**) were screened against *E. faecalis*, it was observed that, among these only compound **3g** exhibited excellent activity at lower concentration (3.125 µg/mL). Whereas, other compounds such as **3c**, **3f** and **3h** were found to be equally active

compared to standard. However, compounds **3a**, **3b**, **3d**, **3e**, **3i** and **3j** were found to be less active. It has been observed that compound **3g** with OCH_3 at C_6 of coumarin and COOEt, NH_2 on pyran nucleus is highly active against both Gram +ve bacterial strains. Similarly, activity against *E. coli,* compound **3d** is found to be highly active at 0.78µg/mL compared to standard. Compound **3c** and **3i** are equally active, however other synthesized compounds were less active. In case of *P. aeruginosa,* compound **3b** (OCH_3 substitution at C_6 of coumarin and CN, NH_2 substations on pyran) and **3d** (CH_3 substitution at C_7 of coumarin and CN, NH_2 substations on pyran) are highly active. Whereas, other compounds are less active and results obtained are summarized in Table 6.

The antibacterial activity of substituted pyrazolylpropanoic acids (**4a-e)** revealed that compounds **4a** (CH_3 substitution at C_6 of coumarin), **4b** (OCH_3 substitution at C_6 of coumarin), **4c** (Cl substitution at C_6 of coumarin) and **4d** (CH_3 substitution at C_7 of coumarin) are highly active against *S. aureus*. Whereas, compound **4e** (benzo substitution at C_7-C_8 of coumarin) shows good activity, the results obtained are summarized in Table 6. Activity against *E. faecalis,* compound **4b** was found to be highly active at lower concentration (1.56µg/mL). However, compounds **4a**, **4c** and **4d** showed good activity (6.25µg/mL) and compound **4e** shows less activity against *E. faecalis*. Whereas, all the compounds **4a-e** were less active against *E. coli* and *P. aeruginosa* bacterial strains.

The antibacterial activity of coumarin substituted 4,5-dihydropyrano[2,3-c]pyrazol-6(1H)-ones **9** were highly active against *S. aureus* bacterial strain. Among these compound **9b** (OCH_3 substitution at C_6 of coumarin) shows activity at lower concentration (3.125 µg/mL) than standard, but compounds **9a** (CH_3 substitution at C_6 of coumarin), **9c** (Cl substitution at C_6 of coumarin) and **9d** (CH_3 substitution at C_7 of coumarin) showed moderate activity compared to standard drug. However compound **9a-e** were less active against *E. faecalis*, *E. coli* and *P. aeruginosa* antibacterial strains, the results obtained are discussed in Table 6.

Insert Table 6

5.2. Anti-inflammatory Studies

5.2.1. Egg albumin denaturation method

All the synthesized compounds were subjected to anti-inflammatory effect against denaturation of hen's egg albumin method [33] at the concentration (31.25-1000 µg/mL) with standard aceclofenac drug (31.25-1000 µg/mL).

The outcome of anti-inflammatory screening of compounds (**3a-j**, **4a-e** and **9a-e**) by using egg albumin denaturation method are summarized in Table 7. The percentage inhibition of all the synthesized compounds showed very high active against the denaturation of protein. Among these compounds **3g**, **4b** and **4c** exhibited an excellent inhibition of heat induced protein denaturation 51.43%, 63.11% and 42.64% respectively and these compounds are almost ten times more active compared to standard aceclofenac drug (5.50%). Whereas, compounds **9c** and **9e** shows less activity and the remaining compounds showed good inhibitory anti-inflammatory activity against the denaturation of protein method.

Insert Table 7

5.2.2. Human Red Blood Cell (HRBC) membrane stabilization method

The anti-inflammatory activity of newly synthesized compounds was also screened by using Human Red Blood Cell (HRBC) membrane stabilization technique [34]. This technique is conventional, sensitive and well accepted for screening of newer anti-inflammatory agents. The activity of compounds was screened at the concentration of 100 µg/mL oral dose and the same dose of standard drug acetyl salicylic acid is used. The results are presented in Table 8.

The bio-screening results disclosed that all the compounds exhibited inhibitions of HRBC membrane stabilization at the concentration of 100 µg/mL and most of the compounds showed more than half maximal (50%) inhibition except compounds **3a**, **3d**, **3e**, **9c** and **9e.** Compounds **3g**, **3h**, **3j**, **4a**, **4b**, **4c**, **4d**, **4e** and **9b** exhibited excellent activity with inhibitions 54.06%, 39.86%, 38.56%, 37.26%, 55.26%, 50.66%, 42.56%, 49.56% and 36.33% respectively in comparison with the standard acetyl salicylic acid inhibition 36.16%. Whereas, compounds **3b**, **3c**, **3f**, **3i**, **9a** and **9d** showed potent inhibition erythrocyte hemolysis membrane stabilization close to the standard drug.

The synthesized compounds have shown significant anti-inflammatory activity in protein denaturation as well as HRBC membrane stabilization methods. Among all the synthesized compounds, **3g** and **4a-e** exhibit very good inhibitions in both the methods.

Insert Table 8

6. Computational studies

The newly synthesized compounds have exhibited excellent antibacterial activity, in particular against Gram +ve bacteria *S. aureus*. The *S. aureus* can cause a range of illnesses from minor skin infections to life-threatening diseases. Today, *S. aureus* has become resistant to many commonly used antibiotics and only 2% of all *S. aureus* isolates are found to be sensitive to penicillin. The β-lactamase resistant penicillins (methicillin, oxacillin, cloxacillin and flucloxacillin) were developed to treat penicillin resistant *S. aureus* and are still used as first-line treatment. *S. aureus* seems to employ specific resistance mechanisms, which include the modification of the antibiotic structure, mutagenesis of key amino acids in the protein targets of specific chemotherapeutics or drug efflux from the cell. In this line Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class and is a second-generation antibacterial agent, which kills bacteria by inhibiting the enzyme DNA-gyrase.

To understand the mechanism of antibacterial activity of newly synthesized compounds, molecular modeling and docking studies were performed on X-ray crystal structure of the dihydropteroate synthetase (DHPS) complexed with OH-CH_2-pterin-pyrophosphate from *S. aureus* (PDB ID:1AD4) [35]. The docking study was obtained from the Protein Data Bank by using Surflex-Dock programme of Sybyl-X software. All the twenty compounds were docked into the active site of the DHPS (Fig. 7), the predicted binding energies and the observed C-score values of all the compounds are ranging from 2.49-5.57, the score values are listed in Table 9.

Insert Figure 4

In order to know, the high activity of synthesized compounds against *S. aureus*, molecular docking study was performed to support the interaction and preferred binding mode of Ciprofloxacin with *S. aureus* DHPS (PDB ID:1AD4). The binding interaction of standard Ciprofloxacin with dihydropteroate synthetase active sites shows five bonding interactions and the docked view of the same has been depicted in Fig. 8.

Insert Figure 5

Insert Table 9

As shown in Fig. 9, compound **3g**, forms eight hydrogen bonding interaction at the active site of the enzyme (PDB ID:1AD4). Oxygen atoms of ester forms three hydrogen bonding interaction with hydrogen of ASN11 & ARG239 (-CH_2CO<u>O</u>---H-ASN11, -CH_2C<u>O</u>O---H-

ASN11 & -CH_2COO---H-ARG239) and hydrogen atom of amine group present at 2nd position of pyran ring forms hydrogen bonding interaction with oxygen atom of ASN11 (-NH_2---O-ASN11). One hydrogen bonding interaction raised from methoxy group oxygen present at C_6 of coumarin ring ASN103 (-CH_3O---H-ASN103). The remaining three interaction are raised from the pyrazole ring, two hydrogen bonding interaction raised from nitrogen atom of pyrazole ring (-N---H-ARG52 & -N---H-SER50) and another from amine of pyrazole ring with oxygen atom of SER50 (-NH---O-SER50).

Insert Figure 6

Compounds **4b** forms six hydrogen bonding interaction with amino acid residues (Fig 10), among them two bonding interactions are due to hydroxyl group present at 3rd position of pyrazole ring with hydrogen of ARG239 & oxygen of ASN11 (-HO---H-ARG239, 2.21 Å; -OH---O-ASN11, 2.10 Å) respectively. Nitrogen of pyrazole ring forms hydrogen bonding interaction with hydrogen of ASN11 (-N---H-ASN11, 2.14 Å) and hydrogen of -NH of pyrazole ring forms a bonding interaction with nitrogen of HIS241 (-NH---N-HIS241, 2.46 Å). Carboxyl group oxygen forms hydrogen bonding interaction with hydrogen of LYS203 (-COOH---H-LYS203, 2.30 Å), and methoxy group oxygen present at 6th position of coumarin ring with hydrogen atom of SER50 (-CH_3O---H-SER50, 2.54 Å).

Insert Figure 7

The comparative molecular docking study of synthesized compounds and standard Ciprofloxacin drug highlighted that the synthesized compounds exhibited high C-score value. Ciprofloxacin C-score value 3.58 whereas the compounds **3b**, **3c**, **3f**, **3g**, **3h**, **3i**, **3j**, **4a**, **4b**, **4c**, **4d**, **4e** and **9b** have higher C-score values than the Ciprofloxacin. The docking study reveals that, the compound **3g** and **4b** are most potent among all the synthesized compounds.

Moreover docking study in Sybyl-X-2.0 software, has been applied for the synthesized compounds to determine the Lipinski's parameters (Table 10). All the synthesized compounds did not indicate any violation against Lipinski rule of five. It was observed that the newly synthesized compounds followed Lipinski's rule of five, indicating more 'drug-like' nature.

Insert Table 10.

7. Experimental protocol

7.1. Materials and methods

All the chemicals were purchased by commercial source and were used without further purification unless otherwise stated. The reactions monitored by thin-layer chromatography (TLC) was performed with Merck Kieselgel 60 F254 plates and visualized using UV light at 254 nm and $KMnO_4$ staining or iodine vapour, the purity of the compound was also checked by TLC. Column chromatography was generally performed on silica gel (60-120 mesh size). The melting points were determined by open capillary method and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 MHz and Jeol solution for innovation 500 MHz spectrometer (400 MHz or 100 MHz, respectively) using DMSO-d_6 as solvent and TMS as an internal standard. Chemical shifts are reported in δ ppm relative to internal tetramethylsilane standard (TMS, δ 0.00). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; q, quartet; dd, doublet of doublets; br, broad. The coupling constants, *J*, is reported in Hertz (Hz). The X-ray diffraction study were performed on a BRUKER AXS SMART APEX CCD diffractometer. The IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer in KBr disc method and mass spectra were recorded using Shimadzu GCMSQP2010S. The CHN elemental analyses of all the compounds were recorded by LECO TRUSPEC CHN analyzer.

7.2. General Procedure and Spectral Characterization of Compounds **3a-j:**

The mixture of hydrazine hydrate 96% (1 mmol) and ethyl acetoacetate (1 mmol) in aqueous ethanol (20%) was stirred for 5-10 min. Aldehyde (1 mmol), malononitrile or ethylcyanoacetate (1 mmol) and base (5 mmol %) were added to it successively at room temperature under open atmosphere with vigorous stirring for 2 h. The progress of the reaction was monitored by TLC. The precipitated solid was filtered and washed with water, to afforded pure product **3** in good yield and no further purification is required

*7.2.1. 6-Amino-1,4-dihydro-3-methyl-4-(6-methyl-2-oxo-2H-chromen-4-yl)pyrano[2,3-c]pyrazole-5-carbonitrile (**3a**):*

White; Yield 94%; m.p: 236-238 ^{o}C; IR (KBr) cm^{-1} 3339, 3311, 2201, 1707; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.25 (s, 1H, NH of Pyrazole), 7.55 (s, 1H, C_5H of Coumarin), 7.41 (d, 1H, *J*= 8.8 Hz, C_8H of Coumarin), 7.32 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 7.15 (s, 2H, NH_2 of Pyran), 6.34 (s, 1H, C_3H of Coumarin), 5.13 (s, 1H, C_4H of Pyran), 2.28 (s, 3H, CH_3 of

Pyrazole), 1.87 (s, 3H, CH_3 of Coumarin); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.62, 160.07, 154.71, 151.89, 136.19, 133.17, 132.98, 124.71, 120.01, 116.96, 114.96, 112.98, 108.16, 101.92, 94.69, 53.95, 20.67, 9.90; MS (*m/z*): 334. Elemental Analysis for $C_{18}H_{14}N_4O_3$ (%), Calcd: C, 64.66; H, 4.22; N, 16.76; found: C, 64.59; H, 4.18; N, 16.71.

*7.2.2. 6-Amino-1,4-dihydro-4-(6-methoxy-2-oxo-2H-chromen-4-yl)-3-methylpyrano[2,3-c]pyrazole-5-carbonitrile (**3b**):*

Light green; Yield 91%; m.p: 204-206 ^{o}C; IR (KBr) cm^{-1} 3378, 3308, 2186, 1708; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.28 (s, 1H, NH of Pyrazole), 7.39 (d, 1H, *J*= 8.8 Hz, C_8H of Coumarin), 7.20 (d, 1H, *J*= 8.4 Hz, C_7H of Coumarin), 7.13 (s, 1H, C_5H of Coumarin), 7.06 (s, 2H, NH_2 of Pyran), 6.47 (s, 1H, C_3H of Coumarin), 5.09 (s, 1H, C_4H of Pyran), 3.68 (s, 3H, OCH_3 of Coumarin), 1.90 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.59, 160.05, 159.99, 154.88, 154.66, 154.61, 148.17, 136.50, 136.43, 119.93, 119.17, 119.11, 118.28, 107.56, 107.50, 55.33, 53.79, 9.84; MS (*m/z*): 350. Elemental Analysis for $C_{18}H_{14}N_4O_4$ (%), Calcd: C, 61.71; H, 4.03; N, 15.99; found: C, 61.67; H, 3.98; N, 15.92.

*7.2.3. 6-Amino-4-(6-chloro-2-oxo-2H-chromen-4-yl)-1,4-dihydro-3-methylpyrano[2,3-c]pyrazole-5-carbonitrile (**3c**):*

White; Yield 89%; m.p: 240-242 ^{o}C; IR (KBr) cm^{-1} 3433, 3315, 2197, 1719; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.30 (s, 1H, NH of Pyrazole), 7.45 (d, 1H, *J*= 2.4 Hz, C_5H of Coumarin), 7.34 (d, 1H, *J*= 8.8 Hz, C_8H of Coumarin), 7.26 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 7.21 (s, 2H, NH_2 of Pyran), 6.37 (s, 1H, C_3H of Coumarin), 5.17 (s, 1H, C_4H of Pyran), 2.30 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 160.11, 159.32, 155.38, 151.92, 134.26, 126.72, 119.89, 119.13, 118.70, 116.17, 111.71, 108.16, 107.73, 106.92, 94.90, 53.87, 9.96; MS (*m/z*): 354. Elemental Analysis for $C_{17}H_{11}ClN_4O_3$ (%), Calcd: C, 57.56; H, 3.13; N, 15.79; found: C, 57.51; H, 3.09; N, 15.74.

*7.2.4. 6-Amino-1,4-dihydro-3-methyl-4-(7-methyl-2-oxo-2H-chromen-4-yl)pyrano[2,3-c]pyrazole-5-carbonitrile (**3d**):*

White; Yield 92%; m.p: 230-232 ^{o}C; IR (KBr) cm^{-1} 3343, 3305, 2190, 1719; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.04 (s, 1H, NH of Pyrazole), 7.31 (d, 1H, *J*= 8.8 Hz, C_5H of Coumarin), 7.23 (d, 1H, *J*= 2.4 Hz, C_8H of Coumarin), 7.17 (d, 1H, *J*= 8.8 Hz, C_7H of

Coumarin), 6.90 (s, 2H, NH_2 of Pyran), 6.41 (s, 1H, C_3H of Coumarin), 5.12 (s, 1H, C_4H of Pyran), 2.32 (s, 3H, CH_3 of Pyrazole), 1.93 (s, 3H, CH_3 of Coumarin); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.73, 160.11, 155.22, 151.87, 136.20, 133.15, 132.74, 125.01, 120.85, 117.26, 115.09, 113.13, 108.19, 102.31, 94.57, 54.65, 21.07, 9.94; MS (*m/z*): 334. Elemental Analysis for $C_{18}H_{14}N_4O_3$ (%), Calcd: C, 64.66; H, 4.22; N, 16.76; found: C, 64.60; H, 4.18; N, 16.71.

*7.2.5. 6-Amino-1,4-dihydro-3-methyl-4-(2-oxo-2H-benzo[h]chromen-4-yl)pyrano[2,3-c]pyrazole-5-carbonitrile (**3e**):*

Light brown; Yield 90%; m.p: 270-272 ^oC; IR (KBr) cm^{-1} 3432, 3314, 2196, 1712; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.30 (s, 1H, NH of Pyrazole), 8.40 (d, 1H, *J*= 8.8 Hz, C_5H of Coumarin), 8.07 (d, 1H, *J*= 8.6 Hz, $C_{10}H$ of Coumarin), 7.70-7.81 (m, 4H, C_6H, C_7H, C_8H and C_9H of Coumarin), 7.26 (s, 2H, NH_2 of Pyran), 6.55 (s, 1H, C_3H of Coumarin), 5.21 (s, 1H, C_4H of Pyran), 1.89 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.50, 159.75, 154.58, 150.89, 136.38, 134.08, 129.06, 127.86, 127.55, 123.73, 122.58, 121.75, 120.32, 119.93, 114.73, 112.98, 108.16, 101.92, 94.67, 53.83, 9.86; MS (*m/z*): 370. Elemental Analysis for $C_{21}H_{14}N_4O_3$ (%), Calcd: C, 68.10; H, 3.81; N, 15.13; found: C, 68.06; H, 3.76; N, 15.10.

*7.2.6. Ethyl-6-amino-1,4-dihydro-3-methyl-4-(6-methyl-2-oxo-2H-chromen-4-yl)pyrano[2,3-c]pyrazole-5-carboxylate (**3f**):*

White; Yield 92%; m.p: 206-208 ^oC; IR (KBr) cm^{-1} 3337, 3313, 1727, 1711; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.40 (s, 1H, NH of Pyrazole), 7.72 (s, 1H, C_5H of Coumarin), 7.64 (s, 2H, NH_2 of Pyran), 7.43 (d, 1H, *J*= 8.4 Hz, C_8H of Coumarin), 7.31 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 6.64 (s, 1H, C_3H of Coumarin), 5.16 (s, 1H, C_4H of Pyran), 4.09 (m, 2H, CH_2 of Ethyl group), 2.33 (s, 3H, CH_3 of Pyrazole), 2.09 (s, 3H, CH_3 of Coumarin), 1.08 (m, 3H, CH_3 of Ethyl group); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.90, 159.75, 153.11, 151.43, 138.15, 133.73, 133.00, 124.55, 117.57, 116.76, 116.13, 113.15, 112.44, 96.11, 94.95, 62.60, 34.63, 20.50, 13.44, 10.53; MS (*m/z*): 381. Elemental Analysis for $C_{20}H_{19}N_3O_5$ (%), Calcd: C, 62.99; H, 5.02; N, 11.02; found: C, 62.95; H, 4.97; N, 10.98.

*7.2.7. Ethyl-6-amino-1,4-dihydro-4-(6-methoxy-2-oxo-2H-chromen-4-yl)-3-methylpyrano[2,3-c] pyrazole-5-carboxylate (**3g**):*

White; Yield 89%; m.p: 210-212 ^{o}C; IR (KBr) cm^{-1} 3387, 3278, 1720, 1711; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.75 (s, 1H, NH of Pyrazole), 7.40 (s, 1H, C_5H of Coumarin), 7.24 (d, 1H, *J*= 8.4 Hz, C_8H of Coumarin), 7.18 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 7.15 (s, 2H, NH_2 of Pyran), 6.44 (s, 1H, C_3H of Coumarin), 5.10 (s, 1H, C_4H of Pyran), 4.06 (m, 2H, CH_2 of Ethyl group), 3.67 (s, 3H, OCH_3 of Coumarin), 2.37 (s, 3H, CH_3 of Pyrazole), 1.07 (m, 3H, CH_3 of Ethyl group); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.67, 160.03, 153.14, 151.49, 138.17, 133.77, 132.98, 124.57, 120.18, 117.60, 116.81, 114.89, 112.58, 97.16, 95.93, 64.67, 55.67, 53.33, 13.68, 10.60; MS (*m/z*): 397. Elemental Analysis for $C_{20}H_{19}N_3O_6$ (%), Calcd: C, 60.45; H, 4.82; N, 10.57; found: C, 60.39; H, 4.77; N, 10.50.

*7.2.8. Ethyl-6-amino-4-(6-chloro-2-oxo-2H-chromen-4-yl)-1,4-dihydro-3-methylpyrano[2,3-c]pyrazole-5-carboxylate (**3h**):*

White; Yield 86%; m.p: 238-240 ^{o}C; IR (KBr) cm^{-1} 3407, 3368, 1723, 1712; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.85 (s, 1H, NH of Pyrazole), 7.45 (s, 1H, C_5H of Coumarin), 7.34 (d, 1H, *J*= 8.8 Hz, C_8H of Coumarin), 7.27 (d, 1H, *J*= 8.4 Hz, C_7H of Coumarin), 7.17 (s, 2H, NH_2 of Pyran), 6.32 (s, 1H, C_3H of Coumarin), 5.11 (s, 1H, C_4H of Pyran), 4.10 (m, 2H, CH_2 of Ethyl group), 2.30 (s, 3H, CH_3 of Pyrazole), 1.17 (m, 3H, CH_3 of Ethyl group); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.61, 159.87, 154.67, 150.84, 136.19, 133.23, 132.86, 123.17, 120.09, 116.71, 114.92, 112.96, 108.20, 102.02, 94.71, 54.63, 53.95, 20.67, 9.90; MS (*m/z*): 401. Elemental Analysis for $C_{19}H_{16}ClN_3O_5$ (%), Calcd: C, 56.80; H, 4.01; N, 10.46; found: C, 56.76; H, 3.96; N, 10.38.

*7.2.9. Ethyl-6-amino-1,4-dihydro-3-methyl-4-(7-methyl-2-oxo-2H-chromen-4-yl)pyrano[2,3-c]pyrazole-5-carboxylate (**3i**):*

White; Yield 91%; m.p: 212-214 ^{o}C; IR (KBr) cm^{-1} 3350, 3321, 1722, 1709; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.05 (s, 1H, NH of Pyrazole), 7.42 (d, 1H, *J*= 8.4 Hz, C_5H of Coumarin), 7.35 (d, 1H, *J*= 2.4 Hz, C_8H of Coumarin), 7.31 (d, 1H, *J*= 8.4 Hz, C_6H of Coumarin), 7.12 (s, 2H, NH_2 of Pyran), 6.30 (s, 1H, C_3H of Coumarin), 5.13 (s, 1H, C_4H of Pyran), 3.84 (m, 2H, CH_2 of Ethyl group), 2.23 (s, 3H, CH_3 of Pyrazole), 1.45 (s, 3H, CH_3 of Coumarin), 1.02 (m, 3H, CH_3 of Ethyl group); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 162.52, 160.17, 155.73, 151.91, 136.23, 133.13, 132.78, 124.87, 120.71, 116.98, 113.16, 112.98, 107.15,

101.72, 94.69, 54.95, 34.86, 20.67, 13.64, 10.13; MS (*m/z*): 381. Elemental Analysis for $C_{20}H_{19}N_3O_5$ (%), Calcd: C, 62.99; H, 5.02; N, 11.02; found: C, 62.93; H, 4.96; N, 10.94.

*7.2.10. Ethyl-6-amino-1,4-dihydro-3-methyl-4-(2-oxo-2H-benzo[h]chromen-4-yl)pyrano[2,3-c]pyrazole-5-carboxylate (**3j**):*

White; Yield 88%; m.p: 207-209 ^oC; IR (KBr) cm^{-1} 3367, 3288, 1720, 1712; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.28 (s, 1H, NH of Pyrazole), 8.37 (d, 1H, *J*= 8.4 Hz, C_5H of Coumarin), 8.34 (d, 1H, *J*= 8.8 Hz, $C_{10}H$ of Coumarin), 8.23 (m, 2H, C_8H and C_9H of Coumarin), 7.85 (d, 1H, *J*= 8.4 Hz, C_7H of Coumarin), 7.68 (d, 1H, *J*= 8.8 Hz, C_6H of Coumarin), 7.28 (s, 2H, NH_2 of Pyran), 6.34 (s, 1H, C_3H of Coumarin), 5.18 (s, 1H, C_4H of Pyran), 4.08 (m, 2H, CH_2 of Ethyl group), 2.28 (s, 3H, CH_3 of Pyrazole), 1.87 (s, 3H, CH_3 of Coumarin), 1.58 (m, 3H, CH_3 of Ethyl group); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 161.92, 160.21, 157.41, 149.67, 134.21, 133.29, 133.00, 128.65, 127.81, 124.04, 122.82, 121.53, 120.29, 117.07, 114.86, 113.08, 108.16, 101.90, 94.67, 54.72, 34.78, 20.71, 10.11; MS (*m/z*): 417. Elemental Analysis for $C_{23}H_{19}N_3O_5$ (%), Calcd: C, 66.18; H, 4.59; N, 10.07; found: C, 66.14; H, 4.53; N, 10.02.

7.3. General Procedure and Spectral Characterization of compounds **4a-e:**

A mixture of compound **3** (1 mmol) and formic acid (10 mL) was prepared and refluxed on oil bath for about 4-5 h. The reaction completion was confirmed by TLC, after that reaction mixture was allowed to cool and poured into ice cold water with stirring. Solid separated was filtered, repeatedly washed with water and dried to obtain the pure white product **4** in excellent yield and no purification required further.

*7.3.1. 3-(5-hydroxy-3-methyl-1H-pyrazol-4-yl)-3-(6-methyl-2-oxo-2H-chromen-4-yl)propanoic acid (**4a**):*

White; Yield 83%; m.p: 268-270 ^oC; IR (KBr) cm^{-1} 3327, 1727, 1712; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.26 (s, 2H, NH of Pyrazole and OH of Propanoic acid), 7.69 (d, 1H, , *J*=1.2 Hz C_5H of Coumarin), 7.38 (d, 1H, *J*= 8.4 Hz, C_8H of Coumarin), 7.27 (d, 1H, *J*= 8.4 Hz, C_7H of Coumarin), 6.41 (s, 1H, C_3H of Coumarin), 4.57 (t, 1H, *J*= 7.6 Hz, C_3H of Propanoic acid), 3.30 (s, 1H, OH of Pyrazole), 3.08 (dd, 1H, *J*= 16.4 & 8.0 Hz, C_2H of Propanoic acid), 2.89 (dd, 1H, *J*= 16.0 & 7.2 Hz, C_2H of Propanoic acid), 2.34 (s, 3H, CH_3 of Pyrazole), 2.07 (s,

3H, CH_3 of Coumarin); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.65, 161.88, 154.73, 151.86, 136.22, 133.17, 132.59, 124.39, 120.01, 116.37, 111.96, 108.16, 101.92, 36.46, 30.36, 20.22, 10.26; MS (*m/z*): 328. Elemental Analysis for $C_{17}H_{16}N_2O_5$ (%), Calcd: C, 62.19; H, 4.91; N, 8.53; found: C, 62.14; H, 4.85; N, 8.48.

*7.3.2. 3-(5-hydroxy-3-methyl-1H-pyrazol-4-yl)-3-(6-methoxy-2-oxo-2H-chromen-4-yl)propanoic acid (**4b**):*

Light green; Yield 80%; m.p: 256-258 ^oC; IR (KBr) cm^{-1} 3328, 1725, 1705; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.21 (s, 1H, NH of Pyrazole), 11.44 (s, 1H, OH of Propanoic acid), 7.42 (d, 1H, *J*= 8.4 Hz, C_8H of Coumarin), 7.26 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 7.17 (s, 1H, C_5H of Coumarin), 6.48 (s, 1H, C_3H of Coumarin), 4.59 (t, 1H, *J*= 7.4 Hz, C_3H of Propanoic acid), 3.72 (s, 3H, OCH_3 of Coumarin), 3.31 (s, 1H, OH of Pyrazole), 3.04 (dd, 1H, *J*= 16.0 & 8.4 Hz, C_2H of Propanoic acid), 2.90 (dd, 1H, *J*= 16.4 & 7.6 Hz, C_2H of Propanoic acid), 2.04 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 162.89, 161.27, 155.09, 151.88, 136.26, 133.16, 132.56, 125.10, 120.03, 117.39, 111.97, 109.04, 101.95, 53.79, 36.51, 30.39, 10.14; MS (*m/z*): 344. Elemental Analysis for $C_{17}H_{16}N_2O_6$ (%), Calcd: C, 59.30; H, 4.68; N, 8.14; found: C, 59.24; H, 4.62; N, 8.08.

*7.3.3. 3-(6-chloro-2-oxo-2H-chromen-4-yl)-3-(5-hydroxy-3-methyl-1H-pyrazol-4-yl)propanoic acid (**4c**):*

White; Yield 77%; m.p: 266-268 ^oC; IR (KBr) cm^{-1} 3338, 1727, 1711; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.81 (s, 1H, NH of Pyrazole), 11. 37 (s, 1H, OH of Propanoic acid), 7.44 (d, 1H, *J*= 2.4 Hz, C_5H of Coumarin), 7.35 (d, 1H, *J*= 8.4 Hz, C_8H of Coumarin), 7.28 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 6.36 (s, 1H, C_3H of Coumarin), 4.53 (t, 1H, *J*= 7.4 Hz, C_3H of Propanoic acid), 3.36 (s, 1H, OH of Pyrazole), 3.07 (dd, 1H, *J*= 16.4 & 8.4 Hz, C_2H of Propanoic acid), 2.97 (dd, 1H, *J*= 16.4 & 7.8 Hz, C_2H of Propanoic acid), 2.19 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.12, 161.34, 154.81, 151.92, 136.23, 133.19, 132.58, 124.96, 120.07, 117.26, 112.05, 108.78, 101.92, 35.55, 30.25, 10.31; MS (*m/z*): 348. Elemental Analysis for $C_{16}H_{13}ClN_2O_5$ (%), Calcd: C, 55.10; H, 3.76; N, 8.03; found: C, 55.06; H, 3.73; N, 7. 98.

*7.3.4. 3-(5-hydroxy-3-methyl-1H-pyrazol-4-yl)-3-(7-methyl-2-oxo-2H-chromen-4-yl)propanoic acid (**4d**):*

White; Yield 82%; m.p: 260-262 ^{o}C; IR (KBr) cm^{-1} 3375, 1719, 1710; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.05 (s, 1H, NH of Pyrazole), 11.39 (s, 1H, OH of Propanoic acid), 7.46 (d, 1H, *J*=8.4 Hz C_5H of Coumarin), 7.32 (d, 1H, *J*= 2.4 Hz, C_8H of Coumarin), 7.23 (d, 1H, *J*= 8.4 Hz, C_6H of Coumarin), 6.31 (s, 1H, C_3H of Coumarin), 4.56 (t, 1H, *J*= 7.6 Hz, C_3H of Propanoic acid), 3.37 (s, 1H, OH of Pyrazole), 3.11 (dd, 1H, *J*= 16.0 & 7.6 Hz, C_2H of Propanoic acid), 2.93 (dd, 1H, *J*= 16.0 & 8.4 Hz, C_2H of Propanoic acid), 2.24 (s, 3H, CH_3 of Pyrazole), 2.06 (s, 3H, CH_3 of Coumarin); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.22, 160.17, 155.11, 152.09, 136.21, 133.20, 132.73, 124.91, 120.81, 117.30, 112.06, 108.86, 102.12, 36.49, 30.95, 20.65, 10.36; MS (*m/z*): 328. Elemental Analysis for $C_{17}H_{16}N_2O_5$ (%), Calcd: C, 62.19; H, 4.91; N, 8.53; found: C, 62.13; H, 4.86; N, 8.46.

*7.3.5. 3-(5-hydroxy-3-methyl-1H-pyrazol-4-yl)-3-(2-oxo-2H-benzo[h]chromen-4-yl)propanoic acid (**4e**):*

Light brown; Yield 79%; m.p: 233-235 ^{o}C; IR (KBr) cm^{-1} 3364, 1727, 1712; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.79 (s, 1H, NH of Pyrazole), 11.27 (s, 1H, OH of Propanoic acid), 8.21 (d, 1H, *J*= 8.8 Hz, C_5H of Coumarin), 8.15 (d, 1H, *J*= 8.8 Hz, C_{10}H of Coumarin), 8.07 (m, 2H, C_8H and C_9H of Coumarin), 7.89 (d, 1H, *J*= 8.6 Hz, C_6H of Coumarin), 7.83 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 6.43 (s, 1H, C_3H of Coumarin), 4.50 (t, 1H, , *J*= 7.6 Hz, C_3H of Propanoic acid), 3.28 (s, 1H, OH of Pyrazole), 3.10 (dd, 1H, *J*= 16.4 & 8.4 Hz, C_2H of Propanoic acid), 2.98 (dd, 1H, *J*= 16.0 & 7.6 Hz, C_2H of Propanoic acid), 2.31 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.90, 161.17, 158.23, 153.19, 149.67, 134.24, 133.19, 132.87, 128.71, 127.89, 123.97, 121.96, 120.21, 116.92, 113.72, 108.20, 100.97, 35.79, 30.47, 10.43; MS (*m/z*): 364. Elemental Analysis for $C_{20}H_{16}N_2O_5$ (%), Calcd: C, 65.93; H, 4.43; N, 7.69; found: C, 65.88; H, 4.39; N, 7.61.

7.4. General Procedure and Spectral Characterization of compounds **9a-e:**

Compound **4** (1 mmol) in acetonitrile was charged into round bottomed flask to this $SOCl_2$ (5 mmol) was added in cold condition and the reaction mixture was refluxed on oil bath for about 3

h. The progress of the reaction was monitored by TLC, then poured into ice cold water and neutralized by $NaHCO_3$. The separated solid was filtered, washed with water and dried. The crude product obtained was purified by column chromatography on silica 60–120, eluent: EtOAc-hexane, 4:6.

*7.4.1. 4,5-dihydro-3-methyl-4-(6-methyl-2-oxo-2H-chromen-4-yl)pyrano[2,3-c]pyrazol-6(1H)-one (**9a**):*

White; Yield 85%; m.p: 215-218 ^{o}C; IR (KBr) cm^{-1} 3327, 1748, 1722; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.23 (s, 1H, NH of Pyrazole), 7.67 (s, 1H, C_5H of Coumarin), 7.40 (d, 1H, *J*= 8.4 Hz, C_8H of Coumarin), 7.28 (d, 1H, *J*= 8.4 Hz, C_7H of Coumarin), 6. 43 (s, 1H, C_3H of Coumarin), 4.60 (t, 1H, *J*= 7.6 Hz, C_4H of Pyran), 3.16 (dd, 1H, *J*= 14.8 & 6.8 Hz, C_3H of Pyran), 3.01 (dd, 1H, *J*= 14.2 & 6.7 Hz, C_3H of Pyran), 2.34 (s, 3H, CH_3 of Pyrazole), 2.06 (s, 3H, CH_3 of Coumarin); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 162.72, 160.91, 158.71, 150.24, 138.17, 134.57, 128.91, 125.73, 121.01, 120.46, 118.96, 115.98, 112.16, 34.36, 30.05, 20.37, 10.31; MS (*m/z*): 310. Elemental Analysis for $C_{17}H_{14}N_2O_4$ (%), Calcd: C, 65.80; H, 4.55; N, 9.03; found: C, 65.75; H, 4.49; N, 8.93.

*7.4.2. 4,5-dihydro-4-(6-methoxy-2-oxo-2H-chromen-4-yl)-3-methylpyrano[2,3-c]pyrazol-6(1H)-one (**9b**):*

Light green; Yield 82%; m.p: 221-224 ^{o}C; IR (KBr) cm^{-1} 3379, 1720, 1707; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.27 (s, 1H, NH of Pyrazole), 7.59 (d, 1H, *J*= 8.4 Hz, C_8H of Coumarin), 7.43 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 7.31 (s, 1H, C_5H of Coumarin), 6.48 (s, 1H, C_3H of Coumarin), 4.68 (t, 1H, *J*= 7.6 Hz, C_4H of Pyran), 3.67 (s, 3H, OCH_3 of Coumarin), 3.13 (dd, 1H, *J*= 16.8 & 7.8 Hz, C_3H of Pyran), 2.99 (dd, 1H, *J*= 16.4 & 7.6 Hz, C_3H of Pyran), 2.38 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.11, 161.03, 159.69, 154.81, 145.62, 140.23, 138.17, 123.54, 122.46, 116.83, 114.15, 111.71, 110.08, 53.79, 34.45, 29.89, 10.24; MS (*m/z*): 326. Elemental Analysis for $C_{17}H_{14}N_2O_5$ (%), Calcd: C, 62.57; H, 4.32; N, 8.59; found: C, 62.54; H, 4.29; N, 8.52.

*7.4.3. 4-(6-chloro-2-oxo-2H-chromen-4-yl)-4,5-dihydro-3-methylpyrano[2,3-c]pyrazol-6(1H)-one (**9c**):*

White; Yield 81%; m.p: 228-230 ^{o}C; IR (KBr) cm^{-1} 3403, 1722, 1711; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.33 (s, 1H, NH of Pyrazole), 7.55 (s, 1H, *J*= 2.4 Hz, C_5H of Coumarin), 7.38 (d, 1H, *J*= 8.4 Hz, C_8H of Coumarin), 7.30 (d, 1H, *J*= 8.8 Hz, C_7H of Coumarin), 6.40 (s, 1H, C_3H of Coumarin), 4.65 (t, 1H, *J*= 7.6 Hz, C_4H of Pyran), 3.11 (dd, 1H, *J*= 16.0 & 7.6 Hz, C_3H of Pyran), 3.00 (dd, 1H, *J*= 16.8 & 8.0 Hz, C_3H of Pyran), 2.38 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.63, 161.47, 159.71, 147.80, 139.19, 135.06, 132.08, 128.71, 125.01, 122.96, 122.17, 112.88, 109.14, 35.05, 28.64, 10.37; MS (*m/z*): 330. Elemental Analysis for $C_{16}H_{11}ClN_2O_4$ (%), Calcd: C, 58.11; H, 3.35; N, 8.47; found: C, 58.08; H, 3.31; N, 8.40.

*7.4.4. 4,5-dihydro-3-methyl-4-(7-methyl-2-oxo-2H-chromen-4-yl)pyrano[2,3-c]pyrazol-6(1H)-one (**9d**):*

White; Yield 84%; m.p: 198-200 ^{o}C; IR (KBr) cm^{-1} 3393, 1719, 1705; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.25 (s, 1H, NH of Pyrazole), 7.49 (d, 1H, *J*=8.4 Hz, C_5H of Coumarin), 7.38 (d, 1H, *J*= 2.4 Hz, C_8H of Coumarin), 7.25 (d, 1H, *J*= 8.4 Hz, C_7H of Coumarin), 6. 34 (s, 1H, C_3H of Coumarin), 4.62 (t, 1H, *J*= 7.6 Hz, C_4H of Pyran), 3.13 (dd, 1H, *J*= 16.4 & 8.0 Hz, C_3H of Pyran), 2.97 (dd, 1H, *J*= 16.0 & 7.6 Hz, C_3H of Pyran), 2.27 (s, 3H, CH_3 of Pyrazole), 2.03 (s, 3H, CH_3 of Coumarin); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.07, 161.31, 158.96, 148.14, 138.87, 134.83, 132.11, 128.73, 123.01, 122.46, 118.73, 115.89, 112.13, 34.26, 30.25, 20.42, 10.21; MS (*m/z*): 310. Elemental Analysis for $C_{17}H_{14}N_2O_4$ (%), Calcd: C, 65.80; H, 4.55; N, 9.03; found: C, 65.77; H, 4.51; N, 8.96.

*7.4.5. 4,5-dihydro-3-methyl-4-(2-oxo-2H-benzo[h]chromen-4-yl)pyrano[2,3-c]pyrazol-6(1H)-one (**9e**):*

Light brown; Yield 83%; m.p: 207-210 ^{o}C; IR (KBr) cm^{-1} 3412, 1723, 1712; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 11.29 (s, 1H, NH of Pyrazole), 8.31 (d, 1H, *J*= 8.4 Hz, C_5H of Coumarin), 8.23 (d, 1H, *J*= 8.8 Hz, C_{10}H of Coumarin), 8.16 (m, 2H, C_8H and C_9H of Coumarin), 8.02 (d, 1H, *J*= 8.6 Hz, C_6H of Coumarin), 7.89 (d, 1H, *J*= 8.4 Hz, C_7H of Coumarin), 6.53 (s, 1H, C_3H of Coumarin), 4.66 (t, 1H, , *J*= 7.8 Hz, C_4H of Pyran), 3.09 (dd, 1H, *J*= 16.0 & 7.8 Hz, C_3H of Pyran), 2.95 (dd, 1H, *J*= 16.4 & 7.6 Hz, C_3H of Pyran), 2.30 (s, 3H, CH_3 of Pyrazole); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.09, 161.13, 159.21, 154.24, 139.67, 135.04, 133.96, 127.47, 126.81, 126.59, 126.04, 124.27, 121.97, 120.71, 120.12, 115.72,

109.20, 34.12, 29.47, 10.33; MS (*m/z*): 346. Elemental Analysis for $C_{20}H_{14}N_2O_4$ (%), Calcd: C, 69.36; H, 4.07; N, 8.09; found: C, 69.32; H, 4.03; N, 8.02.

7.5. Biological evaluation

7.5.1 Antibacterial screening

The susceptibility of the test organisms to synthetic compounds were assessed using broth dilution assay, as minimum inhibitory concentration (MIC) Triplicates were performed for each of the standard strains.

Culture media: Brain Heart Infusion (BHI) broth Test organisms: Four microorganisms were selected for the study: *S. aureus* microbial type culture collection MTCC 12598, *E. faecalis* MTCC 35550, *E. coli* MTCC 443 and *P. aeruginosa* MTCC 25668. All microorganisms were previously sub cultured in appropriate media and under gaseous conditions to confirm their purity at 35 °C for 48 h prior to testing of the vehicles.

Inoculum preparation: The growth method or the log phase method was performed as follows. At least three to five well isolated colonies of the same morphological type were selected from an agar culture plate. Top of each colony was scooped with a loop, and the growth was transferred into a tube containing 4–5 mL of BHI broth. The broth culture was incubated at 35°C for 2–6 h until it achieved the turbidity of the 0.5 McFarland standard. The turbidity of actively growing broth culture was adjusted with broth to obtain a final turbidity optically comparable to that of the 0.5 McFarland standard, done visually by comparing the inoculum tube and the standard against a white card with contrasting black lines.

Broth dilution method: A total of 10 tubes were taken and nine dilutions of the vehicle were done with BHI for MIC. In the initial tube, only 200 μL of vehicle was added. For further dilutions, 200 μL of BHI broth was added to the next nine tubes separately. In the second tube, 200 μL of vehicle was added which already contained 200 μL of BHI broth. This was considered as 10 dilution. From the 10 diluted tube, 200 μL was transferred to the second tube to make 10 dilution. The serial dilution was repeated up to 10 dilution for each vehicle. From the maintained stock cultures of the required microorganisms, 5 μL was taken and added to 2 mL of BHI broth. In each serially diluted tube, 200 μL of the above culture suspension was added. The last tube contained only the media and the culture suspension, i.e. the negative control. The tubes were kept for incubation for 24 h at 37 °C in bacteriological incubator and observed for turbidity.

7.5.2. Anti-inflammatory activity

7.5.2.1. Egg albumin denaturation method

The mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of coumarin based pyrano[2,3-c]pyrazole compounds so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000 µg/mL and similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) °C in an incubator (Bio-technics, India) for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV-1800 Spectrophotometer) by using vehicle as blank. Aceclofenac sodium at the final concentration of (31.25, 62.5, 125, 250, 500, 1000 µg/mL) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula

% Inhibition = 100 x (Abs of control -Abs of sample) /Abs of control.

7.5.2.2. Human Red Blood Cell (HRBC) membrane stabilization method

Collection of blood samples: Human RBCs were collected for the study. 7 ml of blood was collected from healthy male human volunteers (aged 24-26 years) without a history of oral contraceptive or anticoagulant therapy and free from diseases using protocol issued by Institutional Ethics Committee. The collected RBCs were kept in a test tube with an anticoagulant EDTA under standard conditions of temperature 23±2 °C and relative humidity 55±10%.

Assay of membrane stabilization

Erythrocyte suspension: The blood was washed three times using isotonic solution (0.9% saline). The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4) which contained in 1 L of distilled water: $NaH_2PO_4 \cdot 2H_2O$, 0.26 g; Na_2HPO_4, 1.15 g; NaCl, 9 g (10 mM sodium phosphate buffer). Thus the suspension finally collected was the stock erythrocyte (RBC) suspension.

Heat-induced hemolysis: Aliquots (5 ml) of the isotonic buffer, containing 0.1 mg/mL of synthesized compounds were put into two duplicate sets of centrifuge tubes [36].The vehicle, in

the same amount, was added to another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54 °C for 20 min in a water bath. The other pair was maintained at 0-5 °C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 rpm and the absorbance of the supernatant was measured at 540 nm using UV-VIS spectrometer (SHIMADZU, UV-1800). The percentage inhibition or acceleration of hemolysis in tests and was calculated using the following equation.

$$\textbf{\% Inhibition of Hemolysis} = 100 \times 1 - [OD_2 - OD_1 / OD_3 - OD_1]$$

Where, OD_1=Test sample unheated,

OD_2=Test sample heated and

OD_3=Control sample heated.

7.5.3. Computational Studies

For the docking of ligands to protein active sites and for estimating the binding affinities of docked compounds, Surflex-Dock module, a fully automatic docking tool available on Sybyl X-2.0 version was used in this study.

Docking simulations: The X-ray Crystal Structure of *S. aureus* dihydropteroate synthetase (PDB ID:1AD4) enzyme [35] was obtained from protein data bank in PDB format as starting point. The synthesized compounds and the standard compounds tested in this study were docked to *S. aureus* dihydropteroate synthetase (PDB ID:1AD4) enzyme using Surflex-Dock programme in Sybyl software by incremental construction approach of building the structure in the active site so as to favour the binding affinity [37,38]. Finally, the docked ligands were ranked based on a variety of scoring functions that have been compiled into the single consensus score (C-score) [39].

8. Conclusions

To explore different scaffold structures, we have designed and synthesized a series of coumarin based pyrano[2,3-c]pyrazole derivatives with yields ranging from 77-94%. Most of them exhibited excellent activity toward antibacterial against gram +ve bacterial strains and also anti-inflammatory activity. Among all these synthesized scaffolds, compounds **3g** and **4b** are highly active and more potent in both biological as well as molecular docking simulation studies. These

results also suggested a new and potential route in the discovery of drug against antibacterial and anti-inflammatory activities.

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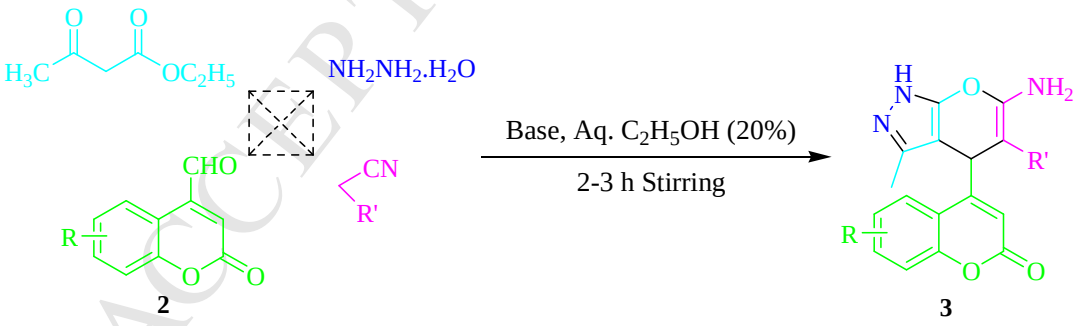
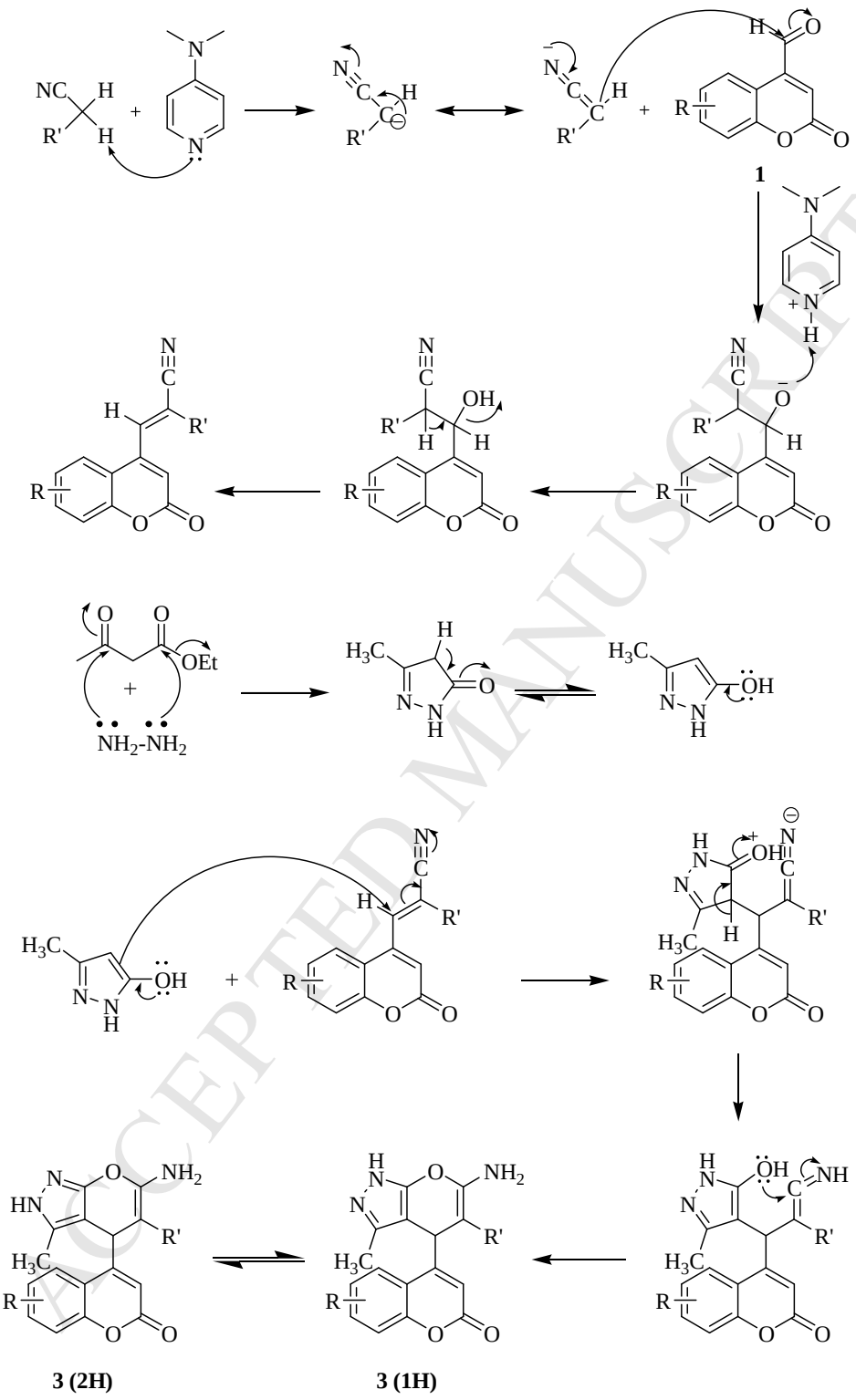
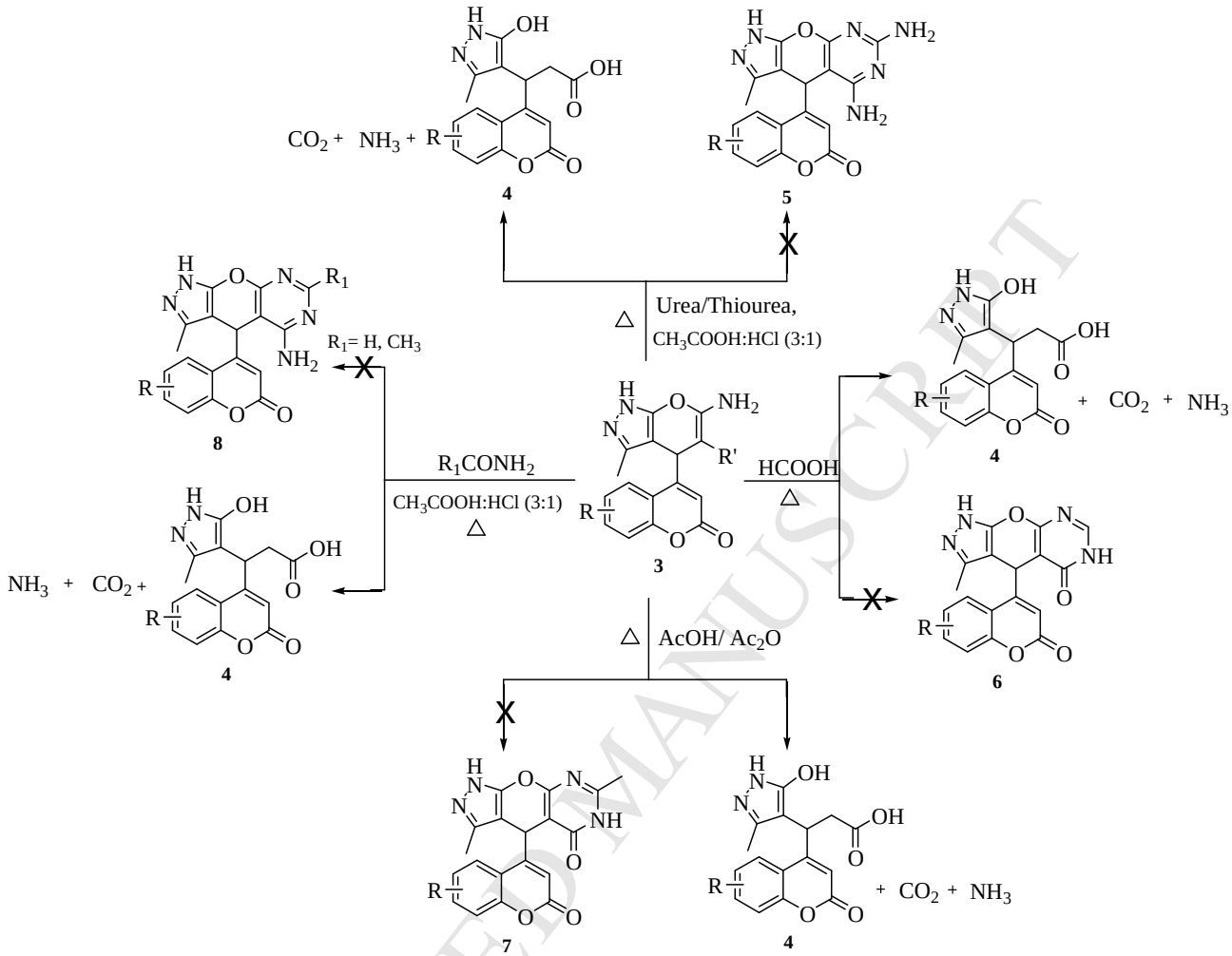


R= 6-Me, 6-OMe, 6-Cl, 7-Me, 7,8-Benzo; R'= CN, COOEt

Scheme 1: Construction of various coumarin substituted pyrano[2,3-c]pyrazoles **3a-j**

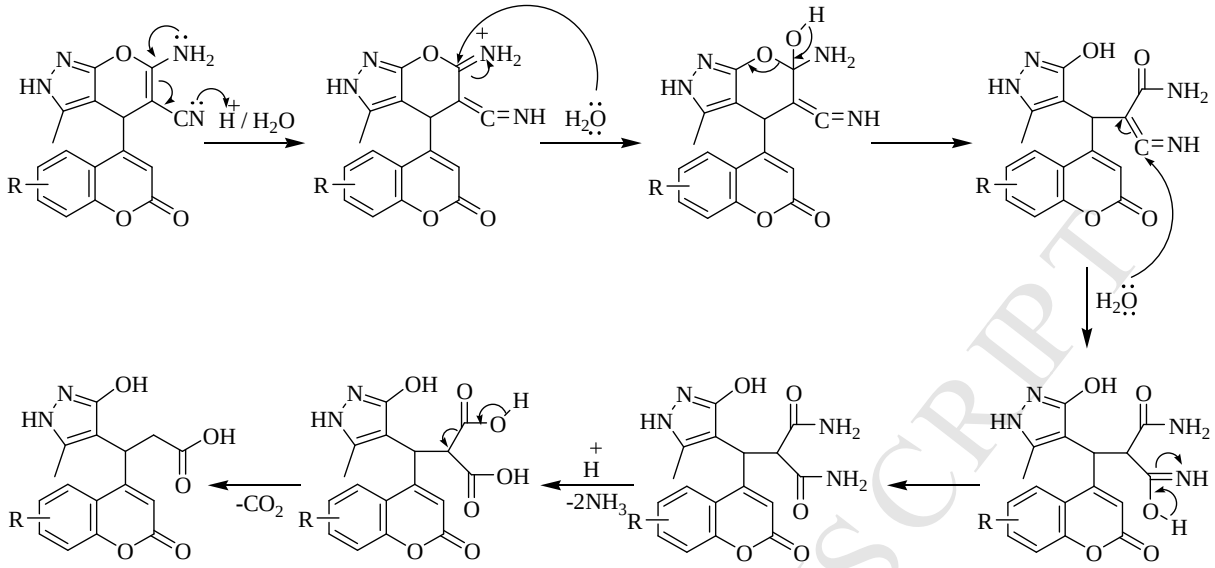


Scheme 2: Mechanism for the synthesis of 4-coumarinyl-6-amino-1,4-dihydro-3-methylpyrano[2,3-c]pyrazole-5-carbonitriles.

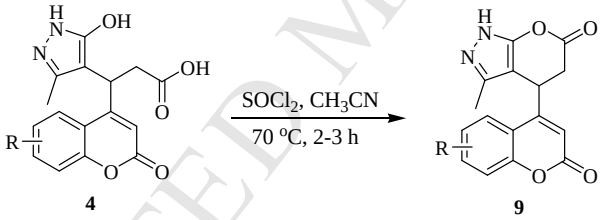


R= 6-Me, 6-OMe, 6-Cl, 7-Me, 7,8-Benzo; R'= CN, COOEt

Scheme 3: Synthesis of substituted 3-coumarinyl-3-(5-hydroxy-3-methyl-1H-pyrazole-4-yl) propanoic acid derivatives **4a-e**.



Scheme 4: Mechanism for the synthesis of substituted 3-coumarinyl-3-(5-hydroxy-3-methyl-1H-pyrazole-4-yl)propanoic acid derivatives.



R= 6-Me, 6-OMe, 6-Cl, 7-Me, 7,8-Benzo

Scheme 5: Synthesis of substituted 4,5-dihydro-3-methyl-4-(2-oxo-2H-chromen-4-yl)pyrano [2,3-c]pyrazol-6(2H)-one derivatives **9a-e**.

Table

Table 1: Optimization effect for the synthesis of pyrano[2,3-c]pyrazoles **3**:

Entry	Base	Solvent	Temp (^{o}C)	Time (h)	Yield (%)
1	Et_3N	Ethanol	RT	2.0	59
2	Et_3N	50 % Ethanol	RT	2.0	70
3	Et_3N	20 % Ethanol	RT	2.0	87
4	Et_3N	Milli-Q Water	RT	2.0	72
5	Et_3N	Milli-Q Water	75 ^{o}C	2.0	82
6	DMAP	Ethanol	RT	2.0	67
7	DMAP	50 % Ethanol	RT	2.0	78
8	DMAP	20 % Ethanol	RT	2.0	94
9	DMAP	Milli-Q Water	RT	2.0	75
10	DMAP	Milli-Q Water	75 ^{o}C	2.0	89
11	Piperidine	Ethanol	RT	2.0	53
12	Piperidine	50 % Ethanol	RT	2.0	67
13	Piperidine	20 % Ethanol	RT	2.0	80
14	Piperidine	Milli-Q Water	RT	2.0	69
15	Piperidine	Milli-Q Water	75 ^{o}C	2.0	77

Table 2: Yield and melting point of pyrano[2,3-c]pyrazole derivatives **3a-j**:

Entry	R	R'	m.p. (^{o}C)	Time (h)	Yield (%)
3a	6-Me	CN	236-238	2.0	94
3b	6-OMe	CN	204-206	2.0	91
3c	6-Cl	CN	240-242	2.0	89
3d	7-Me	CN	230-232	2.0	92
3e	7,8-Benzo	CN	270-272	2.0	90
3f	6-Me	COOEt	206-208	2.0	92
3g	6-OMe	COOEt	210-212	2.0	89
3h	6-Cl	COOEt	238-240	2.0	86
3i	7-Me	COOEt	212-215	2.0	91
3j	7,8-Benzo	COOEt	188-190	2.0	88

Table 3: Yield and melting point of substituted 3-coumarinyl-3-(5-hydroxy-3-methyl-1H-pyrazole-4-yl)propanoic acids **4a-e**:

Entry	R	m.p. (^{o}C)	Time (h)	Yield (%)
4a	6-Me	268-270	5.0	89
4b	6-OMe	256-258	5.0	80
4c	6-Cl	266-268	5.0	77
4d	7-Me	260-262	5.0	82
4e	7,8-Benzo	233-235	5.0	79

Table 4: Yield and melting point of substituted 4,5-dihydro-3-methyl-4-(2-oxo-2H-chromen-4-yl)pyrano [2,3-c]pyrazol-6(2H)-one derivatives **9a-e**:

Entry	R	m.p. (^{o}C)	Time (h)	Yield (%)
9a	6-Me	215-217	3.0	85
9b	6-OMe	221-224	3.0	82
9c	6-Cl	228-230	3.0	81
9d	7-Me	198-200	3.0	84
9e	7,8-Benzo	207-209	3.0	83

Table 5: The crystal data and structure refinement data of compound **3b.**

Identification code	**3b**	
Chemical formula	$C_{18}H_{14}N_4O_4$, H_2O	
Formula weight	368.35	
Temperature	296(2) K	
Mo$K\alpha$ radiation, λ	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1/c$	
Unit cell dimensions	a=11.4868(5) Å	α=90°
	b=13.7176(6) Å	β=99.737(3) °
	c=11.3680(5) Å	γ=90°
Volume	1765.47(13) Å ^{3}	
Z	4	

Density	1.386 g/cm^3
Absorption coefficient μ	0.104 mm^{-1}
F(000)	768.0
Crystal size	0.00 x 0.19 x 0.09 mm^3
2θ range for data collection/°	3.60 to 50.00°
Index ranges	-8 ≤ h ≤ 12, -12 ≤ k ≤ 14, -12 ≤ l ≤ 12
Reflections collected	8326
Independent reflections	2376 [Rint = 0.0360, Rsigma = 0.0358]
Data / restraints / parameters	2376/12/278
Goodness-of-fit on F^2	1.051
Final R indices [I>=2σ(I)]	R1 = 0.0665, wR2 = 0.1658
R indices (all data)	R1 = 0.0886, wR2 = 0. 1808
Largest diff. peak and hole/ e Å^{-3}	0.57/-0.23 e Å^{-3}
CCDC Number	**1463750**

Table 6: *In vitro* antibacterial activity data of compounds **3a-j**, **4a-e** and **9a-e**:

Entry	**R**	**Minimum inhibitory concentration in μg/mL (MIC)**			
		Gram +ve bacteria		Gram -ve bacteria	
		S.aureus	*E.faecalis*	*E.coli*	*P.aeruginosa*
3a	6-Me	6.25	12.5	12.5	12.5
3b	6-OMe	3.125	12.5	6.25	1.56
3c	6-Cl	3.125	6.25	3.125	12.5
3d	7-Me	6.25	12.5	0.78	3.125
3e	7,8-Benzo	6.25	12.5	25.0	25.0
3f	6-Me	3.125	6.25	12.5	6.25
3g	**6-OMe**	**1.56**	**3.125**	**6.25**	**6.25**
3h	6-Cl	3.125	6.25	6.25	50.0
3i	7-Me	3.125	25.0	3.125	12.5
3j	7,8-Benzo	3.125	12.5	6.25	6.25
4a	6-Me	3.125	6.25	12.5	25.0
4b	**6-OMe**	**0.78**	**1.56**	**3.125**	**6.25**

4c	**6-Cl**	**1.56**	**6.25**	**25.0**	**50.0**
4d	7-Me	3.125	6.25	6.25	12.5
4e	7,8-Benzo	6.25	12.5	6.25	12.5
9a	6-Me	6.25	12.5	3.125	12.5
9b	6-OMe	3.125	6.25	6.25	6.25
9c	6-Cl	6.25	12.5	6.25	12.5
9d	7-Me	6.25	6.25	12.5	6.25
9e	7,8-Benzo	12.5	12.5	12.5	6.25
Ciprofloxacin		**6.25**	**6.25**	**3.125**	**6.25**

Table 7: *In vitro* anti-inflammatory activity in protein denaturation method of compounds **3a-j**, **4a-e** and **9a-e**:

Entry	**R**	**% Inhibition of Egg Albumin in 31.25 µg/mL**	**Entry**	**R**	**% Inhibition of Egg Albumin in 31.25 µg/mL**
Control	-	-	Control	-	-
3a	6-Me	10.33	4a	6-Me	37.43
3b	6-OMe	19.13	**4b**	**6-OMe**	**63.11**
3c	6-Cl	28.04	**4c**	**6-Cl**	**42.64**
3d	7-Me	7.6	**4d**	**7-Me**	**40.59**
3e	7,8-Benzo	14.44	4e	7,8-Benzo	39.83
3f	6-Me	24.82	9a	6-Me	16.02
3g	**6-OMe**	**51.43**	9b	6-OMe	29.31
3h	6-Cl	39.06	9c	6-Cl	3.27
3i	7-Me	28.67	9d	7-Me	15.75
3j	7,8-Benzo	37.65	9e	7,8-Benzo	1.89
Aceclofenac	-	**5.50**	**Aceclofenac**	-	**5.50**

Table 8: *In vitro* anti-inflammatory activity in HRBC membrane stabilization method of compounds **3a-j**, **4a-e** and **9a-e**:

Entry	R	% Inhibition of Erythrocyte in 100 µg/mL	Entry	R	% Inhibition of Erythrocyte in 100 µg/mL
Control	-	-	Control	-	-
3a	6-Me	28.36 ± 0.12*	4a	6-Me	37.26 ±0.11**
3b	6-OMe	32.66 ± 0.11**	**4b**	**6-OMe**	**55.26 ± 0.10****
3c	6-Cl	36.06 ± 0.12**	**4c**	**6-Cl**	**50.66 ± 0.10****
3d	7-Me	28.16 ± 0.12*	**4d**	**7-Me**	**42.56 ± 0.39****
3e	7,8-Benzo	28.86 ± 0.11*	**4e**	**7,8-Benzo**	**49.56 ± 0.41****
3f	6-Me	36.06 ± 0.12**	9a	6-Me	35.93 ± 0.05**
3g	**6-OMe**	**54.06 ± 0.12****	9b	6-OMe	36.33 ± 0.05**
3h	6-Cl	39.86 ± 0.11**	9c	6-Cl	21.16 ± 0.12*
3i	7-Me	36.16 ± 0.11**	9d	7-Me	30.56 ± 0.12**
3j	7,8-Benzo	38.56 ± 0.41**	9e	7,8-Benzo	14.56 ± 0.11
Acetyl salicylic acid	**-**	**36.16 ± 0.11**	**Acetyl salicylic acid**	**-**	**36.16 ± 0.11**

Level of significance * p<0.01**=p<0.05* percent inhibition of migration was calculated relative control.

Table 9. Surflex Docking score (kcal/mol) of compounds **3a–j, 4a-e** and **9a-e** with *S. aureus* protein dihydropteroate synthetase (PDB ID: 1AD4)

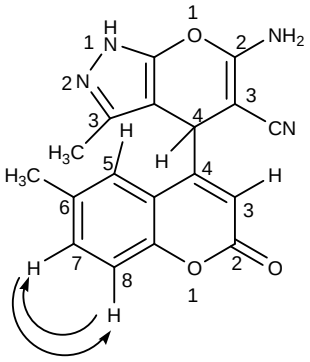
Entry	C-score[a]	Crash score[b]	Polar score[c]	D-score[d]	PMF score[e]	G-score[f]	Chem score[g]
3a	3.11	-0.86	2.85	-1620.94	-25.40	-143.64	-21.02
3b	3.68	-0.70	4.20	-1720.94	-43.25	-106.30	-21.82
3c	3.84	-0.84	4.38	-1712.43	-42.52	-112.57	-22.48
3d	2.83	-1.36	1.72	-1835.22	-70.51	-170.47	-19.91
3e	3.22	-0.60	2.40	-1759.44	-48.28	-131.50	-17.71
3f	3.71	-2.64	2.49	-2209.41	-75.46	-181.26	-26.26
3g	**5.34**	**-1.58**	**3.77**	**-1807.89**	**-51.89**	**-182.59**	**-25.66**
3h	4.09	-1.51	3.86	-1934.28	-47.96	-136.21	-21.46
3i	4.78	-3.34	2.98	-1979.48	-41.88	-209.07	-27.32
3j	4.71	-2.20	0.05	-1641.08	-26.96	-250.15	-21.15
4a	**4.25**	**-0.59**	**4.13**	**-1915.63**	**-59.44**	**-103.01**	**-20.14**
4b	**5.57**	**-1.51**	**3.93**	**-1833.46**	**-46.27**	**-167.89**	**-26.98**
4c	**5.28**	**-0.82**	**3.37**	**-1925.43**	**-25.65**	**-173.98**	**-23.43**
4d	**5.12**	**-1.09**	**3.96**	**-1788.15**	**-27.11**	**-113.69**	**-21.38**
4e	**4.83**	**-1.31**	**3.78**	**-1833.87**	**-48.37**	**-156.83**	**-17.67**
9a	3.50	-0.82	3.11	-1651.86	-31.25	-102.70	-19.89
9b	4.12	-1.53	1.98	-1436.04	-9.74	-135.67	-18.67
9c	2.55	-0.26	3.78	-1559.52	-54.15	-64.52	-19.42
9d	3.40	-0.82	1.04	-1576.38	-40.70	-148.27	-18.04
9e	2.49	-1.79	3.37	-1664.14	-55.32	-136.33	-26.03
Ciprofloxacin	**3.58**	**-1.96**	**3.32**	**-312.22**	**-47.37**	**-188**	**-24.62**

[a]C-Score (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score.
[b]Crash-score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration.
[c]Polar indicating the contribution of the polar interactions to the total score. The polar score may be useful for excluding docking results that make no hydrogen bonds.
[d]D-score for charge and van der Waals interactions between the protein and the ligand.
[e]PMF-score indicating the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF).
[f]G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies.
[g]Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term.

Table 10: Drug likeness property (Lipinski's rule of five) of compounds **3a-j**, **4a-e** and **9a-e**:

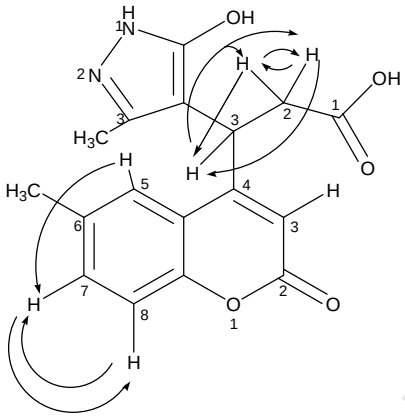
Entry	R	cLogP	Acceptor count	Donor count	Lipinski Violation	MW
3a	6-Me	2.67	7	2	0	334.33
3b	6-OMe	2.51	7	2	0	350.33
3c	6-Cl	2.89	7	2	0	354.75
3d	7-Me	2.67	7	2	0	334.33
3e	7,8-Benzo	3.35	7	2	0	370.37
3f	6-Me	3.48	8	2	0	381.39
3g	6-OMe	3.31	9	2	0	397.39
3h	6-Cl	3.69	8	2	0	401.81
3i	7-Me	3.48	8	2	0	381.39
3j	7,8-Benzo	4.15	8	2	0	417.42
4a	6-Me	1.46	7	3	0	328.32
4b	6-OMe	1.30	8	3	0	344.32
4c	6-Cl	1.45	7	3	0	348.74
4d	7-Me	1.46	7	3	0	328.32
4e	7,8-Benzo	1.91	7	3	0	364.36
9a	6-Me	1.88	6	1	0	310.31
9b	6-OMe	1.72	7	1	0	326.31
9c	6-Cl	2.10	6	1	0	330.73
9d	7-Me	1.88	6	1	0	310.31
9e	7,8-Benzo	2.56	6	1	0	346.34

Figures



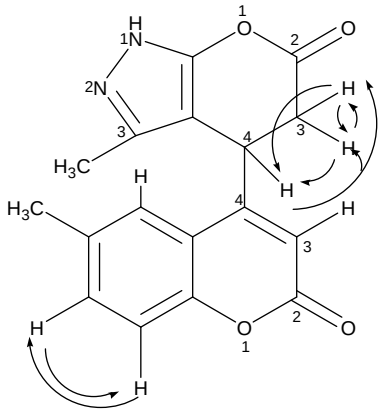
Chemical Shift in δppm		Structural information
12.25	:	Pyrazole NH
7.55 (s, 1H)	:	C_5-H of coumarin
7.41 (d, *J* = 8.8 Hz, 1H)	:	C_8-H of coumarin
7.32 (d, *J* = 8.8 Hz, 1H)	:	C_7-H of coumarin
7.15 (s, 2H)	:	NH_2 of pyran
6.34 (s, 1H)	:	C_3-H of coumarin
5.13 (s, 1H)	:	C4-H of pyran
2.28 (s, 3H)	:	C_6-CH_3 of coumarin
1.87 (s, 3H)	:	C_3-CH_3 of pyrazole

Figure 1. Protons correlations and chemical shift values of compound **3a** by 2D NMR



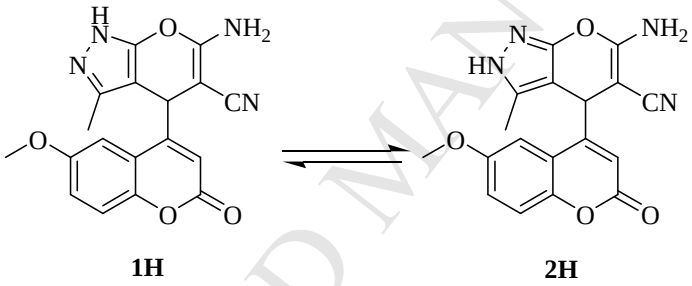
Chemical Shift in δppm		Structural information
11.26 (s, br, 2H)	:	Pyrazole NH and carboxylic acid OH
7.69 (d, *J* = 1.2Hz, 1H)	:	C_5-H of coumarin
7.38 (d, *J* = 8.4 Hz, 1H)	:	C_8-H of coumarin
7.27 (d, *J* = 8.4 Hz, 1H)	:	C_7-H of coumarin
6.41 (s, 1H)	:	C_3-H of coumarin
4.57(t, *J* =7.6 Hz, 1H)	:	C_3-H of propanoic acid
3.30 (s,br, 1H)	:	Pyrazole OH
3.08 (dd, *J* = 16.4 & 8.0 Hz, 1H)	:	C_2-H of propanoic acid
2.89 (dd, *J* =16.0 & 7.2 Hz, 1H)	:	C_2-H of propanoic acid
2.34 (s, 3H)	:	C_6-CH_3 of coumarin
2.07 (s, 3H)	:	C_3-CH_3 of pyrazole

Figure 2. Protons correlations and chemical shift values of compound **4a** by 2D NMR



Chemical Shift in δppm		Structural information
11.23 (s, br, 2H)	:	Pyrazole NH
7.67 (s, 1H)	:	C_5-H of coumarin
7.40 (d, *J* = 8.4 Hz, 1H)	:	C_8-H of coumarin
7.28 (d, *J* = 8.4 Hz, 1H)	:	C_7-H of coumarin
6.43 (s, 1H)	:	C_3-H of coumarin
4.60(t, *J* =7.6 Hz, 1H)	:	C_3-H of propanoic acid
3.28 (dd, *J* = 14.8 & 6.8 Hz, 1H)	:	C_3-H of pyran
2.88 (dd, *J* =14.2 & 6.7 Hz, 1H)	:	C_3-H of pyran
2.48 (s, 3H)	:	C_6-CH_3 of coumarin
2.06 (s, 3H)	:	C_3-CH_3 of pyrazole

Figure 3. Protons correlations and chemical shift values of compound **9a** by 2D NMR



1H **2H**

Figure 4. 1H and 2H tautomers of compound **3b**.

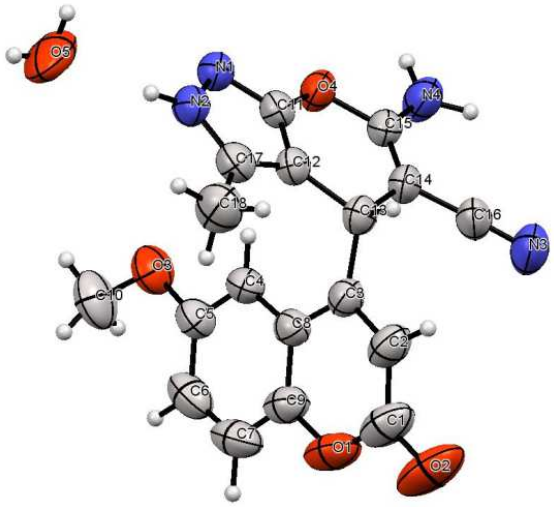


Figure 5. ORTEP diagrams of **3b** with labeling showing 50% displacement ellipsoids.

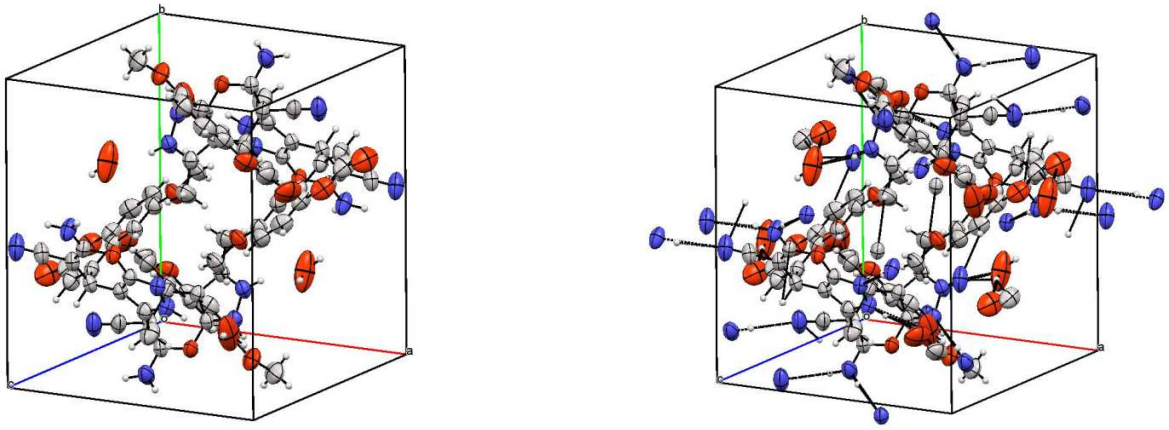


Figure 6. Packing daigram and hydrogen bonding with π....π stacking interactions of **3b**.

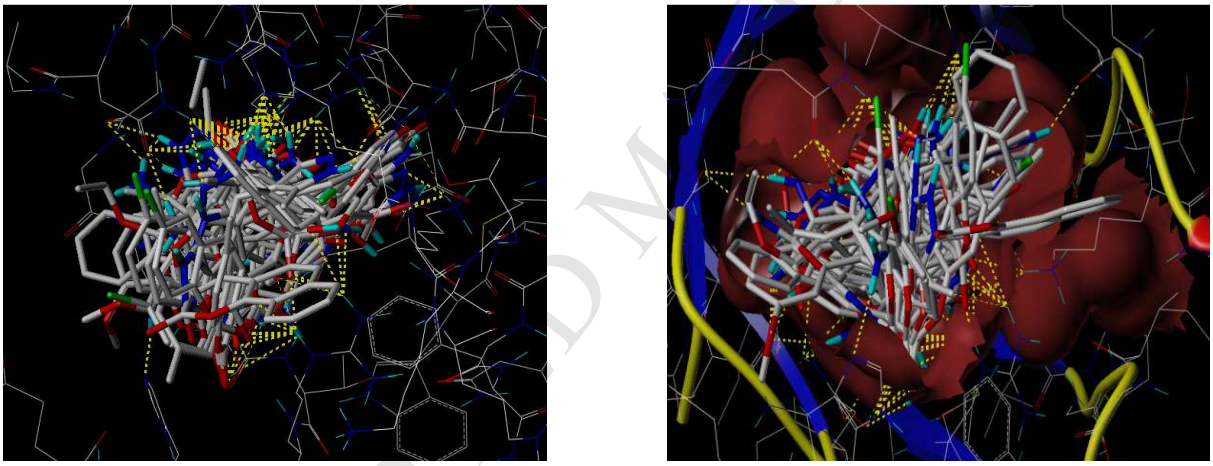
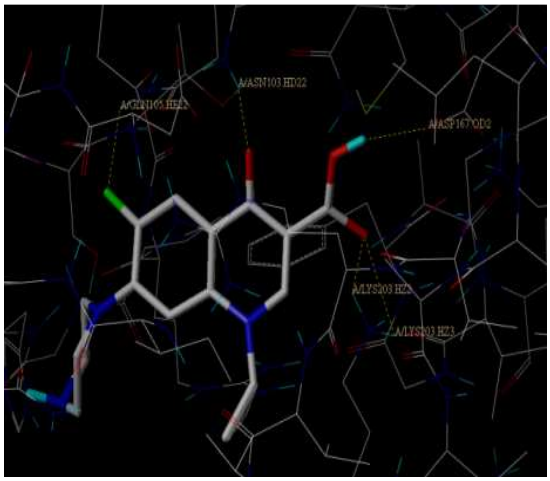


Figure 7. Docked view of all the synthesized compounds at the active site of the *S. aureus* subunit enzyme (PDB ID: 1AD4).



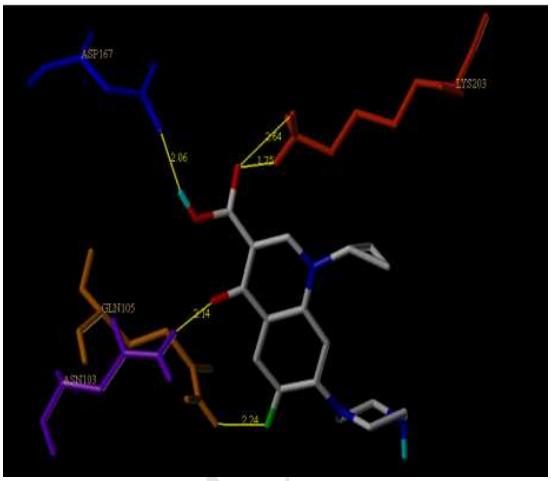
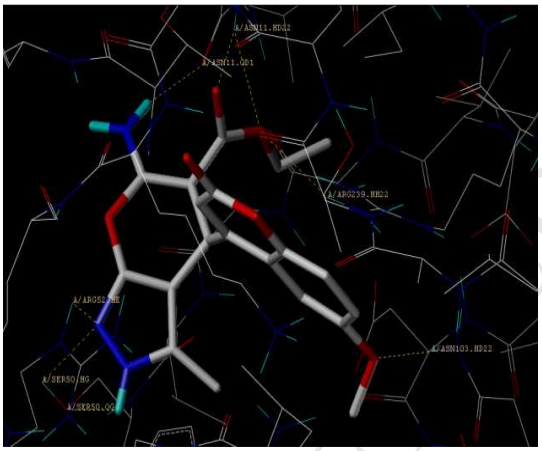
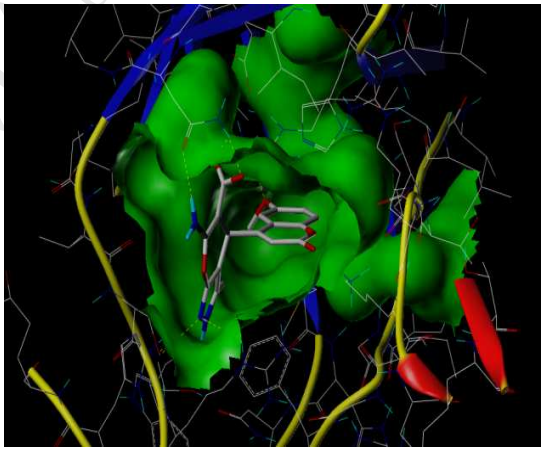
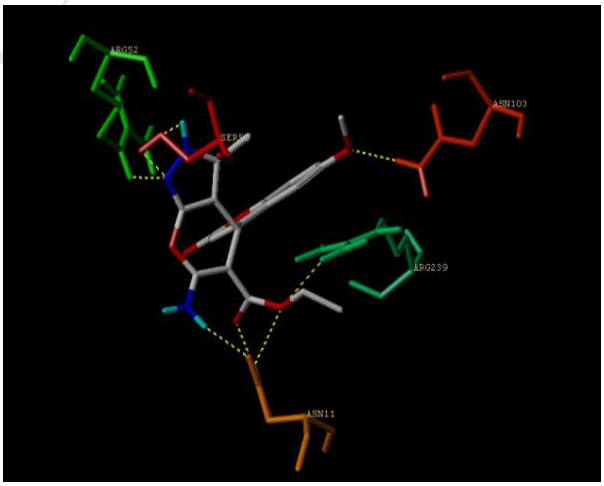


Figure 8. Binding mode of reference Ciprofloxacin in the active site of *S. aureus* protein dihydropteroate synthetase (PDB ID: 1AD4).







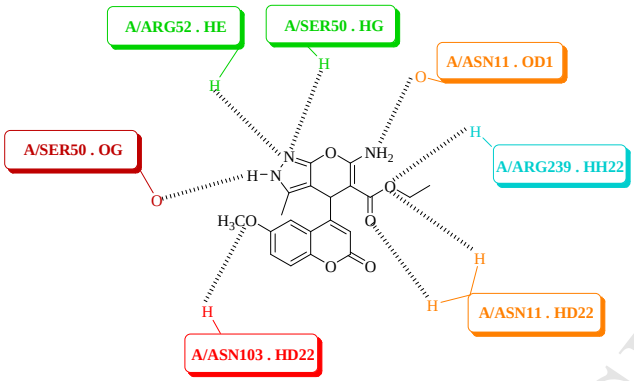
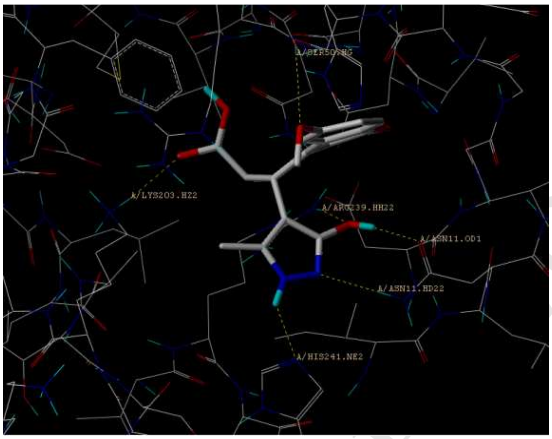
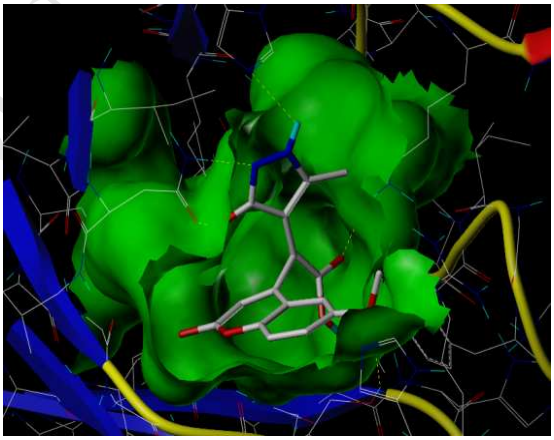
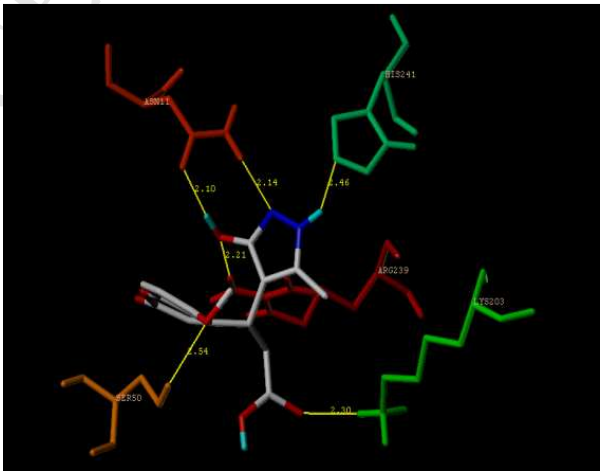


Figure 9. Docked study of the active site of the *S. aureus* subunit enzyme PDB: 1AD4 with compound **3g**, which shows the consensus score (C-score) of 5.34 and schematic representation of compound **3g** bound to the active site of the enzyme PDB: 1AD4 subunits.







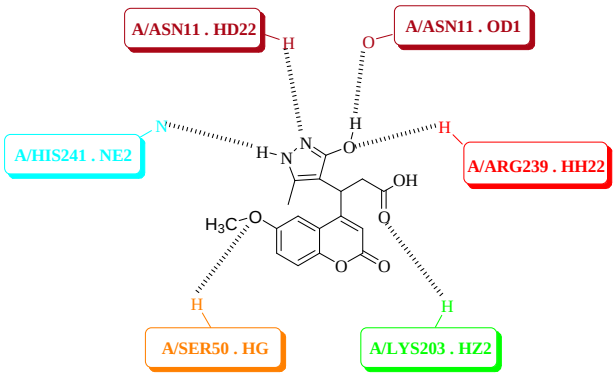


Figure 10. Docked study of the active site of the *S. aureus* subunit enzyme PDB: 1AD4 with compound **4b**, which shows the consensus score (C-score) of 5.57 and schematic representation of compound **4b** bound to the active site of the enzyme PDB: 1AD4 subunits.

Highlights of the Paper

Synthesis, characterization and molecular docking studies of substituted 4-coumarinylpyrano[2,3-c]pyrazole derivatives are potent antibacterial and anti-inflammatory agents

- One-pot four component green method have been developed and synthesized substituted coumarin based pyrano[2,3-c]pyrazole derivatives **3** with excellent yield (>90%).
- The unexpected 3-coumarinyl-3-pyrazolylpropanoic acid derivatives **4** have been isolated by the reaction of compound **3** in acidic conditions.
- The intramolecular cyclization of compound **4** to C_4-C_4 pyranone or C_4-C_4 chromon derivatives **9** have been synthesized.
- All the newly synthesized polyfunctional coumarin based pyrano[2,3-c]pyrazole derivatives are potent antibacterial and anti-inflammatory agents compared to standard.
- The molecular docking study reveals that, all newly synthesized polyfunctional coumarin based pyrano[2,3-c]pyrazole derivatives are well supported for *in vitro* antibacterial activity.
- Among all the synthesized polyfunctional coumarin derivatives, compound **3g** and **4b** are highly active and more potent in both biological (*in vitro*) as well as molecular docking simulation studies.