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Dual evaluation of some novel chalcone annulated pyrazolines as anti-inflammatory and antimicrobial agents via *in-silico* target study on cyclooxygenase-2

Thangavelu Prabha[*1], Selvaraj Jubie[2], Palanisamy Selvamani[3], Subbiah Latha[3], Thangavel Sivakumar[1]

[1]Department of Pharmaceutical Chemistry, Nandha College of Pharmacy, Koorapalayam Pirivu, Pitchandam Palayam Post, Erode-638052, Tamil Nadu, India
[2]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Ooty-643001, Tamil Nadu, India
[3]Department of Pharmaceutical Technology, Centre for Excellence in Nanobio Translational Research, Anna University, Bharathidasan Institute of Technology Campus, Tiruchirappalli-620024, Tamil Nadu, India

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ABSTRACT

A novel series of chalcone bearing pyrazoline moieties were (P1 to P7) synthesized and characterized by various analytical techniques. The anti-inflammatory studies showed the compounds P1, P2, P5, and P6 have produced the noteworthy inhibition on protein denaturation (81.39 - 96.57 %) when compared to standard 98.17% whereas, the antiproteinase activity was in the range of 84.55- 90.44 % when compared to the standard, 95.95 %. The compounds, P1, P2, P5 (2-Cl, 4- Cl & -NO_2 substituent) bearing electron-withdrawing groups and the compounds P3 & P6 (4-$N(CH_3)_2$ & -OH substituent) possessing electron-donating group in its phenyl ring system exhibited the prominent activity. Further, to explore the molecular mechanism, the *in-silico* docking study against COX-2 enzyme was performed. The compounds were also screened for their antibacterial activities. Among them, the compounds P1, P2, P3, P5 and P6 showed the significant antibacterial activity against both gram-positive and gram-negative pathogen such as, *Bacillus cereus*, *Staphylococcus aureus*, *Serratia marcescens* and *Staphylococcus typhi* with maximum zone of inhibition within the range of 12-14 mm and 19-25 mm for 100 and 200 μg/ml concentrations respectively when compared to that of standard drug Gentamycin, whose ranges between 15-18 mm and 25-28 mm correspondingly.

*Corresponding Author

Name: Thangavelu Prabha
Phone:
Email: drtpappa@yahoo.com

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INTRODUCTION

The infectious organism/pathogens growth and their multiplication will be either killed or inhibited by the antibiotics. Owing to multidrug-resistant pathogens there are many antibiotics have been questionable in current medical field (Prabha *et al.*, 2019). On the other hand, the antibiotics have both the immune-modulatory and anti-inflammatory properties (Andre, 2010). Inflammation depends on a various number of factors which reflects the response of the organism to a variety of stimuli and leads to many problems, the importance of anti-inflammatory agents cannot be overstated because of their efficacy frequently as life-saving drugs in many diseases such as cancer, diabetes, arthritis, and rheumatic fever, etc. It is well apparent that the healing property of the anti-inflammatory drug is because it offers anti-bacterial activity.

The enzyme cyclooxygenases catalyze the metabolism of arachidonic acid which causes inflammation through the formation of prostaglandins H_2 that practically affects the diverse biological processes such as regulation of immune function, and maintenance of renal blood flow, reproductive biology, and gastrointestinal integrity. This could be regulated by the COX-2 inhibitor, which possesses 1,3 aryl groups attached to the heterocyclic ring system (Singh *et al.*, 2019; Kiruthiga *et al.*, 2019). However, the production of pro-inflammatory prostaglandins could be blocked by the inhibition of prostaglandins production through COX-2 inhibitors. The inflammation process is maintained by COX-2 by stimulating the prostaglandins such as PGI2, PGF2α, PGD2, and PGE2, with a wide range of actions (Pereira *et al.*, 2013).

Moreover, during the inflammation process, a fluid buildup in the area of injury happens which leads to promoting the bacterial growth because of its increase in the vascular permeability perhaps cause edema which will support the bacterial growth by acting through nutrient media for the bacteria. The mechanism of antibacterial activity of the cox-2 inhibitors was not well understood by the researcher so far. However, it is assumed that the COX-2 inhibitors could help to reduce the bacterial infection through granulocyte function of prostaglandin inhibition. Therefore, we hypothesized that the COX-2 inhibitors might also inhibit the bacterial growth via the inhibition of inflammatory process such as prostaglandin mediated function (Madigan *et al.*, 2000; Mycek *et al.*, 2006). During the inflammatory process, the prostaglandin could elevate the cyclic adenosine monophosphate which inhibits the phosphorylation and translocation of the cytosolic subunit to the cell membrane thereby produced the NADPH oxidase mediated bacterial killing (Stables *et al.*, 2010). Besides, the results of the author Aronoff *et al.* (2004) put forward that the PGE2 inhibits macrophage host defense functions such as phagocytosis and killing against bacterial infections added the association between COX-2 inhibition with the antibacterial activity of the molecules (Aronoff *et al.*, 2005).

The several diaryl heterocyclic compounds substituted on the central heterocyclic ring have been explored as potential scaffolds for the anti-inflammatory activity (Singh *et al.*, 2019). Among the nitrogen-containing five-membered heterocycles, considerable attention has been focused on pyrazolines conjugates owing to their fascinating biological activities (Bhat and Kumar, 2017), which includes anti-tumor (Johnson *et al.*, 2007), anti-tubercular (Sabale *et al.*, 2018), anti-inflammatory (Sudeep *et al.*, 2011), anti-parasitary (Bhat *et al.*, 2009), anti-depressive and anticonvulsant (Özdemir *et al.*, 2007), antimicrobial (Milano *et al.*, 2008), antinociceptives (Singh *et al.*, 2017), antifungal (Chandrashekara *et al.*, 2017), antioxidant (Bhat and Kumar, 2018), and nitric oxide synthase inhibitors, associated with diseases such as Alzheimer's, and inflammatory arthritis (Cara *et al.*, 2009).

Via taking into account, the chalcones are considered as an excellent scaffold as key starting materials for the syntheses of different classes of nitrogen-containing heterocyclic compounds such as pyrazolines, oxazoles, isoxazoles, thiophenes, and pyrimidines, etc. (Sahoo *et al.*, 2017) These chalcone derived heterocyclics compounds possess to have a wide range of pharmaceutical importance which includes antibacterial, antifungal, antiviral, antiparasitic, antitubercular, herbicidal, fungicidal, analgesic, antioxidant, antipyretic, insecticidal, anticancer, antitumor, antidiabetic, anticonvulsant, antidepressant, and anti-inflammatory agents (Kumar *et al.*, 2009).

In this context, and because of our long-standing interest in the chemistry of the privileged chalcone annulated pyrazoline scaffold (Prabha *et al.*, 2019), the study encouraged us to further explore the pyrazoline motif as an active pharmacophore to exploit its anti-inflammatory and antibacterial property. A traditional synthesis involves the base-catalyzed aldol condensation reaction of ketones and aldehydes to give α,β-unsaturated ketones (chalcones), which undergo a subsequent cyclization reaction with hydrazines affording pyrazolines. Herein, the work describes the *in-silico* screening studies against COX-2 enzyme, which is compared with standard drug Celecoxib. The reason for selecting this Celecoxib is, it is a known selective inhibitor of COX-2 enzyme and have pyrazole moiety in it (Singh *et al.*, 2019). The various researches focused on the discovery of novel pyrazoline as a potent COX-2 inhibitor with improved therapeutic and safety consideration is an emerging state in present days. However, there are numerous examples of nitrogen-containing heterocyclic scaffold being used as an anti-inflammatory agent. To support this further, an *in-vitro* anti-inflammatory and antibacterial activities were also performed owing to Inflammation is an unspecific response of the immune system to the pathogen.

The motive to test these synthesized pyrazolines conjugates as an anti-inflammatory agent are (i) to know the efficacy of the chalcone pyrazoline hybrids (P1-P7) on antibacterial and *in-vitro* anti-inflammatory activity; (ii) To study the structure-activity relationship (SAR) of chalcone annulated pyrazolines with various substituents on the benzene core (such as -Cl, $-N(CH_3)_2$, $-NO_2$, $-OCH_3$ and -OH groups) for antibacterial and anti-inflammatory activities. (iii) To investigate the anti-inflammatory potential of the synthesized compounds targeting COX-2 enzyme through *in-silico* molecular docking studies.

MATERIALS AND METHODS

All the chemicals (Merck, Hi-Media and Sigma-Aldrich, SD Fine chem., Mumbai) in this synthesis were of AR and LR grade and were obtained and used without further purification.

Experimental section

The Thomas Hoover apparatus was used to determine the melting points in open capillaries method and the result are uncorrected. The synthesized compounds were characterized by IR spectra were recorded on Shimadzu (8300, Kyoto, Japan) in the range of 4000 cm^{-1} – 400 cm^{-1} using KBr pellet technique. The proton NMR spectra were recorded using BRUKER 300MHz NMR spectrometer using the solvent deuterated chloroform and trimethyl silane used as an internal standard. The chemical shifts (d) were recorded in parts per million (ppm) scale.

Synthesis of chalcone scaffolds

In our research, the chalcones were prepared by the reaction of an equimolar amount of acetophenone and various aromatic aldehydes with activating and deactivating groups in ethanol using a 40% NaOH solution as a catalyst to yield the various chalcone scaffold (Kiruthiga *et al.*, 2018),(Scheme 1).

Synthesis of pyrazoline conjugates

An equimolar mixture of assorted chalcones (0.02 mol), 2,4-dinitro phenylhydrazine (0.02 mol), ethanol (25 ml), followed by the addition of 3 drops of concentrated sulphuric acid and was refluxed for 6-8 hours. The completion of the reaction was monitored by TLC (Hexane: Methanol (9:1), later the mixture was cooled and poured into crushed ice to get the chalcone annulated pyrazoline conjugates as a final product. The precipitate obtained was filtered, washed and recrystallized using absolute ethanol (Prabha *et al.*, 2019; Kiruthiga *et al.*, 2018), (Scheme 1).

Substitution pattern for R= P1: 2- Chloro benzaldehyde; P2: 4- Chloro benzaldehyde; P3: P-dimethyl amino benzaldehyde; P4: 2-Chloro, 4-dimethyl amino benzaldehyde; P5: Nitro benzaldehyde; P6: Vanillin; P7: Anisaldehyde.

Biological Evaluation

Anti bacterial activity

Liquid Mueller Hinton agar media was prepared and 18 h culture of Gram-positive microorganisms such as *Bacillus cereus* (MTCC 430), *Staphylococcus aureus* (MTCC 3160), Gram-negative microorganisms such as *Serratia marcescens* (MTCC 1698) and *Staphylococcus typhi* (MTCC 1430) obtained from IMTECH, Chandigarh were used for this study. The synthesized compounds at different concentrations (100, 200 μg/ml) were dissolved respectively in DMSO and tested for their antibacterial activity (Chandrashekara *et al.*, 2017).

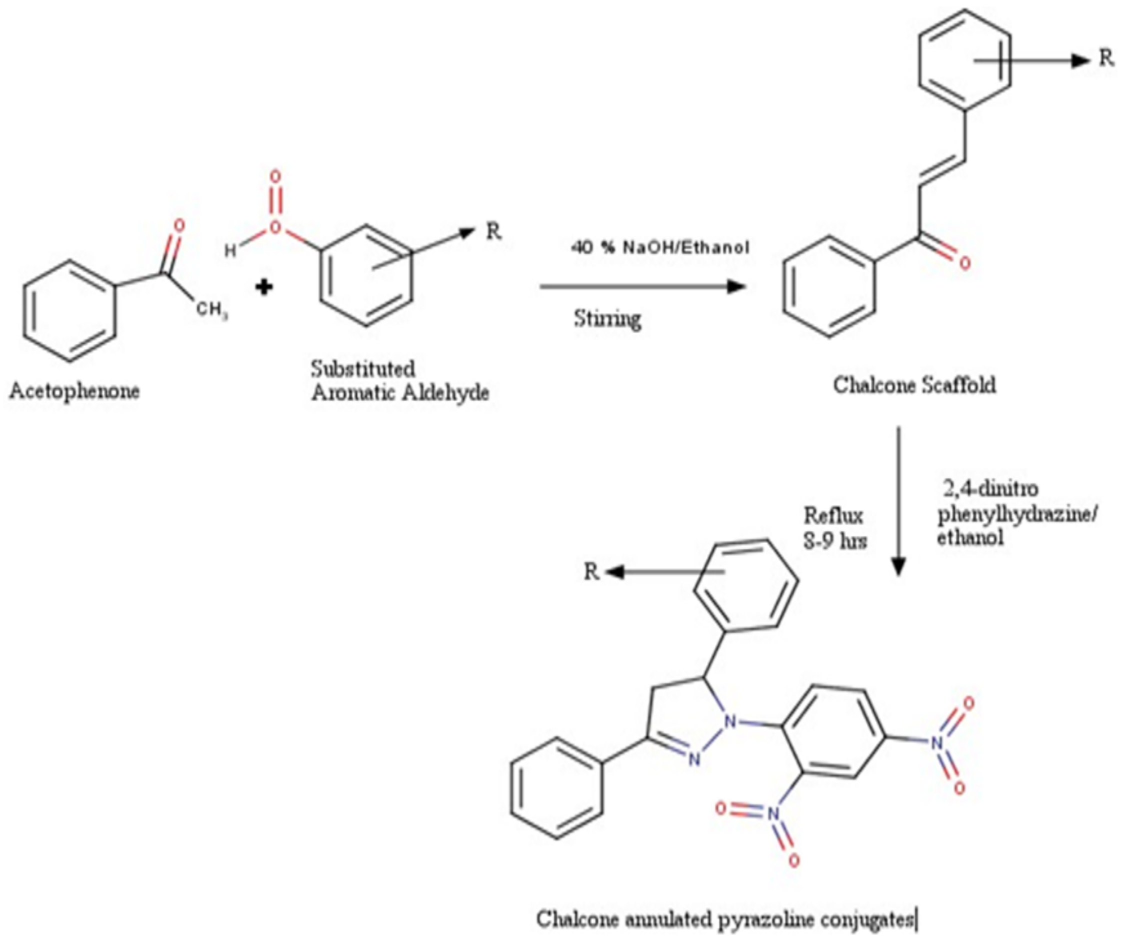
Minimum inhibitory concentration (MIC)

The MIC of synthesized compounds was determined by using serial two folds dilution method (Jubie *et al.*, 2012). A series of test tubes were prepared to contain the same volume of medium inoculated with the test organism. The decreasing concentration of drug was added to the all tubes (200, 100 & 50 μg/ml) except for the one tube which was served as a positive control for the visible growth of the microorganism. The culture was incubated at room temperature for a period of 24 h at 37^{o}C. Based on the turbidity formed, the tubes were inspected visually to determine the growth of the microorganism. The sufficient concentration of the synthesized compound to inhibit the growth was visualized as a tube with clear media.

Assessment of *in-vitro* anti-inflammatory activity

Inhibition of albumin denaturation and proteinase action

The inhibition of albumin denaturation and proteinase action were assessed through previously reported Juvekar *et al.* (2009) method. In concise, the reaction mixture consists 1ml of synthesized compounds and as well a standard drug Aspirin at various concentrations (50, 100, & 200 μg/ml). The remaining procedure was followed as per the above-said method. The protein denaturation and proteinase inhibition were calculated in term of its percentage inhibition by using the following formula. Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control.



Scheme 1: Synthetic scheme of chalcone annulated pyrazoline conjugates

In-silico evaluation

Molecular Docking Studies

The two isoforms of the membrane protein cyclooxygenase are COX-1 and COX-2. The COX-1 is mostly present in tissue and favors the physiological production of prostaglandins, whereas the COX-2 which is induced by cytokines in inflammatory cell and favors the elevated production of prostaglandins during inflammation (Gandhi *et al.*, 2017). The various intra and extracellular stimuli such as lipopolysaccharide, interleukin-1, TNF, EGF, PAF, and arachidonic acid, etc. could activate the inflammation-causing enzyme COX-2. The over-expression of COX-2 leads to metabolize the accumulation of ProstoglandinE_2 along with glucocorticoids. Both are powerful inflammation mediators. However, by targeting this COX-2 enzyme, which favorably catalyzes the first step of arachidonic acid metabolism and thereby causing inflammation.

To explore the potential putative targets of the pyrazoline conjugates as COX-2 inhibitors, a docking analysis was performed by using the Molecular Operating Environment (MOE 2009.10. Suite) software with the biological targets reported so far (Thangavelu *et al.*, 2018). The crystal 3D structure of enzyme cyclooxygenase-2 (prostaglandin synthase-2) complexed with a selective inhibitor, SC-558 (PDB code 1CX2) (Kurumbail *et al.*, 1996) was retrieved from RCSB Protein Data Bank. These PDB files were imported into MOE suite in which the receptor preparation module was used to prepare the protein. All the bound water molecules and hetero atom were removed from the complex by using sequence (SEQ) window, which is default in the MOE program. Both polar and non-polar hydrogen were added, and the 3D structure was corrected for further analysis. Further, the optimized target ligands were built in MOE followed by energy minimized; partial charges and potential energy were corrected and stored in the MOE database as .mdb file. Later the ligand was docked with the cyclooxygenase (PDB: 1CX2) protein using the molecular simulation programme of MOE. For docking simulations, the placement was set as a triangular matcher, rescoring was set as London dG, the number of retaining was set as 10, and the refinement was set as force field on molecular operating environment suite to generate 10 poses of each target ligand conformations. As a result of the docking run, the .mdb output files were created with scoring and multiple conformations of each compound. All the docked conformations were analyzed, and the best-scored pose for the ligand was selected for further interaction studies. Besides, the ligand-receptor interaction followed by the surface analysis of the selected best pose ligand molecule was generated on MOE and viewed for interpretation.

RESULTS AND DISCUSSION

Synthesis

The new series of chalcone annulated pyrazoline conjugates were synthesized and evaluated for their *in-vitro* antibacterial, anti-inflammatory followed by *In-silico* COX-2 inhibition activity. The synthesized compounds were obtained in the reasonable yield. The percentage yield and melting point of the synthesized compounds were recorded and presented uncorrected. The key reactions involved were the intermediate formation of hydrazones and subsequent addition of N-H on the olefinic (CH=CH) bond that forms the ring-closed final products of pyrazoline conjugates. In the ^{1}H-NMR, the signals of the respective protons of the final title compounds were verified based on their chemical shifts and multiplicities. The IR spectra of the compounds show the appearance of C=C (olefinic), C=N, and N-CH stretching bands at 1580–1600, 1530–1600 and 3272-3300 cm-1 respectively due to the ring closure. The previous report of ^{1}H-NMR spectra of chalcone showed an olefinic proton as appeared as doublets at about δ 6.75 and 7.18 ppm respectively. After the ring closure, the CH proton of pyrazoline showed at around δ 5.81-6.02 ppm. (Prabha *et al.*, 2019).

P1: 5-(2-chlorophenyl)-1-(2,4-dinitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole. Orange powder. Yield: 80%; mp. 190-192ºC. IR (νmax in cm-1): 1595 (Aro C=C str); 3020 (Ar-H str); 1514 (Ar-NO2); 1350 (N=O str); 3272 (N-CH); 1459 (-CH2-); 1583 (C=N); 1216 (C-C); 2900 (C-H); 798 (-Cl-). ^{1}H NMR (CDCl3, 300 MHz): 2.99-3.15 (d, 2H, -CH_2), 6.02 (1H, d, -CH), 8.43 (dd, Ar-H), 8.88 (d, Ar-H), 7.12 – 7.72 (m, 10H, Ar-H).

P2: 5-(4-chlorophenyl)-1-(2,4-dinitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole (P2): Reddish Orange powder. Yield: 85%; mp. 194-202ºC. IR (νmax in cm-1): IR(cm-1): 1583 (Aro C=C str); 3031 (Ar-H str); 1545 (Ar-NO2); 1268 (N=O str); 3278 (N-CH); 1483 (-CH2-); 1598 (C=N); 980 (C-C); 2860 (C-H); 800 (-Cl). ^{1}H NMR (CDCl3, 300 MHz): 3.07-3.13 (d, 2H, -CH2), 5.94 (1H, d, -CH), 8.43 (dd, Ar-H), 8.88 (d, Ar-H), 7.12 – 7.72 (m, 10H, Ar-H).

P3: 4-[1-(2,4-dinitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-N,N-dimethylaniline: Crystalline Black powder. Yield: 86%; mp. 212-220^{o}C. IR (νmax in cm-1): 1598 (Aro C=C str); 3010 (Ar-H str); 1567 (Ar-NO2) 1360 (N=O str); 3282 (N-CH);1469 (-CH2-); 1567 (C=N); 1280 (C-C); 2960 (C-H); 1460 (-CH3-); 1220 (N-C).^{1}H NMR (CDCl3, 300 MHz): 2.73 (s, 3H, -CH_3), 2.83-3.65 (d, 2H, -CH2), 5.81 (d, 1H, -CH), 8.43 (dd, Ar-H), 8.88 (d, Ar-H), 6.71 – 7.67 (m, 11H, Ar-H).

P4: 3-chloro-4-[1-(2,4-dinitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-N,N-dimethylaniline: Brownish black. Yield: 65%; mp. 240 ^{o}C. IR (νmax in cm-1): 1600 (Aro C=C str); 3050 (Ar-H str); 1570 (Ar-NO2); 1370 (N=O str); 3285 (N-CH);1485 (-CH2-); 1600 (C=N); 1000 (C-C); 2940 (C-H), 1470 (-CH3-); 1220 (N-C); 800 (-Cl-). ^{1}H NMR (CDCl3, 300 MHz): 2.78 (s, 3H, -CH_3), 2.77-2.92 (dd, 2H, -CH2), 5.86 (dd, 1H, -CH), 8.43 (dd, Ar-H), 8.88 (d, Ar-H), 6.59 – 7.67 (m, 9H, Ar-H).

P5: 1-(2,4-dinitrophenyl)-5-(2-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole: Brick Brown Powder. Yield: 70%; mp. 260-263 ^{o}C, IR (νmax in cm-1): 1614 (Aro C=C str); 3050 (Ar-H str); 1515 (Ar-NO2); 1330 (N=O str.); 3302 (N-CH);1485 (-CH2-); 1589 (C=N); 1000 (C-C); 1301 (C-H), 2853 (-CH2-); 2920 (NO_2). ^{1}H NMR (CDCl3, 300 MHz): 3.08-3.19 (d, 2H, -CH2), 6.14 (d, 1H, -CH); 7.53-8.03 (4H, m, Ar-H); 7.42 -7.67 (5H, m, CH=C-Ar); 8.41 (1H, dd, Ar-H); 8.88 (1H, d, Ar-H).

P6: 4-[1-(2,4-dinitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl]-2-methoxyphenol: Reddish brown powder. Yield: 80%; mp. 198-200 ^{o}C. IR (νmax in cm-1): 1600, ~1500, (Aro C=C str); 3050-3000 (Ar-H str); 1545 (Ar-NO2); 1348 (N=O str); 3300 (N-CH);2860, 1480 (-CH2-); 1530 - 1550 (C=N); 1100 (C-C); 2950 (C-H); 1250-1070 (-OCH3, C-O-C); 1230 (C-O), 3700 (Ar-OH); 1430 (-CH3-) 1526 (NO_2); 1319 (NO_2). ^{1}H NMR (CDCl3, 300 MHz): 2.86-3.06 (d, 2H, -CH2), 3.79 (s, 3H, -CH3), 5.88 (d, 1H, -CH), 7.67 (d, 1H), 6.98 (d, 1H), 7.79- 8.98 (m, 9H, Ar-H), 5.39 (s, 2H, -OH).

P7: 1-(2,4-dinitrophenyl)-5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole: Brick Red powder. Yield: 70%; mp. 222-230 ^{o}C. IR (νmax in cm-1): 1580 (Aro C=C str); 3050 (Ar-H str); 1555, 1368 (Ar-NO2, N=O str); 3298 (N-CH);1457 (-CH2-); 1560 (C=N); 986 (C-C); 2850 (C-H); 1250-1070 (-OCH3, C-O-C); 1050 (C-O). ^{1}H NMR (CDCl3, 300 MHz): 2.84-3.04 (d, 2H, -CH2), 3.74 (s, 3H, -CH3), 5.84 (d, 1H, -CH), 7.14–7.22 (m, 4H, Ar-H), 7.34–7.37 (m, 3H, Ar-H), 7.52 (m, 1H, Ar-H), 7.63–7.67 (m, 2H, Ar-H), 8.13

Table 1: Antibacterial activity (zone of inhibition in mm) of synthesized compounds

Compound Code	Gram-positive Bacteria				Gram-negative Bacteria			
	B.subtilis (μg/ml)		*S.aureus* (μg/ml)		*S.marcescens* (μg/ml)		*S.typhi* (μg/ml)	
	100	200	100	200	100	200	100	200
PI	14	25	11	24	13	22	11	19
P2	12	22	11	20	10	19	10	20
P3	13	25	09	19	10	16	09	18
P4	07	13	-	-	-	06	05	08
P5	10	22	12	19	09	18	13	17
P6	12	19	09	14	11	16	-	15
P7	07	12	-	-	-	-	-	06
Gentamycin	16	28	18	28	16	24	15	25

Table 2: Minimum inhibition concentration (MIC) of pyrazoline derivatives and standard drugs (Gentamycin) (unit, μg/ml)

Compound Code	Gram-positive Bacteria		Gram-negative Bacteria	
	B.subtilis (μg/ml)	S.aureus (μg/ml)	S.marcescens (μg/ml)	S.typhi (μg/ml)
P1	50	50	50	50
P2	50	50	200	200
P3	50	200	100	200
P4	200	NA	NA	100
P5	50	50	50	50
P6	50	100	200	200
P7	200	NA	NA	NA
Gentamycin	50	50	50	50

(d, 1H, CH-), 8.36 (d, 2H, Ar-H).

Result of biological evaluation

Antibacterial study

The close survey of antibacterial efficacy indicated that the inhibition values of all these compounds exhibited a varied range of zone of inhibition against bacterial strains. Among the all seven synthesized compounds, the compounds P1, P2, P3, and P6 showed the significant antibacterial activity against both gram-positive and gram-negative pathogen with maximum zone of inhibition i.e. about within the range of 12-14 mm and 19-25 mm for 100 and 200 (μg/ml) concentrations respectively when compared to that of standard gentamycin, whose value lies between in the range of 15-18 mm and 25-28 mm for 100 and 200 (μg/ml) concentrations respectively, (Table 1).

The two factors influence the Minimal Inhibitory Concentration (MIC) of pyrazoline viz, the rate of penetration into the bacterial cell and its inhibitory activity of DNA gyrase. The compounds were screened for antimicrobial activity against gram-positive and gram-negative bacterial strains (*B.subtilis S.aureus*, *S.marcescens, and S.typhi*) by twofold serial dilution method. (Table 2). Along with the screened compounds viz. P1, P2 & P5 (2-Cl, 4- Cl & -NO2 substituent) (Bahare and Ganguly, 2014) bearing deactivating group i.e. electron-withdrawing groups and the compounds P3 & P6 (4-dimethylamino & -OH substituent) (Awale *et al.*, 2013) bearing strongly activating group i.e. electron-donating group in its phenyl ring system exhibited the prominent antibacterial activity showed good MIC value of 50 μg/ml against both gram-positive and negative pathogen while compared to that of standard drug gentamycin. In general, halogenation improved the antibacterial activity and also due to highly electronegative, and oxidizing potential of chlorine atom leads to the development of bleaches and disinfectants. Further, it indicates the presence of chloro group on the phenyl ring is necessary for the enhanced activity than the nitro group in the ring (P5) (Abdel-Rahman *et al.*, 2007).

Table 3: Effect of synthesized compounds on in-vitro anti-inflammatory activity

Compound Code	Protein Denaturation (% Inhibition)			Antiprotease (% Inhibition)		
	50μg/ml	100 μg/ml	200 μg/ml	50 μg/ml	100 μg/ml	200 μg/ml
P1	47.94	68.49	96.57	47.79	72.42	89.33
P2	36.30	65.63	90.86	41.91	68.75	87.86
P3	32.87	44.74	81.39	35.29	44.48	70.95
P4	20.77	46.68	67.12	29.04	41.91	64.33
P5	34.70	67.12	84.81	37.13	55.51	84.55
P6	46.80	61.98	85.73	36.97	64.33	90.44
P7	15.41	36.52	63.24	19.85	37.13	54.04
Aspirin	49.08	70.50	98.17	42.04	70.95	95.95

Whereas the compounds P4 & P7 showed the modest activity against bacterial infection and the MIC value was 200 μg/ml, and with no activity with this concentration level, this is might be the presence of electron-donating atoms in its phenyl ring system. However, the compound P6 is a phenolic aldehyde possess electron-withdrawing group, whose antibacterial activity has been used in the elimination of pathogens (Bezerra *et al.*, 2017).

In-vitro Anti-inflammatory study

Inflammation is one of the body's most important mechanisms for protecting itself against danger. The anti-inflammatory activity also done owing to inflammation is an unspecific response of the immune system to pathogens, such as assault by bacteria. Infection with pathogenic microbes often results in a significant inflammatory response. Based on this declaration, a microbial infection will cause fluid accumulation in the injured/infected site and leads to inflammation and swelling. By considering the above discussion, an *in-vitro* anti-inflammatory activity was also done. By the application of external stress such as acid, alkali, and heat, etc. the protein loses its tertiary and secondary structure along with their biological function and thereby cause inflammation condition (Deattu *et al.*, 2012). As a part of the investigation on the mechanism of the anti-inflammation activity of the synthesized compound, an additional ability to inhibit the protein denaturation was performed. Besides this, there is an equilibrium between proteinases enzyme in tissue injury, and their inhibitors play a major role in the maintenance of tissue reliability. The inflammatory cells possess the serine proteinases including neutrophils are concerned in diverse inflammatory condition (Hiemstra, 2002) whereas, the leukocytes proteinase during the inflammatory process develop the tissue damage which is significantly protected by the proteinase inhibitors (Leelaprakash and Dass, 2011).

In accordance with this, all the synthesized compounds were evaluated for their protein denaturation activity, the compounds P1, P2, P3, P5 and P6 (50, 100, and 200 μg/ml) showed the significant inhibition in the range of 81.39% - 96.57% while compared to the standard drug aspirin whose inhibition range was 98.17% at 200 μg/ml concentration. The compound P1 & P2 exhibited the highest anti-inflammatory activity among the synthesized compounds compared to that of standard. (Table 4) Whereas, the proteinase inhibitory activity of the synthesized compounds viz. P1, P2, P3, and P6 showed the significant inhibition in the range of 84.55%- 90.44% when compared to the standard drug aspirin whose inhibition range was 95.95% at 200 μg/ml concentration. The compound P1 & P6 exhibited the highest proteinase inhibitory activity among the synthesized compounds compared to that of standard (Table 3).

In-silico evaluation

Results of the docking simulation study

All the docked conformations for the synthesized compounds were found with the most favorable docking poses by means of a maximum number of interactions were ranked by the highest score based on the least binding energy (Table 4). The most favorable docking poses of the 10 docked conformations for each molecule were analyzed for further investigation of the ligand interactions within the active sites. The four ligands viz. P1, P2, P5, and P6 bearing electron-withdrawing groups showed a proper binding pattern and anchored tightly inside the active site canyon (Site I) of the protein. The 2D ligand-protein interactions were visualized for all the compounds were shown in Figure 1 a, Figure 1 b, Figure 1 c, and Figure 1 d. The synthesized compounds have the highest binding affinity

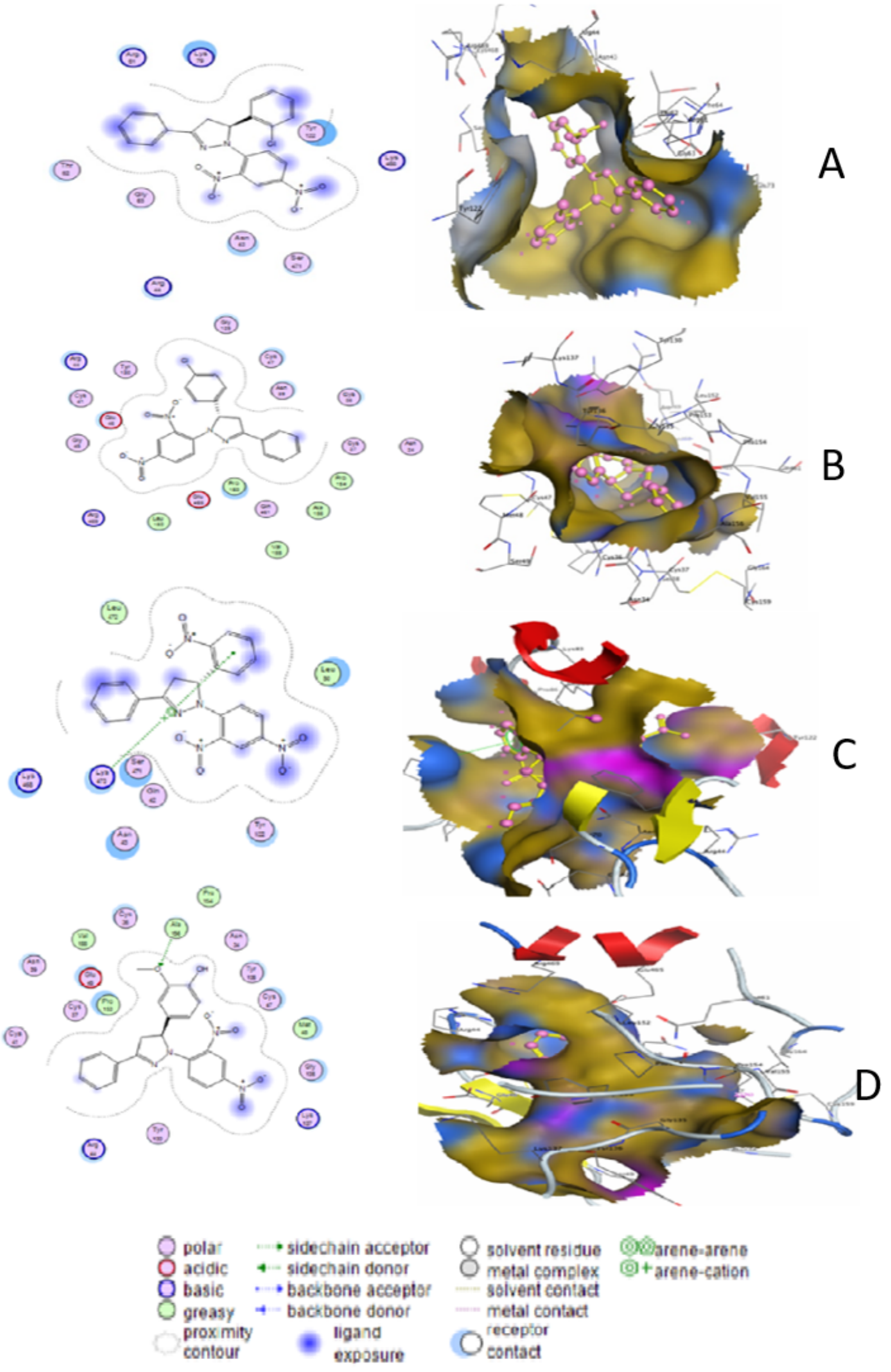


Figure 1: A) LigandReceptor interaction and the binding surface of compound P1 with 1CX2; B) Ligand Receptor interaction and the binding surface of compound P2 with 1CX2; C) Ligand Receptor interaction and the binding surface of compound P5 with 1CX2; D) Ligand Receptor interaction and the binding surface of compound P6 with 1CX2

Table 4: Docking results for chalcone annulated pyrazoline conjugates with protein PDB: 1CX2

Compound Code	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	No. of conf.
P1	-21.4108	3.6576	132.5225	-79.638	-8.9568	-21.4108	10
P2	-10.9936	1.5223	150.5308	-92.3434	-11.0864	-10.9936	10
P3	-	-	-	-	-	-	-
P4	-	-	-	-	-	-	-
P5	-21.7304	1.6544	155.6521	-57.2142	-9.4255	-21.7304	10
P6	-22.4665	2.8879	138.5503	-108.8473	-11.5503	-22.4665	10
P7	-	-	-	-	-	-	-
Celecoxib	-25.8794	2.7206	56.9731	-59.3707	-11.0932	-25.8794	10

S - The final score, rmsd_refine- The root means square deviation between the pose before refinement and the pose after refinement, E_conf- The energy of the conformer. E_place - Score from the placement stage, E_score1-Score from the rescoring stage(s), E_refine-Score from therefinement stage and No. of conf- number of conformations generated byligand

with the receptors, in the narrow range of binding energy for the protein 1CX2 is -10.9936 to -22.4665 kcal/mol: London dG was -8.9568 to -11.5503 kcal/mol when compared to the standard drug celecoxib was -11.0932kcal/mol.

Further, the top docked confirmation of this compound depicted a greater alignment with the native ligand pose. In the compound P6, the Ala 156 acts as sidechain acceptor whereas in compound P5, the Lys 473 connected with an aromatic ring through arene-cation interaction. The number of conformations generated by molecule was 10, which indicate that flexibility is an important parameter for the ligand to dock deeply within the binding pocket of the COX-2 enzyme. The lowest docking score for molecule P6 was -22.4665, which indicate compound is active at this energy of conformation. Further a careful calculation of surface analysis of the binding pocket of this molecule indicated that the compounds P1, P2, P5 and P6 adopted the position in a hydrophobic cage surrounded by the following amino acids residue such as, Pro 153, Glu 45, Met 45, Cys 47, Gly 135, Lys 137, Tyr 136, Asn 34, Ala 156 Val 155, Arg 153, glu 465, Leu 80, etc. and these were approach closely to the ligand for strong interactions.

The preface SAR of the chalcone annulated pyrazoline conjugates reveals that compounds are possessing electron-withdrawing groups such as halogen, nitro atoms in its aromatic ring favor better activity (P1, P2, & P5). This is maybe due to its high electronegativity and the presence of a lone pair of an electron with this substitution (Mujahid *et al.*, 2015).

CONCLUSION

The new series of chalcone annulated pyrazoline conjugates were synthesized and evaluated for their antibacterial, anti-inflammatory followed by *in-silico* cyclooxygenase inhibitory activity. The chief reactions concerned with the chalcone intermediate formation followed by the addition of N-H bond on the olefinic (CH=CH) center discovered the ring closed pyrazoline conjugates. An *in-silico* docking study revealed that, among the seven target compounds, viz. P1, P2, P5, and P6 molecules showed the best docking score with the Cox-2 enzyme when compared to the standard drug score celecoxib. Hence, by introducing the various heterocyclic ring, more polar analogs, deactivating and activating groups in its phenyl system might lead to produce the novel drug course for many infectious diseases.

REFERENCES

Abdel-Rahman, A. A. H., Abdel-Megied, A. E. S., Hawata, M. A. M., Kasem, E. R., Shabaan, M. T. 2007. Synthesis and Antimicrobial Evaluation of Some Chalcones and Their Derived Pyrazoles, Pyrazolines, Isoxazolines, and 5,6-Dihydropyrimidine-2-(1H)-thiones. *Monatshefte Für Chemie - Chemical Monthly*, 138(9):889–897.

Andre, G. B. 2010. Immuno-modulation and anti-inflammatory benefits of antibiotics: The example of tilmicosin. *Canadian Journal of Veterinary Research*, 74(1):1–10.

Aronoff, D. M., Canetti, C., Peters-Golden, M. 2004. Prostaglandin E 2 Inhibits Alveolar Macrophage Phagocytosis through an E-Prostanoid 2 Receptor-Mediated Increase in Intracellular Cyclic AMP. *The Journal of Immunology*, 173(1):559–565.

Aronoff, D. M., Canetti, C., Serezani, C. H., Luo, M., Peters-Golden, M. 2005. Cutting Edge: Macrophage Inhibition by Cyclic AMP (cAMP): Differential Roles of Protein Kinase A and Exchange

Protein Directly Activated by cAMP-1. *The Journal of Immunology*, 174(2):595–599.

Awale, A. G., Gholse, S. B., Utale, P. 2013. Synthesis and Antimicrobial Evaluation of some novel Schiff bases derived from Benzothiazole derivative. *International Journal of Scientific & Engineering Research*, 4(6):1972–1979.

Bahare, R. S., Ganguly, S. 2014. Effect of Electronegative Groups on the. *Antimicrobial Activity*, 3(1):112–119. 5- Disubstituted Thiazolidine-2,4-diones. Anti-Infective Agents.

Bezerra, C. F., Camilo, C. J., Nascimento, M. K., Freitas, T. S., Ribeiro-Filho, J., Coutinho, H. D. M. 2017. Vanillin selectively modulates the action of antibiotics against resistant bacteria. *Microbial Pathogenesis*, 113:265–268.

Bhat, A. R., Athar, F., Azam, A. 2009. New derivatives of 3,5-substituted-1,4,2-dioxazoles: Synthesis and activity against Entamoeba histolytica. *European Journal of Medicinal Chemistry*, 44(2):926–936.

Bhat, K. I., Kumar, A. 2017. Synthesis and Biological Evaluation of Some Novel Pyrazoline Derivatives Derived from Chalcones. *Research Journal of Pharmacy and Technology*, 10(5):1344–1344.

Bhat, K. I., Kumar, A. 2018. Pyrazolines as potent antioxidant agents. *Research Journal of Pharmacy and Technology*, 11(5):1978.

Cara, L. C. L., Camacho, M. E., Carrión, M. D., Tapias, V., Gallo, M. A., Escames, G., Entrena, A. 2009. Phenylpyrrole derivatives as neural and inducible nitric oxide synthase (nNOS and iNOS) inhibitors. *European Journal of Medicinal Chemistry*, 44(6):2655–2666.

Chandrashekara, R., Jisha, M. S., Merugumolu, V. K., Kumar, H. 2017. Synthesis, Antibacterial and Antifungal Evlaution of Novel Pyrazoline Derivatives. *Research Journal of Pharmacy and Technology*, 10(5):1481–1484.

Deattu, N., Narayanan, N., Suseela, L. 2012. Evaluation of anti-inflammatory and antioxidant activities of polyherbal extract by in vitro methods. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 3(4):727–732.

Gandhi, J., Khera, L., Gaur, N., Paul, C., Kaul, R. 2017. Role of Modulator of Inflammation Cyclooxygenase-2 in Gammaherpesvirus Mediated Tumorigenesis. *Frontiers in Microbiology*, 8(538).

Hiemstra, P. S. 2002. Novel roles of protease inhibitors in infection and inflammation. *Biochemical Society Transactions*, 30(2):116–120.

Johnson, M., Younglove, B., Lee, L., Leblanc, R., Holt, H., Hills, P., Lee, M. 2007. Design, synthesis, and biological testing of pyrazoline derivatives of combretastatin-A4. *Bioorganic & Medicinal Chemistry Letters*, 17(21):5897–5901.

Jubie, S., Prabitha, P., Kumar, R. R., Kalirajan, R., Gayathri, R., Sankar, S., Elango, K. 2012. Design, synthesis, and docking studies of novel ofloxacin analogues as antimicrobial agents. *Medicinal Chemistry Research*, 21(7):1403–1410.

Juvekar, A., Sakat, S., Wankhede, S., Juvekar, M., Gambhire, M. 2009. Evaluation of antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata. *Planta Medica*, 75(09):146–155.

Kiruthiga, N., Prabha, T., Selvinthanuja, C., Srinivasan, K., Sivakumar, T. 2018. Multidocking studies on 2-phenyl-4h-chromen-4-one hybrid and evaluation of anti-diabetic activity. *Journal of Pharmaceutical Sciences and Research*, 10(12):3018–3024.

Kiruthiga, N., Prabha, T., Selvinthanuja, C., Srinivasan, K., Sivakumar, T. 2019. Synthesis, biological evaluation, and docking study of novel 2-phenyl-1- benzopyran-4-one derivatives - as a potent cyclooxygenase-2 inhibitor. *Asian Journal of Pharmaceutical and Clinical Research*, 12(3):304–310. SE-Original Article(s)).

Kumar, S., Bawa, S., Drabu, S., Kumar, R., Gupta, H. 2009. Biological Activities of Pyrazoline Derivatives -A Recent Development. *Recent Patents on Anti-Infective Drug Discovery*, 4(3):154–163.

Kurumbail, R. G., Stevens, A. M., Gierse, J. K., Mcdonald, J. J., Stegeman, R. A., Pak, J. Y., Stallings, W. C. 1996. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*, 384(6610):644–648.

Leelaprakash, G., Dass, S. M. 2011. In vitro anti-inflammatory activity of methanol extract of Enicostemma axillare. *International Journal of Drug Development and Research*, 3(3):189–196.

Madigan, M. T., Martinko, J. M., Brock, T. D. 2000. *Brock biology of microorganisms*. Prentice HillInc, USA. Microbial growth control. 9th edition.

Milano, J., Oliveira, S. M., Rossato, M. F., Sauzem, P. D., Machado, P., Beck, P., Bonacorso, H. G. 2008. Antinociceptive effect of novel trihalomethyl-substituted pyrazoline methyl esters in formalin and hot-plate tests in mice. *European Journal of Pharmacology*, 581(1-2):86–96.

Mujahid, M., Yogeeswari, P., Sriram, D., Basavanag, U. M. V., Díaz-Cervantes, E., Córdoba-Bahena, L., Muthukrishnan, M. 2015. Spirochromone-chalcone conjugates as antitubercular agents: synthesis, bio evaluation and molecular modeling studies. *RSC Advantages*, 5(129):1–15.

Mycek, M. J., Harvey, R. A., Champe, P. 2006. Anti-inflammatory drugs, In: Lippincott's llustrated reviews. *Lippincott Williams and Wilkins, USA*, pages 401–420. 2nd edition.

Özdemir, Z., Kandilci, H. B., Gümüşel, B., Çalış, Ü., Bilgin, A. A. 2007. Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. *European Journal of Medicinal Chemistry*, 42(3):373–379.

Pereira, P. A. T., Trindade, B. C., Secatto, A., Nicolete, R., Peres-Buzalaf, C., Ramos, S. G., Faccioli, L. H. 2013. Celecoxib Improves Host Defense through Prostaglandin Inhibition during Histoplasma capsulatum Infection. *Mediators of Inflammation*, pages 1–11.

Prabha, T., Aishwaryah, P., Manickavalli, E., Chandru, R., Arulbharathi, G., Anu, A., Sivakumar, T. 2019. A Chalcone Annulated Pyrazoline Conjugates as a Potent Antimycobacterial Agents: Synthesis and in Silico Molecular Modeling Studies. *Research J. Pharm. and Tech*, 12(8):3857–3865.

Sabale, P., Bhagwat, D., Sabale, V. 2018. Synthesis and Anti-Tubercular Activity of Substituted Phenylpyrazole having Benzimidazole Ring. *Research Journal of Pharmacy and Technology*, 11(8):3599–3608.

Sahoo, B. M., Rajeswari, M., Jnyanaranjan, P., Binayani, S. 2017. Green Expedient Synthesis of Pyrimidine Derivatives via Chalcones and Evaluation of their Anthelmintic Activity. *Indian Journal of Pharmaceutical Education and Research*, 51(4s):700–706.

Singh, G., Kaur, R., Chandel, P., Singla, N., Kumar, A. 2019. Rationally Synthesized Coumarin Based Pyrazolines ameliorates carrageenan induced inflammation through COX-2/Pro-inflammatory cytokine inhibition. *MedChemComm*, 10(3):1–19.

Singh, K., Kumari, S., Gupta, Y. K. 2017. Synthesis and Antimicrobial Activity of New Pyrazoles and Chalcones Derived from Cyclic Imides. *Research Journal of Pharmacy and Technology*, 10(12):4479–4479.

Stables, M. J., Newson, J., Ayoub, S. S., Brown, J., Hyams, C. J., Gilroy, D. W. 2010. Priming innate immune responses to infection by cyclooxygenase inhibition kills antibiotic-susceptible and -resistant bacteria. *Blood*, 116(16):2950–2959.

Sudeep, S., Tathagata, D., Somila, K., Jyothi, Y. 2011. Microwave Assisted Synthesis of Fluoro-Pyrazole Derivatives for Antiinflammatory Activity. *Research J. Pharm. and Tech*, 4(3):413–419.

Thangavelu, P., Cellappa, S., Thangavel, S. 2018. Synthesis, evaluation and docking studies of novel formazan derivatives as an enoyl-acp reductase inhibitors. *International Journal of Pharmacy and Pharmaceutical Sciences*, 10(8).