International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Design and Synthesis of Pyrazoline Derivatives As Anti-Inflammatory Agents

Sonu Singh[1], Dr. Praveen Kumar[2], Dr. Nasiruddin Ahmad Farooqui[3], Madhukar Prabhash[4]

[1,2,3]Translam Institute of Pharmaceutical Education & Research, Meerut U.P. India.
[4]Bhavdiya institute of pharmaceutical sciences and research Ayodhya U.P. India.

ABSTRACT:
The study involved the synthesis and evaluation of several derivatives of 4-(5-phenyl-1H-pyrazol-3-yl) phenol to determine their biological activity. The spectrum analysis validated the structural alterations made to improve the pharmacological characteristics. The compounds underwent molecular docking studies to forecast their binding affinity with the 1B17 protein, a significant target for anti-inflammatory illnesses. The docking tests were conducted using Schrodinger software, and the findings revealed that many derivatives displayed substantial binding interactions with crucial active site residues of the 1B17 protein. An analysis was conducted on the binding modes, which uncovered significant H bonding and π-π stacking interactions, among others, that play a role in stabilizing the ligand-protein complex. Out of all the artificially created molecules, the derivative with the highest docking score was determined to be a potential contender for further advancement. These findings indicate that derivatives of 4-(5-phenyl-1H-pyrazol-3-yl) phenol have the potential to be used as therapeutic drugs that target the 1B17 protein. Further biological evaluations should be conducted to study this.

KEYWORDS: Synthesis, Organic reaction, inflammation, molecular Docking Pyrazoline.

1. INTRODUCTION

Inflammation, often known as the inflammatory process, is a concept that originates from the field of medical rather than engineering. Since the early 1800s, experimental medical research has placed significant emphasis on it. Adam of McGill University was the pioneer in comprehensively covering inflammation in medical textbooks, with separate sections specifically focused on this topic. In the present day, there are now dedicated textbooks and conference proceedings that focus only on the subject of inflammation. The investigation and analysis of inflammatory events mostly take place in the microcirculation. Benjamin W. Zweifach, a trailblazing physiologist in the field of microvascular research and a key figure in the establishment of contemporary bioengineering at the University of California, aimed to create innovative quantitative techniques for investigating inflammation.**[1].** After becoming a member of a group is reported, he actively worked towards achieving this objective. The outcome of his endeavors led to the establishment of a formal partnership between the fields of medicine, effectively creating bioengineering as a structured academic field at UCSD. The citation is from the research paper authored by **[2].**

From a Medicinal perspective, an "inflammatory cascade" could provide a more thorough

understanding of inflammation, which is characterized by sudden pain, heat, redness, swelling, and eventual tissue repair with scar formation. This idea describes a series of responses and biological mechanisms that promote tissue healing. It may be used to a number of situations, including small wounds on the skin, the healing of burn injuries, and the recuperation of postnatal tissue.**[3]** Arterioles and venules widen, blood arteries become more permeable, and blood flow increases as part of the inflammatory cascade that occurs at the tissue and cellular levels. This is followed by a reduction in blood flow and clot formation, white blood cell infiltration, plasma leakage, oxygen radical-induced tissue degradation, apoptosis, phagocytic cell elimination of waste, synthesis of new cell growth regulators, and the regeneration of new connective and functional tissue. **[4]** The resolution of inflammation is the term used to describe the last stage of inflammation. If the process of inflammation does not end, it can result in the malfunction of organs and ultimately, mortality. Almost every tissue possesses its predetermined inflammatory cascade that adheres to a standard pattern. This cascade is the sole recognized mechanism by which tissue may regenerate itself following an injury, rendering it fundamental to medical reasoning. The inflammatory cascade encompasses intriguing cellular processes, including chemotaxis, phagocytosis, mitosis, and cell differentiation. **[5]**

Inflammation is the immune system's reaction to harmful spurs, e.g. infections, toxins, injured cells, or radiation. It operates by removing these harmful elements and commencing the healing process, making it a crucial defence mechanism for sustaining optimal health.**[6]** Cellular and molecular mechanisms successfully stop damage or infection at the outset of inflammation. This stage of the healing process aids in the resolution of acute inflammation and the restoration of tissue homeostasis. Acute inflammation may become a chronic illness and cause a variety of long-lasting inflammatory illnesses if it is not well managed. **[7]**

Tissue inflammation manifests as redness, swelling, heat, pain, and reduced function. This is the outcome of localised immune, vascular, and inflammatory cellular reactions to infection or damage.Alterations in blood artery permeability, leukocyte mobilisation and accumulation, and inflammatory agent production are all important microcirculatory events. **[8]**

Inflammation can be triggered by several events such as infection, tissue injury, or heart attacks, which result in damage to tissues. Inflammation can arise from either infectious or non-infectious factors. Upon tissue injury, the body initiates a cascade of chemical signals that stimulate healing responses in the affected areas. These signals guide white blood cells towards the injured areas, where they secrete cytokines that initiate inflammatory responses. **[9]**

1.1 Pyrazoline

Pyrazolines and their derivatives, which possess many functional groups, hold significant importance in the field of medicine and have been extensively investigated. They are employed for the treatment of tumours, bacterial infections, fungal infections, viruses, parasites, TB, and pests. Certain chemicals in question also possess anti-inflammatory, antidiabetic, analgesic, and anaesthetic properties. In addition, they have the ability to selectively impede Nitric Oxide Synthase and obstruct Cannabinoid CB1 receptors.**[10]** A popular method for synthesising these chemicals involves the reaction of aromatic ketones and aldehydes in the presence of a base. These compounds undergo a reaction with hydrazine to produce 2-pyrazolines. Hydrazones are produced as intermediate compounds in this process and can be converted into 2-pyrazolines by utilising a cyclizing agent such as acetic acid.**[11]** In recent times, there has been a significant amount of scientific research dedicated to the investigation of 2-pyrazolines that possess different aryl groups connected to them, as evidenced by numerous scientific investigations. The

preceding section of the study examined recent progress in the field of pyrazolines and their applications in the field of medicine. The last part of the presentation discussed pyrazoline patents filed with the WIPO and the United States Patent and Trademark Office (USPTO). **[12,13]**

1.2 Chemistry of pyrazoline

Pyrazolines are 5-membered heterocyclic compounds holding two nitrogen atoms at adjacent positions (N1 & N2) & three carbon atoms. The core structure of pyrazoline can exist in three isomeric forms: 1-pyrazoline, 2-pyrazoline, and 3-pyrazoline, with 2-pyrazoline being the most common and widely studied. Among these, 2-pyrazoline exhibits significant biological and chemical properties, making it highly attractive for research. **[14]**

Structure and Stability

Pyrazolines are typically synthesized from α, β-unsaturated carbonyl compounds (chalcones), and hydrazine or its derivatives. The resulting pyrazoline ring is relatively stable due to the resonance between the nitrogen atoms, but its reactivity can be influenced by the nature of the substituents attached to the ring. **[15,16]**

Key Properties

- Tautomerism: Pyrazoline can exist in equilibrium between keto and enol forms, depending on the substitution pattern, temperature, and solvent.
- Basicity: The nitrogen atoms confer basic properties to the compound, and they can participate in hydrogen bonding or act as nucleophiles in various reactions.
- UV-Vis Absorption: Pyrazoline derivatives often show strong absorption in the UV-visible region, which makes them useful in fluorescence applications. **[17]**

Synthesis

Reacting chalcones (α,β-unsaturated ketones) with hydrazine or its substituted derivatives is the most used method for creating pyrazoline derivatives:

1. Pyrazoline is produced when chalcones and hydrazine hydrate react cyclically.
2. The chalcone is first nucleophilically added using hydrazine, and then the reaction is cycled. **[18]**

Chemical Reactions

Pyrazolines undergo a wide range of chemical reactions, including:

Oxidation: Pyrazolines can be oxidized to form pyrazoles, a related heterocyclic compound with an aromatic ring.

Substitution: Electrophilic substitution reactions can occur at carbon atoms in the ring, depending on the substituents present.

Reduction: In the presence of reducing agents, pyrazolines may be reduced to their corresponding hydrazines. **[19]**

Biological and Pharmacological Significance

Derivatives of pyrazoline show a wide range of biological functions, such as::

- Antimicrobial
- Antioxidant
- Antitumor
- Anti-inflammatory**[20,21,22]**

Due to these properties, pyrazolines have garnered interest in medicinal chemistry for drug development.

2. MATERIALS AND METHOD

2.1 Chemical substances and liquids used in the experiment

The compounds utilised for experimental purposes were obtained from various commercial chemical suppliers, including. Sigma Aldrich, HiMedia, CDH, Rankem, TCI, and Merck are the companies mentioned.

2.2 Melting point- The melting point of the synthesised chemical was evaluated using the open capillary method.

2.3 Solubility- The solubility characteristics of the synthesised chemical were assessed by employing different solvents.

2.4 TLC- The after-the reaction was observed by TLC with a mobile phase containing a mixture of N-hexane and EtAoC in the UV chamber.

2.5 NMR- The 1H NMR spectra was acquired using a Bruker and Jeol 400 MHz equipment, with deuterated DMSO and CDCl3 as solvents. The internal standard employed was Tetramethylsilane (TMS).

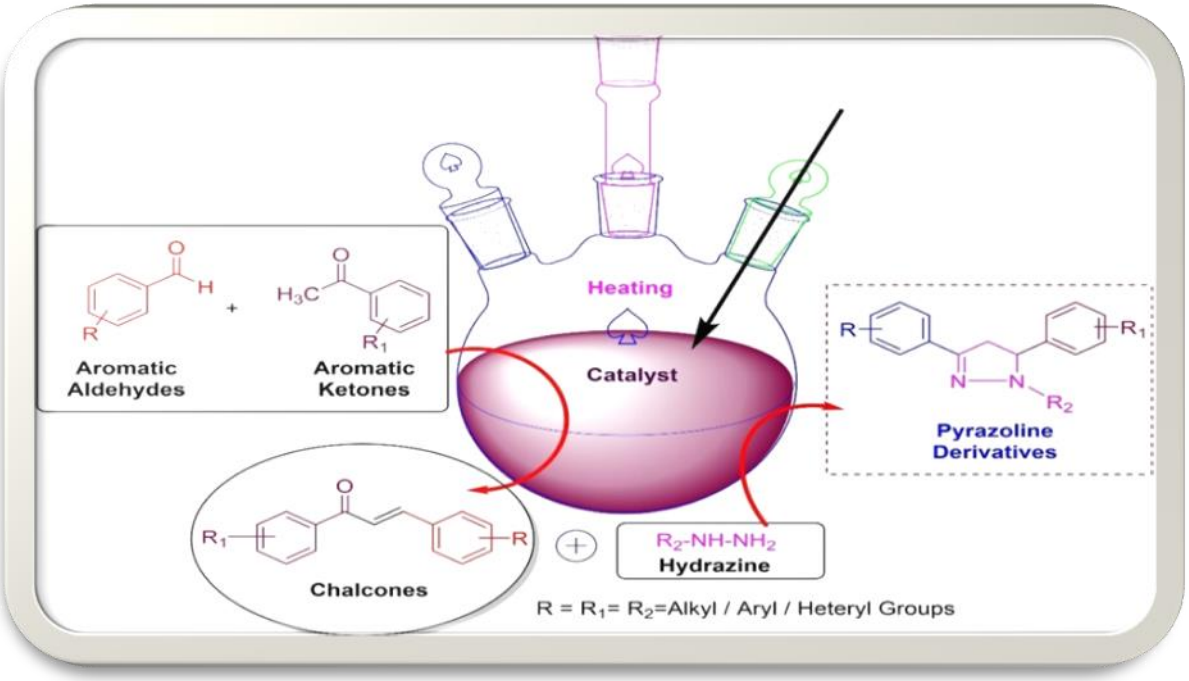
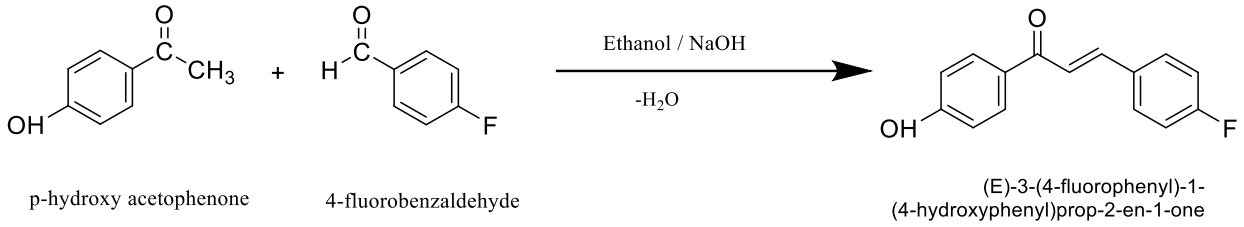


Figure 1 Techniques and equipment

1A. Synthesis of 3-(4-fluorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

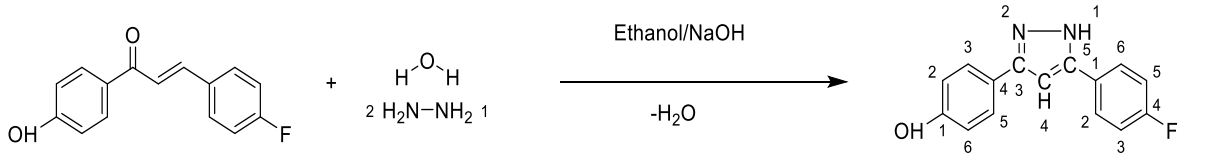
The p-hydroxy acetophenone (1 mmol, 136 mg) and substituted 4-fluorobenzaldehyde (1 mmol, 124 mg) were dissolved in ethanol. A little quantity of NaOH was introduced into the mixture, and the resultant mixture stirred at rt for a period of 8 hours. The progress of the reaction was observed using TLC. The product was identified via a UV chamber. The mobile phase was made up of n-hexane and ethyl acetate as a solvent system. The combination that was left over after the reaction was finished was put into extremely cold water. The resultant solid material was isolated by filtration and subsequently underwent a drying procedure prior to purification by recrystallization using ethanol. (1N HCl was used to neutralise the reaction mixture in the event that no precipitate formed.)



p-hydroxy acetophenone 4-fluorobenzaldehyde (E)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

2A. Synthesis of 4-(5-(4-fluorophenyl)-1H-pyrazol-3-yl) phenol derivative

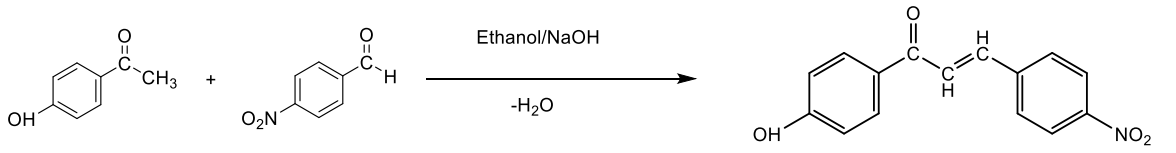
Hydrazine (1.96 ml) was gradually added to a sol^{n} of chalcone (1 mmol, 0.06 g) in ethanol in a RBF. The Rxn mixture underwent reflux at a temperature of 118 oC for a period of 72 hours in an oil bath. The progress of the Rxn observed using thin-TLC. The product was identified by the use of UV-light, employing a solvent solution containing n-hexane and ethyl acetate. After the reaction was completed, the mixture was put into water that had been cooled to a very low temperature. The solid obtained was further isolated by filtering, dried, and then subjected to recrystallization using ethanol.



(*E*)-3-(4-fluorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one hydrazine hydrate 4-(5-(4-fluorophenyl)-1*H*-pyrazol-3-yl)phenol

1B. Synthesis of 3-(4-nitrophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

The p-hydroxy acetophenone (1 mmol, 136 mg) and substituted 4-Nitrobenzaldehyde (1 mmol, 186 mg) were mixed in ethanol. A little quantity of NaOH was introduced into the mixture, and the resultant mixture was stirred at rt for a period 8 hours. The progress of the reaction was observed using TLC. The product was identified via a UV chamber. The mobile phase was made up of n-hexane and ethyl acetate as a solvent system. The combination that was left over after the reaction was finished was put into extremely cold water. The resultant solid material was isolated by filtration and subsequently underwent a drying procedure before purification by recrystallization using ethanol. (1N HCl was used to neutralise the reaction mixture if no precipitate formed.)

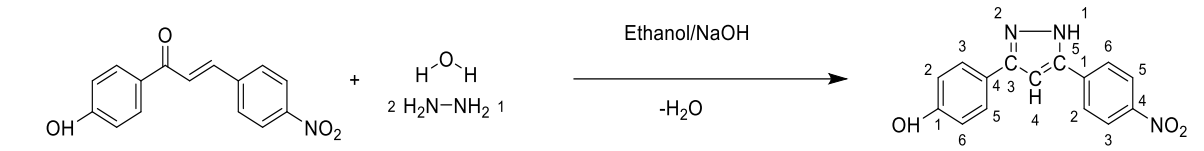


1-(4-hydroxyphenyl)ethan-1-one 4-nitrobenzaldehyde (*E*)-1-(4-hydroxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one

2B – For 4-(5-(4-Nitrophenyl)-1H-pyrazol-3-yl) phenol

In a round bottom flask, hydrazine (1.96 ml) was added dropwise to a Chalcone (1 mmol, 0.06 g) mixed in ethanol. The reaction mixture was subjected to reflux at a temperature of 118 ºC for 72 hours in an oil bath. The Rxn was observed using TLC, and the result was made visible under UV light using a solvent solution consisting of n-hexane and ethyl acetate. Upon the conclusion of the reaction, the reaction mixture was transferred into H_2O that had been chilled to a very low temperature. The resulting solid

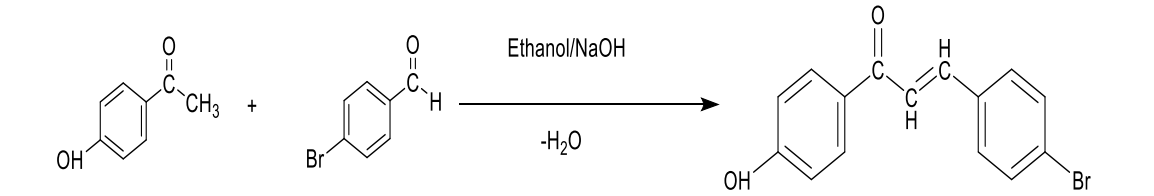
was separated from the mixture using filtration, then dried and subjected to recrystallization using ethanol.



(*E*)-1-(4-hydroxyphenyl)-3-(4-nitrophenyl)prop-2-en-1-one — hydrazine hydrate — 4-(5-(4-nitrophenyl)-1*H*-pyrazol-3-yl)phenol

3A. Synthesis of 3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

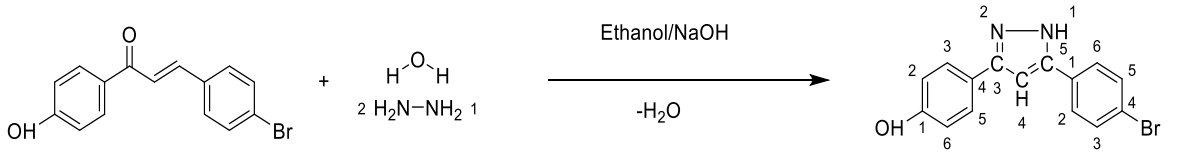
Ethanol was used to dissolve the modified 4-bromobenzaldehyde (1 mmol, 185 mg) and p-hydroxy acetophenone (1 mmol, 136 mg). The mixture was mixed with a small amount of NaOH and let to stand at room temperature for eight hours. TLC was used to monitor the reaction's development. The product was identified via a UV chamber. The mobile phase was made up of n-hexane and ethyl acetate as a solvent system. The combination that was left over after the reaction was finished was put into extremely cold water. The resultant solid material was isolated by filtration and subsequently underwent a drying procedure prior to purification by recrystallization using ethanol. (1N HCl was used to neutralise the reaction mixture in the event that no precipitate formed.)



1-(4-hydroxyphenyl)ethan-1-one — 4-bromobenzaldehyde — (*E*)-3-(4-bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

3B. Synthesis of 4-(5-(4-Bromophenyl)-1H-pyrazol-3-yl) phenol derivative

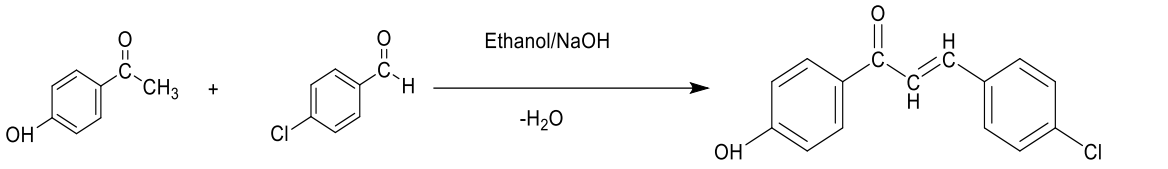
Dropwise additions of hydrazine (1.96 ml) and Chalcone (1 mmol, 0.06 g) dissolved in ethanol were made in a round-bottom flask. Reflux was applied to the reaction mixture in an oil bath for 72 hours at a temperature of 118 ºC. The Rxn was observed using TLC, and the result was made visible under UV light using a solvent solution consisting of n-hexane and ethyl acetate. Upon the conclusion of the reaction, the reaction mixture was transferred into H_2O that had been chilled to a very low temperature. The resulting solid was separated from the mixture using filtration, then dried and subjected to recrystallization using ethanol.



(*E*)-3-(4-bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one — hydrazine hydrate — 4-(5-(4-bromophenyl)-1*H*-pyrazol-3-yl)phenol

4A. Synthesis of 3-(4-Chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

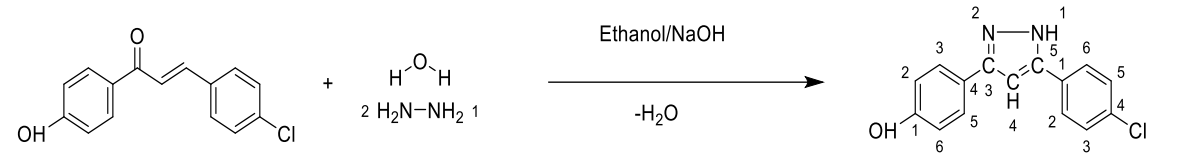
The p-hydroxy acetophenone (1 mmol, 136 mg) and substituted 4-Chlorobenzaldehyde (1 mmol, 140 mg) were dissolved in ethanol. A little quantity of NaOH was introduced into the mixture, and the resultant mixture was stirred at room temperature for a period 8 hours. The progress of the reaction was observed using TLC. The product was identified via a UV chamber. The mobile phase was made up of n-hexane and ethyl acetate as a solvent system. The combination that was left over after the reaction was finished was put into extremely cold water. The resultant solid material was isolated by filtration and subsequently underwent a drying procedure before purification by recrystallization using ethanol. (1N HCl was used to neutralize the reaction mixture in the event that no precipitate formed.)



1-(4-hydroxyphenyl)ethan-1-one 4-chlorobenzaldehyde (*E*)-3-(4-chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

4B. 4-(5-(4-Chlorophenyl)-1H-pyrazol-3-yl) phenol derivative:

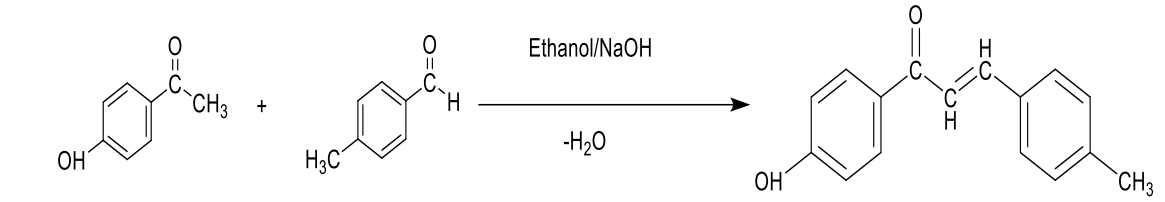
In a round bottom flask, hydrazine (1.96 ml) was added dropwise to a solution of Chalcone (1 mmol, 0.06 g) in ethanol. The reaction mixture was subjected to reflux at a temperature of 118 ºC for 72 hours in an oil bath. The progress of the reaction was observed using thin-layer chromatography (TLC), and the product was made visible using UV light with a solvent solution consisting of n-hexane and ethyl acetate. Upon finishing the reaction, the reaction mixture was transferred into water that had been chilled to a very low temperature. The resulting solid was separated by filtration, dried, and then purified by recrystallization using ethanol.



(*E*)-3-(4-chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one hydrazine hydrate 4-(5-(4-chlorophenyl)-1*H*-pyrazol-3-yl)phenol

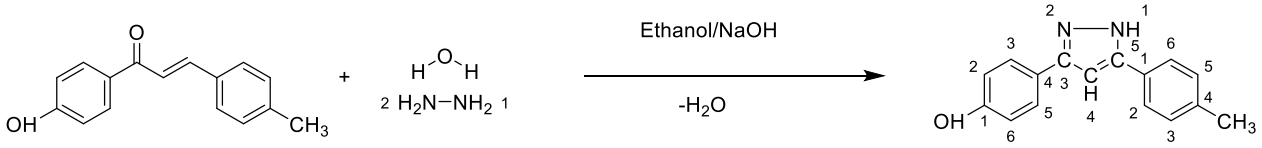
5A. Synthesis of 3-(4-hydroxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

The p-hydroxy acetophenone (1 mmol, 136 mg) and substituted 4-methylbenzaldehyde (1 mmol, 120 mg) were dissolved in ethanol. A little quantity of NaOH was introduced into the mixture, and the resultant mixture was stirred at room temperature for a period 8 hours. The progress of the reaction was observed using thin-layer chromatography (TLC). The product was identified via a UV chamber. The mobile phase was made up of n-hexane and ethyl acetate as a solvent system. The combination that was left over after the reaction was finished was put into extremely cold water. The resultant solid material was isolated by filtration and subsequently underwent a drying procedure prior to purification by recrystallization using ethanol. (1N HCl was used to neutralise the reaction mixture in the event that no precipitate formed.)



5B. Synthesis of 4-(5-(4-Chlorophenyl)-1H-pyrazol-3-yl) phenol derivative:

Hydrazine (1.96 ml) was added dropwise to a Chalcone solution (1 mmol, 0.06 g) in ethanol contained in an RBF. The reaction mixture was subjected to reflux at a temp of 118 ºC for a duration of 72 hours in an oil bath. The progress of the reaction was observed using TLC, and the product was made visible using UV-light with a solvent solution consisting of n-hexane and ethyl acetate. Upon the conclusion of the reaction, the reaction mixture was transferred into water that had been cooled to a very low temperature. The resulting solid was separated from the mixture by filtration, dried, and then purified by recrystallization using ethanol.



3. RESULTS AND DISCUSSION

The newly synthesized compounds underwent docking investigations to determine their docking scores. The docking scores for all the compounds in the series have been documented in a table.

The defining characteristic of the series is the presence of a six-membered substituted aromatic ring at the 4th position of the pyrazoline-4(1H)-phenol.

Compound containing F group at para position in benzene ring showed the good anti-inflammatory effect as compared to the compound containing F position at Meta position.

^{1}H NMR

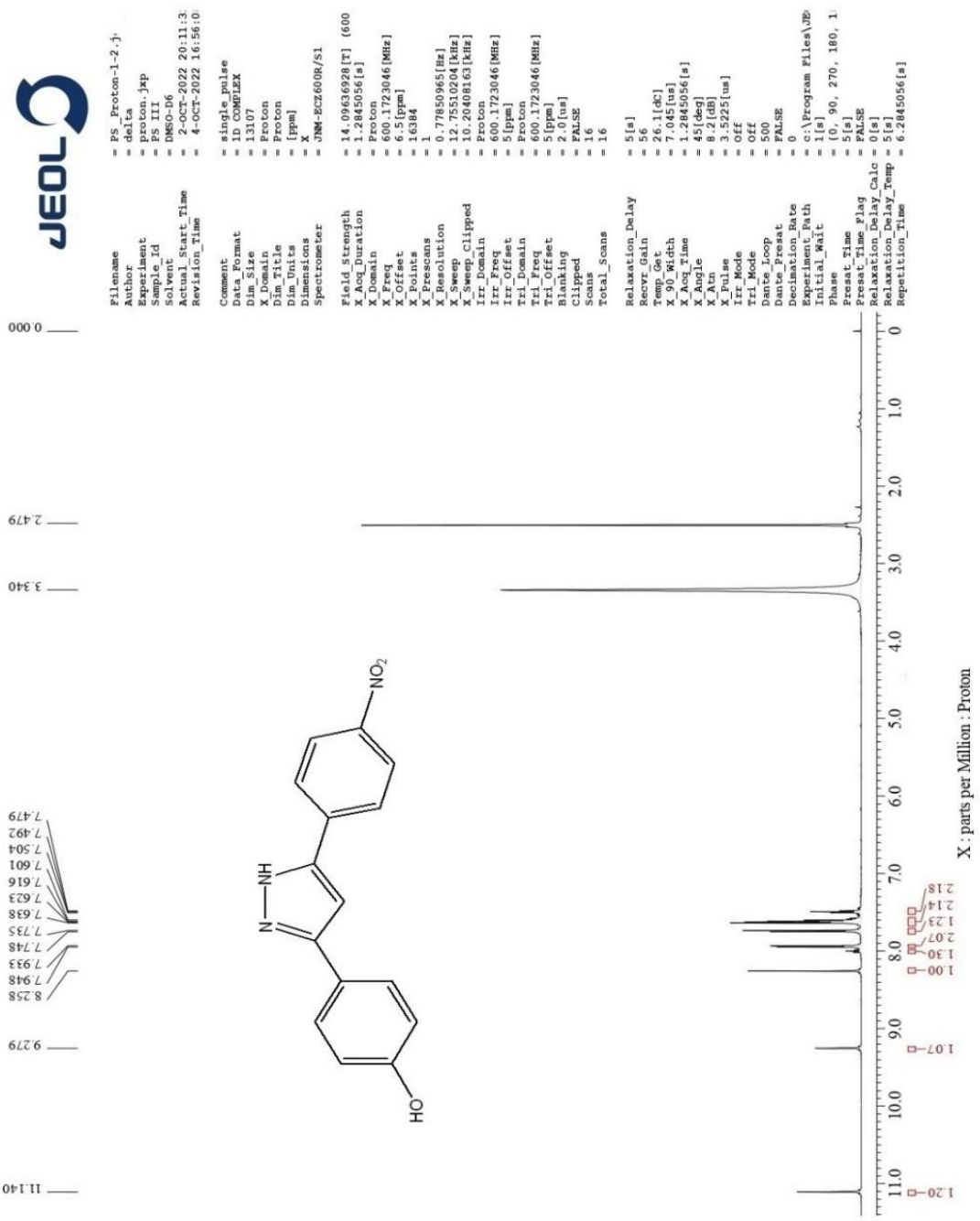


Fig. 2 ^{1}H NMR

^{1}H NMR

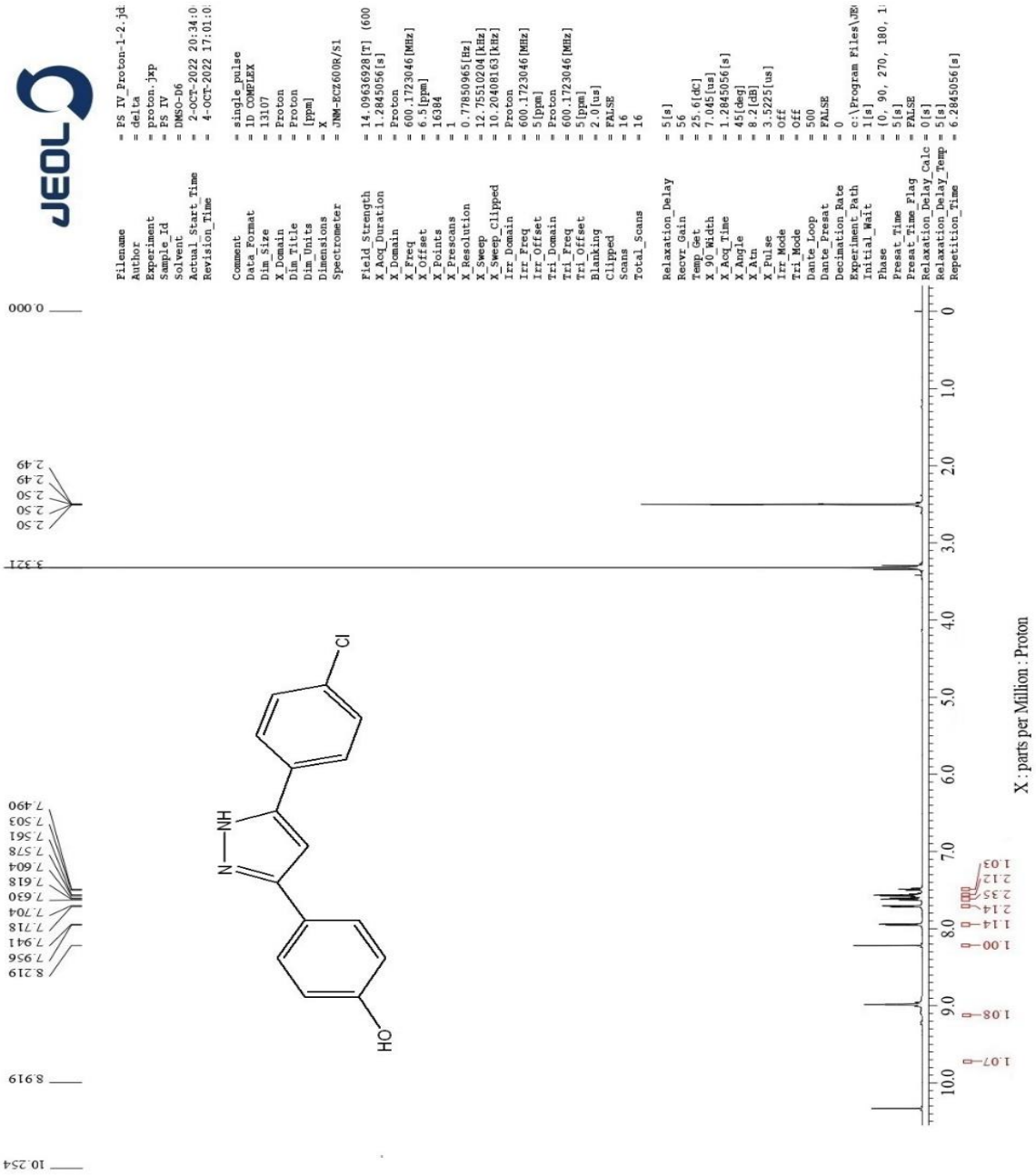


Fig. 3 ^{1}H NMR

^{1}H NMR

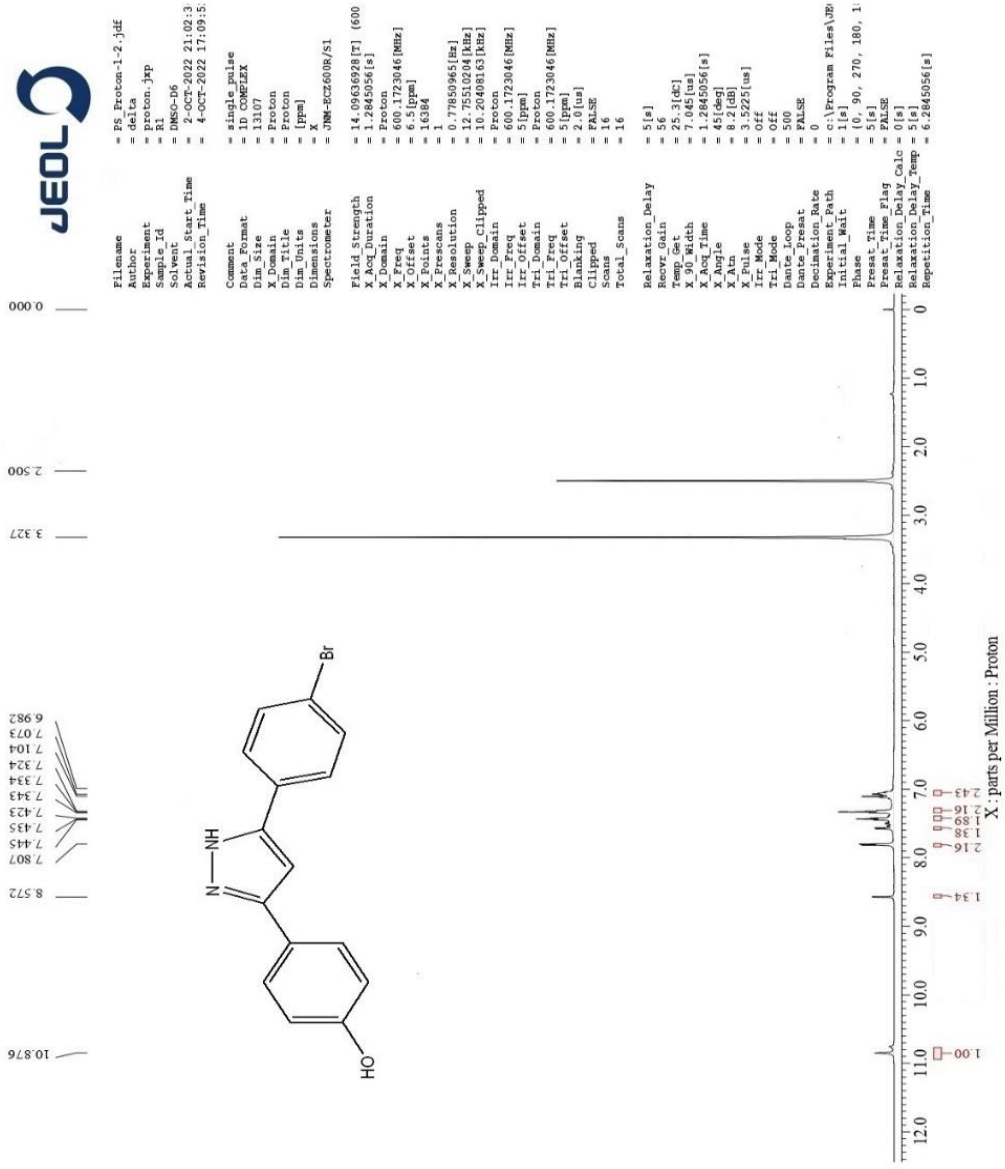


Fig. 4 ^{1}H NMR

^{1}H NMR

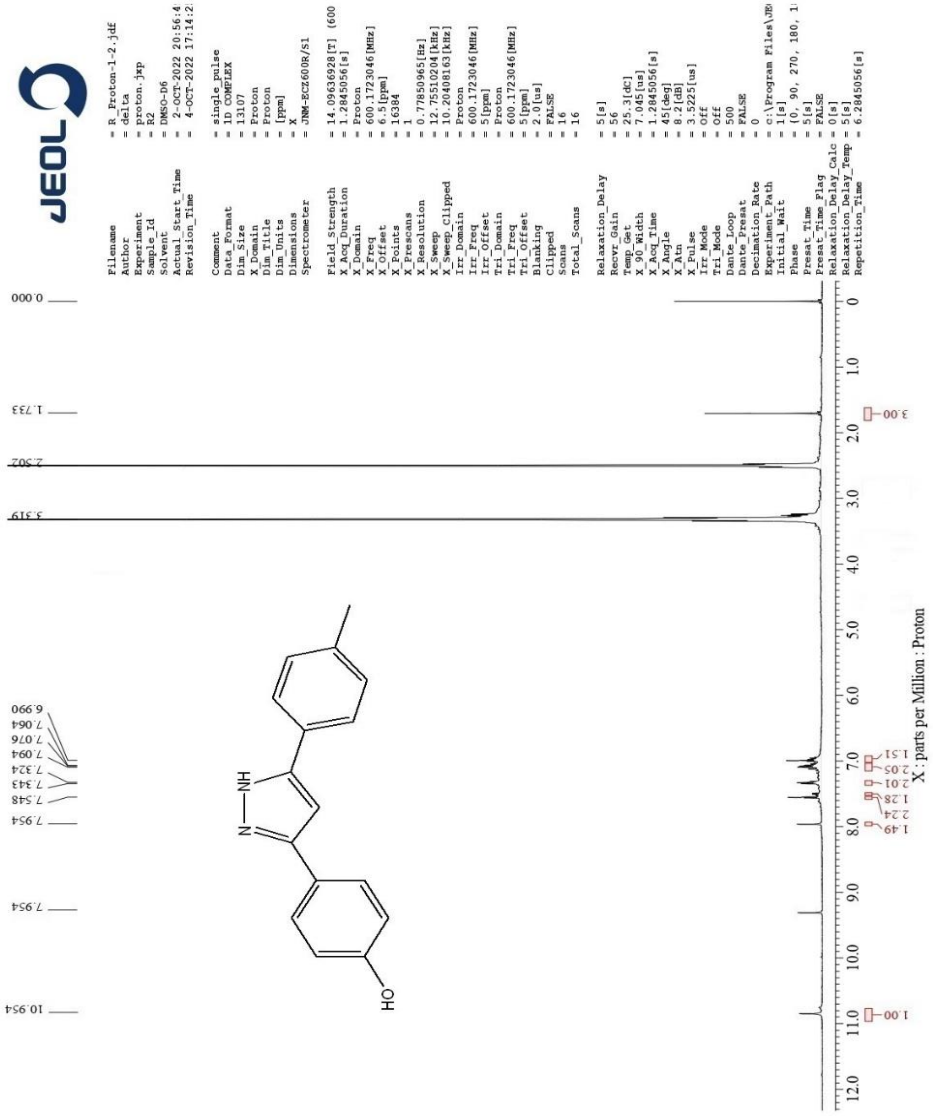


Figure 5 **^{1}H NMR**

Table 1 Percentage yield of 3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

S.N	Compound code	Name of Synthesized Compound	Time of Reaction	Percentage Yield
1.	1A	3-(4-Chloroophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	78.88%
2.	2A	3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	70.63%
3.	3A	3-(4-Flourophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	65.59%
4.	4A	3-(4-Nitrophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	55.71%
5.	5A	3-(P-tolylphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	63.63%

Table 2 Percentage yield of 4-(5-phenyl)-1H-pyrazol-3-yl) phenol

S.N	Compound coc	Name of Synthesized Compound	Time of Reaction	Percentage Yield
1.	1B	4-(5-(4-Fluorophenyl)-1H-pyrazol-3-yl)phenol	72 hours	68.88%
2.	2B	4-(5-(4-Nitrophenyl)-1H-pyrazol-3-yl)phenol	72 hours	63.63%
3.	3B	4-(5-(4-Bromophenyl)-1H-pyrazol-3-yl)phenol	72 hours	65.59%
4.	4B	4-(5-(4-Chlorophenyl)-1H-pyrazol-3-yl)phenol	72 hours	66.71%
5.	5B	4-(5-(P-tolyl)-1H-pyrazol-3-yl)pheno	72 hours	66.63%

Table-1.9.

Table 3 physicochemical properties of 4-(5-phenyl)-1H-pyrazol-3-yl) phenol

Compound code	1B	2B	3B	4B	5B
Molecular formula	$C_{15}H_{12}FN_2O$	$C_{15}H_{12}N_3O_3$	$C_{15}H_{12}BrN_2O$	$C_{15}H_{12}ClN_2O$	$C_{15}H_{12}N_2O$
Molecular weight	250.30	270.72	315.17	254.26	281.08
Colour	Pale yellow	Yellow	Brown	Light yellow	Dark Brown
Melting point	391-385°C	412-410°C	430-425°C	382-380°C	425-422°C

Table 4 Solubility study of 3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

Compound code Solvent	1A	2A	3A	4A	5A
N-Hexane	++	++	++	++	++

DCM	+++	+++	+++	+++	+++
Dietyl ether	++	+++	+++	+++	+++
Ethyl acetate	++++	++++	++++	+++	+++
Acetone	++++	++++	++++	++++	++++
Methanol	++++	++++	++++	++++	++++
DMSO	++	++	++	++	++
Water	---	---	---	---	---

Freely soluble (From 1 to 10 part), +++ soluble (10 to 30 part), ++ sparingly soluble (30 to 100 part), - practically insoluble (≥ 10,000)

Table 5 Solubility study of 4-(5-phenyl)-1H-pyrazol-3-yl) phenol

Compound code Solvent	1B	2B	3B	4B	5B
N-Hexane	++	++	++	++	++
DCM	+++	+++	+++	+++	+++
Dietyl ether	++	+++	+++	+++	+++
Ethyl acetate	++++	++++	++++	+++	+++
Acetone	+++	+++	+++	+++	+++
Methanol	++++	++++	++++	++++	++++
DMSO	+++	+++	+++	+++	+++
Water	---	---	---	---	---

Freely soluble (From 1 to 10 part), +++ soluble (10 to 30 part), ++ sparingly soluble (30 to 100 part), - practically insoluble (more than 10,000)

Molecular docking and its result

After the synthesis and characterization of pyrazoline-4(1H)-phenol. compounds and their derivatives were investigated for molecular docking studies.

Screening for anti-inflammatory potential :

Pyrazoline-4(1H)-phenol is anti-inflammatory potential as shown in previous reports[**23,24,25**] We have considered (PDB.ID)-1B17 the specific binding interaction between drugs NSAIDs and phospholipase A2, focusing on the complex formed with diclofenac. **[26,27]** The protein structure, identified as 1B17, was obtained from the PDB data bank and prepared using the Maestro software's protein preparation wizard from Schrodinger**.[28,29]** The synthesized compounds were drawn in sdf format using ChemDraw 16.0, and a database for virtual screening was created. This ligand database was prepared using Schrodinger's LigPrep tool with default settings.**[58]** A receptor grid was then generated in Maestro by specifying the internal ligand (diclofenac) inside the protein's active pocket. Finally, XP docking was performed for the synthesized ligands against the 1B17 protein at its active binding site. **[30,31,32]** Docking results show the promising anti-inflammatory potential against the target protein in comparison to diclofenac. Compounds 1B,2B,3B,4B, & 5B were found it be more effective against

1B17 protein than internal ligands. The docking score shows ligand molecules, 3B, 5B, 4B, 1B, & 2B are found to be-6.598, -6.097, -6.075, -5.878, -5.778, -5.754 are respectiviely.

Table 7 . Docking score of 4-(5-phenyl)-1H-pyrazol-3-yl) phenol

S.No.	Compound code	Name of Synthesized Compound	Score
1.	Ligand	2-(2-((2,6-dichlorophenyl)amino)phenyl) acetate	-6.598
2.	3B	4-(5-(4-Bromophenyl)-1H-pyrazol-3-yl)phenol	-6.097
3.	5B	4-(5-(P-tolyl)-1H-pyrazol-3-yl)phenol	-6.075
4.	4B	4-(5-(4-Chlorophenyl)-1H-pyrazol-3-yl)phenol	-5.878
5.	1B	4-(5-(4-Fluorophenyl)-1H-pyrazol-3-yl)phenol	-5.778
6.	2B	4-(5-(4-Nitrophenyl)-1H-pyrazol-3-yl)phenol	-5.754

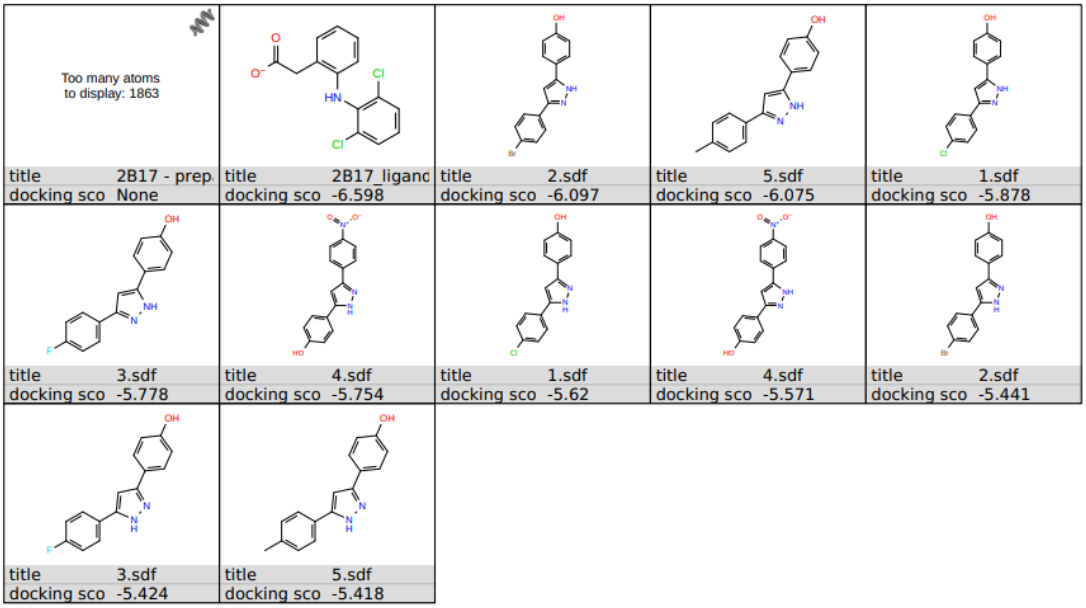


Figure 6 Docking Score Of Compound

The carbonyl oxygen of diclofenac has shown one hydrogen-bonding interaction with the HIP48 residues of protein along with a salt bridge interaction with HIP and one π-π stacking interaction between the aryl ring of extracted ligand molecules from 2B17. (**Fig. 8 and 8A**). Similarly, two

hydrogen-bonding interactions with the TYR22 and ASP49 compound **3b** were observed. Additionally, one π-π stacking interaction with HIP48 residues was observed between the aromatic ring of 4-(5-(4-Bromophenyl)-1H-pyrazol-3-yl)phenol (**Fig. 9 and 9A**). In the case of **5b** same hydrogen bonding and π-π stacking were also observed between the nitrogen and benzene ring of **5b** with TYR22 & ASP49 and HIP48 residues of protein (**Fig. 10 and 10A**). In the case of **4b**, two hydrogen bonds were observed between nitrogen with TYR22 and ASP48 of the target protein. Additionally, one π-π stacking interactions were also seen between aromatic rings of **4b** with HEP48 residue of protein (**Fig. 11 and 11A**).

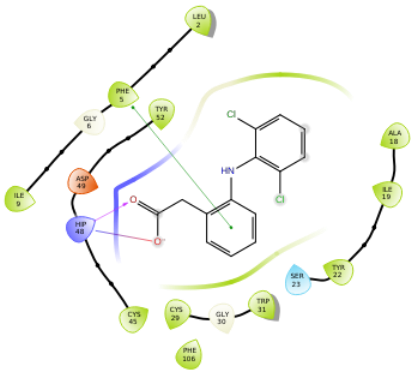


Figure 7(A) 2D interaction diagram of 2B17 with diclofenac

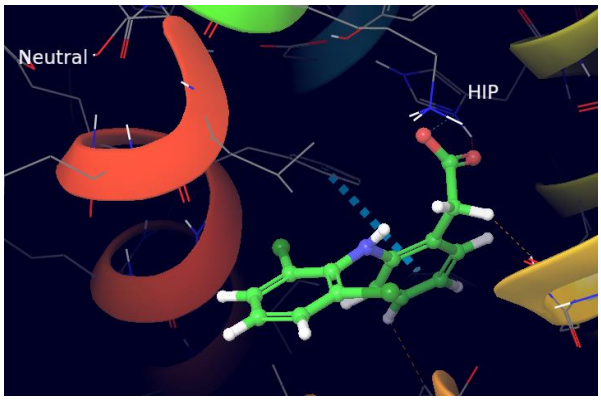


Figure 8(B) 3D interaction diagram of 2B17 with diclofenac

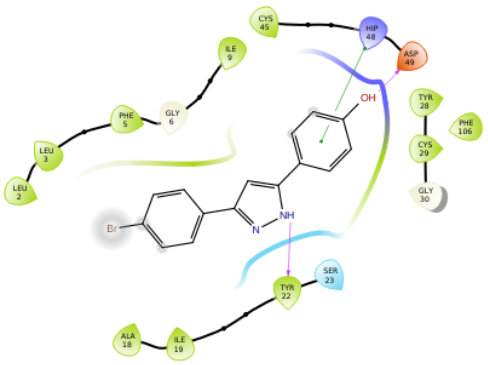


Figure 8(A)2D interaction diagram of 2B17 with 3b

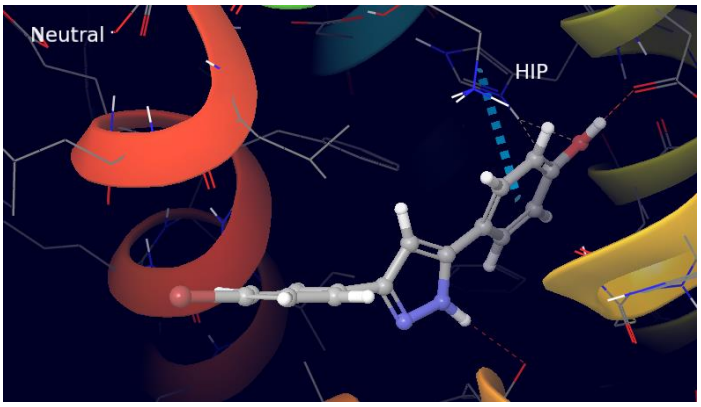


Figure 9(B) 3D interaction diagram of 2b17 with compound 3b

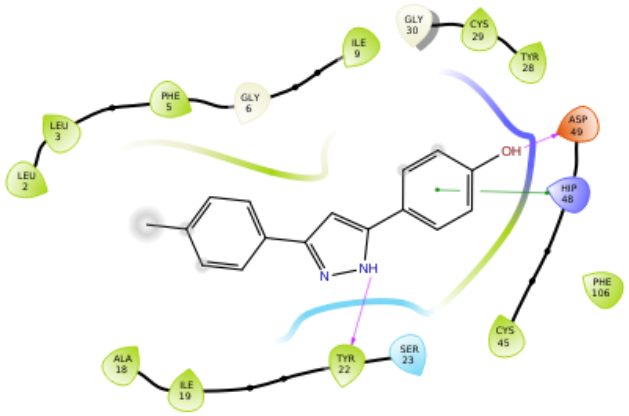


Figure 9(A)2Dinteraction diagram of 2b17 with compound 5b

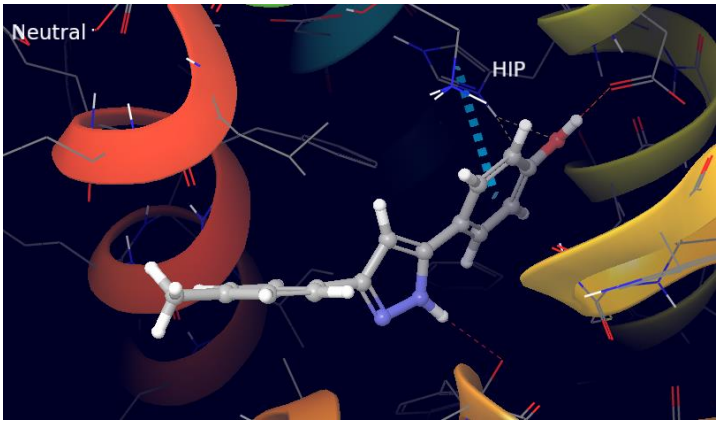


Figure 10(B) 3D interaction diagram of 2b17 with compound 5b

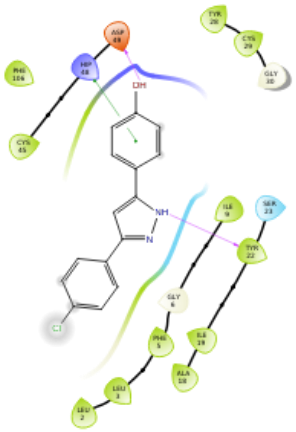


Figure 10(A) 2Dinteraction diagram of 2b17 with compound 4b

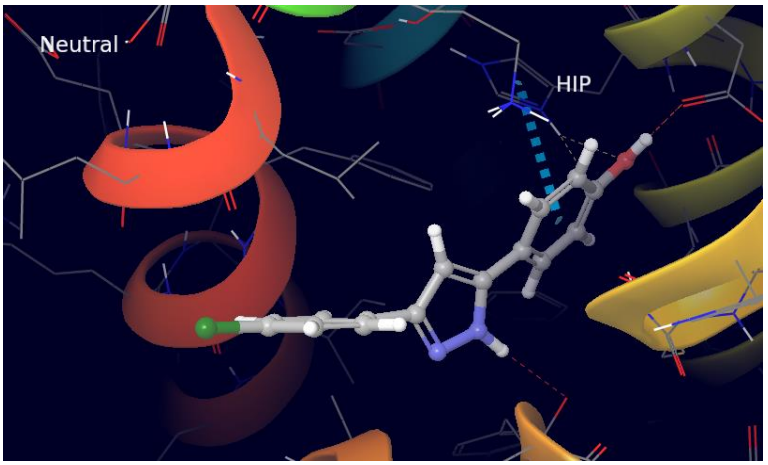


Figure 11(B)3D interaction diagram of 2b17 with compound 4b

Overall, docking analysis gave three promising molecules (**3b**, **5b,** and **4b**), which have good binding potential with the 2B17 protein. Further, these hits can be investigated for in-vitro and in-vivo activity to validate the computational results.

4. CONCLUSION

In conclusion, we have successfully synthesised a library of 4-(5-phenyl)-1H-pyrazol-3-yl) phenol substrates. The synthesised compounds were characterized using various physical and analytical techniques. Moreover, the synthesised compounds were also inspected for ant-inflammatory potential via in-silico studies. Overall, docking analysis gave three promising molecules (**3b**, **5b,** and **4b**), which have good binding potential with the 2B17 protein. Further these hits can be investigated for in-vitro and in-vivo activity to validate the computational results.

7. REFERENCES

1. Schmid-Schönbein, G. W. (2006). Analysis of inflammation. Annu. Rev. Biomed. Eng., 8(1), 93-151.
2. Abdel-Rahman AA, Abdel-Megied AE, Hawata MA, Kasem ER, Shabaan MT. 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. 2004 Sep;138(9):889-97.
3. Chapman, C. R., & Vierck, C. J. (2017). The transition of acute postoperative pain to chronic pain: an integrative overview of research on mechanisms. The Journal of pain, 18(4), 359-e1.
4. Oršolić, N. (2022). Allergic inflammation: Effect of propolis and its flavonoids. Molecules, 27(19), 6694.
5. Ajish Kumar KS, Haj-Yahya M, Olschewski D, Lashuel HA, Brik A. Highly efficient and chemoselective peptide ubiquitylation. Angewandte Chemie. 2009 Oct 12;121(43):8234-8.
6. Medzhitov, R. (2008). Origin and physiological roles of inflammation. Nature, 454(7203), 428-435.
7. Amir LR, Li G, Schoenmaker T, Everts V, Bronckers AL. Effect of thrombin peptide 508 (TP508) on bone healing during distraction osteogenesis in rabbit tibia. Cell and Tissue Research. 2007 Oct;330(1):35-44.
8. Tellides, G., & Pober, J. S. (2015). Inflammatory and immune responses in the arterial media. Circulation research, 116(2), 312-322.
9. Öztürk, A. A., Namlı, İ., Güleç, K., & Kıyan, H. T. (2020). Diclofenac sodium loaded PLGA nanoparticles for inflammatory diseases with high anti-inflammatory properties at low dose: Formulation, characterization and in vivo HET-CAM analysis. Microvascular research, 130, 103991..
10. 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.
11. Hassan, S. Y. (2013). Synthesis, antibacterial and antifungal activity of some new pyrazoline and pyrazole derivatives. Molecules, 18(3), 2683-2711.
12. Shaaban, M. R., Mayhoub, A. S., & Farag, A. M. (2012). Recent advances in the therapeutic applications of pyrazolines. Expert opinion on therapeutic patents, 22(3), 253-291.
13. Marella, A., Ali, R., Alam, T., Saha, R., Tanwar, O., Akhter, M., ... & Mumtaz Alam, M. (2013). Pyrazolines: a biological review. Mini reviews in medicinal chemistry, 13(6), 921-931.
14. Matsubara Y, Tada H, Nagase S, Yoshida ZI. Intramolecular charge transfer interaction in 1, 3-diphenyl-2-pyrazoline ring-fused C60. The Journal of Organic Chemistry. 1995 Sep;60(17):5372-3.
15. de la Hoz, A., Alkorta, I., & Elguero, J. (2021). The mechanism of the reaction of hydrazines with α, β-unsaturated carbonyl compounds to afford hydrazones and 2-pyrazolines (4, 5-dihydro-1H-pyrazoles): Experimental and theoretical results. Tetrahedron, 97, 132413.
16. Varghese, B., Al-Busafi, S. N., Suliman, F. O., & Al-Kindy, S. M. (2017). Unveiling a versatile heterocycle: pyrazoline–a review. RSC advances, 7(74), 46999-47016. Mótyán G, Kovács F, Wölfling J, Gyovai
17. A, Zupkó I, Frank É. Microwave-assisted stereoselective approach to novel steroidal ring D-fused 2-pyrazolines and an evaluation of their cell-growth inhibitory effects in vitro. Steroids. 2016 Aug 1;112:36-46.
18. Varghese, B., Al-Busafi, S. N., Suliman, F. O., & Al-Kindy, S. M. (2017). Unveiling a versatile heterocycle: pyrazoline–a review. RSC advances, 7(74), 46999-47016.
19. Farooq, S., & Ngaini, Z. (2020). One-pot and two-pot synthesis of chalcone based mono and bis-pyrazolines. Tetrahedron Letters, 61(4), 151416.
20. Shringare, S. N., Chavan, H. V., Bhale, P. S., Dongare, S. B., Mule, Y. B., Patil, S. B., & Bandgar, B. P. (2018). Synthesis and pharmacological evaluation of combretastatin-A4 analogs of pyrazoline

and pyridine derivatives as anticancer, anti-inflammatory and antioxidant agents. Medicinal Chemistry Research, 27, 1226-1237.
21. Nehra, B., Rulhania, S., Jaswal, S., Kumar, B., Singh, G., & Monga, V. (2020). Recent advancements in the development of bioactive pyrazoline derivatives. European journal of medicinal chemistry, 205, 112666.
22. Khalil, N. A., Ahmed, E. M., El-Nassan, H. B., Ahmed, O. K., & Al-Abd, A. M. (2012). Synthesis and biological evaluation of novel pyrazoline derivatives as anti-inflammatory and antioxidant agents. Archives of pharmacal research, 35, 995-1002.
23. Das N, Dash B, Dhanawat M, Shrivastava SK. Design, synthesis, preliminary pharmacological evaluation, and docking studies of pyrazoline derivatives. Chemical Papers. 2011 Jan;66(1):67-74.
24. Joshi, R. S., Mandhane, P. G., Diwakar, S. D., Dabhade, S. K., & Gill, C. H. (2010). Synthesis, analgesic and anti-inflammatory activities of some novel pyrazolines derivatives. Bioorganic & medicinal chemistry letters, 20(12), 3721-3725.
25. Sabah, R. S., Al-Garawi, Z. S., & Al-Jibouri, M. N. (2022). The utilities of pyrazolines encouraged synthesis of a new pyrazoline derivative via ring closure of chalcone, for optimistic neurodegenerative applications. Al-Mustansiriyah Journal of Science, 33(1), 21-31.
26. Verrall, G. M. (2020). Scientific rationale for a bottom-up approach to target the host response in order to try and reduce the numbers presenting with adult respiratory distress syndrome associated with COVID-19. Is there a role for statins and COX-2 inhibitors in the prevention and early treatment of the disease?. Frontiers in Immunology, 11, 2167.
27. https://www.rcsb.org/structure/2b17
28. https://www.ncbi.nlm.nih.gov/Structure/pdb/2B17
29. Singh, N., Jabeen, T., Sharma, S., Somvanshi, R. K., Dey, S., Srinivasan, A., & Singh, T. P. (2006). Specific binding of non-steroidal anti-inflammatory drugs (NSAIDs) to phospholipase A2: structure of the complex formed between phospholipase A2 and diclofenac at 2.7 Å resolution. Acta Crystallographica Section D: Biological Crystallography, 62(4), 410-416.
30. Gupta, S. S., Kumar, A., Shankar, R., & Sharma, U. (2021). In silico approach for identifying natural lead molecules against SARS-COV-2. Journal of molecular graphics and modelling, 106, 107916.
31. Desai, N., Mahto, M. K., Alekhya, B., Naveen, C. R., & Bhaskar, M. (2012). Comparative docking studies of estrogen receptor inhibitors and their binding interaction analysis. Int. J. Pharm. Sci. Rev. Res, 16(9), 91-95.
32. Corbeil, C. R., & Moitessier, N. (2009). Docking ligands into flexible and solvated macromolecules. 3. Impact of input ligand conformation, protein flexibility, and water molecules on the accuracy of docking programs. Journal of Chemical Information and Modeling, 49(4), 997-1009.