Assessing Binding Affinities of COX-2 Inhibitors: A Comparative Analysis of LeDock 1.0 and PyRx 0.8 Programs

Sanhajutha Puangmala[1,*], Poruthai Sroypetch[1], and Prasan Tangyuenyongwatana[2]

[1]College of Oriental Medicine, Rangsit University, Pathum Thani, Thailand
[2]Department of Pharmaceutical Industry, School of Pharmacy, Eastern Asia University, Pathum Thani, Thailand
[*]Corresponding author, E-mail: sanhajutha.p@rsu.ac.th

Abstract

Several options exist for molecular docking with good performance and free availability. AutoDock Vina is a popular choice, and it is known for its accuracy and speed. LeDock 1.0 is another option known for its speed and accuracy, particularly in pose prediction. This study compares the performance of LeDock 1.0 and PyRx 0.8 docking programs with AutoDock Vina as the docking engine, focusing on redocking accuracy and correlation with experimental data. Redocking experiments revealed RMSD values ranging from 0.158 to 0.283 Å for LeDock 1.0 and 0.116 to 0.166 Å for PyRx 0.8. Subsequent docking of a set of COX-2 inhibitors demonstrated a superior linear correlation coefficient for LeDock 1.0 *(r^2 = 0.8244)* compared to PyRx 0.8 *(r^2 = 0.5227)*. Analysis of ligand-binding site interactions, benchmarked against the co-crystallized inhibitor SC-558 in the 1CX2 structure, provided further insights into the docking poses. While these initial results suggest a higher performance of LeDock 1.0 in this context, further evaluation across diverse systems is warranted. This work serves as a reintroduction of LeDock 1.0, a freely available docking program characterized by its efficiency, ease of use, and user-friendly graphical interface, highlighting its potential as a valuable tool for molecular docking studies.

Keywords: *LeDock 1.0, PyRx 0.8, COX-2 inhibitor, molecular docking, redock*

1. Introduction

Cyclooxygenase-2 (COX-2), also known as prostaglandin-endoperoxide synthase 2 (PTGS2), is an enzyme that plays a crucial role in the inflammatory response. It catalyzes the conversion of arachidonic acid to prostaglandin H2 (PGH2), a precursor for various pro-inflammatory mediators, including prostaglandins, thromboxanes, and prostacyclin (Berbecka et al., 2021; Funk, 2001). Unlike cyclooxygenase-1 (COX-1), which is constitutively expressed in most tissues and involved in maintaining physiological functions such as gastric mucosal protection and platelet aggregation, COX-2 is primarily induced in response to inflammatory stimuli (Stiller, & Hjemdahl, 2022; FitzGerald, 1991). These stimuli include cytokines (e.g., interleukin-1β, tumor necrosis factor-α), growth factors, lipopolysaccharides (LPS), and tumor promoters. The induction of COX-2 leads to a rapid increase in prostaglandin synthesis at sites of inflammation, contributing to the cardinal signs of inflammation: pain, heat, redness, and swelling (Zarghi, & Arfaei, 2011).

The regulation of COX-2 expression is complex and involves multiple signaling pathways. Nuclear factor-kappa B (NF-κB) is a key transcription factor that regulates COX-2 gene expression. Inflammatory stimuli activate NF-κB, which then translocates to the nucleus and binds to the COX-2 promoter, enhancing its transcription. The expression of COX-2 is also regulated by a wide array of mediators implicated in inflammation. Generally, lipopolysaccharides and proinflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor (TNF), and growth factors, promote COX-2 induction; conversely, IL-4, IL-13, and the anti-inflammatory cytokine IL-10 suppress the enzyme's production (Huang et al., 2000). Furthermore, post-transcriptional mechanisms, including mRNA stability and translation efficiency, can modulate COX-2 expression. The role of COX-2 in inflammation extends beyond acute responses. Chronic overexpression of COX-2 has been implicated in the pathogenesis of various diseases, including arthritis, cardiovascular diseases, neurodegenerative disorders, and cancer (Vane, & Botting, 1998).

Given the limitations and potential adverse effects of synthetic COX-2 inhibitors, there has been increasing interest in identifying natural compounds from plant sources that can selectively inhibit COX-2 activity. Plant-derived compounds, also known as phytochemicals, offer diverse chemical structures and biological activities, making them promising candidates for drug discovery and development (Aggarwal et al., 2009). Numerous phytochemicals, including flavonoids, terpenoids, phenolic acids, alkaloids, and lignans, have demonstrated significant COX-2 inhibitory activity *in vitro* and *in vivo*. These compounds exert their anti-inflammatory effects through various mechanisms, including direct inhibition of the COX-2 enzyme, modulation of upstream signaling pathways, and antioxidant activity (Jurenka, 2009). Curcumin, a major component of turmeric (*Curcuma longa*), is one of the most extensively studied plant-derived COX-2 inhibitors. Curcumin inhibits COX-2 expression by interfering with NF-κB signaling, reducing the production of pro-inflammatory cytokines and prostaglandins. Resveratrol, found in grapes (*Vitis vinifera*) and red wine, also exhibits COX-2 inhibitory activity. It suppresses COX-2 expression by modulating NF-κB and AP-1 signaling pathways. Gingerols, present in ginger (*Zingiber officinale*), have been shown to inhibit COX-2 activity and reduce inflammation in various experimental models. Other plant-derived COX-2 inhibitors include quercetin (found in onions and apples), epigallocatechin-3-gallate (EGCG) (found in green tea), and boswellic acids (found in *Boswellia serrata*). These compounds have demonstrated anti-inflammatory, antioxidant, and anticancer properties.

LeDock is a freely available molecular docking program (https://www.lephar.com/software) known for its accuracy and speed in predicting the binding modes of ligands to proteins (Wang et al., 2016). It employs a hybrid scoring function that combines physics-based and knowledge-based terms, aiming to provide a balance between computational efficiency and accuracy. LeDock's algorithm is based on a combination of simulated annealing and evolutionary optimization techniques to explore the conformational space of the ligand within the protein's binding site (Kamal, & Chakrabarti, 2023). This involves iteratively adjusting the ligand's position, orientation, and rotatable bonds to minimize the overall binding energy. One of the key features of LeDock is its ability to perform flexible ligand docking, allowing for conformational changes in the ligand during the docking process (Liu, & Xu, 2019). This is crucial for accurately modeling the induced-fit phenomenon, where the ligand and protein adjust their shapes upon binding. LeDock has demonstrated high accuracy in pose prediction and virtual screening, making it a valuable tool for computational drug design. A comprehensive evaluation of ten docking programs showed that LeDock had high accuracy and good speed, slightly faster than AutoDock Vina, making it a recommended program for virtual screening tasks (Wang et al., 2016).

Various research studies have used LeDock, including the prediction of ligand binding modes to dopamine D3 receptors (Liu, & Xu, 2019). Xu and his team studied 195 high-quality protein-ligand complexes and tested how well LeDock worked, along with three different scoring methods. The study showed that the best position of the free docking tool, LeDock, had a success rate of 89.20%, indicating it is very effective at sampling (Xu et al., 2021). Wang and his team used LeDock to see how flavonoids attach to the estrogen receptor alpha and checked how well they bind using a method called molecular dynamics simulation (Wang et al., 2022). Another study looks for possible inhibitors of the S6K1 protein using LeDock and other docking programs. It also analyzes how these inhibitors bind and their strength using molecular dynamics simulations (Zhang et al., 2023). However, the number of research reports on using LeDock is far less than PyRx 0.8, a molecular docking program using AutoDock Vina as a docking engine. Our study wants to evaluate the LeDock program, which is free and straightforward to use, to see the performance and accuracy of docking compared to PyRx 0.8.

2. Objective

This research focused on evaluating the binding affinity performance of the LeDock 1.0 program by docking with COX-2 inhibitors (compounds 1–5) against the cyclooxygenase-2 protein (1CX2) and comparing them with docking results from the PyRx 0.8 program.

3. Materials and Methods

3.1 Cyclooxygenase-2 Protein for Docking

COX-2 (PDB ID: 1CX2) was obtained from the Protein Data Bank (www.rcsb.org). The proteins were checked by using PROCHECK (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) to assess the stereochemical quality of the protein structure. Protein preparation involves adding hydrogen atoms or filling in missing parts, removing water, and adjusting the pH to about 7.0, give or take 0.5, for the other atoms involved. The x-ray ligand (SC-558) was put back into the protein, and the difference in position between the redock ligand and the x-ray ligand was measured as the RMSD using the VEGA-ZZ program (https://www.ddl.unimi.it/). The binding site position for docking of both programs to 1CX2 is X = 23.96, Y = 21.51, and Z = 15.24. The box dimensions were in the range of ±7.5, ±10.0, ±12.5, and ±15.0 Å of each coordinate of the binding site above. For PyRx 0.8, the box dimensions were performed in the same box size as the LeDock 1.0 experiment.

3.2 Compounds for Docking Study and Docking Process

We obtained the structure of compounds 1-5 and IC_{50} (nM) from the review of COX-2 inhibitors by Ju et al., (2022) and Riendeau et al., (1997). First, the structure was drawn by using ChemSketch and saved as mol files. Next, the 2D structures were converted to 3D structures with Avogrado software using MMFF94 force field. The 3D structure was save as mol2 format. Molecular docking studies were performed using LeDock 1.0 to evaluate and validate the binding interactions between ligands and the target protein. In LeDock 1.0, ligands are required to be converted to mol2 format. The binding site position is in the middle of the grid box, which has lengths that are plus and minus the specific length to make a box that can cover the part of the protein's binding pocket, as discussed in Section 3.1. The parameters for PyRx 0.8 (AutoDock Vina) were configured to their default settings, with an exhaustiveness level of 8, resulting in the generation of the top 9 poses for each ligand.

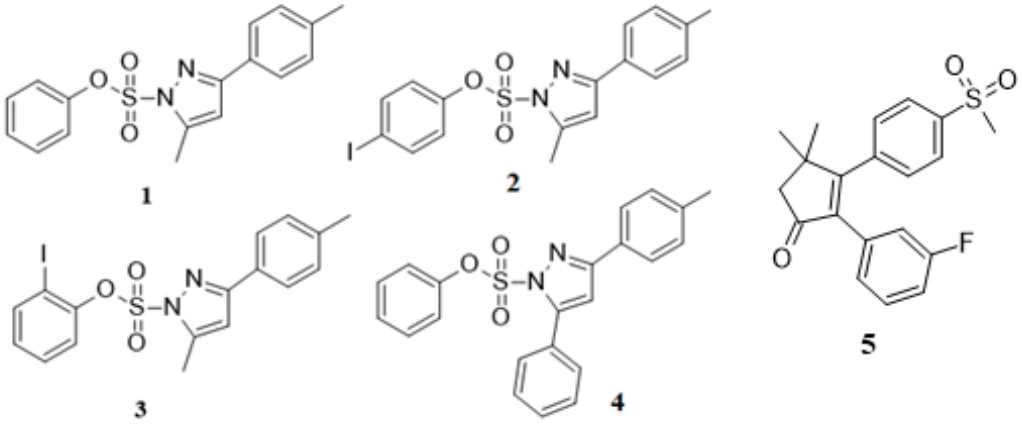

Figure 1 Structures of COX-2 inhibitors (compound 1–5)

Normally, it is simple to dock with LeDock 1.0 on the Windows operating system. However, the special part of LeDock is the output file. It sent a .dok file that composed all pose results of one docking. There are three methods for extracting a .dok file from a pdb file. The first one involves using Python to parse the .dok file and extract the required information; a Python script needs to be written to convert the file. Next, Windows 11 has the ability to run a Linux environment directly on Windows, called the Windows Subsystem for Linux (WSL). Users need to install the Linux version of LeDock 1.0 within the WSL environment and use the LeDock command-line tools within the WSL environment to extract the .pdb file from the .dok file.

The third way is to use LeDock 1.0 in the Linux version in the Linux OS, which can directly extract the PDB file from the .dok file.

The binding energy and the IC_{50} value were plotted by using the GraphPad Prism 10 program, which includes statistical analysis, and the plotted graph.

4. Results and Discussion

4.1 Docking Accuracy: Redocking Study

The x-ray ligands of the 1CX2 protein were returned to their binding sites under the different box size conditions, and we used VEGA-ZZ (Italy) software to calculate RMSD; the results can be seen in Table 1.

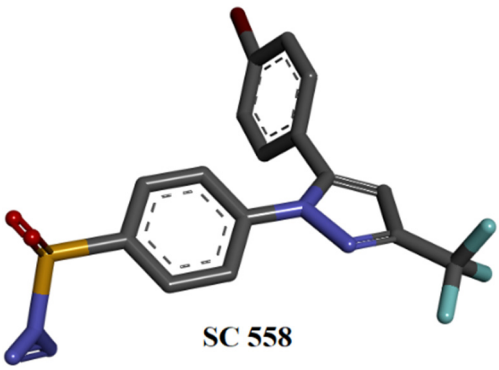

Figure 2 Structures of SC 558 (1CX2 inhibitor)

Table 1 The RMSD of redock results

X-ray ligand of proteins	Box size (Å)	RMSD (Å) LeDock 1.0	RMSD (Å) PyRx 0.8
1CX2	±7.5	0.179	0.141
	±10.0	0.283	0.166
	±12.5	0.225	0.116
	±15.0	0.158	0.165

From the redock results in Table 1, the RMSD of four box sizes is below 2 Å, and the LeDock 1.0 gives the RMSD range from 0.158 to 0.283 Å, while the PyRx 0.8 gives the RMSD range from 0.116 to 0.166 Å. These findings indicate that both LeDock 1.0 and PyRx 0.8 provide reliable docking results, with PyRx demonstrating slightly better precision in RMSD values. On the other hand, a paired t-test of both sets of data showed no significant difference in RMSD between the two programs, with a p-value of 0.1188 and the test was performed at a significant level of $\alpha = 0.05$. Redocking refers to the process of re-evaluating a ligand's binding pose to a target protein using molecular docking software, typically after the initial docking has been performed. The accuracy of redocking is crucial for validating docking predictions and improving drug discovery processes (Agarwal, 2023). An RMSD value below 2 Å signifies a good docking procedure and demonstrates the effectiveness of the utilized docking approach.

The SC 558 overlay with the redock structure in different box sizes from LeDock 1.0 is shown in Figure 3, showing that the best box size for the next step docking with LeDock 1.0 is ±15.0 Å.

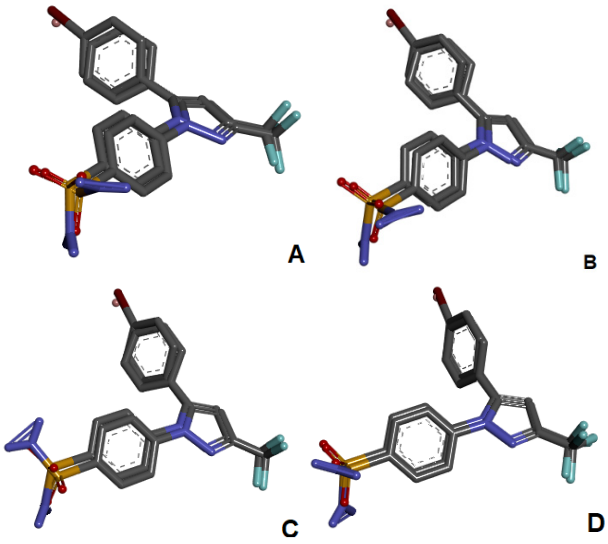

Figure 3 Redock X-ray ligand (SC 558) structure of 1CX2 protein by LeDock 1.0 (A) Box size ±7.5 Å, (B) Box size ±10.0 Å, (C) Box size ±12.5 Å, and (D) Box size ±15.0 Å

4.2 Docking with LeDock 1.0 and PyRx 0.8

4.2.1 Docking COX2 inhibitors with cyclooxygenase-2 (1CX2)

It was found that compounds 1–5 bind to 1CX2 with an energy range of -5.58 to -6.57 kcal/mol in the LeDock 1.0 program, but the same set of compounds bind with a lower energy range of -7.1 to -10.1 kcal/mol in PyRx 0.8. The different docking programs have different algorithms and scoring functions that make the binding energy different. When we plot the binding energy with IC_{50} values, the LeDock 1.0 docking gives the linear correlation with a correlation coefficient (r^2) equal to 0.8244, while the PyRx 0.8 gives the linear correlation with r^2 equal to 0.5227.

There are several reasons why a less popular molecular docking program like LeDock might outperform a more popular one (PyRx) in terms of correlation coefficient. LeDock employs a hybrid algorithm that combines systematic search and stochastic sampling to explore the conformational space of the ligand (Wang et al., 2016). LeDock might have a scoring function that is better parameterized or, more specifically, optimized for the particular target protein or ligand set that we are using. The scoring functions themselves can differ significantly. One program might emphasize certain energy terms (e.g., electrostatics, van der Waals) more than another. If the one program's scoring function places more weight on the interactions that are truly important for binding in this docking system, it could lead to better correlation.

Similar to scoring functions, the search algorithm in the less popular program might be better suited to finding the correct binding pose for a specific system. Different algorithms (e.g., genetic algorithms, Monte Carlo, simulated annealing) explore the conformational space differently (Kitchen et al., 2004).

Table 2 The LeDock 1.0 and PyRx 0.8 docking results

Ligand	Binding energy (kcal/mol) LeDock 1.0	Binding energy (kcal/mol) PyRx 0.8	IC_{50} (nM)
Compound 1	-6.40	-10.1	1.1
Compound 2	-6.57	-7.7	9.2
Compound 3	-5.58	-7.1	92.0
Compound 4	-6.24	-8.7	10.0
Compound 5	-6.31	-8.3	41.0

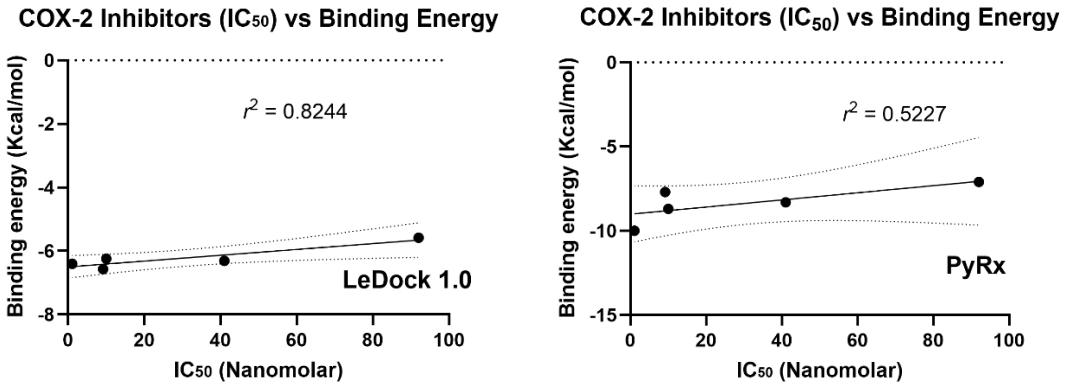

Figure 4 Linear correlation between binding energy (kcal/mol) and COX-2 inhibition (IC50, nM). LeDock with r^2 = 0.8244 (Y = 0.009159*X - 6.801) and PyRx with r^2 = 0.5227 (Y = 0.02118*X - 9.009)

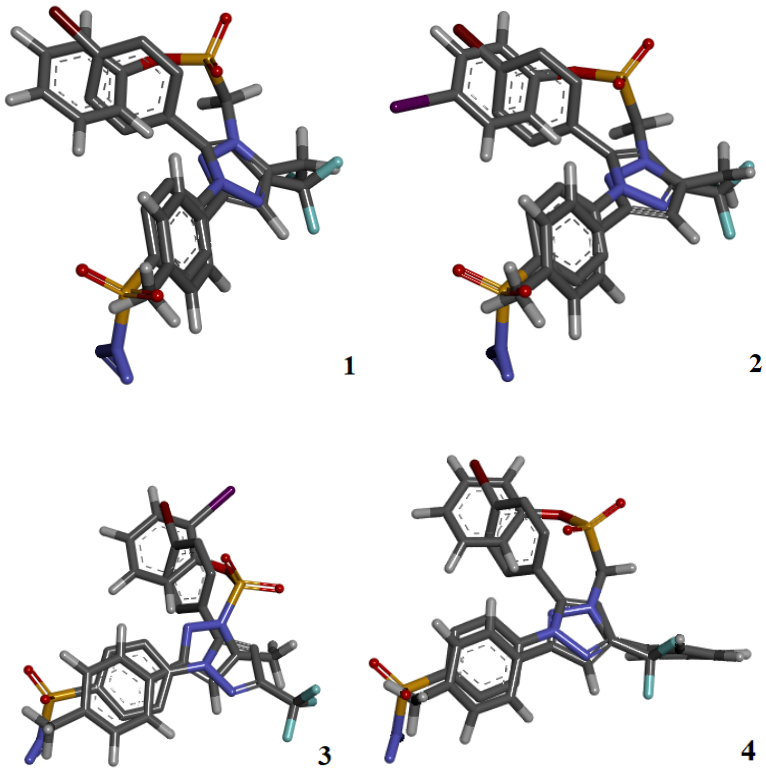

Figure 5 Overlay structure of docking compounds from LeDock 1.0 superimpose with SC558 (**1**) compound-1, (**2**) compound-2, (**3**) compound-3 and (**4**) compound-4

When compound 1-5 is docked, it can be seen that one of its side chains has been inserted into the COX-2 enzyme's selective cavity, which interacts with VAL523, ARG513, and HIS90. The PyRx 0.8 docking pose in Figure 6 looks quite similar to the LeDock docking; however, for compound 2, the molecule poses differently compared to the same molecule in Figure 5. Five compounds have interactions with amino acids in the binding pocket, which are shown in Table 3. All of them have some specific, similar amino acid interactions when compared to SC 558, which the 3D interaction structure depicts in Figure 7 (A), and the 2D interaction map is shown in Figure 7 (B).

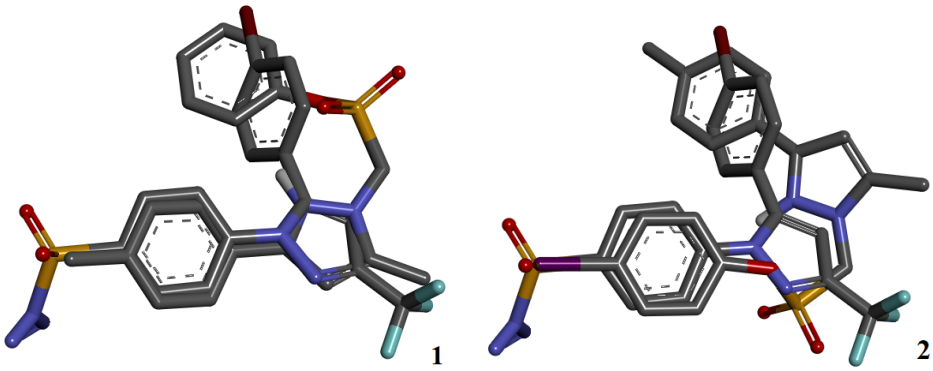

Figure 6 Overlay structure of docking compounds from PyRx 0.8 superimpose with SC558 (**1**) compound-1, and (**2**) compound-2

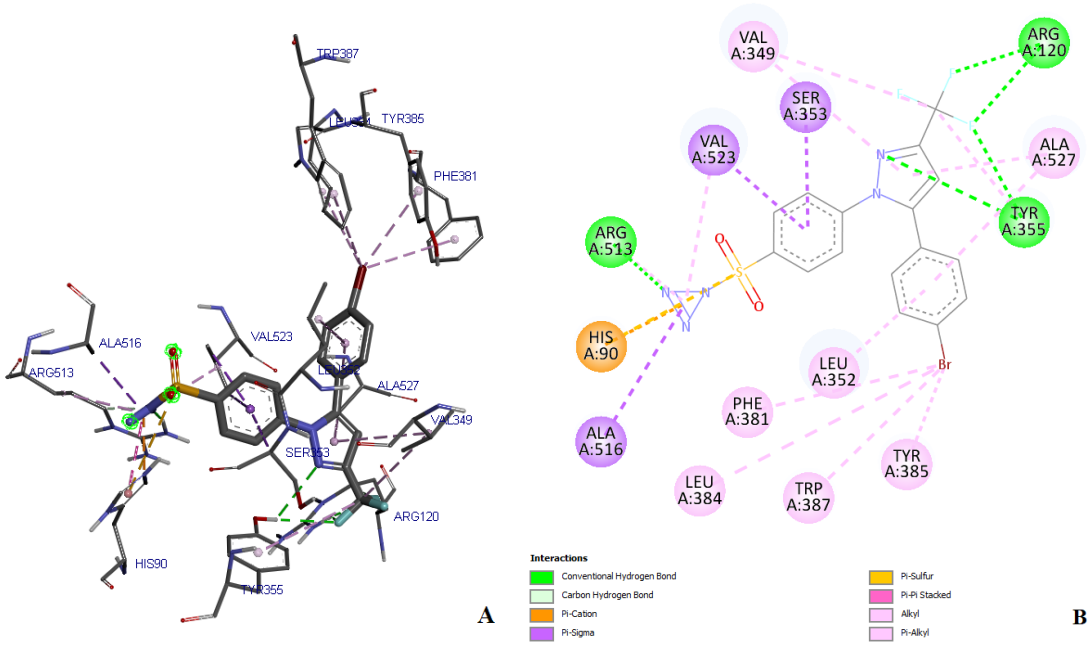

Figure 7 Ligand SC-558 of 1CX2 interacts with amino acids in 1CX2 binding site (A) 3D structure (B) 2D map

Table 3 Amino acid residues in 1CX2 interacted with ligands in LeDock docking

Ligand	Amino acids
SC-558	HIS90, ARG120, VAL349, LEU352, SER353, TYR355, PHE381, LEU384, TYR385, TRP387, ARG513, ALA516, VAL523, ALA527
Compound 1	HIS90, ARG120, VAL349, LEU352, SER353, ALA516, PHE518, MET522, VAL523, ALA527
Compound 2	HIS90, GLN192, LEU352, TYR385, ARG513, ASP515, ALA516, VAL523, TYR355
Compound 3	HIS90, VAL349, SER353, PHE381, TYR385, ARG513, ALA516, PHE518, MET522, VAL523, ALA527, LEU531
Compound 4	HIS90, ARG120, VAL116, VAL349, SER353, TYR355, ALA516, ILE517, MET522, VAL523, ALA527, LEU531
Compound 5	HIS90, VAL349, LEU352, SER353, TYR355, LEU359, TYR385, ARG513, ALA527, VAL523, GLY526

From the experimental survey during this research, LeDock 1.0 has some limitations on metalloproteins that contain zinc or copper atoms, such as carbonic anhydrase or superoxide dismutase. There are some explanations for this phenomenon, such as metal ions having a high positive charge (e.g., Zn^{2+}, Cu^{2+}), which can polarize nearby atoms in the ligand or protein. The docking program typically models interactions using non-covalent force fields, so it may not accurately represent the strength or directionality of metal-ligand bonds. Nonetheless, the scarcity of accessible software for the straightforward docking of metal compounds to established proteins has now impeded the clinical advancement of these compounds. Indeed, a primary impediment to the advancement of metallodrugs is the scarcity of computational tools for in silico metallodrug screening (Hakkennes et al., 2023).

5. Conclusion

In conclusion, while LeDock 1.0 demonstrated superior performance in this specific experiment compared to PyRx 0.8 (which utilizes AutoDock Vina), it is essential to acknowledge that these results may be context-dependent. Further validation across a diverse range of enzyme-ligand systems is necessary to establish the robustness and general applicability of LeDock 1.0. Despite this, the potential of LeDock 1.0 as a freely available docking program with an efficient algorithm, user-friendly graphical interface, and promising performance warrants further investigation and consideration within the scientific community. The reintroduction of such a tool could provide researchers with a valuable alternative for molecular docking studies.

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7. References

Agarwal, R., T, R. R., & Smith, J. C. (2023). Comparative assessment of pose prediction accuracy in RNA-ligand docking. *Journal of Chemical Information and Modeling*, *63*(23), 7444-7452. https://doi.org/10.1021/acs.jcim.3c01533

Aggarwal, B. B., Shishodia, S., Takada, Y., Banerjee, S., Newman, R. A., Bueso-Ramos, C. E., Price, J. E., & Jacobberger, J. W. (2009). Inflammation and cancer: how hot is the link?. *Annals of the New York Academy of Sciences*, *1171*(1), 159-195. https://doi.org/10.1016/j.bcp.2006.06.029

Berbecka, M., Forma, A., Baj, J., Furtak-Niczyporuk, M., Maciejewski, R., & Sitarz, R. (2021). A systematic review of the cyclooxygenase-2 (COX-2) expression in rectal cancer patients treated with preoperative radiotherapy or radiochemotherapy. *Journal of Clinical Medicine*, *10*(19), 4443. https://doi.org/10.3390/jcm10194443

FitzGerald, G. A. (1991). Mechanisms of platelet activation: thromboxane A2 as an amplifying signal for other agonists. *The American Journal of Cardiology*, *68*(16), 11B-15B. https://doi.org/10.1016/0002-9149(91)90379-Y

Funk, C. D. (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science*, *294*(5548), 1871-1875. https://doi.org/10.1126/science.294.5548.1871

Hakkennes, M. L. A., Buda, F., & Bonnet, S. (2023). MetalDock: An open access docking tool for easy and reproducible docking of metal complexs. *Journal of Chemical Information and Modeling*, *63*(24), 7816-7825. https://doi.org/10.1021/acs.jcim.3c01582

Huang, Z. F., Massey, J. B., & Via, D. P. (2000). Differential regulation of cyclooxygenase-2 (COX-2) mRNA stability by interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) in human in vitro differentiated macrophages. *Biochemical Pharmacology*, *59*(2), 187-194. https://doi.org/10.1016/s0006-2952(99)00312-3. PMID: 10810453.

Ju, Z., Li, M., Xu, J., Howell, D. C., Li, Z., & Chen, F. E. (2022). Recent development on COX-2 inhibitors as promising anti-inflammatory agents: The past 10 years. *Acta Pharmaceutica Sinica B*, *12*(6), 2790-2807. https://doi.org/10.1016/j.apsb.2022.01.002

Jurenka, J. S. (2009). Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: A review of preclinical and clinical research. *Alternative Medicine Review*, *14*(2), 141-153.

Kamal, I. M., & Chakrabarti, S. (2023). MetaDOCK: A Combinatorial Molecular Docking Approach. *ACS Omega*, 8(6), 5850–5860. https://doi.org/10.1021/acsomega.2c07619

Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Reviews Drug Discovery*, *3*(11), 935–949.

Liu, N., & Xu, Z. (2019). Using LeDock as a docking tool for computational drug design. *IOP Conference Series: Earth and Environmental Science*, *218*(1), 012143.

Riendeau, D., Percival, M. D., Boyce, S., Brideau, C., Charleson, S., Cromlish, W., Ethier, D., Evans, J., Falgueyret, J. P., Ford-Hutchinson, A. W., Gordon, R., Greig, G., Gresser, M., Guay, J., Kargman, S., Léger, S., Mancini, J. A., O'Neill, G., Ouellet, M. Ple., …, Chan, C. C. (1997). Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. *British Journal of Pharmacology*, *121*(1), 105-117. https://doi.org/10.1038/sj.bjp.0701076

Stiller, C & Hjemdahl, P. (2022). Lessons from 20 years with COX-2 inhibitors: Importance of dose–response considerations and fair play in comparative trials. *Journal of Internal Medicine*, *292*(4), 557-574. https://doi.org/10.1111/joim.13505

Vane, J. R., & Botting, R. M. (1998). Mechanism of action of nonsteroidal anti-inflammatory drugs. *The American Journal of Medicine, 104*(2), 2S-8S. https://doi.org/10.1016/S0002-9343(97)00203-9

Wang, N., Zhang, X., Rothrauff, B. B., Fritch, M. R., Chang, A., He, Y., Yeung, M., Liu, S., Lipa, K. E., Lei, G., Alexander, P. G., & Lin, H. (2022). Novel role of estrogen receptor-α on regulating chondrocyte phenotype and response to mechanical loading. *Osteoarthritis Cartilage*, *30*(2), 302-314. https://doi.org/10.1016/j.joca.2021.11.002

Wang, Z., Sun, H., Yao, X., Li, D., Xu, L., Li, Y., ... & Hou, T. (2016). Comprehensive evaluation of ten docking programs on a diverse set of protein–ligand complexes: the prediction accuracy of sampling power and scoring power. *Physical Chemistry Chemical Physics, 18*(18), 12964-12975. https://doi.org/10.1039/C6CP01555G.

Xu, S., Wang, L., & Pan, X. (2021). An evaluation of combined strategies for improving the performance of molecular docking. *Journal of Bioinformtics and Computational Biology*, *19*(2), 2150003. https://doi.org/10.1142/S0219720021500037

Zarghi, A., & Arfaei, S. (2011). Selective COX-2 Inhibitors: A review of their structure-activity relationships. *Iranian Journal of Pharmaceutical Research*, *10*(4), 655-683. PMID: 24250402; PMCID: PMC3813081.

Zhang, H., Zhang, H. R., Zhang, J., Hu, M. L., Ren, L., Luo, Q. Q., & Qi, H. Z. (2023). Discovery of novel S6K1 inhibitors by an ensemble-based virtual screening method and molecular dynamics simulation. *Journal of Molecular Modeling*, *29*(2), 1-13. https://doi.org/10.1007/s00894-023-05504-9