In-silico molecular docking, ADME study of new azetidin-2-one derivatives having imidazole moiety targeting cyclooxygenase-2 Enzyme

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Abstract:

Background: Non-steroidal anti-inflammatory drugs are commonly used medications as anti-inflammatory, analgesic and antipyretic agents. However, they have many limitations due to the adverse effect, such as gastric irritation and gastric ulceration. Recently, β-lactams have been approved as chosen scaffolds due to their wide range of biological characteristics.

Objective: goal of this research was the development of a series of new azetidin-2-one derivatives (B1-6) and assessment of their function as cox-2 inhibitory agents.
Methods: crystal structure diclofenac complexed with active site of cyclooxygenase-2 from protein data bank, then docked with our new azetidine-2-one derivatives.
Results: molecular docking protocol results proposed that these azetidine-2-one derivatives have sufficiently strong binding interactions within active site of COX-II. they have PLP fitness scores ranges between 66-74 respectively, whereas the fitness score of the standard drug (diclofenac) was (69). Additionally, newly designed ligands show good Swiss-ADME variables, revealing that they might be compounds with good bioavailability when taken orally.
Conclusion: Three ligands exhibit superior PLP fitness compared to the reference ligand, while one demonstrates a comparable score at the active site of COX-II. This suggests that the new derivatives yield encouraging outcomes and may serve as modeling substances for the development of new anti-inflammatory drugs. Nevertheless, more pharmacological assessment is required.

KEYWORDS: Azetidin-2-one, cyclooxygenase-2 inhibitors, imidazole, ADME, anti-inflammatory.

دراسة الارساء الجزيئي حاسوبيا وخصائص (الامتصاص والتوزيع والتأيض والافراز) لمشتقات جديدة من ازيتيدين-2-ون تحتوي على مجموعة الايميدازول التي تستهدف انزيم السيكلواوكسيجينيز-2

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الخلاصة:

الخلفية: تُعدّ الأدوية غير الستيرويدية المضادة للالتهاب من الأدوية الشائعة الاستخدام كمضادات للالتهاب، ومسكنات للألم، وخافضة الحرارة. ومع ذلك، فإن لها العديد من القيود نتيجة لآثارها الجانبية، مثل تهيج المعدة وقرحة المعدة. ومؤخرًا، تم اعتماد مركبات بيتا لاكتام كقوالب بنيوية مفضّلة نظرًا لامتلاكها مجموعة واسعة من الخصائص البيولوجية

الهدف: هدف هذا البحث هو تطوير سلسلة جديدة من مشتقات أزيتيدين-2-ون وتقييم فعاليتها كمثبطات لإنزيم السايكلواوكسيجينيز-2

الطرق :تم الحصول على البنية البلورية لمركب ديكلوفيناك مرتبطًا بالموقع الفعّال لإنزيم سايكلوأوكسجيناز-2 من بنك بيانات البروتينات، ثم تم إجراء الإرساء الجزيئي مع جميع المشتقات الجديدة من أزيتيدين-2-ون.

النتائج :أظهرت نتائج بروتوكول الإرساء الجزيئي أن مشتقات أزيتيدين-2-ون تمتلك ارتباطات قوية داخل الموقع الفعّال لإنزيم COX-2 .تراوحت قيم PLP fitness للمشتقات بين 66 و74، بينما كانت القيمة للدواء القياسي (ديكلوفيناك) تساوي 69. بالإضافة إلى ذلك، أظهرت المركبات الجديدة نتائج جيدة عند تحليلها باستخدامSwissADME ، مما يشير إلى أنها قد تكون مركبات ذات توافر حيوي جيد عند تناولها عن طريق الفم.

الاستنتاج :أظهرت ثلاثة من المركبات نتائج PLP fitness أعلى من العقار القياسي، بينما أظهر مركب واحد نتيجة مماثلة عند الموقع الفعّال لإنزيمCOX-2 .تشير هذه النتائج إلى أن المشتقات الجديدة تُظهر نتائج واعدة، وقد تكون بمثابة نماذج أولية لتطوير أدوية جديدة مضادة للالتهاب. ومع ذلك، هناك حاجة إلى مزيد من التقييمات الدوائية لتأكيد فعاليتها.

الكلمات المفتاحية :أزيتيدين-2-ون، مثبطات إنزيم سايكلواوكسيجيناز، إيميدازول، مضاد الالتهابات, ADME

1. INTRODUCTION

Initially the prominent cause of disability in eastern countries is rheumatic diseases. Typical symptoms of joint diseases include inflammation, pain, joint deterioration, and decreased motor function. Advances in the understanding of cytokine functions have led to the emergence of several therapeutic strategies over the past decade. Despite these developments, current treatments remain largely symptomatic and fail to offer curative outcomes (1,2).

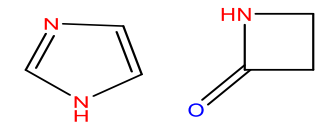
NSAIDs are the most sold medications worldwide that target the two isoforms of membrane proteins cyclooxygenase (COX I and II), which catalyze arachidonic acid metabolism. COX-1 is expressed in tissues, producing prostaglandins, while COX-II is induced in inflammatory cells, causing elevated production during inflammation. NSAIDs account for approximately 30% of medications that are used as antipyretics and analgesics in acute and chronic pain. Both systemic and local side effects are known to occur with the medications. Moreover, long-term use of nonsteroidal anti-inflammatory agents may result in a number of unwanted side effects (3,4).

Thus, in the pharmaceutical industry, heterocyclic systems are one of the best choices owing to their wide variety of pharmacological and biological properties. The available research on substituted imidazole revealed many encouraging therapeutic applications, such as inflammatory inhibition based on their mode of action and significant structure–activity relationships (5).

Azetidine-2-ones substitutes, generally named as β-lactams, are four-membered heterocyclic rings that are widely used in the Design of medicinal products as actives moieties (6) . β-lactams were first synthesized by Hermann Staudinger in the early 20th century through cycloaddition [2 + 2] of imines with ketenes (Staudinger synthesis) (7).

Thus, The development of new medications with a safer anti-inflammatory profile is constantly needed. The synthesis of diverse azetidin-2-one derivatives has garnered significant interest in recent years, as this class of compounds forms the essential structure for numerous naturally occurring substances exhibiting a broad spectrum of biological activities, including, antibacterial, anti-inflammatory,

antioxidant, diuretic, antimicrobial, and analgesic activity. The azetidine-2-one moiety demonstrates anti-inflammatory and analgesic properties, and the incorporation of supplementary heterocyclic rings into β-lactams core significantly enhances its activity (8).



1*H*-imidazole azetidin-2-one

Figure1. chemical structure of imidazole and azetidinone.

To examine the anti-inflammatory and analgesic activity, Daniela *et. al*, in 2016 developed series of new 3-chloro-azetidin-2-one derivatives. diclofenac used as control medication. final results of Anti-inflammatory activity test reveals that the new compounds possess acceptable anti-inflammatory activity, as shown in figure (2), (9).

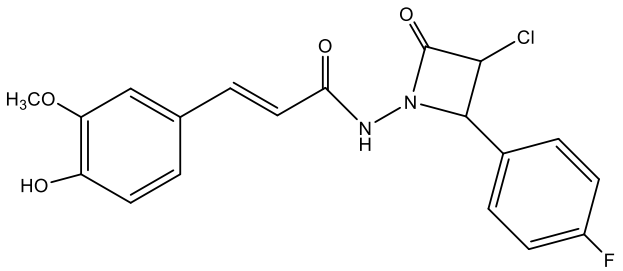


Figure 2. azetidin-2-one derivatives with potent anti-inflammatory activity.

Recent researches finds that theoretical computations facilitate the production of molecules with high potency, that is crucial for the development of drugs across many stages, starting from chemical synthesis to effective comparison (10,11). Moreover, hypothetical methodologies have progressed markedly in the last decade due to technological advancements, providing a complete understanding of how chemicals interact with protein active sites(12).

anti-inflammatory drugs that are currently available have demonstrated acceptable COX-2 inhibitory activity; however, several significant challenges remain. These include undesirable side effects and poor selectivity, which often lead to gastrointestinal complications and other adverse effects. Overcoming these limitations is crucial for the development of safer and more effective COX-2 inhibitors.

2. MATERIALS AND METHODS

In our research, the six suggested compounds were examined by utilizing the docking software, which identifies the desired orientation of a small molecule (like a drug or ligand) that binds to a target receptor protein, and the goal is to model the interaction between two molecules to estimate the strength and binding mode. The three-dimensional structure of diclofenac complexed with the cyclooxygenase-2 active site (PDB code: 1PXX) was chosen for this study figure (3).

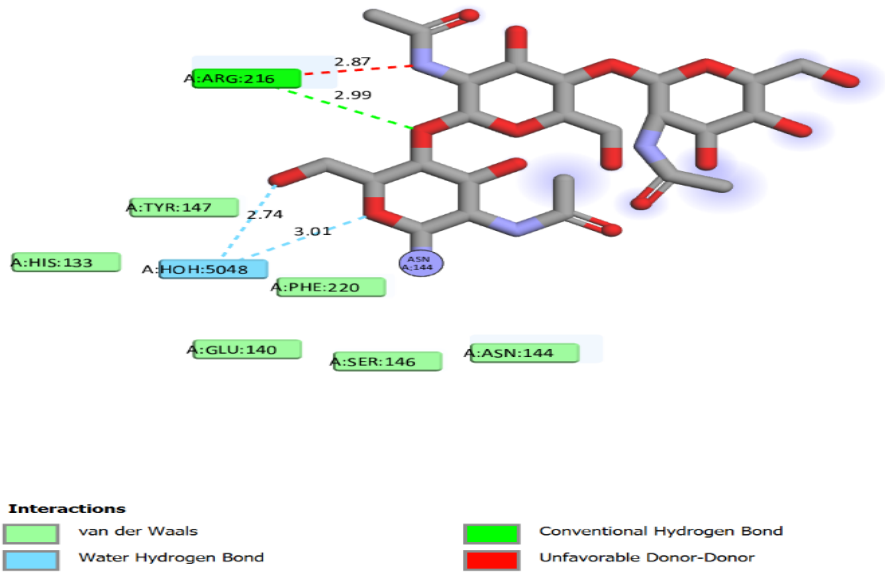


Figure 3. 2D representation of diclofenac structure bound inside active site of COX-2 receptor represented by using Discovery Studio visualizer (PDB code: 1PXX).

2.1. ligands and target receptor Preparation

Six derivatives of 3-chloroazetidin-2-one (β-lactam) was presented. We presented our findings and supported our results with a docking procedure subsequent to confirmation that the recommended ligands had never been synthesized. Ligands were drawn using ChemDraw program and transformed into three dimensional configurations utilizing Chem 3D within the ChemOffice suite software (ChemOffice, 2020). Following that, optimization the lowest energy conformation of the ligands by force field MM2, which was subsequently saved in (. mol2) format.

Additionally, we utilized the COX-2 enzyme structure combined with diclofenac (PDB code: 1PXX) to perform ligand docking. The licensed edition of Genetic Optimization was used for docking from the Cambridge Crystallographic Data Centre (CCDC). The Hermes 2022.3.0 program was utilized to configure the structure of the COX-2 enzyme for the docking and removing extra chains while preserving chain A, and the remaining water molecules were extracted along with a reference ligand. Ultimately, hydrogen atoms were incorporated into the receptor.

2.2. Molecular docking

Subsequent to the preparation of our ligands and receptor active site as previously described, we introduced all six compounds, alongside with the extracted diclofenac structure, to the enzyme. The total number of generated poses has been set at 10, based on docking efficacy. The ChemPLP fitness score indicates that a higher score implies a stronger interaction between the cyclooxygenase-2 enzyme receptor and our ligands. The data was subsequently preserved. The collected data were evaluated to identify the most favourable interactions, prefered docking configurations, binding free energy, and the interactions of amino acid with compounds inside the active site of the enzyme.

2.3. ADME prediction

The pharmacokinetic properties, that includes absorption, distribution, metabolism, and excretion, along with different variables such as permeability of ligand through blood-brain barrier, and drug

similarity characteristics, were assessed using the Swiss-ADME web tool.

In a pharmaceutical application, virtual screening tool like Swiss ADME identifies compounds having high potency and best drug-likeness property among all the proposed ligands. polar surface area considered the most crucial characteristic associated with bioavailability, this suggests a drug with a PSA lower than 140 angstromes squared, has a high bioavailability when taken orally, in addition to the Lipinski rule of five. ADME parameters of six compounds is listed in table (2).

In addition, Swiss-ADME provides BOILED-EGG representation, which is used in investigating blood-brain barrier permeability and intestinal absorption. Likewise, it offers polarity and lipophilicity predictions for the proposed medications.

3. RESULTS AND DISCUSSION

Gold detects the hydrogen bonds, in addition to the short contacts and the bond distance. All six new ligands binding inside COX-II active site show the PLP fitness score varies from 66 to 74, as stated in the table (1), unlike the interaction of standard ligand diclofenac is (69), which binds to SER530 amino acid residue through hydrogen bond, as well as binds to MET522, LEU352, SER530 and TYR385 amino acids through hydrophobic interactions inside cyclooxygenase-2 enzyme, figure (4).

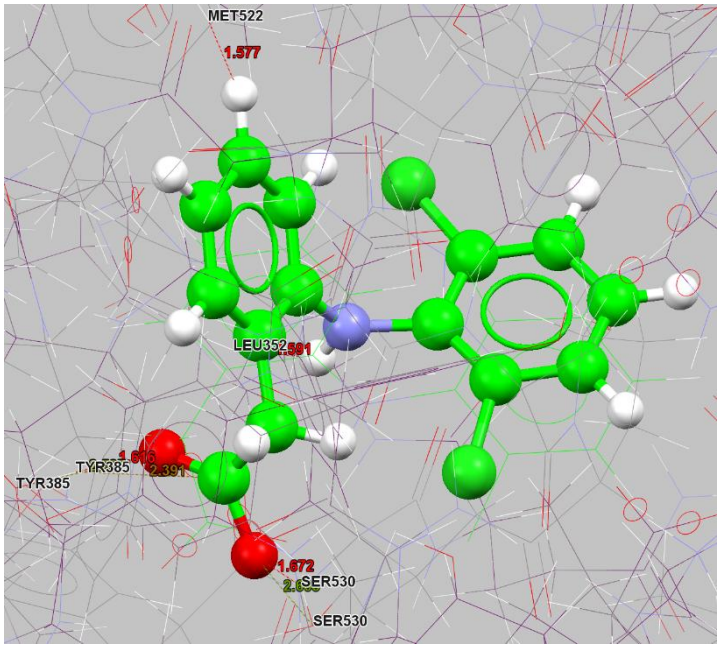
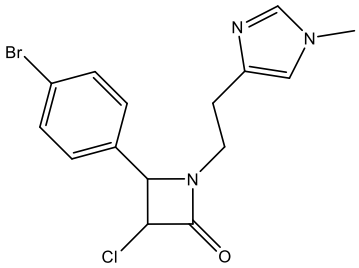
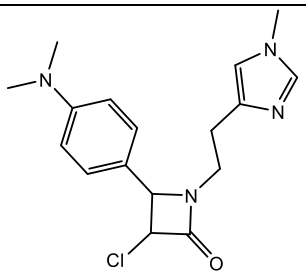
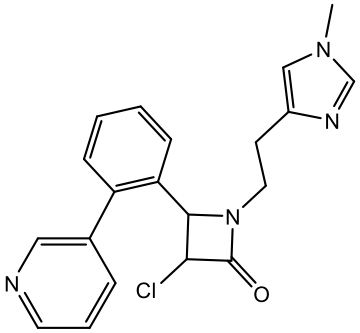
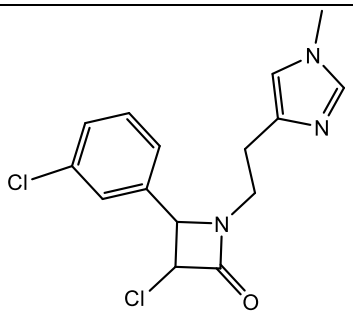
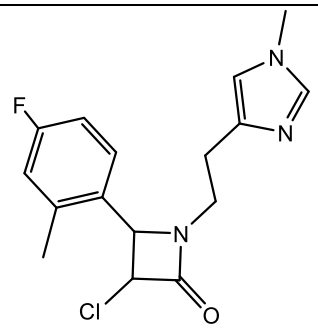
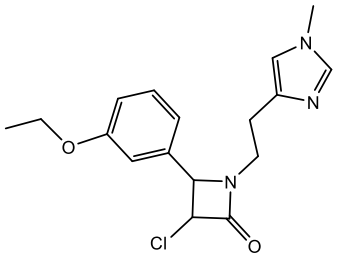
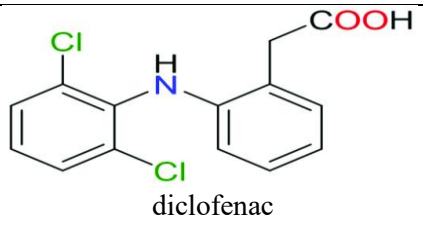


Figure 4. 3D representation from docking showing interactions of diclofenac to amino acids of COX-2 within active site, (PDB code: 1PXX).

The docking results demonstrates that (B1-6) compounds binds inside the active site of cyclooxygenase enzyme receptor by hydrogens bonds through different amino acid residues LEU384, SER353, TYR355, ARG120, VAL349, ALA527 and TYR385, Likewise, the six proposed ligands bind by short contacts inside the active site. specially, compound (B4) have the higher fitness score. In comparison with diclofenac, compounds (B2,4 and 6) exhibit an acceptable binding affinity, as shown in figure (6,8,10).

Table 1: interactions and PLPfitness score of docked Azetidin-2-one derivatives within active site of cyclooxygenase-2 enzyme.

No.	Structure	PLP fitness score	H-bond interactions	Short contact interactions
B-1		66.12	SER353	SER353, VAL349, LEU352, LEU531, VAL523, TRP387, ALA527, PHE381, LEU384
B-2		73.13	TYR355, ARG120	TRP387, PHE381, VAL523, PHE518, SER353, ALA527, TYR355
B-3		69.33	ARG120, SER353, LEU384	SER353, LEU531, VAL349, LEU359, ALA527, VAL349, TRP387, LEU384
B-4		74.49	ARG120	VAL523, VAL349, LEU359

B-5		67.43	SER353, ARG120	MET522, VAL523, ALA527, SER353, VAL349, PHE381
B-6		73.27	TYR385, ALA527	ALA527, SER530, TYR385, TYR348, SER353
	 diclofenac	69.00	SER530 TYR385	SER530, TYR385, LEU352, MET522

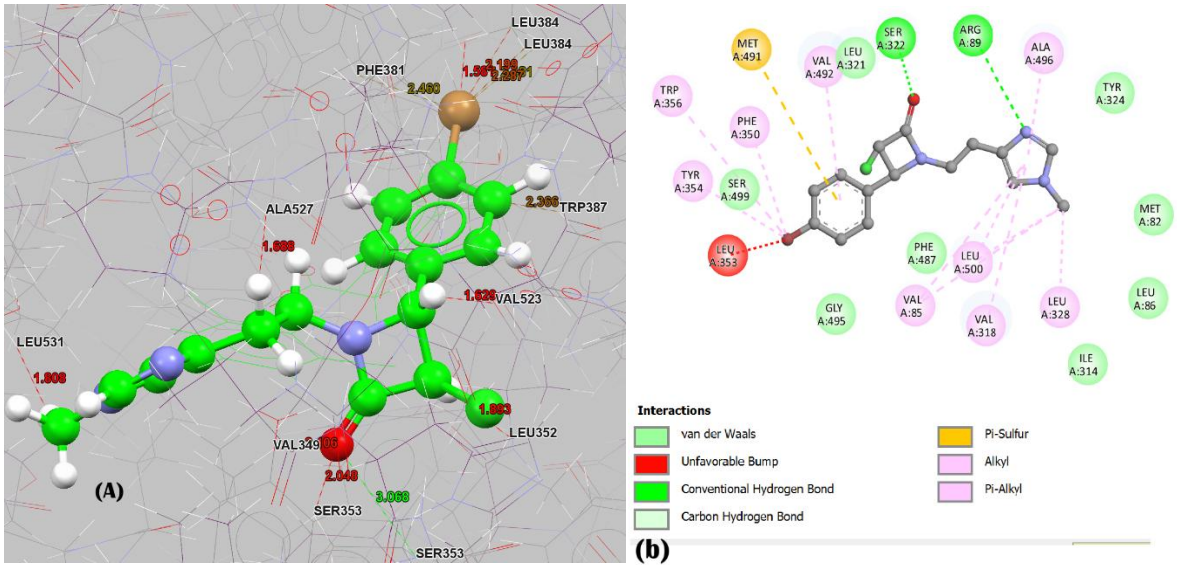


Figure 5: (A) 3D representation showing interactions of B1 to amino acids residues within active site of COX-II generated by using CCDC suit (PDB code: 1PXX). (B) 2D representation of the B1-COX-II complex by utilizing discovery studio visualizer.

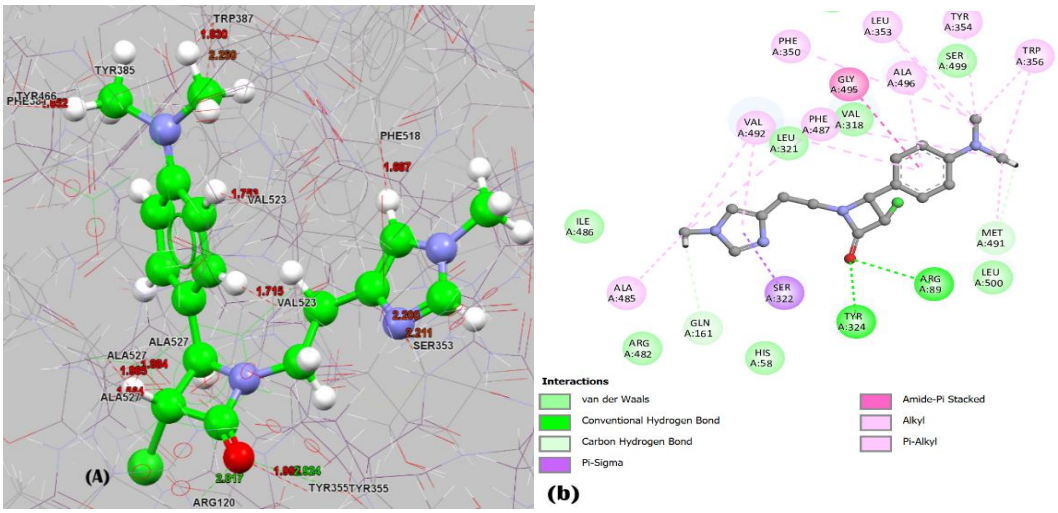


Figure 6: (A) 3D representation showing interactions of B2 to amino acids residues within active site of COX-II generated by using CCDC suit (PDB code: 1PXX). (B) 2D representation of the B2-COX-II complex by utilizing discovery studio visualizer.

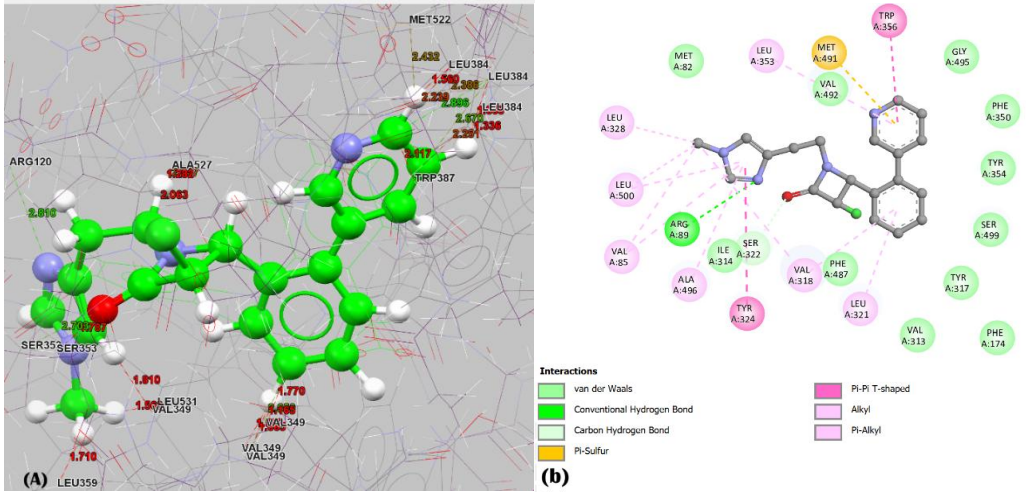


Figure 7: (A) 3D representation showing interactions of B3 to amino acids residues within active site of COX-II generated by using CCDC suit (PDB code: 1PXX). (B) 2D representation of the B3-COX-II complex by utilizing discovery studio visualizer.

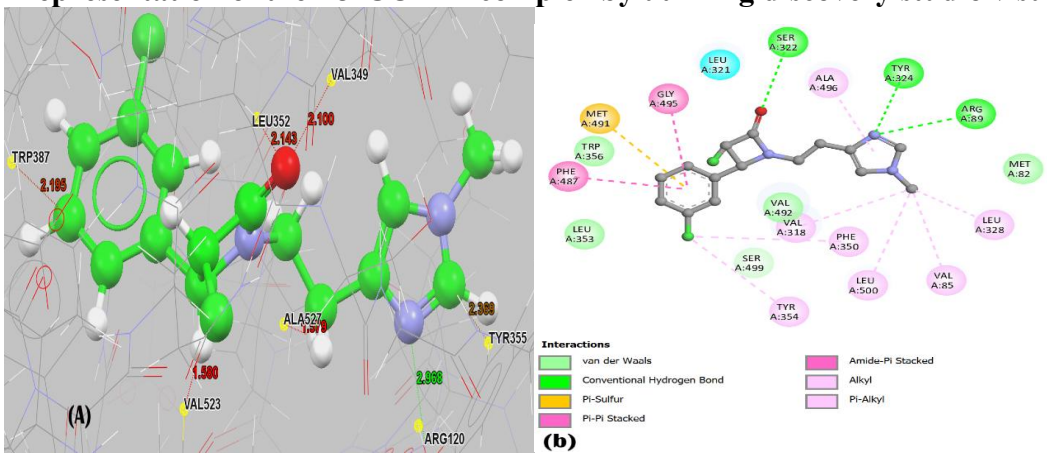


Figure 8: (A) 3D representation showing interactions of B4 to amino acids residues within active site of COX-II generated by using CCDC suit (PDB code: 1PXX). (B) 2D representation of the B4-COX-II complex by utilizing discovery studio visualizer

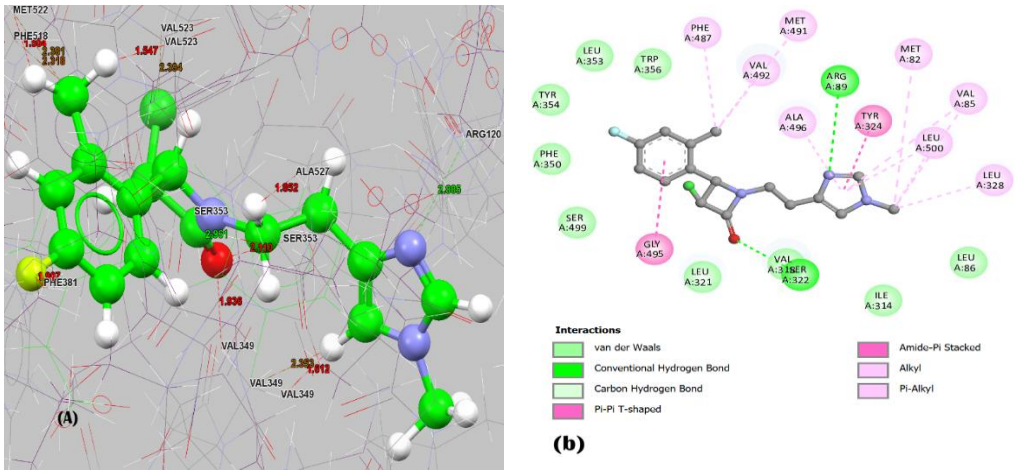


Figure 9: (A) 3D representation showing interactions of B5 to amino acids residues within active site of COX-II generated by using CCDC suit (PDB code: 1PXX). (B) 2D representation of the B5-COX-II complex by utilizing discovery studio visualizer

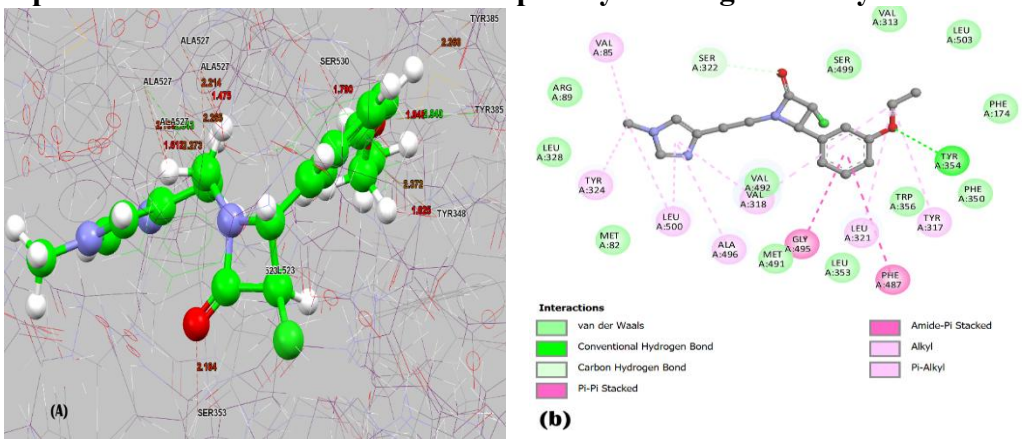


Figure 10: (A) 3D representation showing interactions of B6 to amino acids residues within active site of COX-II generated by using CCDC suit (PDB code: 1PXX). (B) 2D representation of the B6-COX-II complex by utilizing discovery studio visualizer

The newly designed compounds provided with BOILED-egg plot in figure (11) which shows that all ligands can cross BBB. While ligand B3 represented in blue dot have affinity to p-glycoprotein to be ejected from the central nervous system, in contrast, all remaining molecules B1-6 that appears in red dots is not regarded as a substrate for p-glycoprotein.

In the evaluation of drug transportation, bioavailability and intestinal permeability of orally taken medication is described by (TPSA) (13). Poorly bioavailable medications have TPSA > 140 Aº (14). the Lipinski rule of five is essential requirements in drug designing, which claims that pharmaceuticals taken orally must satisfy important parameters: molecular weight (M.Wt.) < 500 Da, an octanol-water partition coefficient [Log P] < 5, less than 5 hydrogen bond donors, and less than 10 hydrogen bond acceptors (15)

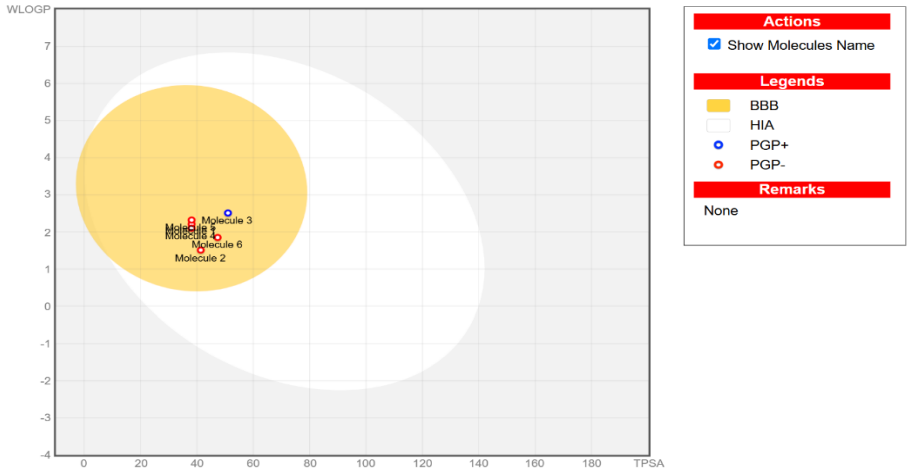


Figure 11. BOILED-Egg model of Azetidin-2-one derivatives, B3 ligand shown in blue dot. The ligands inside the yellow region can passively penetrate through BBB. P-glycoprotein substrates (PGP+), compounds represented by blue dots, can be exported outside the CNS. The ligands that represented by red dots are those which P-glycoprotein unable to eject from the CNS (PGP-).

Swiss-ADME web tool for all newly designed compounds, showing TPSA scores lower than 140 A. In addition, new ligands have a good bioavailability score (0.55), which predicts the ligands penetration from the gastrointestinal tract to bloodstream. Furthermore, they effectively comply with Lipinski's rules. table (2). GI absorption features determine The volume of medication that penetrates through intestinal membrane entering the gastrointestinal system following oral administration. In this research, Assuming that all proposed ligands exhibit a high gastrointestinal absorption score, which means newly designed compounds is effective when taken orally.

Table 2: Lipinski properties and bioavalability of six derivatives of Azetidin-2-one analyzed by Swiss-ADME web tool.

COMP.	MW	H-B.A	H-B.D	TPSA	GI absorption	BBB permeant	P-gp Sub.	log Kp	Lipinski violations	Bio-availability
B-1	368.66	2	0	38.13	High	Yes	No	-6.8	0	0.55
A-2	332.83	2	0	41.37	High	Yes	No	-6.99	0	0.55
A-3	366.84	3	0	51.02	High	Yes	Yes	-6.89	0	0.55
A-4	324.21	2	0	38.13	High	Yes	No	-6.58	0	0.55
A- 5	321.78	3	0	38.13	High	Yes	No	-6.68	0	0.55
A- 6	333.81	3	0	47.36	High	Yes	No	-6.85	0	0.55

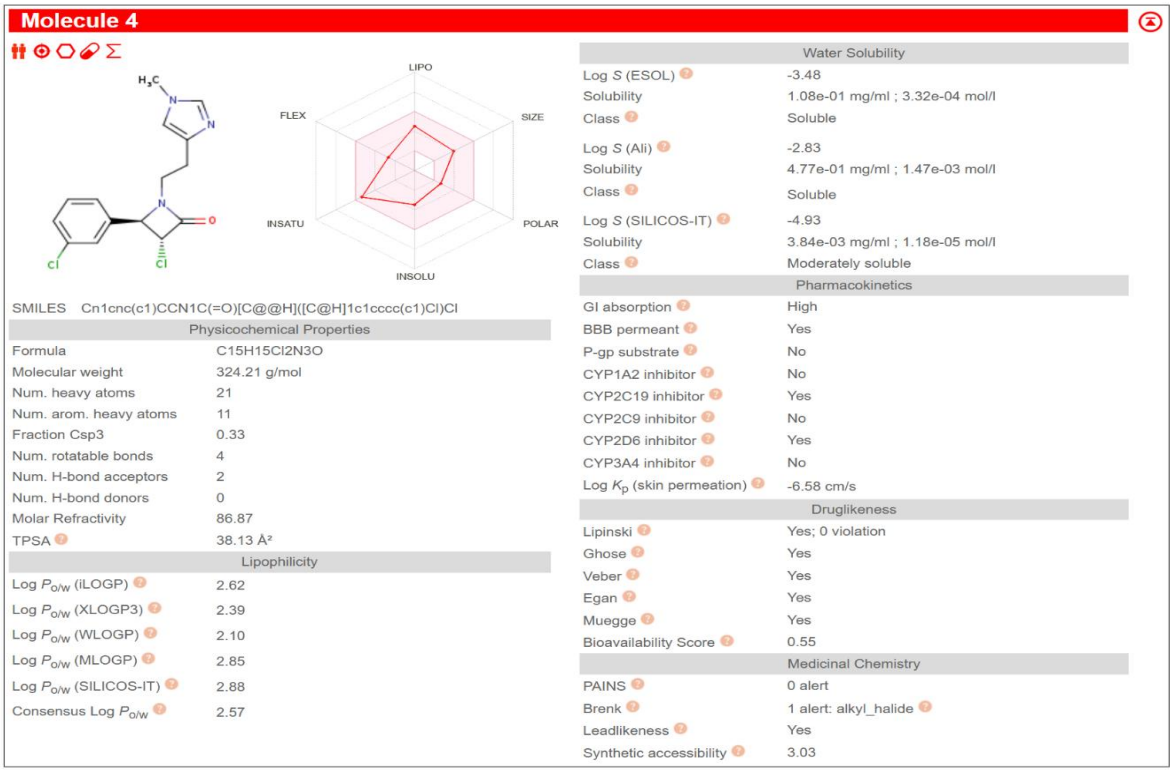


Figure (12): SwissADME virtual characteristics of compound B4.

4.CONCLUSION

Molecular docking protocol among efficient computational techniques for identifying novel pharmaceuticals with enhanced binding affinity and efficacy. This study's innovation lies in the designing of different azetidine-2-one derivatives and the evaluation of their effectiveness. In contrast to diclofenac, our proposed ligands exhibit good inhibitory activity against the cyclooxygenase-2 enzyme as a result of compounds superior binding scores. Moreover, the physicochemical and pharmacokinetic characteristics of six Azetidin-2-one derivatives comply with Lipinski's criteria. Ultimately, these newly formulated compounds exhibited promising outcomes, positioning them as prospective lead candidates for the creation of innovative anti-inflammatory and analgesic medications; despite this, more pharmacological evaluation is needed.

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