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Potent Lead Identification for COX-2 Enzyme: A Computational Approach

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ABSTRACT

Inhibitors of the Clyclooxygenase-2 (COX-2) enzyme are well known for their direct action with steroids along with its non-steroidal anti-inflammatory action, extending to their therapeutic area to treat various cancers and Alzheimer's disease due to their inflammatory action. A series of marketed drugs that act against COX-2 enzyme were selected and subjected to pharmacophore modeling using a pharamagist webserver to get 1462 base structures from ZINC Pharmer, filtered using Data warrior. Filtered compounds were virtually screened and evaluated for ADMET properties using the PKCSM web server and were compared with the standard. ZINC55580105 compound with binding affinity and found to have better ADME properties, which can be further evaluated for in vitro and in vivo anti-inflammatory activity.
Keywords: *Inflammation, COX-2, Pharmacophore Modelling, Molecular docking.*

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INTRODUCTION

The body's inflammatory response is its natural way of recognizing and defending against potentially harmful agents, such as viruses and bacteria. Through the process of inflammation, the body's immune system can identify and remove foreign substances, such as pathogens, and begin the healing process. Inflammation can be either acute or chronic, and the major enzyme responsible for this is COX-2 (cyclooxygenase 2). Anti-inflammatory drugs are used to treat the symptoms of inflammation, and the use of bioinformatics tools, such as the identification of drug targets by analyzing enzymes, nucleic acids and proteins, is becoming increasingly common in the development of new treatments. Inflammatory processes are associated with a wide range of diseases caused by pathogenic microorganisms, such as viruses, bacteria, fungi, and parasites, which can lead to morbidity and mortality. In order to protect the human body from these exogenous agents, it is essential to understand the body's inflammatory response [1-3].

The inflammatory process is a complex sequence of steps that involve several mediators, including neutrophils, mast cells, eosinophils, macrophages, dendritic cells, and epithelial cells. It is characterized by vasodilation, chemotaxis, and increased permeability. Products of pathogens, such as endotoxins and bacterial DNA, activate sensors called Toll-like receptors which can be found in the plasma membrane and endosomes, allowing them to detect microorganisms both inside and outside the cell. Platelets also release complement proteins and mast cells degranulate histamine to induce vasodilation and serotonin for increased permeability and cell movement. Neutrophils are subsequently activated and migrate to the location of injury in response to chemokines. Upon arrival, they phagocytose the foreign organisms, secreting mediators and attracting macrophages while also increasing the release of pro-inflammatory mediators such as prostaglandins and leukotrienes, along with cytokines like interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor (TNFα). Activation of cells triggers the conversion of arachidonic acid (AA) from the cell membrane into prostaglandins and leukotrienes. Lastly, prostaglandins, also referred to as eicosanoids, are synthesized upon stimulation through cell membrane

receptors [4-8]. This process is accompanied by a regulator protein initiating the activation of phospholipase A2 or an increase in Ca+2 concentration. The hydrolysis of phospholipids by the enzyme leads to an influx of arachidonic acid, which serves as a substrate for the synthesis of both beneficial and detrimental prostaglandins [7, 9-11].

It is possible to gain understanding of the structures and regulations of cyclooxygenase (COX)-1 and COX-2 active sites to design more specific inhibitors for the latter and to better analyze the link between the structure and activity of the related compounds. During its action, arachidonic acid binds to an Arg120 and Ser530, while an electron transfer of Tyr385 to oxidized heme commences the reaction catalyzed by cyclooxygenase [12-15]. Researchers have sought to elucidate how certain non-steroidal anti-inflammatory drugs interact with the enzyme to inhibit prostaglandin synthesis. An amino acid difference found at position 523 (valine in COX-2 vs. isoleucine in COX-1) within the hydrophobic channel of COX may be key to the selectivity of several drugs [16-20].

The purpose of this research is to discover a pharmaceutical compound with reduced adverse effects and increased therapeutic benefits. The focus is on determining a moiety that can mitigate the cardio-vascular risks associated with prolonged use of Cox-2 inhibitors.

MATERIAL AND METHODS

Biologically active molecules needed to link the ligand to the appropriate target protein and interact with it are mapped to create a three-dimensional model called a pharmacophore. To use the free online tool, pharmagist, the molecule must first be uploaded in Sybyl Mol 2 format along with a functional email address. Then, the molecule is subjected to pharmacophore modelling utilizing multiple combinations of the input compounds. Subsequently, the service will generate the pharmacophore in Jmol format [21].

The number of aligned molecules generated—for example, seven aligned molecules that were stacked one on top of the other—resulted in a cluster in the JMol format. From there, the most aligned molecule (or the six molecules with the highest scoring) was chosen and downloaded. The molecules were subsequently uploaded to the free online tool ZINC pharma to do pharmacophore modelling. The zinc database generated a query for compounds with the same pharmacophoric characteristics and returned a total of over one lakh hits. To yield a final set of 1024 low molecular weight compounds, the excess hits were filtered out. These molecules were then uploaded to data warrior—a freeware tool used for pharmacophore modelling—and screened according to drug physicochemical characteristics such as molecular weight, log P, H donor, H acceptor, polar surface area, rotatable bond, and steric center. Finally, all the compounds were acquired as an SDF download file in order to be used for molecular docking experiments [22,23].

Molecular docking studies were conducted to assess the binding affinity of drug molecules with a given target protein. This was initially done by obtaining data from Data Warrior and using it to prepare the protein using the Protein Data Bank PDB ID 3ln1 and the X-ray crystallography of the target protein cyclo-oxisyginase2. The preparation of the protein included the deletion of hetero groups, water molecules, and any unwanted ligands by using “Swiss pdb viewer”. The ligands were then minimized and optimized using AutoDock Vina, and then the docking protocol was validated using PyMol version 2.4, a molecular visualization software by Discovery Studio. Additionally, a grid for the location of the ligand-protein binding was established. As a result, the docked output of all the ligands were screened to identify the structures with the highest docking score.

RESULT AND DISCUSSION

In 1997, Lipinski put forward the "Rule of Five," the first rule-based test for assessing drug-likeness which could determine whether a molecule could be effectively absorbed orally or not. This rule stated that a molecule would be considered orally inactive if it broke two or more of the four conditions: the number of hydrogen bond donors (HBDs) is five, the octanol/water partition coefficient (A log P) is five, and the number of hydrogen bond acceptors (HBAs) is ten. Subsequent rules for drug-likeness have since been proposed, such as the study which found that over 80% of the compounds met the criteria of A log P falling between 3.8 and 5.9, molecular weight between 400 and 450, molar refractivity between 40 and 130, and total atom count between 20 and 70. While these rule-based drug-likeness filters based on physicochemical properties have helped to expedite the drug development process, they also have certain drawbacks which have been highlighted by a number of research studies. Such filter criteria can be applied to the comparison of drug properties obtained from the docking of existing drugs and newly obtained drugs through pharmacophore modelling.

Table 1: Binding affinity of the selected drugs and zinc primers with protein pdb 3LN1

Drug Name	Binding Affinity
Valdecoxib	-8.1
Paracoxib	-8.6
Aspirin	-6.2
Celecoxib	-12.2
Diclofenac	-7.6
ZINC15955447	-10.5
ZINC33287116	-10.2
ZINC6495835	-10.7
ZINC72471509	-10.6
ZINC37603698	-9.1
ZINC15955447	-10.5

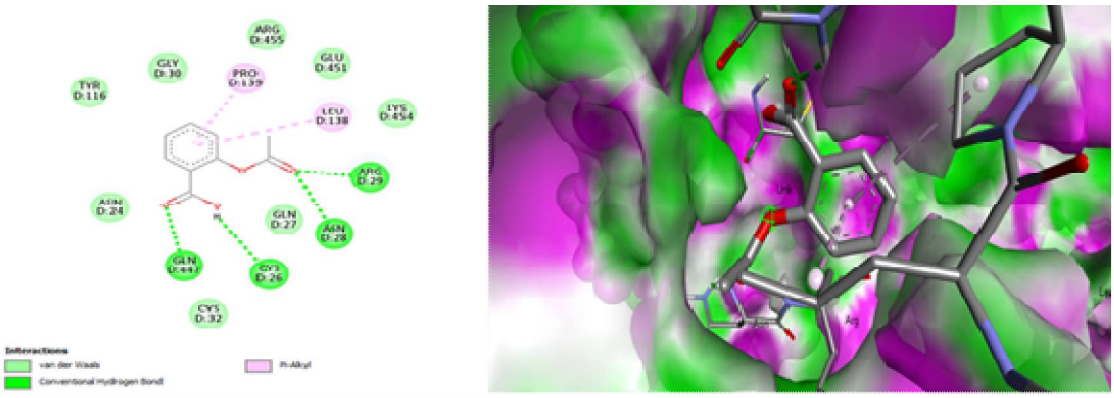

Fig 1: 2D and 3D interaction of aspirin with 3LN1

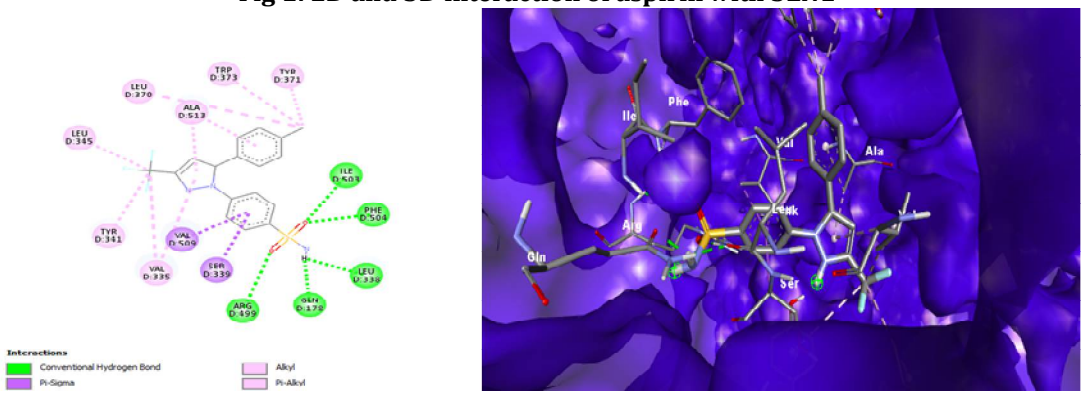

Fig 2: 2D and 3D interaction of celecoxib with 3LN1

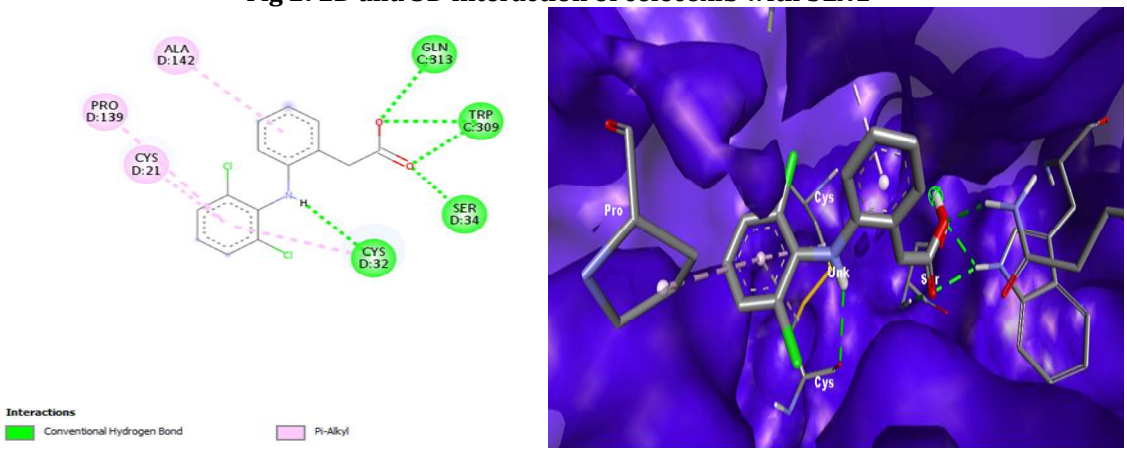

Fig 3: 2D and 3D interaction of diclofenac with 3LN1

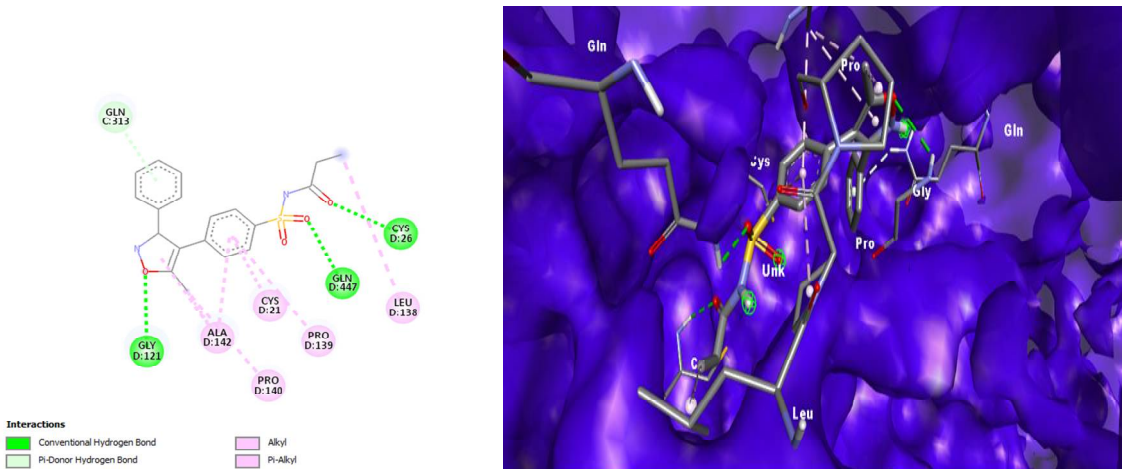

Fig 4: 2D and 3D interaction of parecoxib with 3LN1

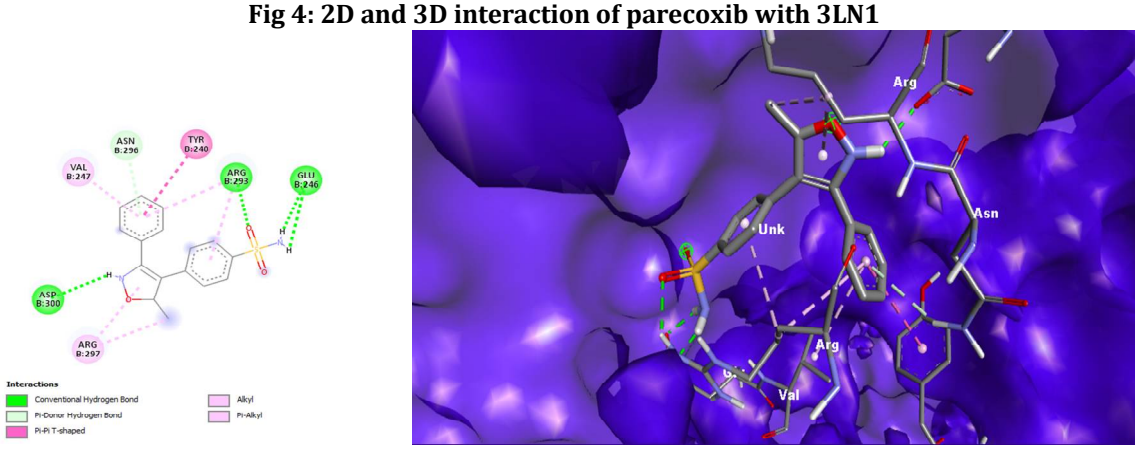

Fig 5: 2D and 3D interaction of valdecoxib with 3LN1

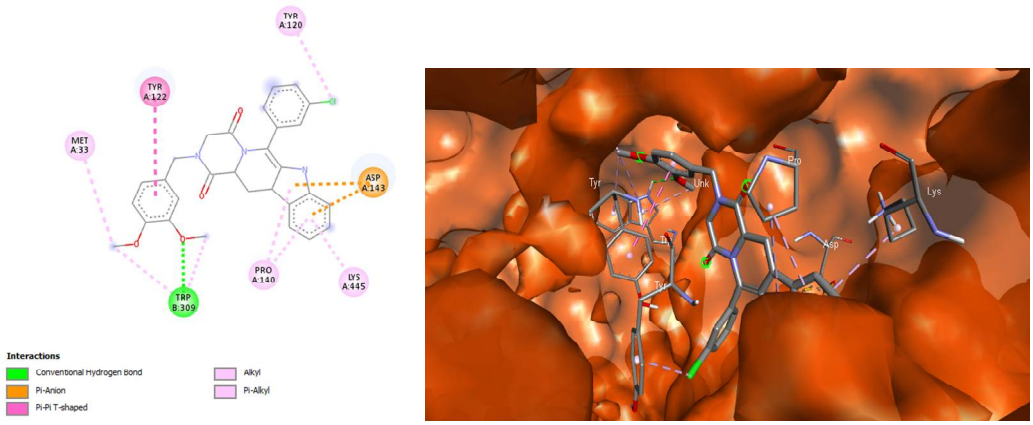

Fig 6: 2D and 3D interaction of ZINC15955447 with 3LN1

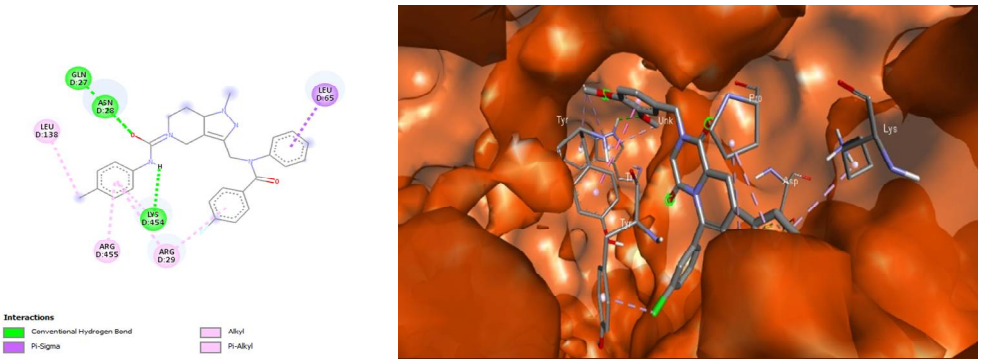

Fig 7: 2D and 3D interaction of ZINC33287116with 3LN1

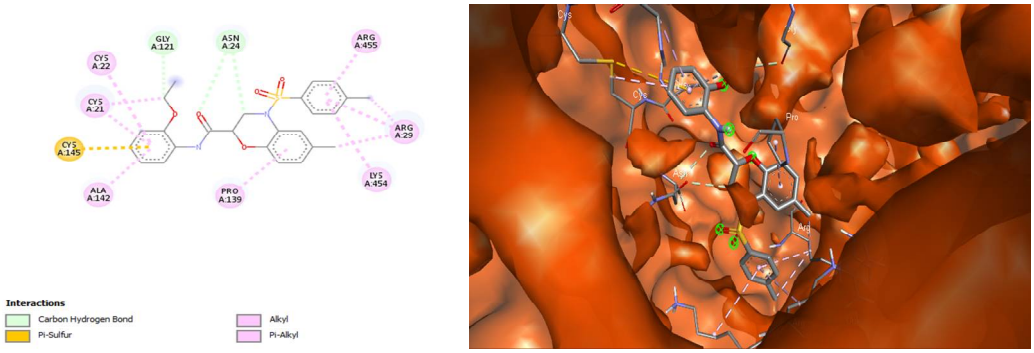

Fig 8: 2D and 3D interaction of ZINC6495835 with 3LN1

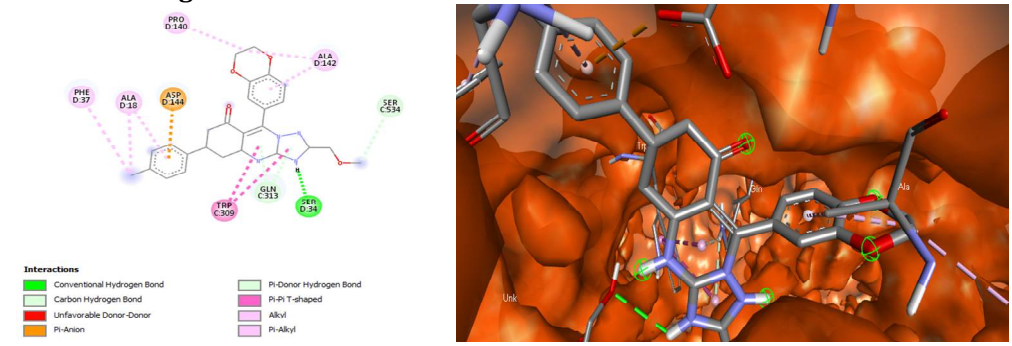

Fig 9: 2D and 3D interaction of ZINC72471509 with 3LN1

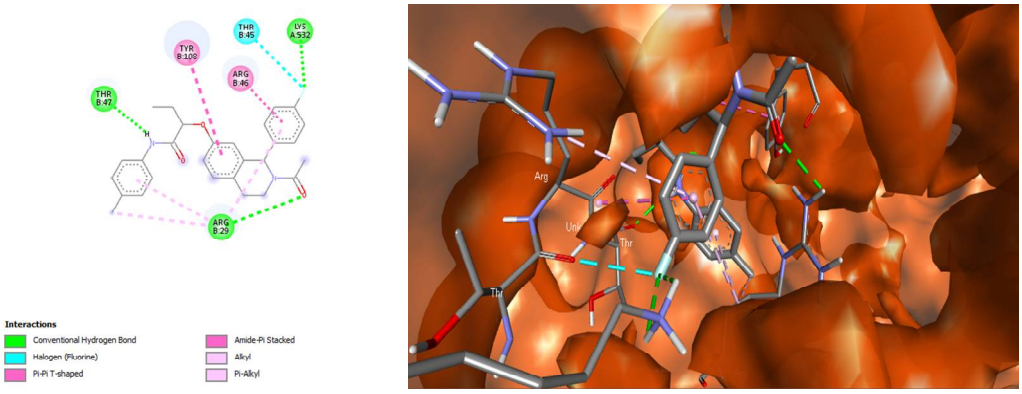

Fig 10: 2D and 3D interaction of ZINC37603698 with 3LN1

In-silico pharmacokinetic and toxicity studies

Absorption

The predicted absorption properties of the selected compounds are shown in Table 2. The results good overall absorption, all the compounds have good intestinal absorption.

Table 2: Absorption properties of the selected drugs and zinc primers

Drug list	Absorption Water solubility	Absorption $Caco_2$ permeability	Absorption Intestinal absorption	Absorption Skin permeability	Absorption P-glycoprotein substrate	Absorption P-glycoprotein 1st inhibitor	Absorption P-glycoprotein 2nd inhibitor
Aspirin	-1.868	0.09	76.938	-2.715	No	No	No
Valdecoxib	-3.777	1.148	94.576	-2.707	Yes	No	Yes
Parecoxib	-4.837	1.278	94.632	-2.74	No	Yes	Yes
Celecoxib	-4.45	0.839	92.995	-2.692	Yes	Yes	Yes
Diclofenac	-3.863	1.379	91.923	-2.724	Yes	No	No
ZINC15955447	-3.696	1.332	98.185	-2.736	No	Yes	Yes
ZINC33287116	-4.175	1.188	99.271	-2.735	No	Yes	Yes
ZINC6495835	-5.665	1.294	97.415	-2.955	No	Yes	Yes
ZINC72471509	-4.996	1.093	94.83	-2.822	Yes	Yes	Yes
ZINC37603698	-5.577	1.173	92.993	-2.758	Yes	Yes	Yes

Distribution

Table 3 depicts the in-silico prediction of in-vivo distribution of the selected compounds and zinc primers, where all the compounds have shown a relatively low steady state volume of distribution, with relatively low BBB and CNS permeability.

Table 3: Distribution properties of the selected drugs and zinc primers

Drug list	Distribution VDss (human)	Distribution Fraction unbound (human)	Distribution BBB Permeability	Distribution CNS Permeability
Aspirin	-1.716	0.481	-0.332	-2.489
Valdecoxib	-0.409	0.167	-0.528	-2.192
Parecoxib	-0.342	0.123	-0.611	-2.442
Celecoxib	-0.273	0.133	-0.931	-2.052
Diclofenac	-1.605	0	0.236	-1.97
ZINC15955447	0.249	0.119	-0.619	-2.188
ZINC33287116	0.215	0.163	-1.095	-2.213
ZINC6495835	0.19	0	-0.407	-2.197
ZINC72471509	-0.023	0.044	-0.537	-2.953
ZINC37603698	0.051	0.013	-0.439	-1.846

Metabolism

Table 4 exhibits the metabolism of the selected compounds and zinc primers, which revels that all the compounds undergo metabolism in either of the CYP's and do not show any negative metabolic ends in-silico, which in turn should be confirmed by in-vitro/in-vivo evaluation.

Table 4: Metabolism properties of the selected drugs and zinc primers

Drug list	Metabolism CYP2D6 Substrate	Metabolism CYP3A4 Substrate	Metabolism CYP1A2 Inhibitor	Metabolism CYP2C19 Inhibitor	Metabolism CYP2C9 Inhibitor	Metabolism CYP2D6 Inhibitor	Metabolism CYP3A4 Inhibitor
Aspirin	No	No	No	No	No	No	No
Valdecoxib	No	Yes	Yes	Yes	Yes	No	Yes
Parecoxib	No	Yes	Yes	Yes	Yes	No	Yes
Celecoxib	No	Yes	Yes	Yes	Yes	No	No
Diclofenac	No	No	No	No	No	No	No
ZINC72279339	No	No	Yes	Yes	No	No	No
ZINC91891618	Yes	Yes	Yes	No	No	Yes	No
ZINC55580150	No	Yes	Yes	No	No	No	No
ZINC78905378	No	Yes	Yes	Yes	No	No	No
ZINC37603698	No	Yes	No	Yes	Yes	No	Yes

Excretion

In-silico prediction of in-vivo clearance of selected compounds and zinc primers are revealed in Table 5

Table 5: Excretion properties of the selected drugs and zinc primers

Drug list	Excretion Total clearance	Excretion Renal OCT2 substrate
Aspirin	0.72	No
Valdecoxib	0.435	No
Parecoxib	0.903	No
Celecoxib	0.84	No
Diclofenac	0.291	No
ZINC72279339	0.216	No
ZINC91891618	0.161	Yes
ZINC55580150	0.253	No
ZINC78905378	0.093	No
ZINC37603698	9.45	No

CONCLUSION

Drug identification was carried out to identify to discovery of a novel drug to overcome the risk factor of COX2 inhibitors to treat inflammation. Based on the study 5 hits show high binding affinity with 3LN1. Celecoxib was selected as standard on the study and then 5 ligands subsequently evaluated for ADMET property using PKCSM webserver ZINC6495835 have the binding affinity with better ADMET property and act as a novel moiety for 3LN1.

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CONFLICT OF INTEREST
The authors declare that no conflict of interest.

REFERENCES
1. Zarghi, A., & Arfaei, S. (2011). Selective COX-2 inhibitors: a review of their structure-activity relationships. Iranian journal of pharmaceutical research: IJPR, 10(4), 655.
2. Brook, R. A., Forsythe, A., Smeeding, J. E., & Lawrence Edwards, N. (2010). Chronic gout: epidemiology, disease progression, treatment, and disease burden. Current medical research and opinion, 26(12), 2813-2821.
3. Lawrence, R. C., Felson, D. T., Helmick, C. G., Arnold, L. M., Choi, H., Deyo, R. A., ... & National Arthritis Data Workgroup. (2008). Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part II. Arthritis & Rheumatism, 58(1), 26-35.
4. Kramer, H. M., & Curhan, G. (2002). The association between gout and nephrolithiasis: the National Health and Nutrition Examination Survey III, 1988-1994. American Journal of Kidney Diseases, 40(1), 37-42.
5. Weaver, A. L. (2008). Epidemiology of gout. Cleveland Clinic journal of medicine, 75, S9-12.
6. Richette, P., & Bardin, T. (2010). Colchicine for the treatment of gout. Expert opinion on pharmacotherapy, 11(17), 2933-2938.
7. Choi, H. K., Atkinson, K., Karlson, E. W., Willett, W., & Curhan, G. (2004). Alcohol intake and risk of incident gout in men: a prospective study. The Lancet, 363(9417), 1277-1281.
8. Choi, H. K., Atkinson, K., Karlson, E. W., Willett, W., & Curhan, G. (2004). Purine-rich foods, dairy and protein intake, and the risk of gout in men. New England Journal of Medicine, 350(11), 1093-1103.
9. Stamp, L., Searle, M., O'Donnell, J., & Chapman, P. (2005). Gout in solid organ transplantation: a challenging clinical problem. Drugs, 65, 2593-2611.
10. Becker, M. A., Schumacher, H. R., Benjamin, K. L., Gorevic, P., Greenwald, M., Fessel, J., ... & Gout Natural History Study Group. (2009). Quality of life and disability in patients with treatment-failure gout. The Journal of rheumatology, 36(5), 1041-1048.
11. Singh, J. A. (2009). Quality of life and quality of care for patients with gout. Current rheumatology reports, 11(2), 154-160.
12. Brook, R. A., Kleinman, N. L., Patel, P. A., Melkonian, A. K., Brizee, T. J., Smeeding, J. E., & Joseph-Ridge, N. (2006). The economic burden of gout on an employed population. Current medical research and opinion, 22(7), 1381-1389.
13. Pillinger, M. H., & Mandell, B. F. (2020, June). Therapeutic approaches in the treatment of gout. In Seminars in Arthritis and Rheumatism (Vol. 50, No. 3, pp. S24-S30). WB Saunders.
14. Leelananda, S. P., & Lindert, S. (2016). Computational methods in drug discovery. Beilstein journal of organic chemistry, 12(1), 2694-2718.
15. Guner, O. F. (2002). History and evolution of the pharmacophore concept in computer-aided drug design. Current topics in medicinal chemistry, 2(12), 1321-1332.
16. Sanders, M. P., McGuire, R., Roumen, L., de Esch, I. J., de Vlieg, J., Klomp, J. P., & de Graaf, C. (2012). From the protein's perspective: the benefits and challenges of protein structure-based pharmacophore modeling. MedChemComm, 3(1), 28-38.
17. Li, H., Sutter, J., & Hoffmann, R. (2000). HypoGen: an automated system for generating 3D predictive pharmacophore models. Pharmacophore perception, development, and use in drug design, 2, 171.
18. Muhammed, M. T., & Esin, A. Y. (2021). Pharmacophore modeling in drug discovery: methodology and current status. Journal of the Turkish Chemical Society Section A: Chemistry, 8(3), 749-762.
19. Muhammed, M. T., & Esin, A. Y. (2021). Pharmacophore modeling in drug discovery: methodology and current status. Journal of the Turkish Chemical Society Section A: Chemistry, 8(3), 749-762.
20. Sharma, S., & Bhatia, V. (2020). Treatment of Type 2 diabetes mellitus (T2DM): Can GLP-1 Receptor Agonists fill in the gaps? Array. Chemical Biology Letters, 7(4), 215-224.
21. Suresh, J., Pushpalatha, C., Parasuraman, P., & Dhananjaya, G. (2022). An In-Silico Approach of the Antibacterial Activity of Coleus Amboinicus Against Streptococcus Mutans. ECS Transactions, 107(1), 18247.
22. Mandhadi, J. R., Panneerselvam, T., & Parasuraman, P. (2020). Design, In-silico Modeling, Toxicity study and Synthesis of Novel Substituted Semicarbazide Derivatives of Pyrimidine: An Antitubercular Agent. Current Bioactive Compounds, 16(3), 294-301.
23. Mahesh, A.R., & Jenita, J.L., (2023). Designing Multi-Epitope Vaccine For SARS-COV-2 Through In-Silico Techniques. International Journal of Biology, Pharmacy and Allied Sciences, 12(3), 1041-1051.

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