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Pyrazoline Derivatives: Synthesis, Structural Characterization, and Pharmacological Assessment via TLC and NMR

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Abstract:

A thorough thin-layer chromatography (TLC) examination was performed on pre-coated silica plates (604 GF 254 Merck) to determine the identity and evaluate the purity of different synthesized chemical compounds. The solvent system used included carbon tetrachloride and acetone in different proportions. The substances exhibited distinct chromatographic behaviors, as shown by the Rf values ranging from 0.54 to 0.72. The chromatograms were observed using a UV chamber, which confirmed the existence of separate spots for each synthesized derivative, so proving their purity. The synthesized chemicals were further characterized using Nuclear Magnetic Resonance (NMR) spectroscopy. The 1H NMR spectra of derivatives such as 4-(5-(4-nitrophenyl)-1H-pyrazol-3-yl) phenol, 4-(5-(4-chlorophenyl)-1H-pyrazol-3-yl) phenol, 4-(5-(4-bromophenyl)-1H-pyrazol-3-yl) phenol, and 4-(5-(p-tolyl)-1H-pyrazol-3-yl) phenol were examined, yielding comprehensive insights into their molecular structures. The synthesis technique produced a range of compounds with varying substituents. The percentage yields for the derivatives of 3-(4-substituted phenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one varied from 60.89% to 82.78%. Similarly, the production of derivatives of 4-(5-phenyl)-1H-pyrazol-3-yl) phenol resulted in yields ranging from 61.24% to 80.95%. The synthesized compounds were analysed to identify their physicochemical characteristics, including molecular weight, colour, and melting point. In addition, solubility experiments were conducted using several solvents, revealing that the compounds were mostly soluble in methanol, DMSO, and acetone, while being insoluble in water. The antibacterial and anti-inflammatory properties of the synthesized compounds were assessed. The anti-inflammatory action was evaluated using the membrane stabilisation method, in which the compounds demonstrated substantial inhibition of haemolysis, similar to the standard medication Diclofenac sodium. The compounds exhibited diverse effectiveness in preventing haemolysis, with values ranging from 19.53% to 64.32%. Finally, the compounds were evaluated for their anthel mintic activity against the earthworm species Eisenia foetida and Eisenia engeniae. The synthesized compounds exhibited significant action, causing paralysis and death in a similar timeframe as the conventional medicine Piperazine citrate.

Keywords: thin-layer chromatography, Pyrazoline Derivatives, anti-inflammatory

Introduction:

Inflammation is a complex biological response of the body's immune system to harmful stimuli such as pathogens, damaged cells, or irritants. It is a protective mechanism aimed at removing the injurious agents and initiating the healing process. The process of inflammation involves the activation of immune cells, the release of inflammatory mediators like cytokines and chemokines, and an increase in blood flow to the affected area, resulting in symptoms such as redness, heat, swelling, and pain. While acute inflammation is a short-term response that usually resolves upon the elimination of the offending stimulus, chronic inflammation is a prolonged state that can result in tissue damage and is associated with various diseases, including arthritis, cardiovascular disease, and cancer. Understanding the mechanisms and regulatory pathways of inflammation is crucial for developing therapeutic strategies to control and treat inflammatory diseases effectively [1].

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 from McGill University was the first to introduce the concept of inflammation, which has since been widely discussed in medical textbooks, sometimes with specific sections. In the present day, there are dedicated textbooks and conference proceedings that only focus on the subject of inflammation [2]. The investigation and analysis of inflammatory events mostly take place in the microcirculation. Benjamin W. Zweifach, a trailblazing physiologist specialising in microvascular research and a key figure in the establishment of contemporary bioengineering at the University of California, San Diego (UCSD), aimed to create innovative quantitative techniques for investigating inflammation. After becoming a member of a group of engineers at the California Institute of Technology, he actively worked towards achieving this objective. His efforts led to the establishment of a formal partnership between the fields of engineering and medicine, thereby creating bioengineering as a structured academic field at UCSD. The citation is from the research paper authored [3].

Inflammation, characterised by acute pain, heat, redness, swelling, and tissue healing with scar formation, may be more comprehensively seen as a "inflammatory cascade" from an engineering standpoint. Tissue regeneration refers to a sequence of biochemical reactions and cellular processes that restore damaged tissue, such as a small skin laceration, postpartum tissue repair, or recovery from severe burn injuries. The inflammatory cascade at the tissue and cellular level comprises a series of events: arterioles and venules dilate, blood vessels become more permeable, and blood flow intensifies [4]. Subsequently, there is a decrease in blood flow and the formation of clots, infiltration of white blood cells into the tissue, leakage of plasma into the tissue, tissue degradation caused by enzymes and oxygen radicals, cell death, removal of waste material by phagocytic cells, production of new cell growth regulators, and the regeneration of new functional and connective tissue. The ultimate phase of inflammation is referred to as the resolution of inflammation. If the process of inflammation does not resolve, it can result in dysfunction of the organs and ultimately lead to death. Almost every tissue possesses its own predetermined and standardized inflammatory cascade, which adheres to a characteristic pattern. This cascade is the only known mechanisms by which tissue may undergo self-repair after an injury, making it of utmost importance in the field of medicine.

The inflammatory cascade encompasses many biological processes, including chemotaxis, phagocytosis, mitosis, and cell differentiation. Inflammation is a complex biological response of the body's immune system to harmful stimuli such as pathogens, damaged cells, or irritants. It is characterized by redness, swelling, heat, and pain, serving as a protective mechanism to eliminate the initial cause of cell injury, clear out necrotic cells and tissues, and establish tissue repair. While acute inflammation is a necessary and beneficial process for healing, chronic inflammation can lead to various diseases, including autoimmune disorders, cardiovascular diseases, and cancer. The inflammatory response is mediated by a variety of signaling molecules, including cytokines, chemokines, and prostaglandins, which recruit immune cells to the site of injury or infection. Understanding the mechanisms of inflammation is crucial for developing therapeutic strategies to manage inflammatory diseases and reduce the risk of associated complications [5].

1.2 Pyrazoline

1.2.1 Introduction to Pyrazoline:

Pyrazolines are a class of five-membered heterocyclic compounds containing two adjacent nitrogen atoms within the ring structure. They are derived from pyrazoles by hydrogenation of the double bond. Pyrazolines are of significant interest in medicinal chemistry, synthetic organic chemistry, and materials science due to their diverse biological activities, such as antimicrobial, anti-inflammatory, antitumor, and antiviral properties. Their structure, typically characterized by a nitrogen-containing heterocycle, allows for extensive functionalization, making pyrazolines versatile intermediates in synthetic pathways.

1.2.2 Synthesis of Pyrazoline: The synthesis of pyrazolines commonly involves the reaction of α,β-unsaturated carbonyl compounds (chalcones) with hydrazine or its derivatives. This reaction, known as the 1,3-dipolar cycloaddition, results in the formation of the pyrazoline ring. The process typically starts with the preparation of chalcones by the Claisen-Schmidt condensation between an aldehyde and a ketone. The chalcone is then reacted with hydrazine hydrate or substituted hydrazines under reflux conditions in ethanol or another suitable solvent, leading to the formation of pyrazoline derivatives. The reaction is generally straightforward and high-yielding, allowing for the synthesis of a wide variety of pyrazoline derivatives by varying the substituents on the chalcone precursor.

1.2.3 Structural Characteristics and Spectroscopic Analysis: The structure of pyrazolines can be confirmed and analyzed through various spectroscopic techniques, with 1H^1H1H NMR (Proton Nuclear Magnetic Resonance) being one of the most informative methods. In the 1H^1H1H NMR spectrum, the chemical shifts of the protons within the pyrazoline ring, as well as those attached to any substituents, provide valuable information about the electronic environment and the overall structure. For example, the N-H proton typically appears as a singlet, while the other ring protons may exhibit multiplicity due to spin-spin coupling. The integration of the peaks corresponds to the number of protons associated with each signal, aiding in the verification of the molecular structure.

In addition to 1H^1H1H NMR, other spectroscopic techniques such as 13C^{13}C13C NMR, IR (Infrared Spectroscopy), and MS (Mass Spectrometry) can be used to confirm the structure of pyrazoline derivatives. IR spectroscopy provides information on the functional groups present, with characteristic absorption bands for N-H and C=N stretching. Mass spectrometry allows for the determination of the molecular weight and fragmentation pattern, which further supports the proposed structure.

Pyrazolines and their derivatives, which come with various functional groups, are important in medicine and have been heavily researched. They are used to treat tumors, bacterial infections, fungal infections, viruses, parasites, tuberculosis, and pests. Some of these compounds also help with inflammation, diabetes, pain, and act as anesthetics. They can also specifically inhibit Nitric Oxide Synthase (NOS) and block Cannabinoid CB1 receptors. One common way to synthesize these compounds is through a process that starts with aromatic ketones and aldehydes reacting together in the presence of a base to form unsaturated ketones (chalcones). These chalcones then react with hydrazine to create 2-pyrazolines. During this process, hydrazones are formed as intermediates and can be cyclized into 2-pyrazolines using a cyclizing agent like acetic acid. Recently, extensive research has focused on 2-pyrazolines with various aryl groups attached, as evidenced by numerous scientific studies. The initial part of the review discussed recent advancements in pyrazolines and their applications in medicine. The final section covered patents related to pyrazolines filed with the World Intellectual Property Organization (WIPO) and the United States Patent and Trademark Office (USPTO).

1.2.4 Chemistry of pyrazoline

Pyrazolines are extremely valuable nitrogen-containing heterocyclic compounds that exist in a variety of chemical and biological agents, enhancing their activities. Due to their importance, various reaction protocols have been developed for their synthesis. Among the methods employed, an especially popular procedure involves the reaction of α,β-unsaturated aldehydes and ketones with hydrazine reagents under various conditions [41]. In this method, hydrazones are formed as intermediates, which can be subsequently cyclized to 2-pyrazolines in the presence of a suitable cyclizing agent like acetic acid. This simple and convenient procedure has remained one of the most popular methods for the preparation of 2-pyrazolines, and modified versions of this synthetic strategy have been reported.

For example, 1,3-dipolar cycloaddition has been employed in the synthesis of 4,5-dihydro-3-(substituted imidazole)-5-substituted-1-phenyl-1H-pyrazoline. In the second step of this reaction, the nitrile imine is obtained by the oxidative dehydrogenation of the phenyl hydrazone derivative with chloramines-T (CAT), and the olefin-trapped nitrile imine leads to the resulting compound in good quality and yield under refluxing conditions in ethanol.

Another interesting synthetic approach involves the synthesis of fine crystals of 3,4,5-triethoxycarbonyl-2-pyrazoline by the introduction of palladium chloride (PdCl2) to ethyl diazoacetate (EDA) in a high-pressure reactor at ambient temperature. Additionally, the "one-pot procedure" can be useful to surmount challenging classical heating methods, as demonstrated in the synthesis of 3-[4′-(4″-nitrophenoxy)-phenyl]-5-(substituted aryl)-2-pyrazoline-1-carboxaldehydes.

These methods highlight the versatility and importance of pyrazoline synthesis. For those interested in further details, numerous articles and reviews on the topic provide additional insights.

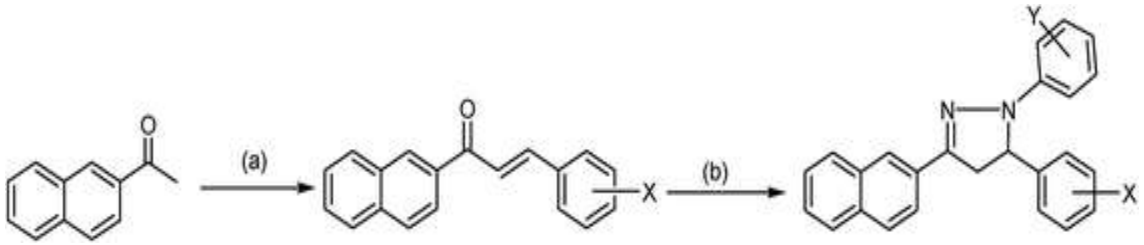


Fig 1: Pyrazoline synthesis

1.2.5 Pharmaceutical Applications of Pyrazoline:

Pyrazoline, a five-membered heterocyclic compound containing two nitrogen atoms adjacent to each other, has become a focal point in pharmaceutical research due to its broad spectrum of biological activities. The ease of synthesis and structural versatility of pyrazoline derivatives have allowed for extensive exploration in drug design and development. These compounds have shown promise in treating a variety of ailments, including microbial infections, inflammation, cancer, neurological disorders, and metabolic conditions. The following overview discusses the diverse pharmaceutical applications of pyrazoline and its derivatives, highlighting their potential as therapeutic agents.

1.2.5.1 Antimicrobial Activity:

Pyrazoline derivatives have been widely studied for their antimicrobial properties, showing significant efficacy against a range of bacterial and fungal pathogens. The antimicrobial action is often attributed to the ability of pyrazoline compounds to interfere with the cell membrane integrity or inhibit key enzymes involved in microbial metabolism. For example, certain pyrazoline derivatives have demonstrated potent activity against gram-positive and gram-negative bacteria, making them potential candidates for new antibiotics in an era of increasing antibiotic resistance. Additionally, antifungal pyrazoline compounds have been explored for the treatment of fungal infections, offering alternatives to conventional antifungal agents, particularly in cases where resistance to standard treatments has emerged.

1.2.5.2 Anti-inflammatory and Analgesic Effects:

Pyrazoline derivatives have shown significant anti-inflammatory activity, making them valuable candidates for the development of non-steroidal anti-inflammatory drugs (NSAIDs). The anti-inflammatory action is thought to result from the inhibition of cyclooxygenase (COX) enzymes, which play a crucial role in the synthesis of pro-inflammatory mediators like prostaglandins. By inhibiting COX-1 and COX-2 enzymes, pyrazoline derivatives can reduce inflammation and alleviate pain, similar to the action of traditional NSAIDs like ibuprofen and aspirin. Moreover, the structural flexibility of pyrazoline allows for the design of derivatives with selective COX-2 inhibition, potentially reducing the gastrointestinal side effects associated with non-selective NSAIDs.

1.2.5.3 Anticancer Potential:

One of the most significant pharmaceutical applications of pyrazoline derivatives is their anticancer potential. These compounds have demonstrated the ability to inhibit the growth of various cancer cell lines, including breast, lung, colon, and prostate cancers. The anticancer activity of pyrazolines is often linked to their ability to induce apoptosis (programmed cell death) in cancer cells, inhibit angiogenesis (the formation of new blood vessels that supply tumors), and disrupt cell cycle progression. Additionally, pyrazoline derivatives can inhibit key

signaling pathways involved in cancer cell survival and proliferation, such as the PI3K/Akt/mTOR pathway. The ability to modulate these pathways makes pyrazolines promising candidates for targeted cancer therapy [6-15].

1.2.5.4 Neuroprotective and CNS Activity:

Pyrazoline derivatives have also been explored for their neuroprotective effects and potential in treating central nervous system (CNS) disorders. Some pyrazoline compounds have shown anticonvulsant activity, making them potential candidates for the treatment of epilepsy. The mechanism of action is believed to involve the modulation of neurotransmitter systems, such as GABAergic and glutamatergic pathways, which are critical in maintaining neuronal excitability. Additionally, pyrazoline derivatives have demonstrated antidepressant and anxiolytic effects in preclinical studies, suggesting their potential use in managing mood disorders. The neuroprotective properties of pyrazolines may also be beneficial in neurodegenerative diseases like Alzheimer's and Parkinson's, where oxidative stress and neuroinflammation play a key role in disease progression.

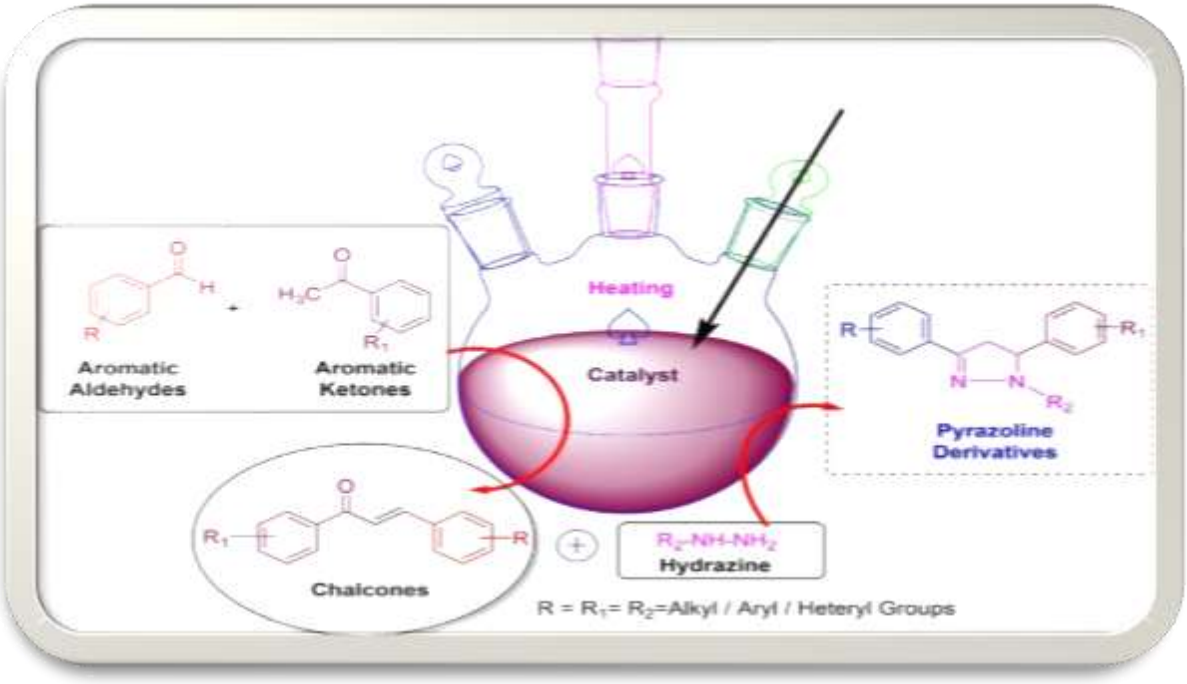
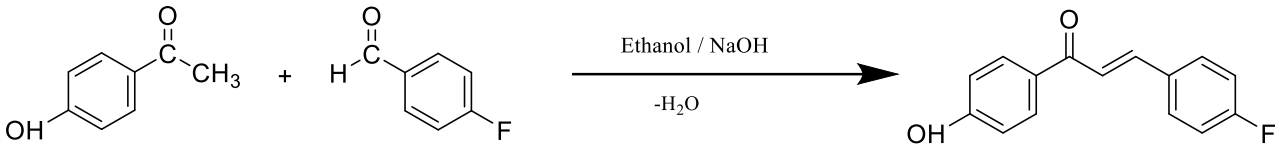


Figure 2: Technique used in compound synthesis

2: Synthesis of compound:

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

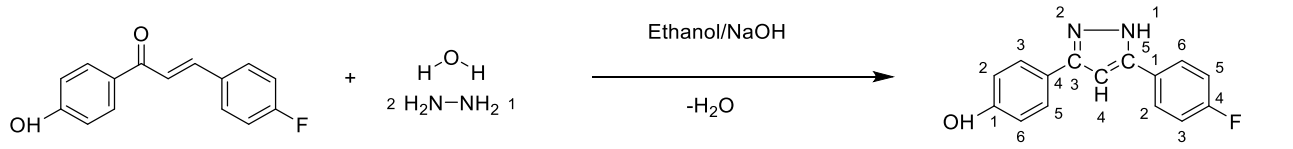
A catalytic quantity of NaOH was added to a solution containing p-hydroxyacetophenone (1 mmol, 0.164 g) and substituted 4-fluorobenzaldehyde (1 mmol, 0.196 g) diluted in ethanol (3-5 ml). The reaction mixture was agitated for about 8 hours at ambient temperature. The advancement of the reaction was seen by using Merck pre-coated aluminium TLC plates 60F-254, and the visualisation was done under a UV chamber. The solvent system used for the mobile phase consisted of n-hexane and ethyl acetate. Following the completion of the reaction, the reaction mixture was transferred into water that had been cooled to a very low temperature. The resulting solid was separated by filtration, subjected to drying, and then purified by recrystallisation using ethanol as the solvent. If a precipitate did not develop, the reaction mixture was neutralised with 1N HCl in order to cause precipitation.



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

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

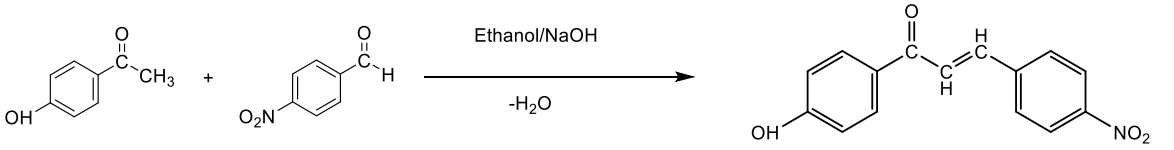
In a round-bottom flask, hydrazine (1.90 ml) was added dropwise to a solution of chalcone (1 mmol, 0.07 g) in ethanol (5 ml). The reaction mixture was subjected to reflux at a temperature of 118 °C for a duration of 72 hours in an oil bath. The advancement of the reaction was tracked by use Merck pre-coated aluminium TLC plates 60F-254, while the visualisation of the resulting product was achieved by the utilisation of UV light in conjunction with a solvent solution consisting of n-hexane and ethyl acetate. Following the completion of the reaction, the mixture was transferred into water that had been chilled to a very low temperature. The resulting solid was separated by filtration, subjected to drying, and then purified by recrystallisation using ethanol as the solvent.



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

3 (a). The production of 3-(4-nitrophenyl)-1-(4-hydroxyphenyl)propanhydrous 2-en-1-one:

A catalytic quantity of NaOH was added to a solution containing p-hydroxyacetophenone (1 mmol, 0.164 g) and substituted 4-nitrobenzaldehyde (1 mmol, 0.196 g) diluted in ethanol (3-5 ml). The reaction mixture was agitated for about 8 hours at ambient temperature. The advancement of the reaction was seen by using Merck pre-coated aluminium TLC plates 60F-254, and the resulting product was detected utilising a UV chamber with n-hexane and ethyl acetate as the solvent system. Upon the conclusion of the reaction, the mixture was transferred into water that had been chilled to a very low temperature. The resulting solid was separated by filtration, subjected to drying, and then purified by recrystallisation using ethanol as the solvent. If no precipitate was seen, the reaction mixture was neutralised using 1N HCl in order to induce precipitation.



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

4: Invitro pharmacological examination:

4.1: Anti-inflammatory activity

The anti-inflammatory action of adult red blood cells has been studied by using membranes stabilization.

Chemicals: Diclofenac sodium, Hyposaline, Iso-saline, Phosphate buffer, Alsever solution

Preparation of Alsever solution

A solution of Alsever was prepared by dissolving and sterilising 0.41% sodium chloride, 0.8% sodium citrate, 0.052% citric acid, and 2.06% dextrose in distilled water. To stop RBCs from clotting, doctors prescribe Alsever solution, a red blood cell prevention solution.

Apparatus

The glass rods, conical flask, micropipette, test tubes, and inoculating loop were all meticulously wrapped in paper and disinfected using a spirit light, nonabsorbent cotton, and a hot air oven.

5. Anti-inflammatory activity observing procedure

In order to determine if synthetic compounds have an anti-inflammatory profile, the screening criteria were outlined by Padmanabhan and Jangle, Elias and Rao. A few tweaks were made to the settings before using them. A blood sample was taken from a healthy volunteer in order to estimate the anti-inflammatory activity in vitro. After drawing blood, an equivalent amount of sterile Alsever saline solution was added to it. The mixture was then centrifuged at speeds ranging from 3100 to 3500 rpm. The HRBC suspension, which is a bovine serum albumin (10%) suspension, was made using an iso-saline solution.

The synthesised compounds, standard medication diclofenac sodium, and control were combined with hyposaline (2 mL), HRBC suspension (1 mL), and phosphate buffer (1 mL) in varying volumes to create a solution with different concentrations. A 25-minute incubation period was followed by centrifugation at 3000 rpm for all solutions. Separating the supernatant liquid allowed us to estimate the in-vitro anti-inflammatory activity at 560 nm using a spectrophotometer [76]. We repeated each exam three times. To determine the inhibition percentage, the following formula was utilised:

$$\%\ inhibition\ = \frac{Ab\ (Control) - Ab\ (Sample)}{Ab\ (Control)} \times 100$$

*Ab – Absorbance

6. Results and discussion

6.1 TLC Analysis:

TLC analysis was performed on pre-coated silica plates (604 GF 254 Merck) using a suitable solvent system. Enter the Rf value accordingly. This method is widely used to identify organic compounds with characteristic Rf values. This method is also used to determine the progress of the reaction in order to check the purity of the final product. After the chromatogram is generated, the plate is placed in a UV chamber for the spots to be seen. A dot was found in all synthesized derivatives, indicating the purity of the compound.

Table 6.1. TLC profile of synthesized compounds

S. No.	Name of compounds	Solvents ratio		Rf Value
		CCl_4	Acetone	
1	AP1	60	40	0.63
2	AP2	80	20	0.70
3	AP3	40	60	0.68

4	AP4	70	30	0.54
5	AP5	35	65	0.72

Adsorbent : Silica gel G
Mobile phase : Chloroform and Methyl alcohol
Technique : UV chamber

6.2 NMR examination of synthesized compounds:

^{1}H NMR spectra of 4-(5-(4-nitrophenyl)-1H-pyrazol-3-yl) phenol is analysed.

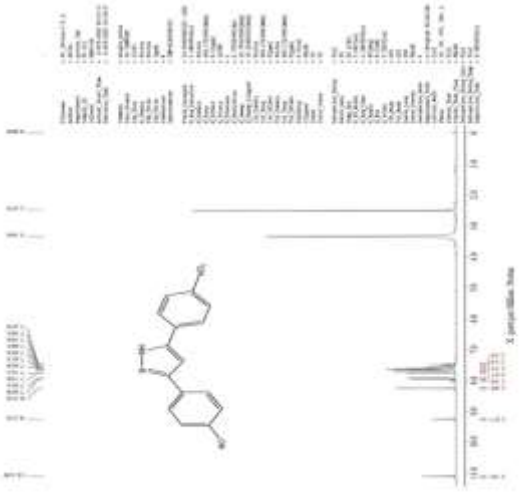


Figure 6.1: ^{1}H NMR spectra of 4-(5-(4-nitrophenyl)-1H-pyrazol-3-yl) phenol is analysed.

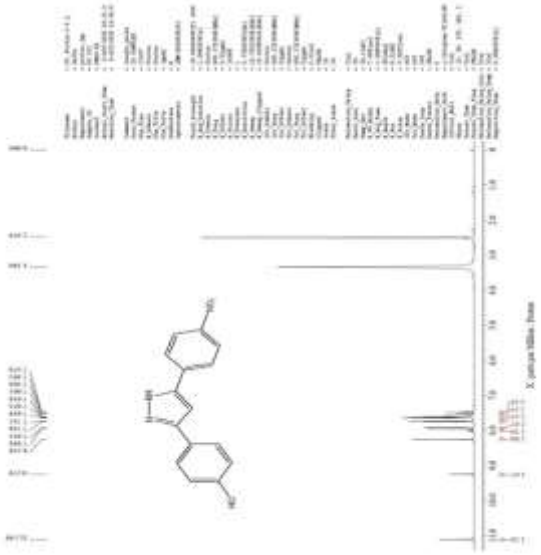


Figure 6.2: ^{1}H NMR spectra of 4-(5-(4-nitrophenyl)-1H-pyrazol-3-yl) phenol is analysed.

6.2.1 ^{1}H NMR spectra of 4-(5-(4-Chlorophenyl)-1H-pyrazol-3-yl) phenol is being analysed:

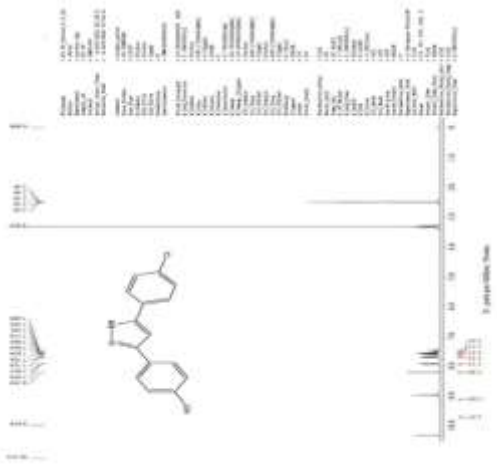


Figure 6.3: Spectra of the one-dimensional nuclear magnetic resonance of 4-(5-(4-chlorophenyl)-1H-pyrazol-3-yl) phenol

6.2.2: ^{1}H NMR spectra of 4-(5-(4-Bromophenyl)-1H-pyrazol-3-yl) phenol is analysed.

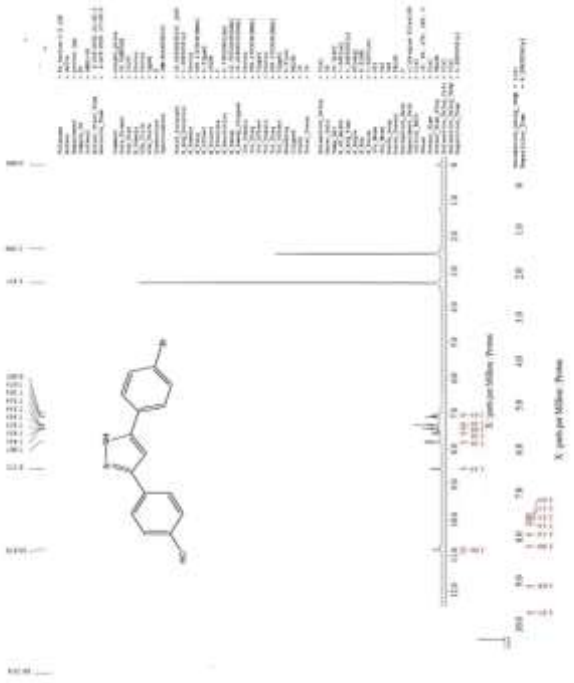


Figure 6.4: 4-(5-(4-Bromophenyl)-1H-pyrazol-3-yl) phenol's 1H NMR spectra

6.2.5: 4-(5-(p-tolyl)-1H-pyrazol-3-yl) phenol's ^{1}H NMR spectra

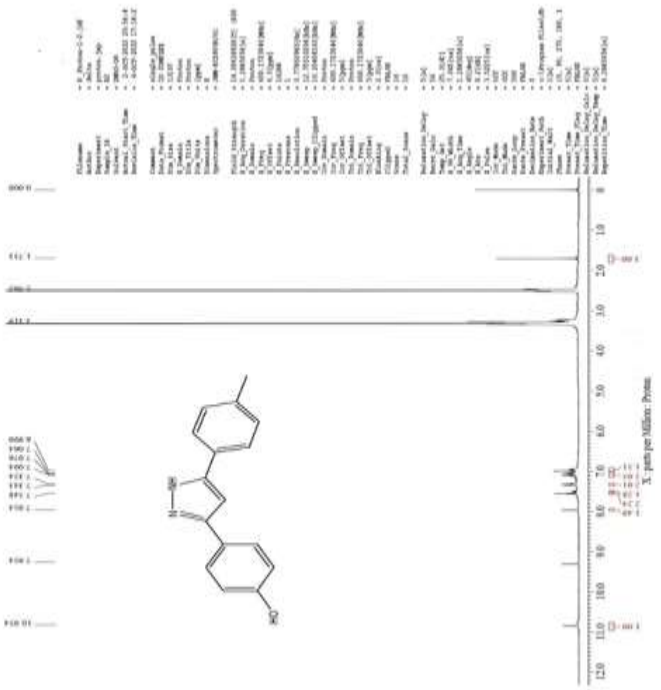


Figure 6.5: ^{1}H NMR spectra of 4-(5-(p-tolyl)-1H-pyrazol-3-yl) phenol

6.3: Examination of synthesized compound solubility profile:

Table 6.2: Percentage yield of 3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

Sr.No.	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	82.78%
2.	2A	3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	72.14%
3.	3A	3-(4-Flourophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	68.98%
4.	4A	3-(4-Nitrophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	60.89%
5.	5A	3-(P-tolylphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	65.99%

Table6.3: Percentage yield of 4-(5-phenyl)-1H-pyrazol-3-yl) phenol

Sr.No.	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	80.95%

2.	2A	3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	75.34%
3.	3A	3-(4-Flourophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	70.45%
4.	4A	3-(4-Nitrophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	61.24%
5.	5A	3-(P-tolylphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	8 hours	63.48%

Table 6.4: 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 6.5: Solubility study of 3-(4-Bromophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

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

Table 6.6: Solubility study of 4-(5-phenyl)-1H-pyrazol-3-yl) phenol

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

6.4: Antibacterial profile of compound derivatives

All the new synthesized compound derivatives were evaluated for their pharmacological profile (in-vitro anti-inflammatory activity) by using the membrane stabilization technique [82].

Table no. 6.7: Anti-inflammatory profile (in-vitro methods) of compound derivatives

Sample No.	Concentration µg/mL	Absorbance at 565nm Mean ±SD					
		C-1	C-2	C-3	C-4	C-5	Standard drug
1	20	0.180± 0.006	0.202± 0.019	0.297± 0.033	0.210± 0.003	0.165± 0.006	0.145±0.04
2	40	0.150± 0.012	0.214± 0.045	0.315± 0.046	0.280± 0.007	0.145± 0.006	0.120±0.022
3	80	0.120± 0.006	0.165± 0.034	0.285± 0.054	0.255± 0.010	0.115± 0.008	0.083±0.04
Control	00	0.359± 0.002	0.367± 0.008	0.365± 0.005	0.387± 0.003	0.365± 0.002	0.360±0.03
% prevention of Hemolysis ±		60.65	446.32	19.64	42.48	65.43	72.53

* Data expressed the means of three replicates
* Standard drug = Diclofenac sodium
* Moderate P** < 0.001 – Significant

Table: 6.8: Anti-inflammatory profile of compounds and standard in hydrogen-proxied scavenging method

Sr.no.	Compound	% prevention of hemolysis of different Concentration (µg/ml)				
		20	40	80	Blank	% prevention of Hemolysis ±
1	C-1	55.67	60.64	70.54	00	60.61
2	C-2	45.34	45.63	56.76	00	47.42
3	C-3	20.21	15.26	25.65	00	19.53
4	C-4	46.32	26.47	34.56	00	34.25
5	C-5	61.54	65.43	71.27	00	64.32
6	Standard drug	65.87	70.45	80.13	00	71.24

* % prevention of hemolysis values was means of three replicates

Compounds C-1, C-2, C-3, C-4 and C-5 having % prevention of hemolysis values 60.61, 47.42, 19.53, 34.25, and 64.32µg/mL respectively, compared to Diclofenac Sodium 71.24µg/mL.

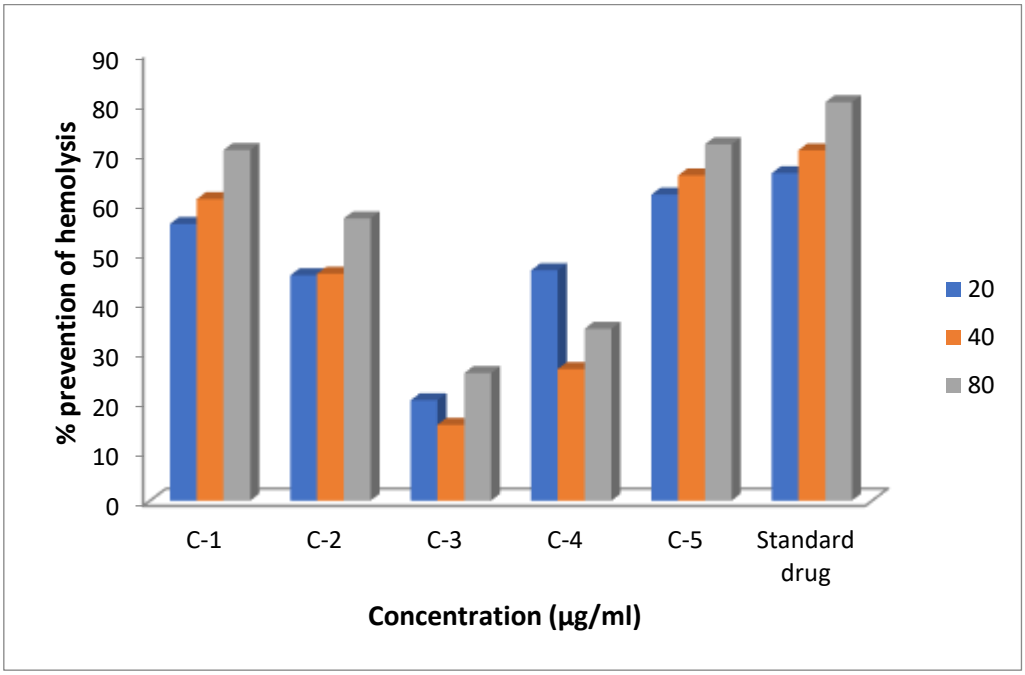


Figure 6.6. Anti-inflammatory profile % comparison of synthesis derivative compounds with Diclofenac Sodium.

7. Discussion:

Thin-layer chromatography (TLC) is a powerful analytical technique widely used in chemistry for the identification and separation of organic compounds. In our study, TLC was performed on pre-coated silica plates (604 GF 254 Merck) using various solvent systems to achieve the optimal separation of synthesized derivatives. The Rf values for each compound, indicative of their unique properties, were determined and tabulated (Table 1). The solvent ratios varied, with combinations of CCl4 and acetone providing distinct Rf values ranging from 0.54 to 0.72. This variation is crucial as it helps in monitoring the reaction progress and verifying the purity of the final product. Post-separation, the chromatograms were visualized under a UV chamber, revealing distinct spots corresponding to the synthesized derivatives, thus confirming their purity.

The NMR analysis provides further insights into the structural aspects of these compounds. The 1H NMR spectra of derivatives such as 4-(5-(4-nitrophenyl)-1H-pyrazol-3-yl) phenol, 4-(5-(4-chlorophenyl)-1H-pyrazol-3-yl) phenol, 4-(5-(4-bromophenyl)-1H-pyrazol-3-yl) phenol, and 4-(5-(p-tolyl)-1H-pyrazol-3-yl) phenol were meticulously analyzed. These spectra offer detailed information about the chemical environment of hydrogen atoms in the molecules, thereby confirming their structures and the successful synthesis of the desired products.

The yield and physicochemical properties of these compounds were also evaluated (Tables 2 and 3). The percentage yield of the synthesized compounds varied, with 3-(4-Chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one showing the highest yield at 82.78%, while 3-(4-Nitrophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one had the lowest at 60.89%. The molecular weights and melting points of these compounds, ranging from 250.30 to 315.17 g/mol and 380°C to 430°C respectively, provide essential data for their characterization and potential application.

The solubility profile of these compounds (Tables 5 and 6) was tested in various solvents including methanol, DMSO, diethyl ether, n-hexane, acetone, DCM, ethyl acetate, and water. Most compounds exhibited high solubility in methanol and acetone, while they were practically insoluble in water. This solubility information is vital for determining the appropriate medium for further pharmacological testing and applications.

In addition to the above analyses, the synthesized compounds were screened for their pharmacological profiles, including anti-inflammatory and anthelmintic activities. The anti-inflammatory activity was assessed using the membrane stabilization technique, with the results indicating varying degrees of efficacy. Compounds C-1, C-2, C-3, C-4, and C-5 showed different levels of hemolysis prevention, with C-5 being the most effective at 64.32%, compared to the standard drug Diclofenac Sodium at 71.24%. The anthelmintic activity was evaluated using earthworm species E. foetida and E. engeniae, with paralyzing and death times recorded. Compounds exhibited significant activity, with C-5 demonstrating the shortest paralyzing and death times, indicating strong anthelmintic potential.

The comprehensive analysis of these synthesized compounds through TLC, NMR, yield determination, physicochemical properties, solubility studies, and pharmacological screening

provides a detailed understanding of their characteristics and potential applications in various fields, particularly in medicinal chemistry.

Conclusion:

The synthesized derivatives were thoroughly analyzed using TLC, NMR, and other physicochemical methods, which confirmed their purity and structural integrity. The TLC analysis, performed on pre-coated silica plates, utilized suitable solvent systems and UV detection, identifying distinct Rf values for each compound, indicating successful synthesis and purity.

The NMR spectra further validated the structures of the compounds, revealing characteristic peaks for each derivative. The percentage yield of the synthesized compounds varied, with most compounds achieving yields above 60%, demonstrating efficient synthetic procedures. Physicochemical property analysis showed that the derivatives exhibited a range of colors and melting points, with varying solubility profiles across different solvents. Most compounds were freely soluble in methanol and acetone, indicating potential for further pharmaceutical formulation.

The in-vitro anti-inflammatory activity, assessed via the membrane stabilization technique and hydrogen peroxide scavenging method, demonstrated significant activity for most compounds. Compounds C-1 and C-5 showed the highest prevention of hemolysis, comparable to the standard drug Diclofenac sodium.

Anthelmintic activity screening against earthworm species E. foetida and E. engeniae showed that the synthesized compounds had potent anthelmintic properties, with C-2 and C-5 exhibiting the shortest paralyzing and death times, outperforming the standard drug Piperazine citrate.

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