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Original research article

An *in-silico* study of structure-based virtual screening of IDO1 inhibitors as candidate compounds for drug development of IDO1-related diseases

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ABSTRACT

Indoleamine 2, 3-dioxygenase 1 (IDO1) is the rate-limiting enzyme of the kynurenine pathway and converts L-tryptophan to form N-formylkynurenine. IDO1 and its targeting pathways have been greatly implicated in immunomodulation and tumor development and more recently also neuroinflammation and neurodegeneration. Therefore, within the central nervous system (CNS), IDO1 and the kynurenine pathway play a role in a variety of conditions, including neurodegenerative diseases, neuropsychiatric disorders, and motor neuron diseases. The main mechanism whereby IDO1 and its pathway exert their effects in these conditions is mainly through the generation of excitotoxic metabolic byproducts downstream of the kynurenine pathway, such as quinolinic acid and kynurenic acid, with some being able to easily pass through the blood-brain barrier (BBB) and causing microglial activation, astrogliosis, oxidative stress and neuronal apoptosis. Therefore, this study aimed to find inhibitors for IDO1 to potentially be used in further therapeutic studies for different conditions. Using AutoDock Vina, docking scores and binding affinities of virtual screening compounds from the drug library on MTiOpenScreen were acquired. Compounds with the highest scores and binding affinities were selected and screened from the drug library to perform molecular docking and 2D-plot analysis with IDO1. Further screening was based on Lipinski's rule of five and seganserin was found to be the overall most appropriate inhibitor from the drug library for IDO1 with BBB permeability. Upon further comparison with its related enzymes, namely indoleamine 2,3-dioxygenase 2 (IDO2) and tryptophan 2,3-dioxygenase (TDO) via molecular docking, it was found that binding affinity to seganserin was highest for IDO1.

Keywords: IDO1, IDO2, TDO, virtual screening, seganserin

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1. Introduction

Indoleamine 2,3-dioxygenase 1 (IDO1) is a 45-kDa monomeric enzyme with a heme molecule contributing to the kynurenine pathway by breaking the 2,3-double bond in tryptophan at its indole ring with the presence of oxygen leading to the formation of N-formyl-kynurenine at the first stage of this pathway.[1] The residues in the active site of IDO1 interact with tryptophan mostly via hydrophobic interactions. These include one basic (Arg231), three aromatic (Phe163, Tyr126, and Phe226), two polar (Ser263 and Thr379) and six nonpolar residues (Val130, Leu234, Gly262, Ala264, Ile354, and Gly378). There are two polar interactions found in the active site where Thr379 and Arg231 form hydrogen bonds with the substrate, tryptophan. IDO1 together with indoleamine 2,3-dioxygenase 2 (IDO2) and tryptophan 2,3-dioxygenase (TDO) are the crucial rate-limiting enzymes in the kynurenine pathway, which is the process of converting L-tryptophan to N-formylkynurenine.[2] However, IDO2 has a considerably lower affinity for tryptophan than IDO1. Significantly, IDO1 has been shown to catabolize most tryptophan throughout the body, whereas TDO is primarily expressed in the liver. IDO1 and the pathway it controls have been implicated in several diseases, such as cancers, diabetes, obesity, liver fibrosis and many others. Recently, intensive interest has been shown in its potential to affect several neurodegenerative and neuropsychiatric diseases with significant underlying neuroinflammation related to the central nervous system (CNS). IDO1 and the kynurenine pathway can influence such a range of conditions within the CNS through the generation of neuro-excitotoxic metabolic byproducts including quinolinic acid, kynurenic acid, anthranilic acid and 3-hydroxykynurenine. For example, quinolinic acid has been found to stimulate brain endothelial microvascular cells, macrophages and microglia.[3] Quinolinic acid can also induce astrogliosis through N-methyl-D-aspartate (NMDA) receptor activation, causing vimentin and glial fibrillary acidic protein (GFAP) upregulation.[1] Blocking IDO1 upregulation under proinflammatory conditions has been shown to interfere with IDO1-dependent beta-amyloid protein-induced neurotoxicity and neuroinflammation in Alzheimer's disease (AD). Moreover, beta-amyloid protein-affected neurons also displayed upregulated IDO1, kynurenine and the aryl hydrocarbon receptor (AhR) signaling pathways.[1] This pathway is able to initiate crosstalk and downregulate the Wnt/beta-catenin signaling pathway, resulting in the upregulation of Tau phosphorylation, another hallmark of AD.

Interestingly, IDO1 inhibition via 1-L-MT, a commonly used IDO1 inhibitor, has been shown to decrease Tau and beta-catenin phosphorylation.[4] To date, most IDO1 inhibitors have been developed for anti-cancer therapy,[5] and there is still a need for the development of suitable IDO1 inhibitors for CNS-related diseases with minimal off-target and other side effects. However, the efficacy of IDO1 inhibitors remains controversial,[6] due to pharmacokinetic or off-target effects. Moreover, not all current IDO1 inhibitors may possess blood brain barrier (BBB) permeability and hence may not be sufficiently effective for CNS-related diseases.

Accordingly, structure-based virtual screening (SBVS) for IDO1 inhibitors can be an important tool for expediting the discovery and development of potential IDO1 inhibitors. Compared to traditional high-throughput screening methods, SBVS, which can be considered an *in-silico* high-throughput screening method, can reduce resource utilization, as there is no need for biological samples of targets and ligands or other laboratory chemicals, while also creating a far quicker workflow. It also allows only the most appropriate compounds to enter into future *in-vitro* and *in-vivo* studies.[7]

In this work, compounds from the MTiOpenScreen drug library are primarily ranked by docking scores. To compare their physicochemical characteristics, the high-rank scoring compounds were further examined using Lipinski's rule of five. Finally, the molecular docking of IDO1 and the selected compound was performed in comparison with IDO2 and TDO, to identify the most selective compound for IDO1.

2. Materials and Methods

2. 1 Structure-based virtual screening of IDO-1 inhibitors

The schematic flow of procedures is as follows: virtual screening was first conducted using MTiOpenScreen, followed by the assessment of the Lipinski's rule of five and finally the conformation was re-validated by the local docking using AutoDock Vina.

The protein structures were retrieved from Protein Data Bank (RCSB PDB) with the PDB code 6E43 for IDO1. This enzyme structure was elucidated by X-ray crystallography at the resolution of 1.71 Å. The protein receptor (target) was prepared by the DockPrep function implemented in University of California San Francisco (UCSF) Chimera. [8] All nonstandard residues, solvents, and water molecules were deleted. The hydrogen atoms and charges and missing atoms were added. The grid box for virtual screening was determined at the active site of IDO1; the box coordinate center and dimension were submitted in MTiOpenScreen server[9] as follows; x = 72.70, y = 18.37 and z = 44.04 Å; x = 20, y = 23 and z = 20 Å. Using MTiOpenScreen, the grid resolution of AutoDock Vina was set to 1 Å and the number of binding modes used was 10, with an exhaustiveness of 8. The structure-based virtual screening of the drug library was used to screen for selective inhibitors of IDO1. Initially, a total of 21,276 compounds were recruited by the drug library construction process. They were further filtered by physicochemical property analysis. Finally, this library contained 7,173 stereoisomers corresponding to 4,574 single isomers. The three-dimensional (3D) structures of all chemical compounds were generated for virtual screening.

To identify the most favorable drug that could bind with IDO1, 50 compounds were initially selected based on the docking scores at a cut-off of -11.0 kcal/mol. The compounds were subsequently subjected to further analysis using Lipinski's rule of five.

2.2 Physicochemical analysis

The fifty highest binding affinity compounds from the drug library underwent further screening using SwissADME[10] for identifying the most appropriate compounds based on Lipinski' s rule of five and other predicted pharmacokinetic properties, including gastrointestinal (GI) absorption and BBB permeability. These properties included lipophilicity, size, polarity, insolubility, insaturation, and flexibility of the compound. The ligands were filtered to twenty compounds according to their scores and properties to pass the BBB. The compound possessing the highest affinity toward IDO1 was selected for further analysis using molecular docking in comparison with IDO2 and TDO.

2.3 Molecular Docking

The PDB files of IDO1, IDO2 and TDO were downloaded from RCSB PDB or AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk). The structures with the highest resolution were selected. The PDB codes of IDO1 and TDO were 6E43 and 6PYZ. IDO-2 structure has not yet been elucidated by X-ray crystallography; thus, its predicted 3D structure was downloaded from AlphaFold Protein Structure Database (https:// alphafold. ebi. ac. uk/ entry/ Q6ZQW0). The protein structures were then prepared for molecular docking in UCSF Chimera. [8, 11] The active site of IDO1 was identified by uploading the amino acid FASTA sequence (Accession

number: NP_002155.1) onto the PROSITE server (https://prosite. expasy. org), which allows the retrieval of active site information of proteins. The x, y, and z coordinates of IDO1/2 and TDO active sites were acquired by defining the grids used in molecular docking. For IDO1, the active site coordinate center and dimensions were as follows: x = 72.70, y = 18.37 and z = 44.04 Å and x = 20, y = 23 and z = 20 Å. For IDO2, the active site coordinate center and dimensions were as follows: x = -5.00, y = -10.50 and z = 3.00 Å and x = 20, y = 23 and z = 20 Å. For TDO, the active site coordinate center and dimensions were as follows: x = 39. 15, y = -59. 12 and z = -32.06 Å and x = 20, y = 23 and z = 20 Å. The chemical structure of seganserin was downloaded from PubChem in SDF format and used as the ligand for molecular docking using Autodock Vina. [8] The ligand-binding mode with the lowest binding energy (kcal/ mol) was selected and subjected to 2D plot analysis.

2.4 2D plot generation and analysis

Using PoseView,[12] the protein-ligand complexes were uploaded to generate 2D-plots of the interaction profiles of seganserin with IDO1, IDO2 and TDO. The analysis of nature and types of molecular interactions were identified and compared to one another.

2.5 Multiple sequence alignment and co-evolution analysis

The human IDO1 amino acid sequence (Accession number: NP_002155.1) was subjected to the GREMLIN Co-evolution Analysis tool (http://openseq. org/submit.php). Overall, 721 amino acid sequences of homology-related enzymes in the IDO1 family were recruited from the database. The degree of conservation was visualized using WebLogo v3 (http:// weblogo.threeplusone.com).

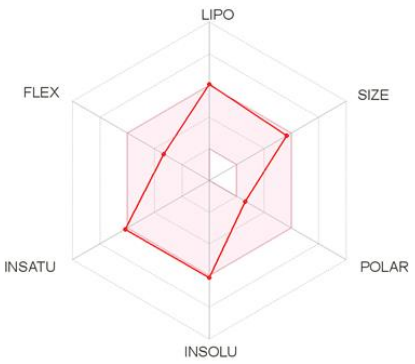

Fig. 1. Physicochemical space for seganserin (SwissADME) represented by LIPO (lipophilicity; -0.7 < XLOGP3 < +5) SIZE (150 g/mol < MV < 500 g/mol), POLAR (Polarity; 20 Å² < TPSA < 130 Å²), INSOLU (Insolubility; 0 < Log S (ESOL) <6), INSATU (Insaturation; 0.25, Fraction Csp3, 1) and FLEX (Flexibility; 0 < Num. rotatable bonds < 9).

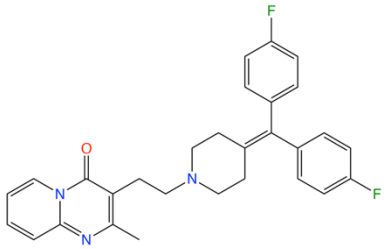

Fig. 2. 2D chemical structure of seganserin (Pubchem CID: 71767; Molecular Formula: $C_{29}H_{27}F_2N_3O$).

Table 1. The list of compounds with the highest binding energies toward the IDO1 active site

Compounds	Energy (kcal/mol)	MW	GI abs.	BBB
Mk3207_ZINC000103760981	-14.2	557.60	High	No
Antrafenine_ZINC000053073961	-13	588.50	Low	No
Ditercalinium_ZINC000004215707	-12.9	718.90	Low	No
Golvatinib_ZINC000043195317	-12.8	633.70	High	No
Nilotinib_ZINC000006716957	-12.8	529.52	Low	No
Merestinib_ZINC000095926668	-12.7	552.53	Low	No
Lifitegrast_ZINC000084668739	-12.6	615.48	Low	No
R428_ZINC000051951668	-12.6	506.64	High	No
Talniflumate_ZINC000000601275	-12.6	414.33	High	No
Seganserin_ZINC000000538333	**-12.6**	**471.54**	**High**	**Yes**
Sb-649868_ZINC000043207236	-12.4	477.55	High	No
Tg100-801_ZINC000029136020	-12.4	580.08	Low	No
Lopinavir_ZINC000003951740	-12.4	628.80	High	No
Duoperone_ZINC000004215745	-12.4	514.58	Low	No
Lumacaftor_ZINC000064033452	-12.3	452.41	High	No
Ag-13958_ZINC000034037183	-12.3	467.50	High	No
Ubrogepant_ZINC000095598454	-12.3	549.54	High	No
Aprepitant_ZINC000027428713	-12.3	534.43	Low	No
Rupatadine_ZINC000000598829	-12.2	415.96	High	Yes
Upidosin_ZINC000003796141	-11.9	511.61	High	Yes
Mk-8033_ZINC000068203670	-11.9	404.54	High	Yes

3. Results

3.1 Structure-based virtual screening of IDO1 inhibitors

Using MTiOpenScreen, 20 best-selected compounds from the drug library with the highest binding affinities and most appropriate physicochemical characteristics were identified. Compound IDs are indicated by the respective numbers following the compound names, all of which can be found in the ZINC database (Table 1). Seganserin was found to fit the physicochemical space deemed most appropriate for drugs with high oral bioavailability indicated in the shaded red zone (Fig. 1). The only violation of Lipinski's rule of five for seganserin was regarding its MLogP value (4. 74), which was greater than the suggestion of 4.15 and below. The structure of seganserin is shown in Fig. 2.

The most appropriate binding conformation of seganserin with IDO1 and its binding site is shown in Fig. 3. The residues in the active site of IDO1 including Phe163, Phe164, Ala264, Phe270, Val264, Leu342 and His346 interacted with seganserin. A hydrogen bond is formed between His346 and seganserin, while most residues in the active site interact with seganserin via hydrophobic forces (Fig. 3).

3.2 Comparison between IDO1, IDO2 and TDO chemical interactions with seganserin

The structural alignment between all three enzymes including IDO1, IDO2 and TDO shows that the main structural characteristics of IDO1 is largely conserved in other enzymes (Fig. 4A). This also includes active site similarities between these enzymes, with the exception most notably of the extended helix found in TDO (Fig. 4A). As shown in Fig. 4B, seganserin formed a hydrogen bond with the His346 imidazole side chain of IDO1, the distance of which was found to be 2.6 Å. In addition, seganserin interacted with Phe163 of IDO1 via π-π stacking between aromatic rings of the two. Hydrophobic interactions could be observed around several residues of the IDO1 active site and seganserin, including Val269, Phe270, Leu342, Ser263, Ala264 and Phe163. For IDO2, there was formation of a hydrogen bond with His360 (Fig. 4C). Additionally, hydrophobic interactions could be identified for numerous residues including Val364, Met234, Leu356, Leu231, Val187, His360, Ala281 and Leu287. For the TDO-seganserin complex, hydrophobic interactions between TDO and seganserin occurred at Phe72, Phe140, Ser151, Phe158 and Leu351 (Fig. 4D). Three π-π interactions between the Phe72 and Phe140 of TDO and the aromatic rings of seganserin moieties could be observed (Fig. 4D). The numbers of hydrophobic interactions and hydrogen bonds for all three enzymes (IDO1, IDO2 and TDO) interacting with seganserin are depicted in Fig. 5. The results indicate that IDO2 possessed the greatest number of hydrophobic contacts at 8, while IDO1 was the second greatest at 7 and for TDO this was 5. Both IDO1 and IDO2 displayed one hydrogen bond and despite this, TDO still displayed higher binding energy with seganserin compared to IDO2, but not IDO1. IDO1 showed the highest binding energy of -12.6 kcal/mol, while for IDO2 it was -10.8 kcal/mol and for TDO, -11.3 kcal/mol (Table 2).

3.3 Coevolution analysis of IDO1

The sequence coevolution analysis of the IDO1 homologous enzymes was performed using 721 related enzymes from different organisms (Fig. 6). The results showed that there are various conserved regions between IDO1 and other members in its family. Most notably, the amino acid residues composing the active site, where IDO1 forms a hydrogen bond at His346 with seganserin (Table 2), are found to be highly conserved in IDO1, IDO2 and TDO and other homologous enzymes.

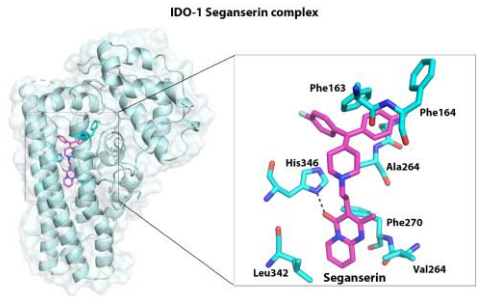

Fig. 3. The complex structure visualization of the IDO-1 (cyan) and Seganserin (pink) using UCSF Chimera is shown in ribbons. The residues in the active site are shown as sticks. The H-bond is represented by a dotted line. The oxygen and nitrogen atoms are represented in red and blue colors.

4. Discussion

In terms of the compounds acquired by performing the structure-based virtual screening for IDO1 ligands using the drug library, the 20 best candidates (Table 1) were selected for closer analysis based on their binding affinity to IDO1 and their physicochemical properties. AutoDock Vina was used to perform molecular docking due to it being open sourced and providing a free educational license for usage. Compared with the previous version (AutoDock 4), AutoDock Vina possesses greater average accuracy of binding mode prediction, as

well as processing its functions at a faster rate. In terms of the physicochemical analysis, Lipinski's rule of five was used as it is able to successfully predict the drug disposition characteristics for compounds both adhering to and violating those criteria which are reflected in the pharmacokinetic properties. It was found that the best performing compound was Mk3207, with a binding affinity of -14.2 kcal/mol. Mk3207 is best known for its role as a highly potent and orally bioavailable calcitonin-gene related peptide (CGRP) receptor antagonist for potential use in treating migraines. [13, 14]

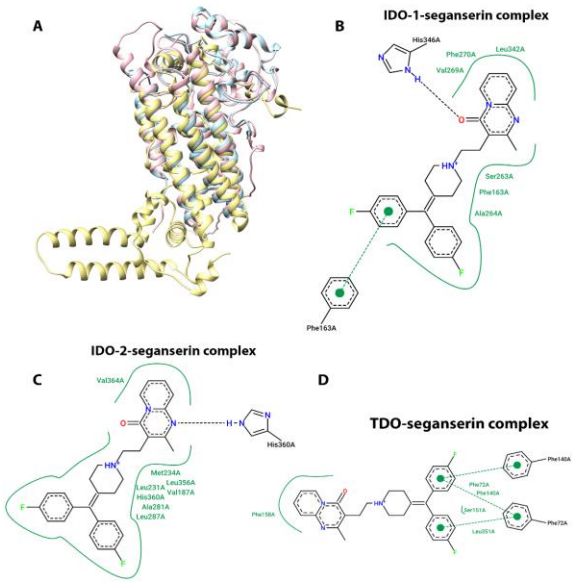

Fig. 4. Illustration of IDO1 (Cyan), IDO2 (Pink) and TDO (Yellow) alignment to show their similarity and conserved regions using UCSF Chimera **(A)**. 2D plot of the IDO1 seganserin complex **(B)**, the IDO2 seganserin complex **(C)** and the TDO seganserin complex (**D**).

Despite some CNS bioavailability of this drug, based on the *in vivo* cerebrospinal fluid/plasma ratio, having been determined at 2 to 3% in cisterna magna-ported rhesus monkeys,[14] this compound was described as a non-permeable BBB compound according to SwissADME. This was possibly owing to its violation of one of Lipinski's rules,[15] where its molecular weight was determined to have been greater than 500 g/mol (557.59 g/mol). For these reasons, seganserin was deemed the most suitable drug candidate for binding to IDO1, due to its more appropriate CNS bioavailability and binding affinity even despite its one violation of Lipinski's rule, namely a higher than 4.15 MLogP value identified to be 4.74 (SwissADME), which may, however, facilitate its BBB permeation. Moreover, the IDO1-seganserin complex boasts the highest binding affinity of -12.6 kcal/mol, which could perhaps be explained by the relatively close hydrogen bond distance between seganserin and IDO1 of 2.6 Å at His346 of the IDO1 active site. Despite the IDO2-seganserin complex possessing one more hydrophobic contact than IDO1, its calculated binding affinity was lower than that of the IDO1-seganserin complex. Furthermore, another study identified a novel class of IDO1 inhibitors based on benzimidazole substructures, shown to extensively bond with the active site of IDO1, which includes the C pocket region not commonly considered in IDO1 inhibitors.[15]

Despite their similar capabilities in regulating the kynurenine pathway and tryptophan levels, IDO1, IDO2, and TDO have a variety of physiologic and pathological roles, including those in immunomodulation, inflammation, cancer development, and cellular proliferation.[16] This might be caused by enzymatic variations in substrate specificity, locoregional control over the production of kynurenine and its metabolites, or the efficiency of kynurenine effector mechanisms, such as the aryl hydrocarbon receptor and other catabolic enzymes in the kynurenine pathway. [17]

Finally, the conservation of IDO1 alongside its related enzymes (Fig. 6) indicates that there are various conserved regions between IDO1 and other members

in its family. Most notably, the active site area, where IDO1 forms a hydrogen bond at His346 with seganserin, has a very high probability of conservation in the residues of that region. In the structural comparison study, His346 from IDO1 was found to be conserved along with the His360 of IDO2 when complexed with seganserin. Similarly, it was found that the Leu342/Leu356 residues, as well as Ala264/Ala281 of IDO1 and IDO2 are conserved and found to interact with seganserin. In addition, the Phe270 from IDO1 and Phe158 from TDO was also found to be conserved and important for the interaction with seganserin. It is therefore a worthwhile consideration for future IDO1 inhibitors to be designed in accordance with these conserved regions.

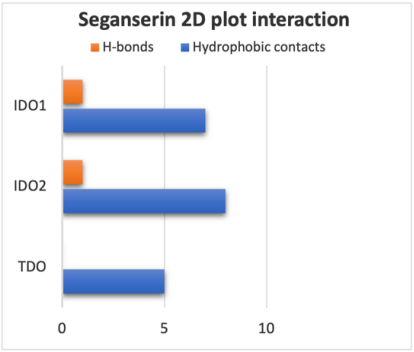

Fig. 5. The number of hydrophobic interactions (blue) and hydrogen bonds (orange) for IDO1, IDO2 and TDO.

Table 2 Comparison of docking scores in kcal/mol and all residues involved in the seganserin complex with IDO1, IDO2 and TDO.

Enzyme	Docking score (kcal/mol)	Residues involved in protein-ligand interactions
IDO-1	-12.6	**His346**, Val269, **Phe270**, **Leu342**, Ser263, **Ala264** and Phe163
IDO-2	-10.8	Val364, Met234, Leu231, **Leu356**, Val187, **His360**, **Ala281**, Leu287
TDO	-11.3	**Phe158,** Phe72, Phe151, Leu351, Phe140

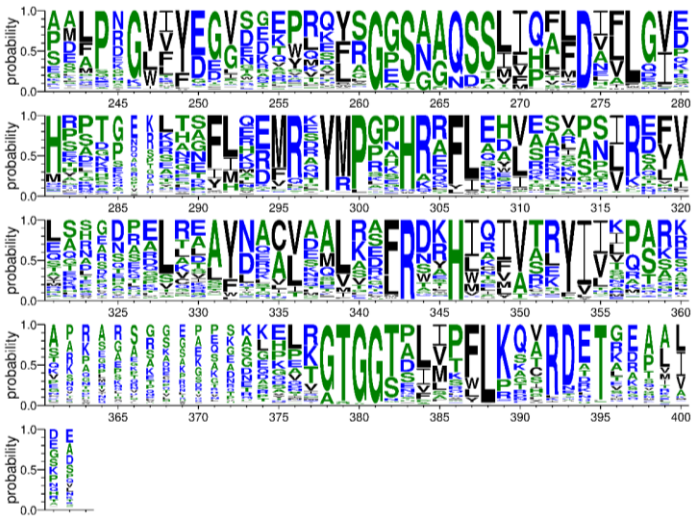

Fig. 6. Active site sequence of coevolution analysis of IDO1 and related enzymes. The Y-axis represents the probability of conservation.

5. Conclusion

Structure-based virtual screening is of significant value by helping to decrease resource utilization, while also enhancing the overall workflow for the preclinical phase of drug discovery. Therefore, both experimentation time and costs can be reduced for both *in vitro* and *in vivo* experiments during drug discovery, along with allowing only the most suitable compound candidates with the best physicochemical characteristics to be included. In this study, compounds were virtually screened from the drug library on MTiOpenScreen server and the best candidates were chosen to be future potential IDO1 inhibitors. In order to identify a selective IDO1 inhibitor, seganserin from the drug library was chosen to perform molecular docking with IDO2 and TDO. Seganserin was shown to be the most suitable candidate from the drug library based on its binding affinity score (-12.6 kcal/mol), as well as its physicochemical properties indicated by various characteristics including Lipinski' s rule of five. Upon comparison between all three enzymes, it was found that seganserin was able to bind with the highest affinity to IDO1, which can in part be explained by its strong hydrogen bond with His346 in the active site of IDO1. Due to the scarcity of research on seganserin and the lack of research on its use for IDO1, future *in vitro* and *in vivo* studies are required to investigate its safety and efficacy, in order to determine its suitability as a therapeutic drug for a variety of IDO-related diseases.

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Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Ting KK, Brew BJ, Guillemin GJ. Effect of quinolinic acid on human astrocytes morphology and functions: implications in Alzheimer's disease. J Neuroinflammation. 2009;6(1):36.

[2] Badawy AAB, Bano S. Tryptophan metabolism in rat liver after administration of tryptophan, kynurenine metabolites, and kynureninase inhibitors. Int J Tryptophan Res. 2016;9:51-65.

[3] Guillemin GJ, Cullen KM, Lim CK, Smythe GA, Garner B, Kapoor V, et al. Characterization of the kynurenine pathway in human neurons. J Neurosci 2007;27(47): 12884-12892.

[4] Duan Z, Zhang S, Liang H, Xing Z, Guo L, Shi L, et al. Amyloid β neurotoxicity is IDO1–Kyn–AhR dependent and blocked by IDO1 inhibitor. Signal Transduct Target Ther. 2020;5(1):96.

[5] Tang K, Wu Y-H, Song Y, Yu B. Indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors in clinical trials for cancer immunotherapy. J Hematol Oncol. 2021; 14(1): 68.

[6] Prendergast GC, Malachowski WP, DuHadaway JB, Muller AJ. Discovery of IDO1 inhibitors: from bench to bedside. Cancer Res. 2017;77(24):6795-6811.

[7] Maia EHB, Assis LC, de Oliveira TA, da Silva AM, Taranto AG. Structure-based virtual screening: from classical to artificial intelligence. Front Chem. 2020;8.

[8] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function,

efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-461.

[9] Labbé CM, Rey J, Lagorce D, Vavruša M, Becot J, Sperandio O, et al. MTiOpenScreen: a web server for structure-based virtual screening. Nucleic Acids Res. 2015;43(W1): W448-W454.

[10] Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7(1):42717.

[11] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605-1612.

[12] Stierand K, Rarey M. PoseView -- molecular interaction patterns at a glance. J Cheminformatics. 2010;2(1):50.

[13] Hewitt DJ, Aurora SK, Dodick DW, Goadsby PJ, Ge Y, Bachman R, et al. Randomized controlled trial of the CGRP receptor antagonist MK-3207 in the acute treatment of migraine. Cephalalgia. 2011; 31(6):712-722.

[14] Salvatore CA, Moore EL, Calamari A, Cook JJ, Michener MS, O'Malley S, et al. Pharmacological properties of MK-3207, a potent and orally active calcitonin gene-related peptide receptor antagonist. J Pharmacol Exp Ther. 2010;333(1):152-160.

[15] Serafini M, Torre E, Aprile S, Grosso ED, Gesù A, Griglio A, et al. Discovery of highly potent benzimidazole derivatives as indoleamine 2,3-dioxygenase-1 (ido1) inhibitors: from structure-based virtual screening to in vivo pharmacodynamic activity. J Med Chem. 2020;63(6):3047-3065.

[16] Ge H, Mao L, Zhao J, Wang Y, Shi D, Yang X, et al. Discovery of novel IDO1 inhibitors via structure-based virtual screening and biological assays. J Comput Aided Mol Des. 2021;35(5):679-694.

[17] Prendergast GC, Malachowski WJ, Mondal A, Scherle P, Muller AJ. Indoleamine 2,3-dioxygenase and its therapeutic inhibition in cancer. Int Rev Cell Mol Biol. 2018;336:175-203.