In-Silico Evaluation of Binding Interaction and ADME Properties of Novel Pyrazoline and Pyrimidine Derivatives Targeting Cyclooxygenase-2 Enzyme.

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Abstract:

Inflammation is a crucial defense mechanism against toxic stimuli and physical traumas. This study aimed to design six new pyrazoline and pyrimidine derivatives and assess their potential anti-inflammatory activity by targeting the cyclooxygenase (COX) enzyme, a key mediator in the inflammation process. Through in-silico methods, the binding affinity of these derivatives to the COX-2 receptor was predicted using computer simulations and compared with the reference drug, meloxicam.

Docking studies employing gold software facilitated the visualization of ligand-protein interactions, utilizing the crystal structure of the COX-2 protein (PDB ID: 4m11). Preceding the docking procedure, the receptor underwent energy minimization by SPDPV software and hydrogen atoms addition. Compounds 1 to 6 exhibited superior docking scores, indicative of strong binding affinities and favorable positioning within the COX-2 enzyme's active site. The results of using this approach are series of products content of pyrazoline and pyrimidine derivatives that had greater potency as anti-inflammatory action and binding in the active location inside the COX-2 protein may potentially serve as a lead for the identification of novel anti-inflammatory medications to decrease the side effects and toxicity that produced from some NSAIDs.

KEYWORDS: Anti-inflammatory, cyclooxygenase, Molecular Docking, Pyrazoline, Pyrimidine.

النمذجة الجزيئية حاسوبيا لتقييم الارتباط ودراسة خصائص (الامتصاص والتوزيع والتايض والافراز) لبعض مشتقات البايرازولين والبيريميدين الجديدة تستهدف انزيم السيكلواوكسيجينيز-2.

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الخلاصة:

الالتهاب هو آلية مهمة للحماية والبقاء على قيد الحياة ضد المنبهات السامة والصدمات الجسدية. كان الهدف من هذه الدراسة استخدام الارساء الجزيئي لتصميم ودراسة ست مشتقات جديدة من البيرازولين والبيريميدين وتحديد تأثيرها المضاد للالتهابات حيث قد يكون لها القدرة على تثبيط إنزيم السايكلواوكساجينيز-2 الذي يلعب دورًا مهمًا أثناء عملية الالتهاب. تم التنبؤ بالتصرف الملزم لستة مشتقات جديدة من البيرازولين والبيريميدين بمستقبلات COX-2 باستخدام المحاكاة الحاسوبية من خلال مقارنتها

بالدواء المرجعي وهو الميلوكسيكام. تم استخدام أسلوب الالتحام باستخدام برنامج Gold بعد ان تم الحصول على بروتين 4m11 من موقع PDB وتم خفض طاقة البروتين باستخدام برنامجSPDBV وكذلك تمت اضافة ذرات الهيدروجين الى البروتين، وحصلت المركبات من 1 إلى 6 على أفضل الدرجات للالتحام نتيجة لارتباطاتها القوية والموضع الأمثل داخل الموقع النشط لإنزيم COX-2. نتائج استخدام هذا النهج هي سلسلة جديدة من المنتجات التي تحتوي على مشتقات البيرازولين والبيريميدين والتي لها فعالية أكبر كمضاد للالتهابات وارتباط في الموقع النشط داخل بروتين COX-2 ومن الممكن ان هذه المواد قد تكون بمثابة بداية لاكتشاف عقاقير جديدة مضادة للالتهابات. لفهم الآثار الجانبية وسمية جميع المشتقات الجديدة المنتجة، تعد أبحاث التقييم البيولوجي والدوائي نقطة ضرورية لاختراع عقاقير جديدة مضادة للالتهابات.

الكلمات المفتاحية: مظاد للالتهابات، سيكلوآكسجيناز ، الارساء الجزيئي،بيرازولين،بيريميدين.

1. Introduction

Inflammation constitutes a pivotal defense mechanism against various forms of toxic stimuli and physical traumas.[1] This complex biological process entails a cascade of reactions involving numerous mediators, leading to manifestations such as heat, redness, edema, discomfort, or impaired functionality within the inflamed tissues .[2] Dysregulated inflammatory responses have been implicated in the pathogenesis of diverse ailments, including but not limited to diabetes mellitus, cardiovascular disorders, and Alzheimer's disease .[3,4] **Figure 1** illustrates the diverse inflammatory effects orchestrated by immune cells, encompassing conditions ranging from hypertension and minor ailments to more severe manifestations such as gastrointestinal toxicities, cardiovascular complications, renal impairments, and hepatotoxicity .[5–7]

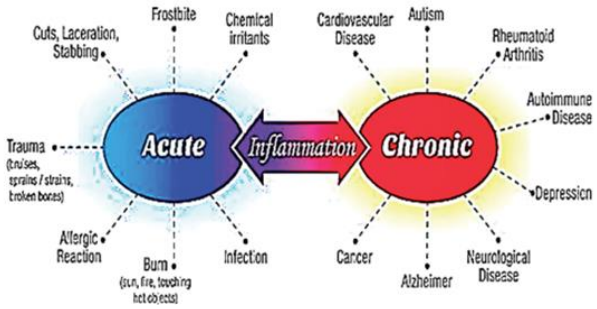


Figure (1): Main medical condition caused by acute and chronic inflammation. [7]

The inflammation mechanism, initiated by the arachidonic acid cascade, represents a fundamental pathway activated by cyclooxygenases (COX) and lipoxygenases (LOX) to generate eicosanoids, thereby presenting potential pharmacological targets for pain alleviation and inflammation reduction[8]. Throughout antiquity, significant shifts have occurred in treatment approaches, all of which revolve around the inhibition of proinflammatory agents.[9]

Prostaglandins (PGs) play a pivotal role in the initiation and progression of inflammatory responses. These bioactive lipid mediators are synthesized from arachidonic acid, a polyunsaturated fatty acid comprising 20 carbon atoms. Arachidonic acid is liberated from the plasma membrane through the action of phospholipases. Upon release, arachidonic acid undergoes metabolism mediated by cyclooxygenase

(COX) enzymes, initially identified as prostaglandin G/H synthases (PGHS).[10]

Arachidonic acid undergoes oxidation at the active site of the cyclooxygenase enzyme within prostaglandin G/H synthases (PGHS), resulting in the formation of the unstable intermediate, prostaglandin G2 (PGG2). Subsequently, PGG2 is rapidly reduced to prostaglandin H2 (PGH2) at the peroxidase active site. PGH2 then undergoes further transformation into specific prostaglandin derivatives through the catalytic activity of synthases and specialized isomerases. Inflammation triggers significant fluctuations in both the quantity and composition of prostaglandin synthesis. The extent of these variations is governed by the activity of two isoforms of the cyclooxygenase enzyme, namely COX-1 and COX-2. [11]

Cyclooxygenase (COX), a important enzyme in the prostaglandin biosynthetic pathway, provide a sequence of critical events. These processes encompass the cyclooxygenation of arachidonic acid, leading to the formation of prostaglandin G2 (PGG2), followed by the peroxidation of PGG2 to generate prostaglandin H2 (PGH2). Notably, these intermediates, PGG2 and PGH2, serve as specific targets of nonsteroidal anti-inflammatory drugs (NSAIDs).[12]

Cyclooxygenase (COX) exists in two distinct isoforms: COX-1 and COX-2, each with unique roles and functions. COX-1 is primarily responsible for the synthesis of prostaglandins essential for maintaining physiological functions such as protecting the stomach lining and regulating internal homeostasis. In contrast, COX-2 represents a variant that plays a significant role in mediating inflammatory responses. Significant homology exists between the COX-1 and COX-2 enzymes, underscoring a high degree of similarity between these isoforms. Specifically, an analysis of amino acid sequences reveals substantial correspondence, with approximately 84% of amino acids exhibiting similarity, among which 61% are identical. This level of similarity suggests evolutionary conservation and functional overlap between COX-1 and COX-2, despite their distinct roles in physiological and pathological processes.The homology within the top active site surpasses 90%, indicating a remarkable degree of similarity in the critical regions. Notably, only a limited number of positions within this active site exhibit variations that could be targeted for specific purposes. A pivotal substitution occurs at position 523, where Ile (in COX-1) is replaced by Val (in COX-2). This single amino acid variation, characterized by the presence of a solitary methyl group, results in the expansion of the active site, commonly referred to as the COX-2 pocket. This structural divergence confers distinct functional properties to COX-2, highlighting its potential as a therapeutic target for the development of selective inhibitors.[13]

Nonsteroidal anti-inflammatory drugs (NSAIDs) stand as prominent and widely utilized medications within the annals of contemporary medicine. Renowned for their efficacy, NSAIDs constitute a cornerstone in the management of conditions characterized by inflammation, fever, and discomfort like rheumatoid arthritis and osteoarthritis.[14]

Among the well-known NSAIDs, a majority are classified as important analogues of salicylic acid, acetic acid, enolic acid, anthranilic acid, or propionic acid.[15]

Certain NSAIDs, such as celecoxib, exhibit selective inhibition of cyclooxygenase-2 (COX-2), while others are non-specific and inhibit both COX-1 and COX-2 enzymes. Previously, there was a prevailing belief that inhibitors of COX-1 could potentially induce gastrointestinal complications by impeding the production of prostaglandins in the gastric mucosa.[16–18]

Treatment with NSAIDs carries a risk of side effects, particularly within the digestive system, including dyspepsia, stomach ulcers, and other adverse reactions. To mitigate these drawbacks, efforts have been directed towards developing NSAIDs with minimal side effects [19] Notably, a variety of pharmacologically active substances containing 2-pyrazolines have been explored for their potential therapeutic effects. These substances encompass phenazone/amidopyrene/methampyrone (utilized for pain relief and fever reduction), azolid/tandearil (employed for inflammation reduction), indoxacarb (an insecticide), anturane (enhancing uric acid excretion), among others. The diverse spectrum of potential pharmacological effects exhibited by 2-pyrazolines underscores their significance in drug discovery and development.[20]

Nitrogen-based heterocyclic analogues hold a pivotal position in medicinal chemistry owing to their profound contributions to the development of therapeutic medications. Nitrogen, characterized by its high affinity for forming hydrogen bonds with biological targets, readily engages in interactions vital for pharmacological activity. A diverse array of nitrogen-containing heterocyclic compounds exhibits therapeutic potential in combating a spectrum of diseases, including cancer, HIV, malaria, tuberculosis, diabetes, and various other ailments. Their versatile pharmacological properties make them indispensable in the quest for novel therapeutic interventions...[21]

The pyrazoline structure has garnered significant attention in the realm of drug development, exemplifying the advancements in modern pharmaceutical research and the unforeseen effects that can emerge from structural modifications of drug prototypes.[22–24] Pyrazoline, a 5-membered heterocyclic molecule characterized by two adjacent nitrogen atoms at positions 1 and 2, along with three carbon atoms, has been extensively studied and synthesized through various methodologies. Its three partly reduced versions, namely 1-pyrazoline, 2-pyrazoline, and 3-pyrazoline as depicted in **Figure 2**, exhibit diverse double bond configurations. Pyrazoline derivatives feature endocyclic double bonds, underscoring their significance in the historical evolution of heterocyclic compounds and their substantial role in biological activity. Consequently, pyrazoline serves as an essential pharmacophore with promising prospects for future drug development efforts.[25]

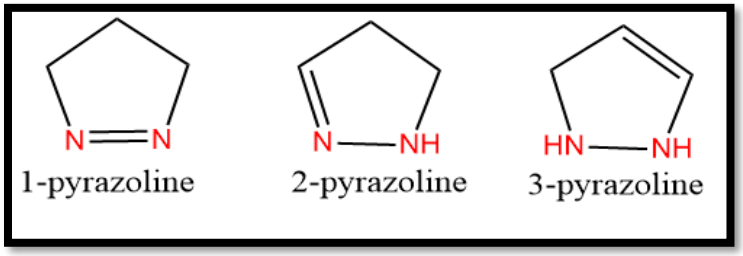


Figure (2): Core structure of pyrazoline [25]

Pyrimidines represent a class of heterocyclic aromatic compounds distinguished by the presence of two nitrogen atoms situated at positions 1 and 3 within the six-membered rings as shown in **Figure 3**, Due to their versatile structural features, substituted pyrimidines fulfill a wide array of biological functions, including but not limited to anti-inflammatory properties, antimicrobial activities, and tuberculosis prevention. Consequently, pyrimidine derivatives are acknowledged as physiologically active chemical entities with considerable therapeutic potential. [26,27]

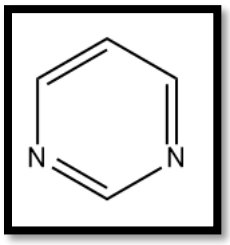


Figure (3): structure of pyrimidine

The current study is focus on the design and evaluation of heterocyclic rings consisting of both five and six members. After verifying that the proposed compounds had not been previously synthesized, our findings were presented alongside a docking analysis. The primary objective of this research was to assess the anti-inflammatory properties of new pyrazoline and pyrimidine core compounds. Specifically, to identify compounds that demonstrate enhanced efficacy, minimized side effects, and increased selectivity against cyclooxygenase-2 (COX-2).

2. Materials and Method

The development of new drugs is a costly and intricate endeavor due to the multitude of steps involved in creating novel compounds with desired properties. The process of synthesizing a new molecule underscores the intricate structure and high expenses associated with contemporary drug development. Embracing a comprehensive approach to drug development, one that takes into account the potential interactions of chemicals with the entire network of biomolecules within cells, holds promise for yielding improved outcomes. This holistic strategy aims to enhance the efficacy, safety, and specificity of novel drug candidates, thereby addressing the complexities inherent in modern drug discovery and development processes.[28]

Figure 4 illustrates the computational strategy employed in this study [29]. The chemicals underwent molecular docking studies utilizing the GOLD Suite (v. 5.6.2), a fully licensed product of CCDC. Visualization of the protein, ligands, hydrogen bonding interactions, short contacts, and bond length calculations was facilitated using CCDC Hermes visualizer software (version 1.9.2). The chemical structures of our ligands were depicted using ChemBioOffice software (v. 17.1). Additionally, simulation was conducted with the assistance of Swiss ADME services to determine the pharmacokinetic profile, encompassing processes such as adsorption, distribution, metabolism, and excretion (ADME), of the synthesized chemicals. taking into account that the six compounds was checked with SCI finder tool and all of them are new and not prepared before.[30,31]

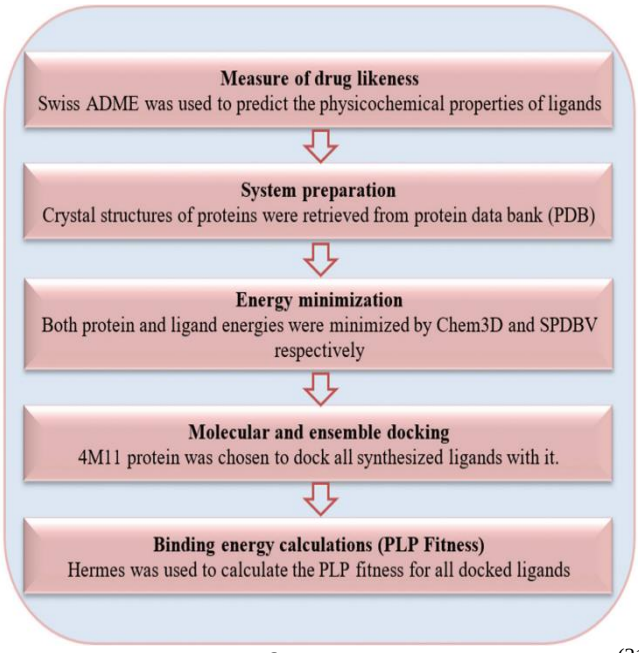


Figure (4): Outline of computational protocol. [31]

The crystal structures of the COX-2 enzymes were retrieved from the Protein Data Bank (PDB). Subsequently, using Swiss PDB Viewer (v. 3.7), missing atoms were inserted into the crystal structures of the enzymes. The obtained protein structures underwent refinement processes, involving the elimination of water molecules and the addition of hydrogen atoms to ensure proper ionization and tautomerization of amino acid residues. Utilizing the MM2 force field, energy minimization for our compounds was carried out and using ChemOffice (v. 15) to draw the structures.

For the molecular docking process, GOLD (version 5.6.2) was employed under a full license. The receptors were prepared for docking using the Hermes visualizer application within the GOLD Suite. The binding site utilized for GOLD docking was defined to encompass all protein residues within 10 Å of the standard ligand, meloxicam. To conduct the ensemble docking procedure, five COX-2 proteins (1pxx, 4m11, 3LN1, 3KK6, and 5kIR) were retrieved from the Protein Data Bank (PDB). Subsequently, 4m11 was selected for the docking study on the compounds. The cavity and active site were identified using CCDC Superstar, and the radius of the active site (10 Å) was calculated based on the reference ligand of the protein. The ChemScore kinase configuration template was employed for the docking procedure.

In this study, we will compare the binding affinity of our compounds with the NSAID drug meloxicam. For this purpose, we will utilize the COX-2 receptor protein, specifically the 4m11 structure, as the binding target for both our compounds and meloxicam. Through molecular docking simulations, we aim to evaluate and compare the interactions of our compounds with the COX-2 receptor against those of meloxicam. This comparative analysis will provide insights into the potential efficacy and binding characteristics of our compounds relative to the known NSAID meloxicam.

In our docking procedure, the scoring function employed was the ChemPiecewise linear potential (CHEMPLP). All parameters utilized in the docking process were maintained at their default levels. The docking solutions were evaluated based on the CHEMPLP fitness function, which calculates the steric interactions between the

protein and the ligand. This scoring function accounts for the impact of hydrogen bonds, considering both their angle and distance, in determining the overall binding affinity between the ligand and the protein.

The objective of the study was to evaluate the interaction between the amino acid residues of the COX-2 protein and our compounds. This comprehensive analysis involved examining the docking data to understand the binding mode, docked posture, and binding free energy of the compounds within the COX-2 active site. By elucidating these aspects, we aimed to gain insights into the molecular interactions between our compounds and the COX-2 receptor, which could inform their potential as anti-inflammatory agents.[32]

The pharmacokinetic properties of the six compounds were assessed using the SwissADME server, which comprehensively evaluates absorption, distribution, metabolism, and excretion (ADME), along with additional characteristics such as the ability to cross the blood-brain barrier (BBB), affinity for P-glycoprotein (P-gp), and bioavailability. The primary aim of this evaluation was to identify the most viable and promising therapeutic candidates by eliminating compounds with unfavorable ADME characteristics that may lead to failure in later stages of medication development. This process helps prioritize compounds with optimal pharmacokinetic profiles for further preclinical and clinical studies, thereby enhancing the likelihood of successful drug development.[33]

The ligands (1-6) were prepared using ChemOffice (v. 15) to draw their chemical structures. The SMILES (Simplified Molecular Input Line Entry System) notation of each ligand was obtained. Subsequently, the Swiss ADME tool, accessible via the website http://www.swissadme.ch/, was utilized. Within the Swiss ADME tool, there is a dedicated window where the SMILES notation of each ligand can be inputted. Once all six SMILES notations were added, the "predict" button was pressed to initiate the operation. This process facilitated the evaluation of various pharmacokinetic parameters and drug-likeness properties of the ligands, aiding in the identification of promising therapeutic candidates for further investigation.[34]

3. Result and Discussion

The primary objective of drug discovery is the identification of new active molecules possessing enhanced pharmacological characteristics against a specific biological target. This goal drives the continual exploration and development of novel compounds with improved therapeutic efficacy, reduced side effects, and enhanced selectivity. Through rigorous research and experimentation, the endeavor aims to expand the repertoire of treatment options available to address unmet medical needs and improve patient outcomes.[35]

The objective was to develop new inhibitory ligands with enhanced binding affinity by employing binding selectivity strategies through molecular docking. Virtual screening, a computational technique, was utilized to identify potential drug candidates from extensive chemical libraries or databases. This approach rapidly evaluates a large number of small molecules to predict their likelihood of binding to a specific target, such as a protein receptor involved in a disease process. The primary aim of virtual screening is to prioritize compounds for experimental testing based on their predicted binding affinity or other relevant properties.

Virtual screening serves as a cost-effective and time-efficient method to identify lead compounds, thereby reducing the need for laborious and expensive experimental screening of large compound libraries. It plays a critical role in early-stage drug discovery by accelerating the identification of lead compounds with potential therapeutic activity. Virtual screening complements

experimental screening efforts and enables researchers to explore a vast chemical space to identify promising drug candidates efficiently.[36]

Table 1 presents the binding affinity scores for all screening agents. According to the virtual screening (VS) results, COX-2 exhibited a range of binding affinity values between 70.21 and 66.3, while meloxicam achieved a score of 65.21. Notably, compounds p1 and p2 attained the highest docking scores due to their exceptional affinity for binding and precise positioning within the active region of the receptor. This region is characterized by a key amino acid that facilitates effective interactions.

Consequently, efforts have been concentrated on the development of inhibitors targeting this enzyme, aiming for optimal efficacy while minimizing potential side effects. The favorable binding characteristics exhibited by compounds p1 and p2 underscore their potential as promising candidates for further evaluation and development as COX-2 inhibitors.[37]

Table (1): Docking score for different pyrazoline and pyrimidine Derivatives located inside the active site of the COX-2 enzyme.

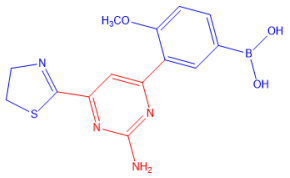
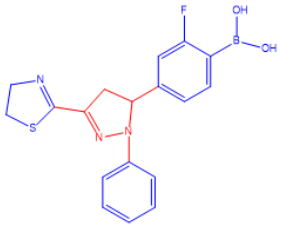
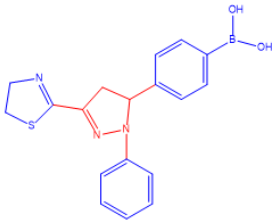
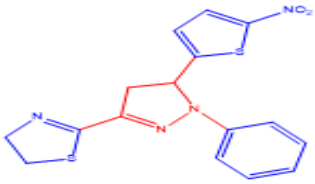
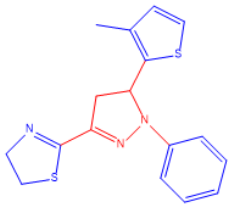
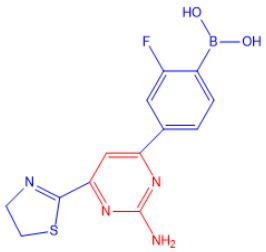
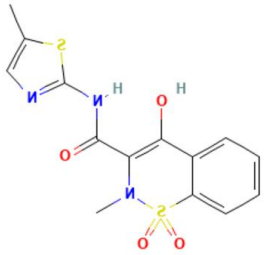
NO.	Structure	Docking score cox 2	Binding interaction for drugs with cox 2
P1		70.21	H-BOND: SER530, TYR 355 and ARG120 Short contact: TRP387, MET522, TYR385, VAL349, SER530, ALA527, TYR355 and ARG120
P2		69.76	H-bonds: VAL523 and MET522. Short contact: PHE470, GLY526, VAL523, MET522, TRP387, ARG120, VAL349, VAL116, SER119, SER530.ALA527 and TYR355
P3		68.03	H-bonds: TYR355, ARG120, MET522 Short contact: GLY526, VAL523, MET522, TRP387, ARG120, VAL523, VAL116, SER119, LEU390, LEU384. ALA527, TYR385 and TYR355
P4		67.41	H-bond: ARG120 Short contact: ALA527, LEU359, LEU531, MET113,TRP387, VAL116, SER119 and TYR355
P5		66.95	H-bond: SER530 Short contact: ALA527, LEU359, LEU531, MET113, TRP387, VAL116, VAL349, SER530 and ARG120

Table (1): Docking score for different pyrazoline and pyrimidine Derivatives located inside the active site of the COX-2 enzyme.

NO.	Structure	Docking score cox 2	Binding interaction for drugs with cox 2
p6		66.3	H-bond: SER119 Short contact: ALA527, LEU117, LEU93, LEU531, MET113, TYR355, VAL116, SER119 and ARG120
Reference meloxicam		65.21	H-bond: SER530 Short contact: TYR355, VAL523, ALA527, VAL349 and SER530

As depicted in **Table 1**, the docking results range between 65.21 and 70.21. Compounds 1 to 6 exhibited the highest docking scores, indicative of their significant affinity for binding and optimal positioning within the active region of the COX-2 enzyme. Notably, compounds p1 and p2 attained the highest scores, indicating strong interactions with the amino acids that meloxicam binds to within the COX enzyme. This suggests that p1 and p2 possess favorable interactions with the COX-2 enzyme, making them promising lead compounds for the development of new anti-inflammatory drugs.

Figures 5 and 6 depict the interactions of compounds **p1** and **p2** with COX-2 amino acids, revealing the formation of hydrogen bonds. These interactions provide insights into the potential of p1 and p2 as effective inhibitors of COX-2 activity. Such observations underscore the significance of compounds p1 and p2 as promising candidates warranting further investigation and development in the pursuit of novel anti-inflammatory agents.

The docking investigations revealed that the amino acids Arg120, Tyr355, Ser530, Val116, Tyr385, Gly526, Val523, Trp387, Ala527, Leu531, Leu534, Leu345, Leu539, Val89, and Val349 of the COX-2 enzyme engage in hydrogen bonding and short-range interactions with our ligands. Figure 7 illustrates the interaction between meloxicam and the amino acids of COX-2. The bonds with a length below 3Å were analyzed to determine the distance between short contacts and hydrogen bonds involving specific atoms of the protein and our ligands. The term "short contacts" encompasses various interacting forces, including van der Waals, electrostatic, steric, π-π stacking, dipole-dipole, and others. These interactions play a crucial role in determining the binding

affinity and stability of the ligand-receptor complex, ultimately influencing the efficacy of the ligands as inhibitors of COX-2 activity. Indeed, hydrophobic contacts and hydrogen bonding interactions play crucial roles in the interaction within the active site of proteins like COX-2. These interactions contribute significantly to the stability and specificity of ligand-receptor complexes. Hydrophobic contacts, in particular, are essential as they promote the formation of stable complexes by maximizing the van der Waals interactions between the ligand and the hydrophobic residues lining the active site pocket.

The quantity of hydrophobic contacts is indeed directly correlated with the biological activity of a compound. When a ligand forms hydrophobic interactions with the hydrophobic residues lining the active site of a protein, it contributes significantly to the stability of the ligand-receptor complex. This enhanced stability increases the binding affinity of the compound for the target enzyme, consequently improving its potency as an inhibitor.

Hydrophobic interactions play a vital role in stabilizing the ligand within the active site, thereby enhancing the likelihood of successful inhibition of the target enzyme. As a result, compounds with a higher number of hydrophobic contacts tend to exhibit stronger binding affinity and greater biological activity, making them more effective as therapeutic agents in the treatment of various diseases, including inflammation.

Therefore, optimizing hydrophobic contacts through rational drug design strategies holds significant promise for the development of more potent and effective inhibitors of COX-2 activity. By strategically designing compounds to maximize hydrophobic interactions with the hydrophobic residues within the active site of COX-2, researchers can enhance the stability and binding affinity of the ligand-receptor complex. This optimization process may involve modifying the chemical structure of existing compounds or designing entirely new molecules that possess favorable hydrophobic moieties. By doing so, researchers can improve the potency and efficacy of COX-2 inhibitors, ultimately leading to the discovery of novel anti-inflammatory agents with enhanced therapeutic benefits. Overall, the rational design of compounds to optimize hydrophobic contacts represents a promising approach in the quest for more effective treatments for inflammatory conditions, offering potential advancements in the field of drug discovery and development..[38]

From the results of the docking study, it is evident that most of our compounds exhibit a higher binding affinity score than the reference ligand, meloxicam. This superiority can be attributed to several factors, including the chemical structure and substituents of our compounds.

For the purpose of this study, we will focus on product p2, which demonstrated highes score in the docking study, and compare it with the reference ligand, meloxicam. Meloxicam has a core structure of 1,1-dioxo-1λ6,2-benzothiazine-3-carboxamide, with substituents including 4-hydroxy, 2-methyl, and N-(5-methyl-1,3-thiazol-2-yl).

On the other hand, our compound, product p2, features a core structure of 4,5-dihydrothiazol-2-yl-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl, with substituents consisting of 2-fluorophenylboronic acid. The differences in core structure and substituents between meloxicam and our compound may contribute to the variations observed in their binding affinity and interaction with the COX-2 enzyme. Further analysis of the molecular interactions and binding modes of p2 and meloxicam with the COX-2 enzyme will provide valuable insights into the potential mechanisms underlying their differential binding affinities and may inform future drug design strategies for the development of novel anti-inflammatory agents. Now if we compare the **Structural**

Diversity of the tow products: Compound P2 contains a structurally diverse and complex heterocyclic core compared to the reference compound. This diversity may allow compound P2 to access a broader range of binding interactions within the target protein's binding site. **Functional Groups**: The boronic acid moiety in compound P2 may form specific interactions (e.g., hydrogen bonding or reversible covalent bonds) with residues in the target protein, enhancing its binding affinity. **Steric Considerations**: Compound P2 structure may have fewer steric clashes or better complementarity with the target protein's binding site compared to the reference compound, leading to a more favorable binding mode.

By considering these factors and analyzing the specific interactions observed in the docking study, we can gain insights into the structure-activity relationship (SAR) of our compounds and understand why it exhibited better results compared to the reference compound.

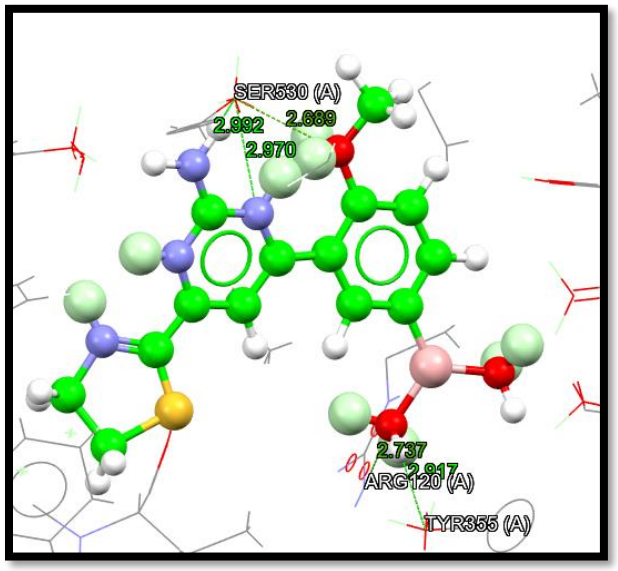


Figure (5): Highest scoring molecule (compound p1) inside COX-2 enzyme.

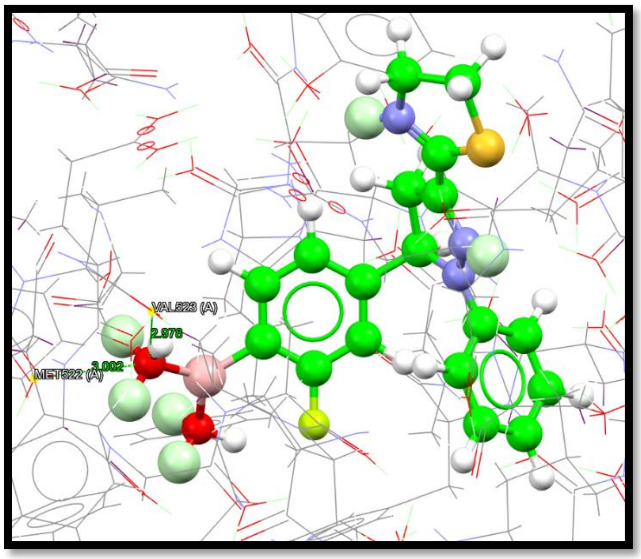


Figure (6): Second highest scoring molecule (compound p2) inside COX-2 enzyme.

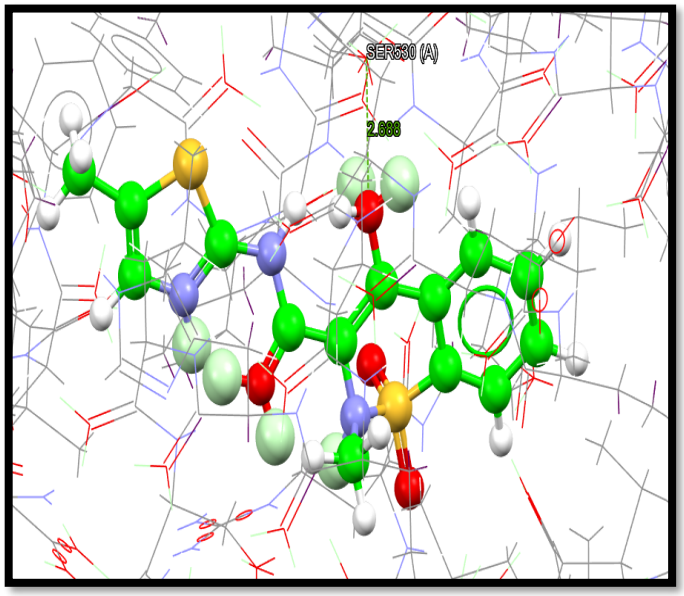


Figure (7): Meloxicam (Reference) inside COX-2 enzyme.

4. ADME Studies

The SwissADME online service offers free access to a wide range of efficient prediction simulations encompassing pharmacokinetics, physicochemical characteristics, medicinal chemistry attributes, and medicinal efficacy. These models include advanced approaches such as log P and bioavailability. Utilizing this tool enables the efficient and expedient conduct of absorption, distribution, metabolism, and excretion (ADME) research, which is essential in the early stages of drug development.

Figure 8 illustrates the BOILED-Egg plot for the two molecules with the highest scores. The plot reveals that neither compound can cross the blood-brain barrier (BBB), and both are cleared from the central nervous system (CNS). Additionally, Compound 1 is predicted to have poor gastrointestinal (GIT) absorption, while Compound 2 demonstrates strong passive absorption from the GIT.

These insights provided by the SwissADME tool aid in the assessment and optimization of pharmacokinetic properties, helping researchers make informed decisions regarding the selection and further development of promising drug candidates.

The Topological Polar Surface Area (TPSA) is a critical parameter used to characterize the drug transport mechanism, particularly regarding the absorption and bioavailability of orally administered medications within the intestines. It quantifies the sum of the contributions of polar atoms to the molecular surface area, including nitrogen, oxygen, and their attached hydrogen atoms.

It is widely recognized that molecules with a TPSA greater than 140 Å² tend to have low oral bioavailability. Additionally, the Lipinski's Rule of Five is commonly employed as a guideline for assessing the drug-likeness of potential compounds intended for oral administration. According to this rule, orally administered medications should ideally have:

1. No more than five hydrogen bond donors.

2. No more than ten hydrogen bond acceptors.
3. A molecular weight (M.Wt.) of 500 Daltons or less.

Adhering to these criteria increases the likelihood of a compound's success in terms of oral bioavailability and overall pharmacokinetic profile. Therefore, when designing orally administered medications, it is crucial to consider the TPSA value and comply with the Lipinski's Rule of Five to enhance the compound's suitability for oral delivery and potential efficacy as a therapeutic agent..[41]

ADME (Absorption, Distribution, Metabolism, and Excretion) factors play a crucial role in drug development. Understanding and optimizing these factors are essential for ensuring the efficacy, safety, and pharmacokinetic profile of a drug candidate. Here's how ADME factors can impact drug development:

1. **Absorption**:

- **Oral Bioavailability**: The extent to which a drug is absorbed after oral administration affects its systemic exposure and therapeutic efficacy. Factors such as solubility, permeability, and interactions with transporters and efflux pumps influence oral bioavailability.
- **Routes of Administration**: Different routes of administration (e.g., oral, intravenous, transdermal) have varying absorption profiles, affecting the onset, duration, and intensity of drug action.
- **Formulation Optimization**: Formulation strategies can enhance drug absorption, stability, and bioavailability through approaches like nanoparticle formulations, lipid-based formulations, and controlled-release formulations.

2. **Distribution**:

- **Plasma Protein Binding**: The degree of binding of a drug to plasma proteins influences its distribution, metabolism, and elimination. Highly bound drugs may have limited tissue penetration and reduced efficacy.
- **Blood-Brain Barrier Penetration**: The ability of a drug to cross the blood-brain barrier affects its efficacy in central nervous system (CNS) disorders. CNS-active drugs require sufficient brain penetration to reach their targets.
- **Tissue Distribution**: Drugs distribute unevenly among tissues due to variations in blood flow, tissue composition, and affinity for specific receptors or transporters.

3. **Metabolism**:

- **Metabolic Stability**: The susceptibility of a drug to metabolism by enzymes, particularly cytochrome P450 enzymes in the liver, influences its systemic exposure, half-life, and efficacy. Metabolism can lead to the formation of active, inactive, or toxic metabolites.
- **Drug-Drug Interactions**: Drugs may inhibit or induce metabolic enzymes, altering the metabolism of co-administered drugs and potentially leading to adverse effects or therapeutic failure.
- **Metabolic Activation/Inactivation**: Some drugs require metabolic activation to exert their pharmacological effects, while others are prodrugs that undergo metabolic conversion to their active form.

4. **Excretion**:

- **Renal Clearance**: Renal excretion plays a significant role in eliminating drugs and their metabolites from the body. Impaired renal function can prolong drug half-life and increase the risk of toxicity.
- **Biliary Excretion**: Drugs and metabolites may be excreted via bile into the gastrointestinal tract, where they can undergo enterohepatic circulation or be eliminated via feces.

- **Other Routes of Elimination**: Drugs can also be eliminated through sweat, saliva, tears, and respiratory secretions.

Optimizing ADME properties early in drug development helps identify promising candidates with desirable pharmacokinetic profiles and reduces the risk of late-stage failures due to poor absorption, metabolism, distribution, or excretion. Integration of ADME studies with other aspects of drug discovery, such as pharmacology, toxicology, and formulation, is essential for successful drug development.

Figures 9 and 10 display the results from the SwissADME tool for compounds 1 and 2, indicating TPSA scores of 139.15 Å² and 93.72 Å², respectively. These values are below the threshold of 140 Å², suggesting that both compounds exhibit hydrophobic properties and prefer interactions with nonpolar environments. Hydrophobic molecules with low TPSA values are typically associated with better membrane permeability. This property is advantageous as it enhances the molecules' ability to traverse biological barriers such as cell membranes, which is crucial for oral absorption and distribution to target tissues.

While hydrophobic molecules with low TPSA values may possess favorable properties for absorption and distribution, they may also be more susceptible to metabolism by cytochrome P450 enzymes in the liver. This metabolic process can influence the compound's pharmacokinetic profile and may impact its overall efficacy and safety.

Overall, the TPSA values obtained for compounds 1 and 2 suggest that they possess hydrophobic characteristics that could facilitate their absorption and distribution. However, further investigation is warranted to assess their metabolic stability and potential for oral bioavailability in vivo.

The metabolism of a compound can indeed have significant effects on its systemic exposure, half-life, and overall pharmacokinetic profile. Compounds with a bioavailability of 0.55 indicate that a substantial portion of the administered dose reaches systemic circulation and is potentially active. Bioavailability, influenced by factors such as solubility, permeability, stability, formulation, and interactions with physiological barriers, plays a crucial role in determining the efficacy and safety of a drug candidate.

In the case of compounds 1 and 2, their bioavailability of 0.55 suggests that they have the potential to be effective in systemic circulation. Additionally, both compounds satisfy the Lipinski's Rule of Five, a crucial criterion for determining drug-likeness, which, along with considerations of physicochemical and pharmacokinetic features, contributes to their potential as drug candidates.

However, it's important to note that while satisfying the Lipinski's Rule of Five is indicative of favorable drug-like properties, it is not a definitive determinant of a compound's success as a drug candidate. Further studies are necessary to evaluate the compounds' efficacy, safety, and overall pharmacological profile in preclinical and clinical settings. Additionally, investigations into potential drug interactions, metabolic pathways, and toxicity profiles are essential aspects of drug development that warrant careful consideration.[42]

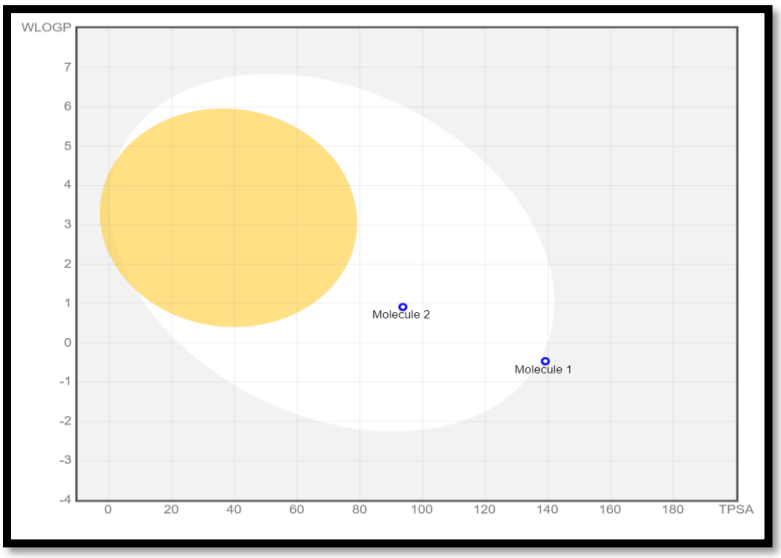


Figure (8): BOILED-Egg for both compound 1 and compound 2 (blue dots). It shows that (P2) inside the white ovule has the ability to passively absorb throw the GIT, while P1 is outside the white area which mean it is poor GIT absorption. P-glycoprotein: Both of P1 and P2 are able to eliminate from the central nervus system. BBB: Blood–brain barrier, GIT: Gastrointestinal tract, CNS: Central nervous system

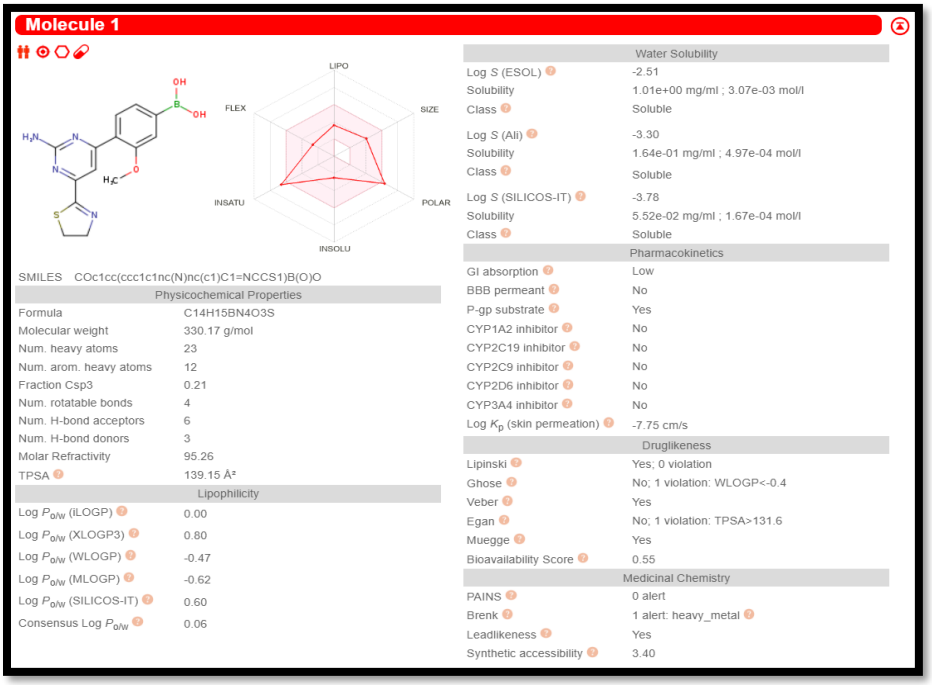


Figure (9): SwissADME virtual characteristics of compound 1.

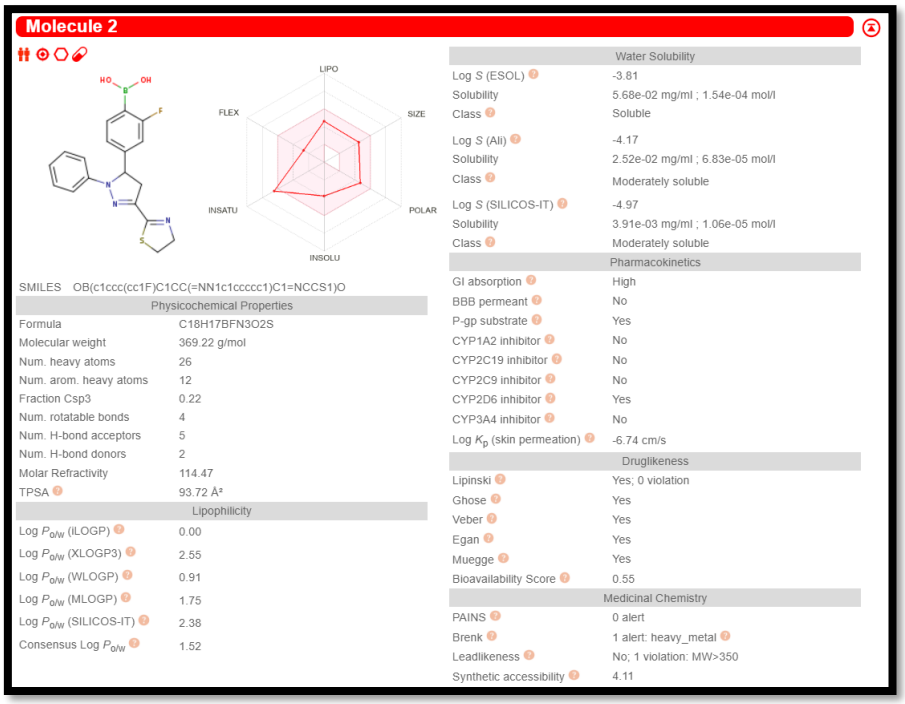


Figure (10): SwissADME virtual characteristics of compound 2.

5. Conclusion

Molecular docking stands as one of the most effective methods for drug discovery, aiming to enhance the effectiveness and medicinal benefits of current medications by increasing enzyme binding affinity while reducing costs and time. In this study, docking analyses were successfully conducted for the six proposed compounds, comparing their interactions with the COX-2 enzyme against meloxicam as a reference. The results revealed promising binding interactions for the compounds, indicating improved effectiveness in reducing inflammation and a stronger ability to bind to target molecules compared to meloxicam.

Moreover, the physicochemical and pharmacokinetic properties of compounds 1 and 2 were found to meet the criteria outlined by Lipinski's rules. Additionally, their favorable bioavailability for gastrointestinal absorption, coupled with their inability to penetrate the blood-brain barrier, further highlights their potential as promising anti-inflammatory agents.

In conclusion, this study has identified six compounds comprising pyrazoline and pyrimidine derivatives that exhibit potential anti-inflammatory activity. These compounds could serve as models for the development of novel therapeutic agents. Overall, these findings contribute to the ongoing efforts in drug discovery and offer promising avenues for the development of improved anti-inflammatory treatments.

6. Limitations and Recommendations

6.1.limitations

Molecular docking studies, while powerful and widely used in drug discovery and design, have several limitations that should be considered:

1. **Scoring Function Limitations**: Docking software relies on scoring functions to predict the binding affinity of ligands to the target receptor. However, scoring functions have inherent limitations and may not always accurately capture the complex interactions between ligands

and receptors. They often oversimplify molecular interactions and may fail to account for factors such as solvent effects, protein flexibility, and entropic contributions.

2. **protein Flexibility**: Most docking studies assume a rigid protein structure, neglecting protein flexibility. In reality, proteins undergo conformational changes upon ligand binding, which can affect the accuracy of docking predictions.

3. **Ligand Flexibility**: Docking studies typically explore the conformational space of ligands by sampling different conformers. However, this sampling may be incomplete, especially for flexible ligands with many rotatable bonds or conformational states. As a result, docking may miss relevant binding modes or underestimate the ligand's affinity.

4. **Water Molecules and Solvent Effects**: Docking studies often ignore the presence of water molecules in the binding site and neglect solvent effects. Water molecules can mediate important interactions between ligands and proteins, and their exclusion may lead to inaccuracies in docking predictions, particularly for polar ligands or binding sites.

Overall, while molecular docking is a valuable tool for drug discovery and design, it is essential to recognize its limitations and interpret the results with caution. Integrating docking studies with other computational and experimental techniques can help mitigate these limitations and provide more robust predictions.

6.2. Recommendations

to enhance the reliability and effectiveness of molecular docking studies:

1. **Use Multiple Docking Software**: Employ multiple docking programs with different algorithms and scoring functions to cross-validate results and reduce bias. Each program may have strengths and weaknesses, so using a combination can provide a more comprehensive analysis.

2. **Consider Protein Flexibility**: Account for protein flexibility by incorporating techniques such as induced fit docking or molecular dynamics simulations. These methods allow the protein structure to adapt to ligand binding and can improve the accuracy of docking predictions.

3. **Validate Docking Results Experimentally**: Validate docking predictions experimentally using techniques such as X-ray crystallography, NMR spectroscopy, or binding assays. Experimental validation confirms the predicted binding modes and affinities and provides confidence in the computational predictions.

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