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# In Silico Study: Molecular Docking and Toxicity Prediction of Pyrazoline Derivatives with Potential as Anti-Inflammatory

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***Abstract—*** Research on heterocyclic compounds suggested that pharmacologically active agents featuring pyrazoline played a crucial role in medicinal chemistry. When fused with other heterocycles, pyrazoline, as a quiescent heterocyclic moiety, resulted in the enhancement of biological properties. Therefore, synthesizing these compounds had attracted the attention of researchers focused on designing novel drugs. The addition of substituents to the N-pyrazoline atom and modifications to the benzene ring of pyrazoline compounds were essential for the identification of pyrazoline derivatives exhibiting enhanced biological activity. Extensive research had shown that pyrazoline compounds had significant biological effects, including anti-inflammatory effects. Inflammation was the body's reaction to infection or injury and marked by symptoms such as redness, heat, swelling, and pain. This research involved a computational analysis of pyrazoline compounds utilizing molecular docking with AutoDock Tools and AutoDock Vina software on four pyrazoline derivative compounds (pyrazolines 1-4). Simultaneously, their toxicity was assessed through online pkCSM to evaluate their potential as anti-inflammatory drug candidates. The interaction between the active site of cyclooxygenase-2 (COX-2) receptor (PDB: 4PH9) and pyrazoline derivatives showed that pyrazoline 2 (1-benzoyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline) exhibited the highest binding affinity of -8.0 kcal/mol compared to pyrazoline derivatives 1, 3, 4 and ibuprofen as native ligands also in the molecular docking test with values of -7.1; -7.7; -7.6; and -7.1 kcal/mol, respectively. Toxicity evaluation for pyrazoline 2 also suggested that this compound was non-toxic, non-hepatotoxic, and did not induce skin sensitization, with an Oral Rat Acute Toxicity (LOAEL) score of 1.417 log (mg/kg_bw/day).

***Keywords—*** *Anti-Inflammatory; Molecular docking; Pyrazoline; Toxicity*

## 1. INTRODUCTION

Pyrazoline is a significant five-membered heterocyclic compound that has been widely utilized in the agrochemical and pharmaceutical sectors as herbicides and active pharmaceutical agents [1]. Investigations on this category of heterocyclic compounds have demonstrated that pharmacologically active agents featuring pyrazoline play a significat role in medicinal chemistry. When pyrazoline, as a dormant heterocyclic component, is combined with other heterocycles, it enhances biological properties, thereby attracting researchers' interest in synthesizing these compounds to develop innovative pharmaceuticals [2].

The widespread occurrence of pyrazoline cores in biologically active compounds has prompted the demand for sophisticated and effective methods to synthesize these heterocyclic precursors [3]. Multiple studies have indicated that pyrazoline compounds exhibit promising biological activities, including anti-inflammatory properties [4,5], antimicrobial effects [6], anticancer capabilities [7], and antidiabetic properties [8].

Inflammation represents the body's response to infection or other forms of injury, characterized by symptoms including redness, heat, swelling, and discomfort [1]. Inflammation is significantly associated with cancer, contributing to the growth and advancement of tumors [2]. Chronic inflammation undermines the immune system by enhancing cell proliferation, promoting angiogenesis, and facilitating

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metastasis, which ultimately contributes to oncogenesis [9].

Modifications of substituents on the N-pyrazoline atom, as well as the variations in substituents on the benzene ring of pyrazoline compounds, significantly contribute to the identification of pyrazoline derivative compounds with enhanced biological activity [10]. Studies have shown that numerous pyrazolines demonstrate significant anti-inflammatory properties [1], [4,5]. Many research studies indicate that differences in substituents on the nitrogen atom and benzene ring of pyrazoline compounds lead to varying degrees of anti-inflammatory activity [1], [3], [10]. Consequently, the diversity of substituents in pyrazoline compounds is crucial for determining their effectiveness as anti-inflammatory agents.

Currently, various methods are used to develop drug compounds. One approach to drug development involves computational chemical simulations. Computational medicinal chemistry enables the three-dimensional (3D) representation of compounds, allowing for comparative analysis of their similarity and energy levels in relation to other known active compounds through a pharmacophore query [11,12]. A range of derivatives and analogs may be generated using computational methods, often referred to as theoretical compounds. This software application computationally examines the interactions between these theoretical compounds and receptors for which 3D structural data are available. Such research can forecast the efficacy of these theoretical compounds while also discarding those with minimal activity [13]. Molecular docking employs computational methods for drug design, allowing for the prediction of interactions between receptors and ligands through 3D visualization [14]. This study explores the interactions of pyrazoline derivatives using molecular docking and assesses their toxicity as anti-inflammatory agents.

## 2. EXPERIMENTAL SECTIONs

### 2.1. Protein Ligand Preparation

The molecular docking procedure was conducted on an Asus Vivobook with Windows 11 Home Single Language. This laptop features an AMD Ryzen 5 3500U CPU, a Radeon Vega Mobile Gfx GPU operating at 2.10 GHz, and is equipped with 8 GB of RAM. The 3D representations of Pyrazoline Derivatives were generated using Avogadro and underwent geometric optimization with Orca. The crystallographic structure of cyclooxygenase-2 (COX-2) in complex with the ligand ibuprofen (PDB: 4PH9) [10] was obtained from the protein data bank and subsequently processed utilizing Discovery Studio Visualizer.

### 2.2. Molecular Docking of Pyrazoline Derivatives

Molecular docking was subsequently conducted using AutoDock Tools and AutoDock Vina, with a precision range of 8 to 264 [15]. A suitable grid box was defined as a parameter to improve the thoroughness of the conformational search [16,17]. A grid box with dimensions of 30x30x30 Å was utilized, incorporating a grid spacing of 0.375 Å to cover the entire structure of both the standard and proposed ligands. Ibuprofen was utilized as the reference ligand, and a redocking process was executed to confirm the accuracy of the molecular docking method. The root mean square deviation (RMSD) from the re-docking was calculated, alongside a comparison of the binding energy between ibuprofen as the reference ligand and the proposed compound. The dimensions of the grid box correspond to the control ligand (native ligand) associated with the PDB protein.

### 2.3. Toxicity Prediction using online pkCSM. pharmacokinetics

The online pkCSM application could be used to forecast the toxicity and characteristics of pyrazoline derivative compounds [18]. The structure of the compound in SDF file format was translated into a SMILES file via the SMILES translator. The results encompassed predictions of acute rat oral LD50s [19]. The chemicals were presented alongside their respective SMILES codes.

## 3. RESULT AND DISCUSSION

### 3.1. Protein Ligand Preparation

The three-dimensional structure of the COX-2 receptor bound to the Ibuprofen inhibitor was obtained from the Protein Data Bank (PDB ID: 4PH9). Solvent molecules present in the receptor were eliminated, after which the receptor structure and the standard ligand (Ibuprofen) were isolated using Discovery Studio Visualizer software, each saved in PDB format. Four pyrazoline derivative compounds (pyrazolines 1-4) depicted in **Fig. 1** were created using the Avogadro application and subsequently optimized with the Orca application employing DFT/B3LYP.

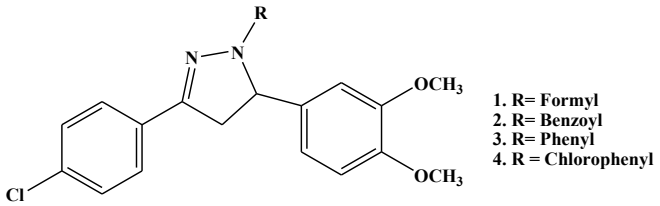



**Fig. 1.** Pyrazoline derivatives structure

Density Functional Theory (DFT) is a rigorously precise yet practically empirical approach based on first principles for addressing the fermionic many-electron problem, which is fundamental to the majority of chemistry as well as significant areas of biology and physics [20]. DFT/B3LYP is a hybrid DFT standard computational method that provides sufficiently accurate results for organic molecules without

consuming significant amounts of computing resources [21].

### 3.2. Molecular Docking Analysis of Pyrazoline Derivatives

The docking procedure was executed using the standard ligand ibuprofen through Autodock Vina software, focusing on the receptor's active site (COX-2), consisting of an amino acid residue that interacts with the ligand. A grid box measuring 30x30x30 Å was employed, featuring a grid spacing of 0.375 Å to encompass the complete structure of both standard and the proposed ligands. The exhaustiveness parameter was varied from 8 to 264 to enhance the thoroughness of the conformation search. Prior to molecular docking on four pyrazoline derivative compounds, a redocking process was performed on the standard ligand ibuprofen, resulting in a binding affinity of -7.6 kcal/mol. This binding affinity indicates the energy required to establish a bond with the receptor; thus, it serves as a predictive measure of a compound's activity. A lower binding affinity correlates with increased stability in the ligand-receptor interaction, suggesting that compounds with lower affinity values demonstrate superior activity compared to those with higher affinity values.

The docking procedure was validated using the native ligand and protein structures, resulting in a root mean square deviation (RMDS) of 2 Å. The RMDS value presented in **Fig. 2** was 1.272 Å, demonstrating the method's validity with exceptional resolution [22].

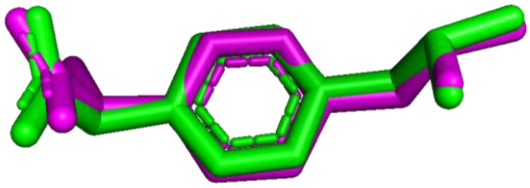


**Fig. 2.** The overlay structure of the standard Ibuprofen ligand is presented prior to docking (in green) and subsequent to docking (in purple), with a Root Mean Square Deviation (RMSD) value of 1.272 Å

Molecular docking analysis of four pyrazoline derivative compounds revealed differing binding affinity values, suggesting that variations in the substituents of the pyrazoline molecule influence the interaction between the ligand and the receptor. A summary of the binding affinity values and their interactions with the receptor amino acids is presented in **Table 1**. Several important amino acids that play a role in ligand binding to the COX-2 receptor are ARG121, TYR356, and VAL117 [23]. The interactions between the amino acid residues of the receptor and the tested ligand are illustrated in **Fig. 3**.

Pyrazoline 2, featuring a benzoyl substituent on its ring, exhibited the highest binding affinity of -8.0 kcal/mol, surpassing the standard ligand ibuprofen, which had a binding affinity of -7.6 kcal/mol. The interactions between the ligand (pyrazoline 2) and the receptor include Van der Waals forces involving SER472, GLU525, PHE471, PRO86, LEU124, SER120, LEU93, and TYR356. Additionally, a Carbon-Hydrogen bond was formed with GLU525, while Pi-Cation interactions occured with ARG121 and LYS83. The Pi-Pi T-Shaped interaction involved TYR116, and Pi-Alkyl interactions were noted with TYR123, VAL117, VAL89, and PRO84. The docking simulation results suggested that the interaction between pyrazoline compound 2 and the COX-2 receptor was stronger than that of ibuprofen, indicating a superior stability of the bond between pyrazoline 2 and the receptor compared to that of standard ligands and other pyrazoline derivatives.

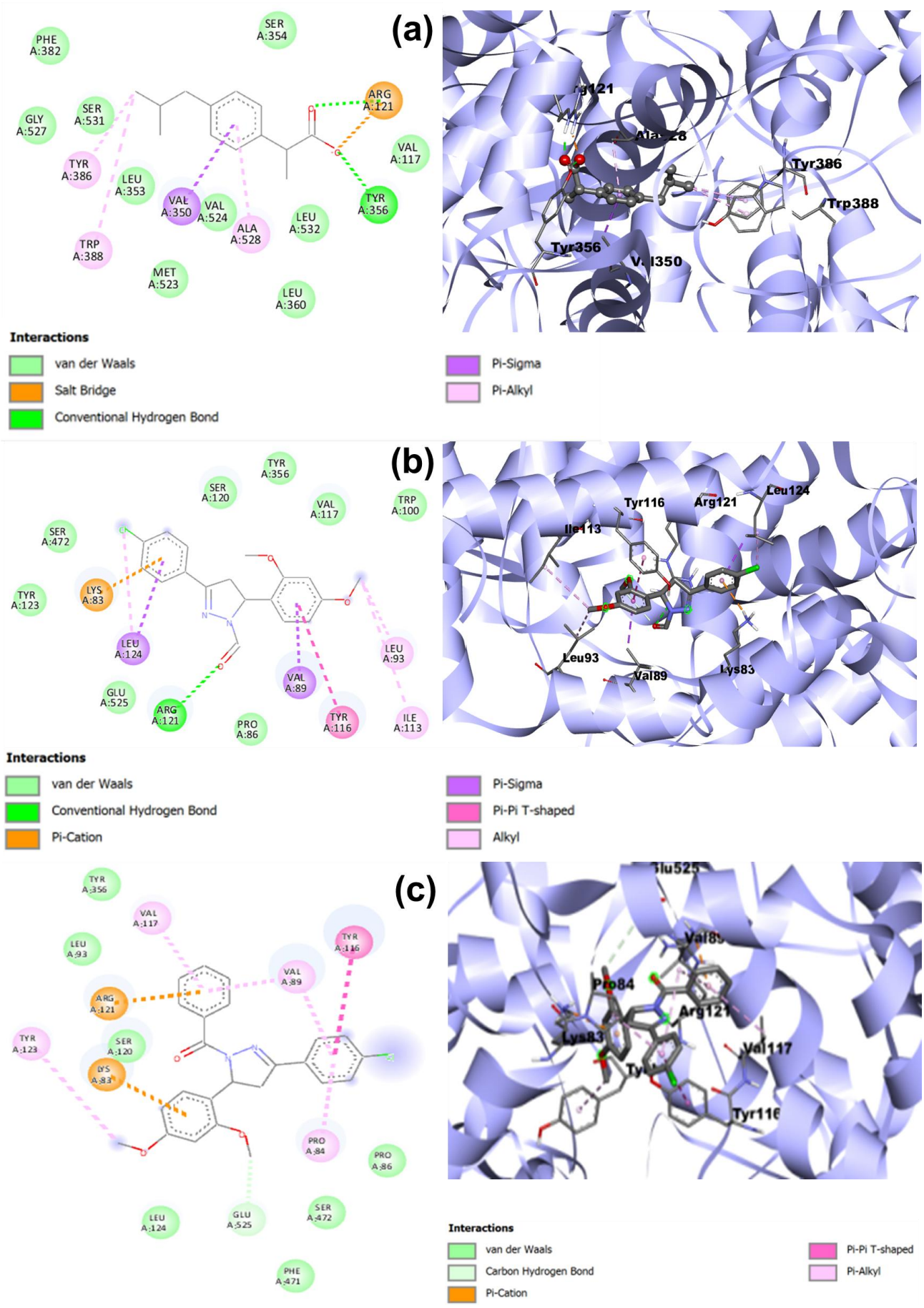
**Table 1.** Molecular docking results of pyrazoline derivatives.

| Compound | BA* | Interaction |
|---|---|---|
| Standard Ligan (Ibuprofen) | -7,6 | Van der Waals: PHE382, GLY527, SER531, LEU353, MET523, VAL524, LEU360, LEU532, SER354, VAL 117; Hydrogen Bond: TYR356; Salt Bridge: ARG121; Pi-Sigma : VAL350; Pi-Alkyl: TYR386, TRP388, ALA528. |
| Pyrazoline 1 | -7,1 | Van der Waals : SER472, TYR123, PRO86, TRP100, VAL117, TYR356, SER120; Hydrogen Bond: ARG121; Pi-Cation: LYS83; Pi-Sigma : LEU124, VAL89; Pi-Pi T-Shaped: TYR116; Alkyl : ILE113, LEU93. |
| Pyrazoline 2 | -8.0 | Van der Waals: SER472, GLU525, PHE471, PRO86, LEU124, SER120, LEU93, TYR356; Carbon Hydrogen Bond: GLU525; Pi-Cation : ARG121, LYS83; Pi-Pi T-Shaped: TYR116; Pi-Alkyl: TYR123, VAL117, VAL89, PRO84. |
| Pyrazoline 3 | -7.7 | Van der Waals : ARG121, SER120, THR88, LEU112, ILE113; Pi-Sigma : VAL89; Pi-Pi Stacked: TYR116; Pi-Pi T-Shaped : TYR116; Alkyl and Pi-Alkyl: VAL117, TYR356, LEU93, ILE92, PRO84. |
| Pyrazoline 4 | -7.6 | Van der Waals: ARG121, SER120, THR88, ILE113; Pi-Sigma : VAL89, LEU93; Pi-Pi Stacked : TYR116; Alkyl and Pi-Alkyl: VAL117, TYR356, LEU93, ILE92, PRO84, LEU112. |

*BA = Binding Affinity (kcal/mol)

### 3.3. Toxicity Prediction of Pyrazoline Derivative

In silico toxicity predictions were conducted to assess the toxicity of pyrazoline derivative compounds, focusing particularly on pyrazoline 2, which demonstrated the highest binding affinity according to molecular docking analyses. The toxicity evaluation results for pyrazoline compound 2, derived from the online pkCSM platform, are presented in **Table 2**.


(a)
PHE A:382
SER A:354
ARG A:121
GLY A:527
SER A:531
VAL A:117
TYR A:386
LEU A:353
VAL A:350
VAL A:524
TYR A:356
ALA A:528
LEU A:532
TRP A:388
MET A:523
LEU A:360
Tyr386
Trp388
Tyr356
Interactions
van der Waals
Salt Bridge
Conventional Hydrogen Bond
Pi-Sigma
Pi-Alkyl
(b)
TYR A:356
SER A:120
VAL A:117
TRP A:100
SER A:472
TYR A:123
LYS A:83
LEU A:124
LEU A:93
VAL A:89
GLU A:525
ARG A:121
PRO A:86
TYR A:116
ILE A:113
Tyr116
Arg121
Leu124
Ile113
Leu93
Val89
Lys83
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Pi T-shaped
Alkyl
(c)
TYR A:356
VAL A:117
LEU A:93
TYR A:116
VAL A:89
ARG A:121
TYR A:123
SER A:120
LYS A:83
PRO A:84
PRO A:86
LEU A:124
GLU A:525
SER A:472
PHE A:471
Pro84
Arg121
Lys83
Val117
Tyr116
Interactions
van der Waals
Carbon Hydrogen Bond
Pi-Cation
Pi-Pi T-shaped
Pi-Alkyl

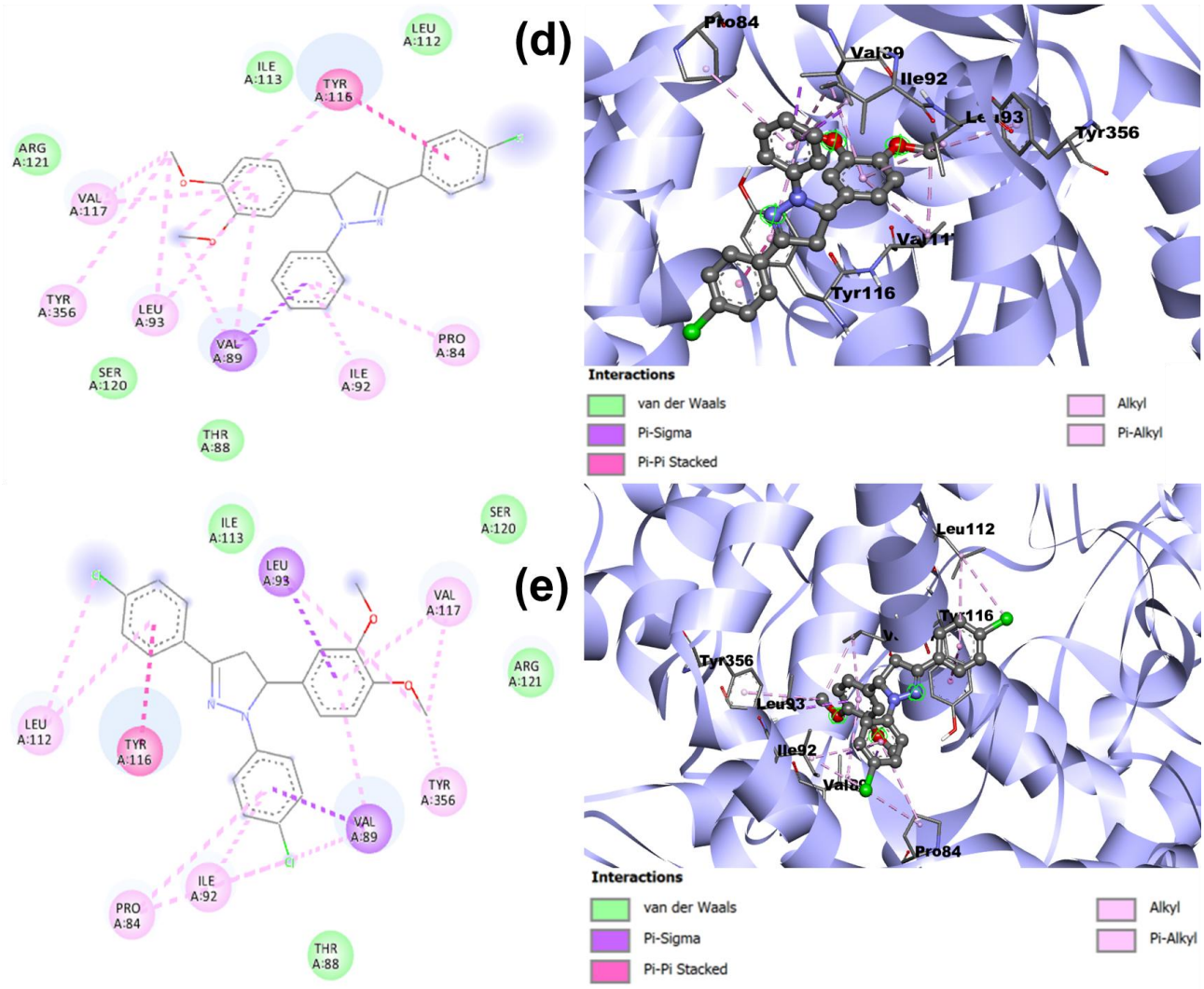



**Fig. 3.** Illustration of the interaction of (a) native ligand and receptor, (b) pyrazoline 1 and receptor, (c) pyrazoline 2 and receptor, (d) pyrazoline 3 and receptor, and (e) pyrazoline 4 and receptor.

Pyrazoline 2 is confirmed to be non-toxic, non-hepatotoxic, and does not cause skin sensitization. The compound had an LD50 value of 2,563 mol/kg, indicating the dosage that results in the death of 50% of the test subjects. This LD50 value is a standard measure for acute toxicity, allowing for the comparison of toxicity levels across various substances [19]. Based on the LD50 results, the pyrazoline is categorized as non-toxic. The threshold dose that could potentially lead to adverse effects was represented by a log dose value of 1,417 mg/kg_bw/day. Drug-induced liver injury is a major safety concern in pharmaceutical development and is a leading cause of drug attrition. The online pkCSM evaluation confirm that pyrazoline 2 does not show hepatotoxicity. Additionally, pyrazoline 2 is also determined to be non-mutagenic based on the Ames test results from the online pkCSM platform. The Ames test is a well-established method for assessing the mutagenic potential of compounds through bacterial assays [19], [24]. The toxicity prediction findings for pyrazoline 2 suggest that the maximum tolerated dose is safe for the organism.

**Table 2.** Toxicity prediction of pyrazoline 2

| Model Name | Prediction Value | Unit | Interpretation |
|---|---|---|---|
| AMES | No | Yes/No | Non- Mutagenic |
| Max tolerated dose (human) | 0.589 | Log mg/kg/ day | High MRTD |
| Oral Rat Acute Toxicity (LD50) | 2.563 | Mol/kg | Non-toxic |
| Oral Rat Chronic Toxicity (LOAEL) | 1.417 | Log mg/kg_ bw/day | Low Log Dose |
| Hepatoxicity | No | Yes/No | The compound does not cause side effects |
| Skin Sensiti-zation | No | Yes/No | The compound does not induce skin sensitization. |

## CONCLUSION

The findings from the conducted study revealed that pyrazoline 2 (1-benzoyl-(3-(4-chlorophenyl)-5-(3,4-dimethoxy)-4,5-dihydro-2-pyrazoline) exhibited optimal ligand-receptor interaction, as reflected by its binding energy of -8.0 kcal/mol, surpassing that of the standard ligand, ibuprofen, which had a binding energy of -7.6 kcal/mol. Additionally, toxicity assessments indicated that pyrazoline 2 was nontoxic, lacks hepatotoxicity, and did not induce skin sensitization, with an Oral Rat Acute Toxicity (LOAEL) score of 1.417 log (mg/kg_bw/day). It was advisable for future research to include an in silico molecular dynamics investigation to analyze these interactions through simulation, as well as to synthesize pyrazoline 2, and evaluate its effects both in vitro and in vivo to assess the potential of its derivatives as anti-inflammatory agents.

## SUPPORTING INFORMATION

This article contains no supporting material. Supporting information can be provided by request to Corresponding Author (MW).

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## CONFLICT OF INTEREST

There was no conflict of interest in this study.

## AUTHOR CONTRIBUTIONS

This research was developed by MW, AR, MBH, and AKH. MW, MBH, and AKH were responsible for the preparation of compounds and receptors, and the optimization of pyrazoline derivatives. MW conducted the molecular docking analysis. Toxicity prediction was carried out by MW, AZ, and AKH. The manuscript was drafted and reviewed by MW, AZ, MBH, and AKH. All authors conveyed their approval for the final version of the manuscript.

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