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Molecular Docking of Acetylacetone-Based Oxindole Against Indoleamine 2,3-Dioxygenase: Study of Energy Minimization

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Abstract

Molecular docking plays an essential role in drug discovery because it is more efficient and more affordable compared to traditional synthesis methods and biological assays. Molecular docking determines the conformation and affinity of non-covalent bonds between macromolecules (receptors) and small molecules (ligands) computationally. Energy minimization carried generally out by using the Merck Molecular Force Field 94 (MMFF94) force field produces ligands with the most stable conformation. MarvinSketch and Open Babel for energy minimization were utilized in this docking study of acetylacetone-based oxindole derivatives to 2,3-dioxygenase indoleamine macromolecules (IDO-1, PDB: 2D0T). The results showed that MarvinSketch provides better binding energy than energy minimization with Open Babel. Molecular docking indicated different interactions between 2D0T macromolecule residues with ligands that have been prepared using MarvinSketch, Open Babel, and without energy minimization.

Keywords: *molecular docking; energy minimization; oxindole; IDO-1*

Introduction

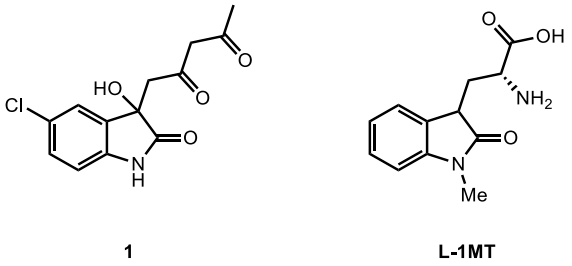
The kynurenine pathway involving *indolamine 2,3-dioxygenase 1* (IDO1) plays an important role in cancer immunotherapy as it regulates tryptophan metabolism, which is associated with immune and neurological enhancement (Platten et al., 2019). IDO1, which catalyzes tryptophan catabolism and causes local immunosuppression in T cells, is continuously expressed in various types of tumors, causing poor predictions of tumor development (prognosis) (Zhou et al., 2020). Thus, research has continuously been conducted to find inhibitors that can suppress IDO1 expression (Platten et al., 2019).

The development of active compounds as IDO1 inhibitors takes place gradually started by in silico study, in vitro and in vivo experiments, and ended in clinical trials. Virtual screening is performed on a collection of compounds that may have biological activity based on their structural form (Zheng et al., 2018). Molecular docking as a part of in silico study predicting non-covalent bonds, conformation and bond affinity between ligands and target proteins computationally. This technique is more effective than traditional synthesis and bio-assay methods

(Zhang et al., 2018). The docking procedure includes validation by via re-docking followed by cross-docking and works after ligands and protein preparation which is generally represented by the energy of minimization. The binding affinity resulted from docking process illustrates binding energy between ligands and proteins, where the low binding affinity indicates a strong, high affinity, and more stable conformations (Hari, 2019; Nisha et al., 2016).

The minimization energy of ligands is an important step in molecular docking because it determines the ligands stability. The Universal Force Field (UFF) and the Merck Molecular Force Field 94 (MMFF94) are the two prevalent molecular mechanics methods used in minimization step. The parameters of elements, hybridization, and connectivity are generally used by the UFF method, while the MMFF94 calculation is divided into seven types of energy which are independently calculate the partial energies of various interactions in the molecule. It is known that the UFF method is less precise than other molecular mechanics due to its ability in the calculation of electrostatic interactions in hydrogen bonding. On the contrary, the MMFF94 method is generally applied in molecular simulations and organics calculations because it is more precise and computationally intensive than the UFF (Jász, Rák, Ladjánszki, & Cserey, 2019). The minimization of energy using MMFF94 method can be performed with several applications such as *MarvinSketch* (ChemAxon, 2020, http://www.chemaxon.com) and *Open Babel* (O'Boyle et al., 2011) in *Pryx* (Dallakyan & Olson, 2015). The *MarvinSketch* program utilizes the divide and conquer algorithm (Imre, Veress, Volford, & Farkas, 2003), while *Open Babel* program in *Pryx* applies the conjugate gradient algorithm (Cormen, Leiserson, & Rivest, 2009).

The potential of oxindole derivatives as IDO1 inhibitors has been studied by molecular docking. (2S)-2-Acetamide-3-(2-oxoindolin-3-yl)propanoic acid, (Z)-1-(5-chloro-2-oxoindolin-3-ylidene)pentane-2,4-dione, and 1-(5-chloro-3-hydroxy-2-oxoindolin-3-yl)pentane-2,4-dione are oxindole derivatives inhibited IDO1 in breast cancer (MDA-MB -231) (Paul et al., 2017). 1-(5-Chloro-3-hydroxy-2-oxindoline-3-yl) pentane-2,4-dione **(1)** (Figure 1) with similar backbone as previously reported oxindole derivatives was then studied in this research by evaluating the influence of energy minimization. The study was conducted using the *MarvinSketch* and *Open Babel* in *Pryx* for energy minimization, and without minimization, followed by docking of ligand **1** to IDO1 (PDB ID: 2D0T) using the *Autodock Vina* (Trott & Olsson, 2012) in *Pryx* (Dallakyan & Olson, 2015). The docking results were compared to 1-methyltryptophan (**L-1MT**) (Figure 1), a common inhibitor of IDO1 using the same treatment (Zhang et al., 2018). The results were evaluated based on the minimization energy, binding affinity, and the interaction of ligands with the macromolecule.



1 L-1MT

Figure 1. The structure of ligand **1** and **L-1MT**

Methods

General

This study was performed on Acer Notebook running on Intel (R) Core i5-5200U CPU @ 2.20 GHz 2201 MHz 2 core (s) specification with 4 GB RAM and Nvidia GeForce 820M graphics using *Microsoft Windows 10 Enterprise* as the operating system. Ligand preparation and optimization were executed using *MarvinSketch 20.8.0* (ChemAxon, 2020, http://www.chemaxon.com) and *Open Babel Pryx* (O'Boyle et al., 2011) in Pryx (Dallakyan & Olson, 2015). Macromolecule preparation, visualization, and data analysis were carried

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out with *PyMOL 2.3.4* (Schrodinger LLC, 2015, https://pymol.org). The molecular docking was achieved using *Autodock Vina* (Trott & Olsson, 2012) in *Pyrx* (Dallakyan & Olson, 2015).

Molecular Docking

Indoleamine 2,3-dioxygenase 1 (IDO-1) at a resolution of 2.3 Å was retrieved from wwPDB (PDB ID: 2D0T) (Sugimoto et al., 2006) and prepared using *PyMOL*. The ligands were optimized by energy minimization using the MMFF94 force field on *MarvinSketch 20.8.0* and *Open Babel Pryx*. As an initial step, the redocking of PIM co-crystal ligand was performed as validation step and followed by cross-docking of ligands using *Autodock Vina* in *Pyrx*. Molecular docking was carried out with a grid size of 12 x 12 x 12 Å and x, y, z dimensions of 60, 53, and 18 Å. *PyMOL* was used for visualization of molecular docking results.

Result and Discussion

Preparation and Optimization

Macromolecule 2D0T

Indoleamine 2,3-dioxygenase 1 (PDB ID: 2D0T) at a resolution of 2.3 Å was chosen for this study. This dimer-cross-linked macromolecule contains two identical chains and each chain bind to co-crystallized ligands namely 4-phenyl imidazole (PIM), 2-(*N*-cyclohexylamino) ethane sulfonate (NHE), and protoporphyrin IX containing Fe (HEM) (Sugimoto et al., 2006). Preparation was initially started by removing water molecules in order to avoid interference and complexity during docking process (Cole, Nissink, & Taylor, 2005). The two chains was then separated and docking process can be done only with one chain (Greco et al., 2016). The PIM co-crystallized ligand was removed from the binding site for creating docking position and this was centered at the coordinate x = 60; y = 53; and z = 18 Å. The 2D0T preparation was finished by the addition of hydrogen atoms for macromolecule-ligand interactions.

Energy Minimization of Ligands

The 2D structure of ligand **1** and **L-1 MT** was drawn using *MarvinSketc*h and was converted into 3D structure based on the Minkowski matrix approach (Imre et al., 2003). The energy of the ligands was minimized by MMFF94 on *MarvinSketch* (**a**) and *Open Babel* (**b**), and without energy minimization (**c**). The minimization provided stable conformation with lowest potential energy. This step gave strucutre with energy of 16.64, 21.86, and 23.52 Kcal/mol for **1a**, **1b**, and **1c**, respectively. A slightly different pattern was found for L-1MT with energy of 73.90, 53.88, and 54.94 Kcal/mol for **L-1MTa**, **L-1MTb**, and **L-1MTc** (Figure 2). These results indicated that different algorithms combined with different bond angles and torsion of ligand structures produced divergent energy. The lowest energy of ligand **1a** resulting from minimization using *MarvinSketch* implies that divide and conquer approach of *MarvinSketch* works better on ligand **1a**. This algorithm resolves the problems into smaller sub-problems which are then recursively solved and combined to break the real problem (Cormen et al., 2009). Otherwise, the conjugate gradient algorithm of *Open Babel* developed for symmetric and non-symmetric systems, where the algorithm simplifies code and reduces storage space, was fit to **L-1MTa**. The later algorithm solves linear problems by finding the minimum point of the quadratic function of a vector (Hestenes & Stiefel, 1952). These data demonstrated that the proper molecular arrangement with low potential energy depends on the algorithm used in the minimization step and it cannot be generalized.

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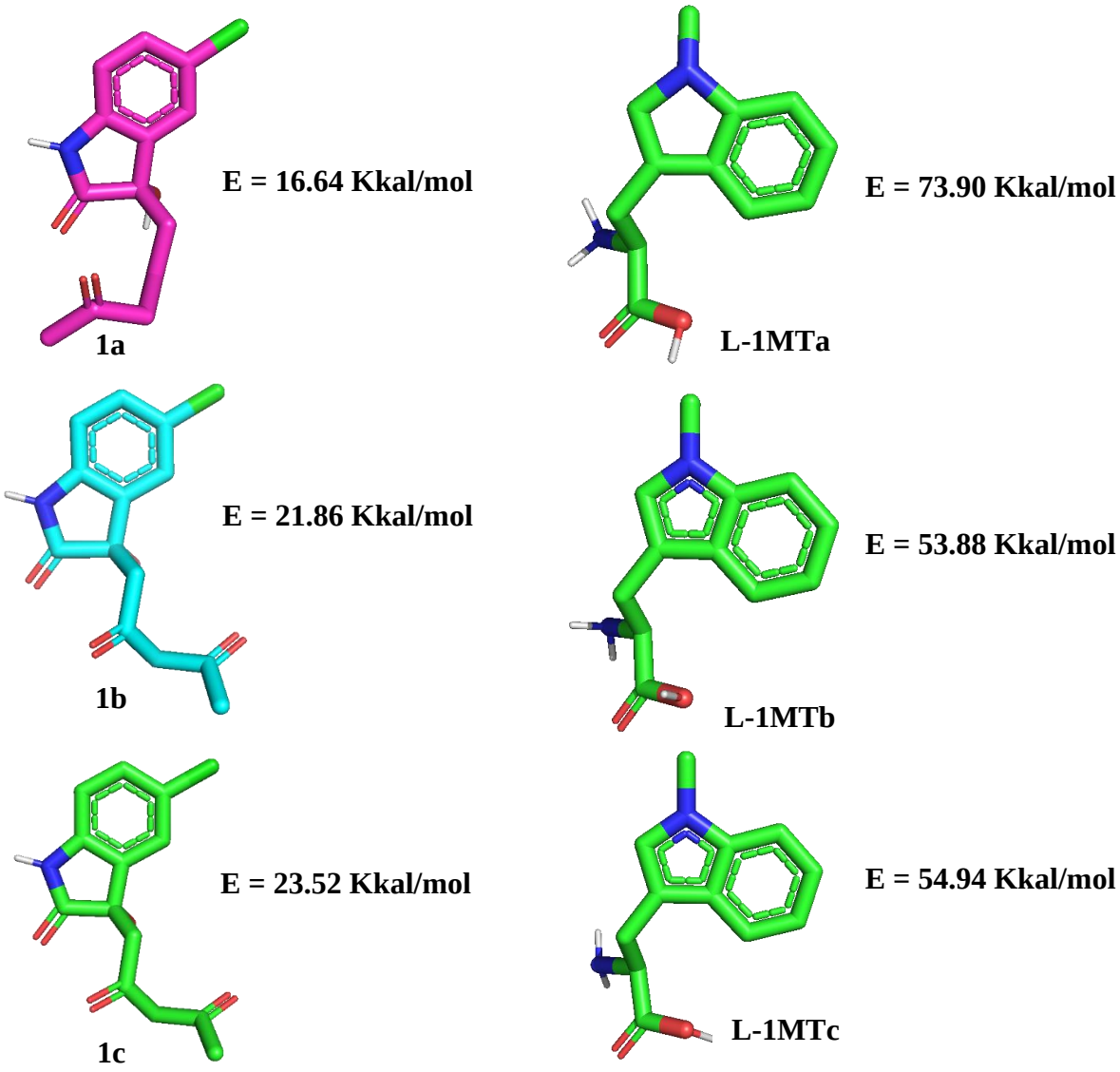

Figure 2. The structure conformation of ligand **1** and **L-1MT** resulting from minimization using **(a)** *MarvinSketch,* **(b)** *Open Babel*, and **(c)** without minimization

Molecular Docking

Docking Validation

The docking procedure was verified using the redocking method by removing the PIM ligand from 2D0T binding site and inserted back into the same site using *Autodock Vina* in *Pyrx* (Ramírez & Caballero, 2018; Tangyuenyongwatana, 2017). The *MarvinSketch* energy-minimized PIM ligand was successfully reattached to its binding site and gave root mean square deviation (RMSD) value of 0.019, resulting from alignment of all atoms of redocked PIM and co-crystallized ones. Further, visualization of redocked and co-crystallized PIM ligands showed similar interaction through amide group with the nitrogen atom of HEM in addition to phi-phi stacking between the pyrrole ring to HEM (Figure 3). The RMSD value (< 2.00 Å) and the resulting interactions indicate that redocking method is acceptable and the docking process can be used for the next evaluation (Bell & Zhang, 2019).

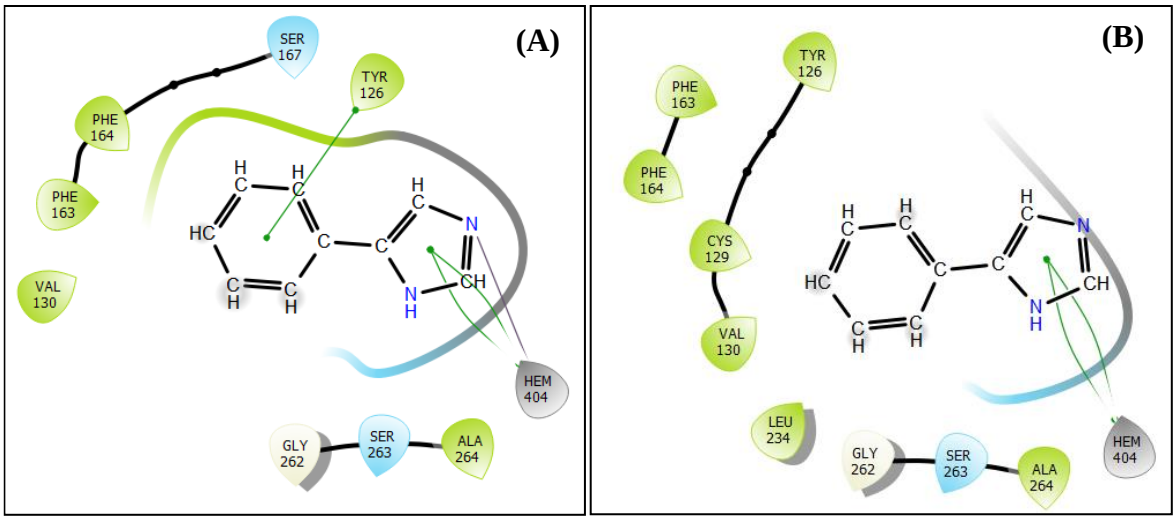

Figure 3. Representation of co-crystallized (**A**) and redocked PIM (**B**) to 2D0T

Table 1. Binding energy of ligand **1** and **L-1MT**

Ligand	(Pose) Binding Affinity (Kcal/mol)
1a	(1) -0.4; (2) 2.4; (3) 2.4
1b	(1) 0.2; (2) 2.4
1c	(1) 0.3; (2) 2.3; (3) 2.7; (4) 2.9
L-1MTa	(1) -2.8; (2) -2.1; (3) -1.3; (4) -0.9; (5) -0.7; (6) -0.1
L-1MTb	(1) -1.0; (2) -0.9; (3) -0.7; (4) -0.6; (5) -0.2; (6) -0.2; (7) 0.6
L-1MTc	(1) -1.1; (2) -1.1; (3) -0.8; (4) -0.8; (5) -0.2; (6) 1.2; (7) 1.5; (8) 1.8

Docking of The Ligands

The docking process was executed in the binding site of PIM co-crystallized ligand at x, y, z coordinates of 60, 53, and 18 Å using a grid of 12 × 12 × 12 Å. The evaluation of binding affinity is important because this paramatere shows the strength of ligand-protein interactions. The lower binding affinity means the greater ligand-protein strength and interactions (Hari, 2019; Nisha et al., 2016). As shown in **Table 1**, the ligands energy minimization differences resulted in various binding affinity values. Both ligands **1** and **L-1MT** displayed comparable arrangement where ligands with *MarvinSketch* minimization showed the lowest binding energy at the best pose (pose 1). This fact indicated that energy minimization affected the docking process and is required to provide more stable ligand conformations. Moreover, the lower binding afinity values could be related to the different algorithms used in *MarvinSketch* and *Open Babel* as previously described (Imre et al., 2003; Samdani & Vetrivel, 2018). The **L-1MT** docking evaluation supported the results of ligand **1** and ensured that energy minimization affects the molecular docking process as expressed by the energy minimization and binding affinity values.

Interactions Analysis

The docking results were then visualized to determine the interactions

between ligands and 2D0T residues. Visualization at a distance of ≤ 3.2 Å which indicates moderate bond strength (Yu, Chen, Wu, & Chen, 2014) pointed out that **1a** and **1b** interact with Tyr126, Ser167, and 7-propanoate HEM residues, but no Tyr126 connection with **1c** (Figure 4). The Tyr126 residue is bound with the carbonyl group on the acetylacetone chain, while the amide group on the oxindole ring is bound to Ser167 residue through hydrogen-bonding interactions. The 5-chloro group on the benzene ring connects with 7-propanoate HEM by halogen-bonding interactions. Furthermore, phi-phi stacking interactions also occurred between benzene ring and HEM.

Visualization of **L-1MT** indicated different modes (Figure 4). **L-1MT** interacted with Gly262 residue through hydroxyl part of the carboxyl group and connects with Ser167 via the oxindole amide. The different minimization of **L-1MT** provided distinct interactions as shown by phi-phi stacking interactions between benzene and heme rings, the oxindole groups with heme, and the benzene rings with Tyr126 residues. The hydrogen-bonding interactions also occured between the amide groups of propanoate chain with Cys129 residue and between the hydroxyl groups of carboxyl group and Gly262 residue.

These visualizations illustrate that ligands minimization step has a significant impact on the value of energy minimization, binding affinity, and interactions. The MMFF94 minimization using *MarvinSketch* and *Open Babel* resulted in conformational structures of **1a** and **L-1MTa** with lower energy minimization, respectively. *MarvinSketch* minimization gave lower binding affinity than others. **1** and **L-1MT** showed varied interactions with 2D0T macromolecular residues.

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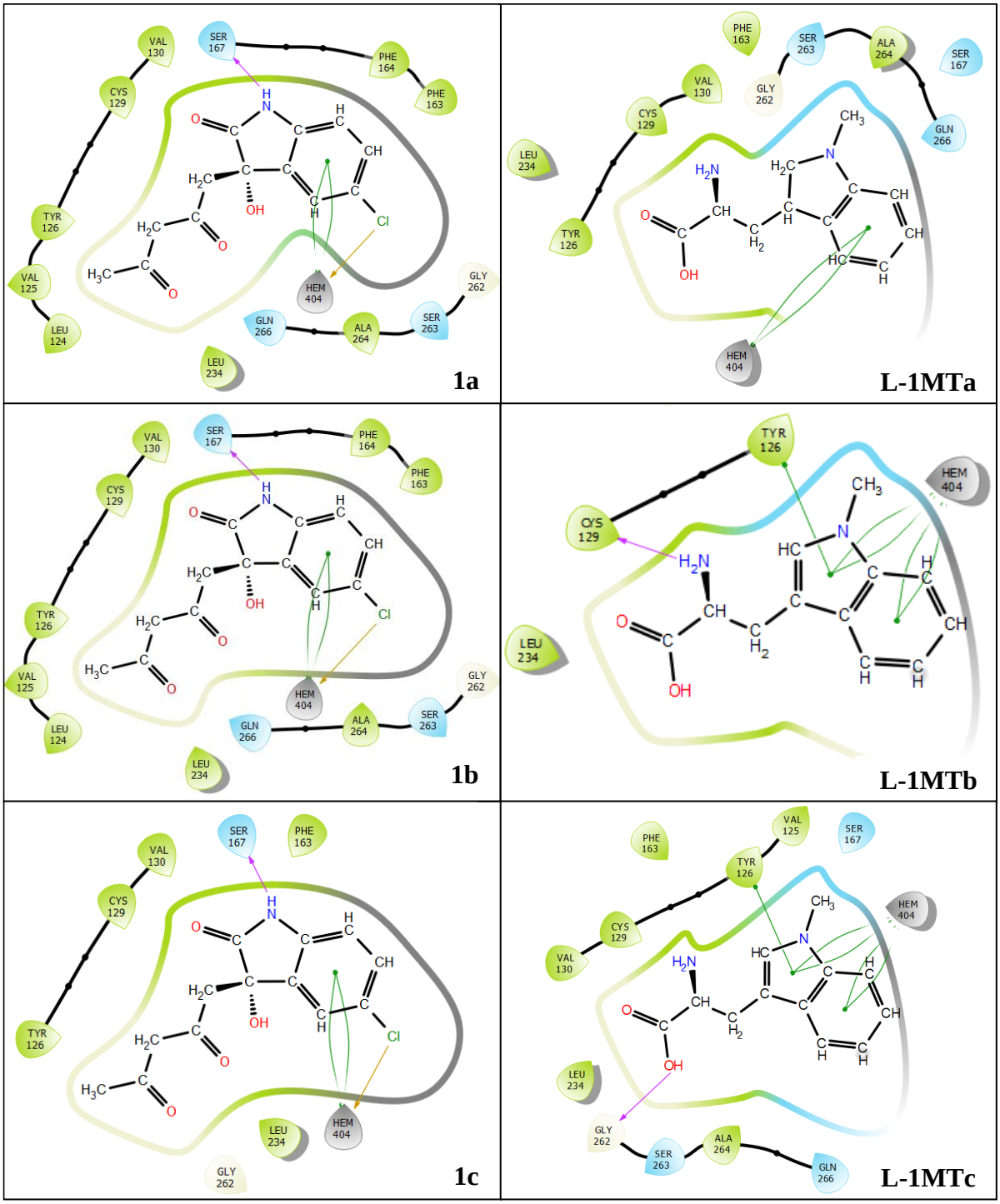

Figure 4. Interactions of ligand **1** and **L-1MT** with 2D0T

Conclusion

In this study, we explored the influence of MMFF94 energy minimization by *MarvinSketch* and *Open Babel*, and with no minimization, on the molecular docking of ligand **1** and **L-1MT** using *AutodockVina* in *Pyrx*. The redocking experiment was acceptable confirmed by RMSD value and the resulted interactions. The docking results indicated that minimization step affects the minimization energy, binding affinity, and

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interactions as well. The MMFF94 minimization by using *MarvinSketch* provided the lowest binding affinity.

References

Bell, E. W., & Zhang, Y. (2019). DockRMSD: An Open-source Tool for Atom Mapping and RMSD Calculation of Symmetric Molecules Through Graph Isomorphism. *Journal of Cheminformatics*, *11*(40), 1–9. https://doi.org/10.1186/s13321-019-0362-7

ChemAxon. (n.d.). MarvinSketch (Marvin 20.13). ChemAxon. www.chemaxon.com

Cole, J. C., Nissink, J. W. M. and Taylor, R. (2005). *Protein-Ligand Docking and Virtual Screening with GOLD*. In J. Alvarez & B. Shoichet (Eds.), *Virtual Screening in Drug Discovery* (pp. 379–415). CRC Press.

Cormen, T. H., Leiserson, C. E. and Rivest, R. L. (2009). *Instructor's Manual to Accompany Introduction to Algorithms 3ed*.

Dallakyan, S., & Olson, A. J. (2015). Small-Molecular Library Screening by Docking with PyRx. *Methods in Molecular Biology*, *1263*, 243–250.

Greco, F. A., Bournique, A., Coletti, A., Custodi, C., Dolciami, D., Carotti, A., & Macchiarulo, A. (2016). Docking Studies and mmcmurMolecular Dynamic Simulations Reveal Different Features of IDO1 Structure. *Molecular Informatics*, *35*, 449–459. https://doi.org/10.1002/minf.201501038

Hari, S. (2019). In Silico Molecular Docking and ADME/T Analysis of Plant Compounds Against IL17A and IL18 Targets in Gouty Arthritis. *Journal of Applied Pharmaceutical Science*, *9*(7), 018–026. https://doi.org/10.7324/JAPS.2019.90703

Hestenes, M. R., & Stiefel, E. (1952). Methods of conjugate gradients for solving linear systems. *Journal of Research of the National Bureau of Standards*, *49*(6), 409. https://doi.org/10.6028/jres.049.044

Imre, G., Veress, G., Volford, A., & Farkas, O. (2003). Molecules from the Minkowski Space: An Approach to Building 3D Molecular Structures. *Journal of Molecular Structure: THEOCHEM*, *666–667*, 51–59. https://doi.org/10.1016/j.theochem.2003.08.013

Jász, Á., Rak, A., Ladjanszki, I., & Cserey, G. (2019). Optimized GPU Implementation of Merck Molecular Force Field and Universal Force Field. *Journal of Molecular Structure*, *1188*, 227–233. https://doi.org/10.1016/j.molstruc.2019.04.007

Nisha, C. M., Kumar, A., Nair, P., Gupta, N., Silakari, C., Tripathi, T., & Kumar, A. (2016). Molecular Docking and in silico ADMET Study Reveals Acylguanidine 7a as A Potential Inhibitor of β -secretase. *Advances in Bioinformatics*, *2016*, 1–6. https://doi.org/10.1155/2016/9258578

O'Boyle, N. M., Banck, M., James, A. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An Open Chemical Toolbox. *Journal of Cheminformatics*, *3*(33), 1–14. https://doi.org/10.1186/1758-2946-3-33

Paul, S., Roy, A., Deka, S. J., Panda, S., Srivastava, G. N., Trivedi, V., & Manna, D. (2017). Synthesis and evaluation of oxindoles as promising inhibitors of the immunosuppressive enzyme indoleamine 2,3-dioxygenase 1. *MedChemComm*, *8*(8), 1640–1654.

https://doi.org/10.1039/c7md00226b

Platten, M., Nollen, E. A. A., Röhrig, U. F., Fallarino, F., & Opitz, C. A. (2019). Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. *Nature Reviews Drug Discovery*, *18*(5), 379–401. https://doi.org/10.1038/s41573-019-0016-5

Ramírez, D., & Caballero, J. (2018). Is It Reliable to Take the Molecular Docking Top Scoring Position as the Best Solution without Considering Available Structural Data?. *Molecules*, *23*(5), 1–17. https://doi.org/10.3390/molecules23051038

Samdani, A., & Vetrivel, U. (2018). POAP: A GNU parallel based multithreaded pipeline of open babel and AutoDock suite for boosted high throughput virtual screening. *Computational Biology and Chemistry*, *74*, 39–48. https://doi.org/10.1016/j.compbiolchem.2018.02.012

Schrödinger, L. (2015). The PyMOL Molecular Graphics System (2.0). Schrödinger, LLC. www.pymol.org

Schrödinger. (2020). Maestro (Schrodinger Release 2020-2). Schrödinger, LLC.

Sugimoto, H., Oda, S-i., Otsuki, T., Hino, T., Yoshida, T., & Shiro, Y. (2006). Crystal Structure of Human Indoleamine 2,3-dioxygenase: Catalytic Mechanism of O2 Incorporation by A Heme-containing Dioxygenase. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(8), 2611–2616. https://doi.org/10.1073/pnas.0508996103

Tangyuenyongwatana, P. (2017). Cross-docking Study of Flavonoids Against Tyrosinase Enzymes Using PyRx 0.8 Virtual Screening Tool. *Journal of Pharmaceutical Sciences*, *41*, 189–192.

Trott, O. and Olsson, A. J. (2012). Software News and Updates Gabedit — A Graphical User Interface for Computational Chemistry Softwares. *Journal of Computational Chemistry*, *32*, 174–182. https://doi.org/10.1002/jcc

Yu, J., Chen, W., Wu, C., & Chen, H. (2014). PEG-Protein Interaction Induced Contraction of NalD Chains. *PLoS ONE*, *9*(5), 3–8. https://doi.org/10.1371/journal.pone.0096616

Zhang, G., Xing, J., Wang, Y., Wang, L., Ye, Y., Lu, D., Zhao, J., Luo, X., Zheng, M., & Yan, S. (2018). Discovery of Novel Inhibitors of Indoleamine 2,3-dioxygenase 1 Through Structure-Based Virtual Screening. *Frontiers in Pharmacology*, *9*(MAR), 1–10. https://doi.org/10.3389/fphar.2018.00277

Zheng, M., Zhao, J., Cui, C., Fu, Z., Li, X., Liu, X., Ding, X., Tan, X., Li, F., Luo, X., Chen, K., & Jiang, H. (2018). Computational chemical biology and drug design: Facilitating protein structure, function, and modulation studies. *Medicinal Research Reviews*, *38*(3), 914–950. https://doi.org/10.1002/med.21483

Zhou, C., Lai, F., Sheng, L., Chen, X., Li, Y., & Feng, Z. (2020). Design, synthesis, and biological evaluation of phenyl urea derivatives as IDO1 inhibitors. *Molecules*, 25(6). https://doi.org/10.3390/molecules25061447