

Identification of Paulownin as a Potential Monoamine Oxidase-B (MAO-B) Inhibitor by Screening the Traditional Chinese Medicine (TCM) Database

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One of the prominent neurodegenerative ailments that can inflict the elderly population is the Parkinson's disease (PD). This disease is marked by the reduction of dopamine concentration inside brain due to the gradual destruction of the dopaminergic neurons. The treatment of PD is primarily related to symptoms mitigation, yet no treatment is available to restrain or stop the development of neuronal degeneration. In this regard, it is believed that the selective block of monoamine oxidase type B (MAO-B) enzyme can't only reduce the metabolism of neuronal dopamine but also lower the production of the side product hydrogen peroxide. Thus, the inhibition of MAO-B enzyme may have a neuroprotective influence by limiting oxidative stress related to the release of hydrogen peroxide. Therefore, the goal of this in-silico research is to specify a selective MAO-B inhibitor by screening the Traditional Chinese Medicine (TCM) database through application of docking as well as molecular dynamics (MD) tools. As a result, it is expected that the herbal compound Paulownin may have the capacity to selectively inhibit MAO-B enzyme. In this regard, the MD simulation indicates that Paulownin has a close proximity to MAO-B active site with a high binding energy as compared to that of MAO-A. Additionally, docking image examination refers to the fact that this compound may be involved in binding with important residues of MAO-B active site. Further, prediction of several chemical features indicates that the compound may have good oral absorption. Also, Paulownin is expected to be water soluble, non-mutagenic, safe and it may have a moderate volume of distribution and CNS permeability inside the body. Lastly, the compound Paulownin was able to record an IC₅₀ (half maximal inhibitory concentration) of 42.6 μ M when tested against MAO-B enzyme in the monoamine oxidase inhibition assay.

Keywords: Docking; Dynamics simulation; Paulownin; MAO-B; TCM.

Parkinson's disease (PD) is a multisystem and a gradual disorder where the dopaminergic neurons in the midbrain region are specifically degenerated.¹ As the incidence of PD is growing with age, this neurodegenerative disease is

mainly affecting elderly population.² Clinically, PD diagnosis depends on the manifestation of fundamental motor symptoms and these are: rigidity of muscles, posture imbalance, bradykinesia and body tremor. Also, non-motor

symptoms like cognition and sleep disorders may develop during the disease course. And sometimes these non-motor signs may predate the incidence of PD motor features by several years.³ In addition to the degeneration of dopaminergic nerve cells, PD is also marked by the accumulation of misfolded proteins inside neurons. These neuronal protein inclusions are known as Lewy bodies that composed of α -synuclein protein.⁴ Pathologically, the buildup of α -synuclein can disrupt normal functions of the neurons and eventually lead to progressive degeneration.⁵ It is thought that this neuronal degeneration can precede the onset of PD clinical symptoms by several decades.⁶ The diagnosis of PD is mainly dependent on the clinical characteristics of the disease in addition to the employment of specialized brain imaging tools.⁷ The exact reason for PD neurodegeneration is not defined yet, and the underlying pathology framework appears to be complicated with the interplay of multiple risk factors.⁸ Mutations in specific genes like those encoding for α -synuclein (SNCA) or leucine rich repeat kinase-2 (LRRK2) can predispose to the incidence of familial PD.⁹ Moreover, the exposure to head trauma or other environmental agents like pesticides can increase the likelihood of idiopathic PD.¹⁰ While the available therapeutic options like the stimulation of deep brain or the use of dopaminergic agents can only alleviate PD motor symptoms, no treatment is still available to pause or slow the progress of PD degeneration.^{11,12} It is assumed that no protective agent was yet identified because all the related clinical trials were focused on volunteers diagnosed with PD at the advent of motor symptoms. It is well-known that the PD motor symptoms usually emerge when about 75% of the dopaminergic neurons are already destructed in the substantia nigra region of the brain. So, the neuronal degeneration will be at an advanced level when PD is clinically diagnosed and as such the effect of protective agents will be limited at that point.¹ As the PD pathology is complex, then the presentation of a drug candidate that can target more than one molecular target or pathway seems to be an interesting strategy. In this perspective, it is believed that the selective block of the monoamine oxidase-B (MAO-B) enzyme can have a dual effect on PD pathology by limiting the oxidative deamination process of both endogenous and exogenous dopamine while reducing the oxidative

stress caused by the hydrogen peroxide byproduct. Therefore, the administration of selective MAO-B inhibitors is expected to both lower the daily dose of L-dopa and offer some level of neuroprotection in PD patients.¹³ Due to this dual beneficial effect of MAO-B inhibition, this computational study was designed to screen the Traditional Chinese Medicine (TCM) online compounds for their probable capacity to selectively block the MAO-B enzyme. The current in-silico study has utilized several tools that encompass molecular docking, dynamics simulation and in-vitro enzyme inhibition assay to evaluate the MAO-B inhibition potential for these TCM compounds.

MATERIAL AND METHODS

Planning of virtual screening paradigm

The computerized screening of TCM compounds for their MAO-B inhibition potential in this study is made up of four major steps: molecular docking of the TCM phytocompounds against MAO-B enzyme, then anticipation of pharmacokinetics, toxicity and chemical characteristics for only the best five hits, followed by a 50 nanoseconds (ns) dynamics simulation study for these five compounds, with a determination of the MAO-B median inhibitory concentration (IC_{50}) for the best hit as a final step by using an in-vitro enzyme inhibition assay. Additionally, these selected top hits were subjected to a molecular docking and dynamics simulation against MAO-A enzyme. The generated docking and dynamics simulation results for these hit compounds were compared between MAO-A and MAO-B enzymes to ensure the potential selectivity of these compounds. As a summary, the applied methodology in this screening is very identical to what was implemented in our formerly published studies.^{14,15}

Molecular docking study

At start, the TCM database of herbal compounds were screened against MAO-B active site by using the DrugRep online server.¹⁶ For this step, only chain A of the human monoamine oxidase B crystal (PDB: 2BK3) was submitted to the virtual screening server.¹⁷ Then, the docking coordinates were specified as X: 56, Y: 153 and Z: 23 while measurements of the grid box were 22*22*22 Angstrom (Å). It is worth mentioning

that these applied coordinates and grid box dimensions are the same to what had been used in previously published studies.^{18,19} After that, the docking of 2,390 compounds in the TCM library was performed by the DrugRep server which utilize both the AutoDockTools (ADT) 1.5.6 and AutoDock Vina 1.1.2 for this purpose.^{20,21} Finally, the output hits were tabulated and ordered based on their computed energy of binding to target enzyme. And as such, only the best five TCM compounds with the smallest energy were picked for more computerized assessment. Furthermore, the selectivity of these best hits for MAO-B inhibition was considered by also docking of these compounds against MAO-A enzyme (PDB: 2BXR) using docking measurements of X: 21, Y: 2 and Z: 6.²² Then, the predicted affinity of these compounds towards MAO-A and MAO-B enzymes were compared in terms of docking energy and orientation of docking pose. And for each docking complex, the orientation of the compound pose with the lowest energy of binding was observed by using discovery studio visualizer 21.1.0 and PyMOL 2.4.1. It is worth mentioning that the precision of this docking step was calculated for both MAO-A and MAO-B targets by applying the redocking procedure. In this process, the co-crystallized ligand was first taken out of target enzyme then docked again by using the same virtual conditions. After that, the conformational changes between co-crystallized and docked compound was computed as the root mean square deviation (RMSD) value.

Prediction of the pharmacokinetics, chemical and toxicity qualities

For this stage of the virtual study, both pkCSM and SwissADME websites were used to expect the chemical and pharmacokinetics features for the best hit compounds.^{23,24} Also, the ProTox 3.0 online tool was employed to predict each hit toxicity potential while the Molsoft L.L.C. site was utilized to compute drug-likeness score.²⁵ In addition, the herbal origin and documented pharmacological effect were reviewed for these selected hits.

Study of molecular dynamics (MD) simulation

It is widely known that molecular docking only offers a static or a frozen representation for the interaction between ligand under assessment and target protein. And to obtain a dynamic view for the ligand binding, the molecular dynamics

(MD) study usually applies multiple forcefields to compute and track molecules and atoms movement during simulation time. Consequently, the MD study was utilized in this in-silico screening to get a better understanding for the interaction between each ligand and target.²⁶ For this objective, the YASARA Dynamics 20.12.24 software was used to do the simulation study.²⁷ In this stage, the docking complex between each hit and MAO-B with the lowest energy pose was subjected for 50 ns simulation. The applied simulation parameters and methodology are identical to what was used in previously published manuscripts.^{14,15} Once more, the MD simulation of each hit was also evaluated against MAO-A throughout 50 ns duration and results were compared with that of MAO-B. The aim of this comparison is to estimate each compound selectivity to inhibit MAO-B enzyme. In addition, the docked Farnesol and Clorgyline were included as a control for the simulation of MAO-B and MAO-A respectively. Then, the final simulation results were presented as mean RMSD value of ligand movement in relation to enzyme active site, while the binding energy was calculated as average molecular mechanic Poisson Boltzmann surface area (MM-PBSA).

Monoamine oxidase inhibition assay

In this in-vitro assay, the IC₅₀ value was measured for the best hit as an indicator for the compound's inhibition potential against MAO-B. The basic principle of the monoamine oxidase inhibition assay depends on the ability of the enzyme to metabolize the non-fluorescent kynuramine into the fluorescent 4-hydroxyquinoline. As such, any inhibitor compound can interfere with this metabolic pathway and reduce the concentration of the fluorescent product.²⁸ In this assay, the compound under investigation was purchased from Cayman chemical online store and serially diluted with dimethyl sulfoxide (DMSO) through eight points of a 96-well plate. Then, an assay kit was purchased from Sigma-Aldrich (MAK136) and utilized to carry out the monoamine oxidase inhibition test. In this assay, MAO-B enzyme was added to these eight points in a concentration of 0.015 mg/ml with the presence of 20 iM of the non-fluorescent kynuramine. This mixture was incubated for twenty minutes at a temperature of 37 °C then the fluorescence activity of the 4-hydroxyquinoline was recorded by a fluorometer

at a wavelength pair of 310/ 380 nm. Additionally, the well-known antiparkinsonian agent Zonisamide was included in this assay as a positive control. The measurement of each point in the dose-inhibition curve was repeated as a duplicate and plotted as mean $\pm$ standard deviation.

RESULTS

Before screening compounds database in the TCM library, the redocking approach was utilized to ensure the fitness of the applied docking procedure. In this validation approach, the co-crystallized Farnesol was taken off the MAO-B active site. Then, the removed Farnesol was docked again into MAO-B using the same docking protocol employed in the screening of TCM library. And by aligning the co-crystallized and docked Farnesol, the precision of the docking protocol can be concluded as RMSD value of the conformational variations. As noted in Figure 1 (A), the computed RMSD of conformational changes was only 0.8 Å when comparing the co-crystallized Farnesol versus the docked one. Also, the energy of binding was recorded to be -7.96 Kcal/ mol for the docking of Farnesol against MAO-B. As mentioned before, the MAO-A enzyme was used as an additional target during docking and simulation to assess the selectivity of the best screening hits against MAO-B. As such, the redocking method was used also to evaluate the accuracy of the employed protocol against MAO-A. According to Figure 1 (B), the conformational changes RMSD was 0.2 Å for the docked and co-crystallized Clorgyline while the docking energy was reported to be -6.67 Kcal/ mol.

After conducting the virtual screening of the TCM compounds against MAO-B, the generated hit compounds were listed based on the calculated energy of binding. In this computational study, as seen in Table 1, only the best five herbal compounds against MAO-B with the least docking energy were presented. In this table, the docking energy of these best five compounds was also computed toward MAO-A to consider ligand selectivity against target enzyme. As noted from Table 1, the docking energy for these five hits seem to be slightly higher for MAO-B as compared to MAO-A. Moreover, all the listed docking energy values in Table 1 appear to be more than 10 Kcal/

mol regardless of enzyme subtype.

Then, the best screening hits against MAO-B were reviewed for their existing herbal sources and the documented pharmacological activities as listed in Table 2. It is obvious from this table that these listed hits have anticancer, anti-inflammatory or antimicrobial actions. And with the exception of the lignan Paulownin, these hits are either flavonoid or alkaloid medicinal compounds.

A predictive summary for the important chemical properties that can affect the oral bioavailability of the five hit compounds is presented in Table 3. According to this table, it is very easy to recognize that all the hit compounds but Licoflavone B do have chemical features that align with the rule of five (RO5) conditions.⁴⁴ And when considering the RO5 cutoff limits, then it is clear that all these natural compounds do have a molecular weight less than 500 g/ mol. Also, all these compounds are expected to have below than 5 H-bond donor groups and also lower than 10 H-bond acceptor groups. In addition, all the listed hits with the exception of Licoflavone B seem to have an octanol/ water partition coefficient of no more than 5. Besides, all the compounds in Table 3 are predicted to have a polar surface area (PSA) of less than 120 Å² and also less than 10 rotatable bonds in its structure. Finally, the listed molecular formula column reveals that both Licoflavone B and Corylin are flavonoids while Oxysanguinarine and Rutaecarpine are alkaloids. On the other hand, the fourth hit Paulownin appears to be a lignan compound.

After that, the drug-likeness numerical score was calculated for each of the five hits as seen in Table 4. As shown in this table, all of the hit compounds with the exception of Licoflavone B are anticipated to have physicochemical features that not resemble those of known drug molecules as was inferred by their low drug-likeness scores. Regarding the pharmacokinetics profile, it is predicted that all these hits are highly or moderately soluble in water with the exception of Licoflavone B. Moreover, all the listed hits are believed to have a high gastrointestinal absorption however the expected volume of distribution inside body was moderate for compounds Oxysanguinarine and Paulownin. Also, the prediction of the ratio for the brain/ blood concentration points to the

possibility that all listed compounds in Table 4 are CNS permeable with Log BB value of more than -1. Finally, the prediction of toxicity potential points out to the likelihood that these herbal compounds are slightly toxic with an LD₅₀ value of 1000 mg/ Kg or more. Nonetheless, the listed hits but Paulownin are anticipated to have a mutagenic ability as indicated by Ames toxicity column in Table 4. In this direction, it was predicted that all these hits except Paulownin may have the ability to block multiple variant forms of cytochrome P450.

And to get a better understanding for the ligand-target interaction beyond the scope of

docking study, the MD process was done to assess the evolution of each ligand-enzyme complex over 50 ns duration. A tabular summary for the output of the MD simulation is reported in Table 5. As observed in this table, for each hit, the mean ligand movement and the average binding energy were compared between MAO-A and MAO-B isoenzymes to assess the selectivity of each compound. Based on Table 5, it is obvious to note that all the hits have a closer proximity to MAO-B active site as compared to that of MAO-A. Also, the average binding energy computed during simulation was higher for the binding of

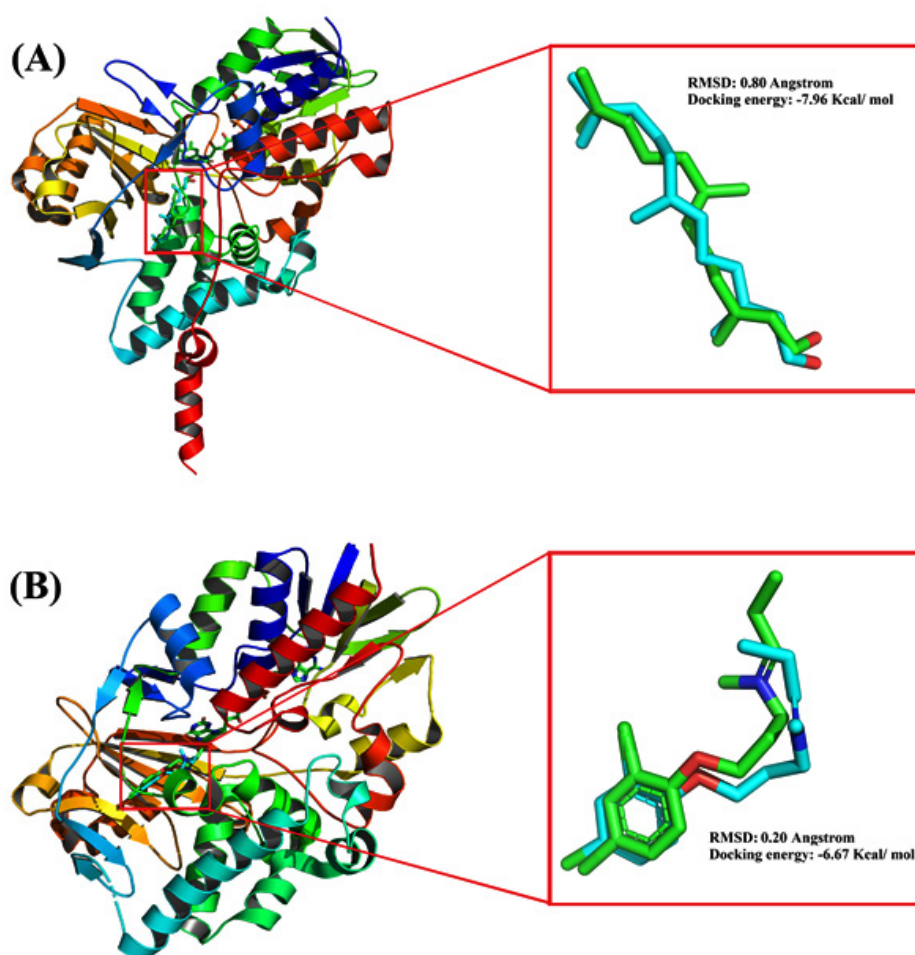


Fig. 1. Alignment analysis of the co-crystallized Farnesol with the docked one into the active site of MAO-B as depicted in (A). While the co-crystallized Clorgyline was aligned with its docked form into MAO-A as seen in (B). In both (A) and (B), the co-crystalline ligand is shown in cyan color while the docked one is colored by green

these hits to MAO-B enzyme. But the highest MAO-B binding selectivity was recorded to the compound Paulownin with more than 2.2- and 2.9-folds of MAO-B preference when considering ligand proximity and MM-PBSA binding energy respectively. As indicated in Table 5, both Farnesol and Clorgyline were included as positive controls for MAO-B and MAO-A simulations. And it is worth to notice that the MM-PBSA binding energy for both Farnesol and Clorgyline were higher than that computed for the listed five compounds.

In order to obtain a deep understanding for the attitude of each compound during its simulation, stepwise plots for the ligands' movement RMSD throughout 50 ns duration were presented in Figure 2 for MAO-B and MAO-A. According to Figure 2, it is very clear to say that the compound Paulownin did record a remarkable MAO-B binding selectivity of more than 2 times as compared to MAO-A especially after 15 ns simulation duration. On the contrary, the compounds Licoflavone B and Corylin have shown a lower binding preference to MAO-B enzyme when considering the average

ligand movement RMSD in Figure 2. Moreover, both Oxysanguinarine and Rutaecarpine did present the least preference to MAO-B binding with significant fluctuations in ligand proximity to enzyme active site during MD simulation.

Then, the most selective hit (Paulownin) was validated in-vitro for its ability to inhibit MAO-B enzyme. According to the dose-inhibition curve in Figure 3, the calculated IC_{50} for Paulownin was 42.6 μ M. This inhibition capacity of Paulownin seemed to be less than that of Zonisamide which was able to achieve an IC_{50} of 28.9 μ M in the MAO inhibition assay. Moreover, the R^2 (goodness of fit) for the nonlinear curve fitting in Figure 3 were 94% and 99% for Paulownin and Zonisamide respectively.

As a result for the prediction and simulation analyses, the compound Paulownin appears to have a non-mutagenic nature and a high MAO-B binding selectivity. As such, Paulownin was selected for an in-depth evaluation of its docking complex as illustrated in Figure 4. Interestingly, according to this figure, the compound Paulownin seems to be engaged in specific interactions with essential MAO-B active site residues like Ile 199, Tyr 326, Tyr 398 and Tyr 435.

DISCUSSION

Generally, the management of PD is primarily focused on the alleviation of motor manifestations through administration of L-dopa to replace the dopamine level inside CNS. However, the replacement of the dopamine can be linked with motor complications like dyskinesia. One of the pharmacotherapeutic options to overcome these

Table 1. Comparison of docking energy for the best screening compounds against MAO-A and MAO-B monomers

No.	Hit ID	Docking energy (Kcal/ mol)	
		MAO-A	MAO-B
1	Licoflavone B	-11.4	-12.7
2	Corylin	-10.9	-11.9
3	Oxysanguinarine	-10.9	-11.7
4	Paulownin	-11.0	-11.5
5	Rutaecarpine	-10.7	-11.3

Table 2. A tabular summary of the herbal origin and the probable pharmacological effects for the best hits acquired by the structure-based computational screening

No.	Phytomedicine name	Herbal origin	Pharmacological effect
1	Licoflavone B	<i>Glycyrrhiza inflata</i>	Anti-inflammatory, ²⁹ anticancer, ³⁰ anti-schistosomiasis ³¹
2	Corylin	<i>Psoralea corylifolia</i>	Osteogenic, ³² anticancer, ³³ anti-hyperlipidemia ³⁴
3	Oxysanguinarine	<i>Macleaya cordata</i> , <i>Meconopsis simplicifolia</i> , <i>Sanguinaria canadensis</i>	Anti-inflammatory, ³⁵ anticancer, ³⁶ antiviral, ³⁷ antimalarial ³⁸
4	Paulownin	<i>Paulownia tomentosa</i>	Anticancer, ³⁹ antimicrobial ⁴⁰
5	Rutaecarpine	<i>Evodia rutaecarpa</i>	Cardiotonic, ⁴¹ anti-inflammatory, ⁴² antioxidant ⁴³

motor complications is to use a selective MAO-B inhibitor as an additive agent. Adding an inhibitor of MAO-B to PD treatment regimen can extend the duration of the administered L-dopa dose and also boost its effect.⁴⁵ MAO enzyme is present in two isoform types, MAO-A and MAO-B, that utilize a flavin adenine dinucleotide (FAD) moiety as a cofactor to participate in dopamine metabolism into the corresponding aldehyde product.⁴⁶ During this oxidative deamination pathway, the hydrogen peroxide (H_2O_2) is formed as a side product. The formation of H_2O_2 can participate in the cellular oxidative stress. Unlike MAO-A, the activity of MAO-B enzyme is usually upregulated with age advances and progress of neurodegeneration. As

a result, it was hypothesized that the selective inhibition of only MAO-B enzyme in PD patients can elevate the CNS concentration of dopamine and also lower the production of H_2O_2 .⁴⁷ Consequently, in the current study, a library of herbal compounds in the TCM database was screened against MAO-B enzyme. Both docking and MD simulation programs were utilized in this computational project to recognize a potential MAO-B inhibitor. Moreover, a machine learning based tool was utilized to predict MAO-B IC_{50} values for the best screening hits.

At first, the accuracy of the applied virtual screening methodology and parameters were evaluated by using the redocking approach.

Table 3. A tabular presentation of the chemical properties for the best phytomedicines acquired by the computerized screening of the Monoamine Oxidase B (MAO-B) with the Traditional Chinese Medicine (TCM) database. These TCM compounds were listed based on the lowest docking energy.

No.	Hit name	Molecular formula	M.W. (g/mol)	HBD	HBA	PSA ($\text{\AA}^2$)	Log P	Rotatable bonds no.
1	Licoflavone B	$C_{25}H_{26}O_4$	390.5	2	4	70.67	5.19	5
2	Corylin	$C_{20}H_{16}O_4$	320.3	1	4	59.67	3.53	1
3	Oxysanguinarine	$C_{20}H_{13}NO_5$	347.3	0	5	58.92	3.31	0
4	Paulownin	$C_{20}H_{18}O_7$	370.4	1	7	75.61	2.10	2
5	Rutaecarpine	$C_{18}H_{13}N_3O$	287.3	1	2	50.68	3.02	0

M.W.: the molecular weight; PSA: the polar surface area; HBA: the H-bond acceptor group; HBD: the H-bond donor group; $\text{\AA}$: degree angstrom; Log P: the logarithmic value of partition coefficient.

Table 4. A prediction summary of the pharmacokinetics, toxicity characteristics and drug-likeness score for the best screening hits. These compounds were organized in this table according to the lowest energy of binding as computed by docking process

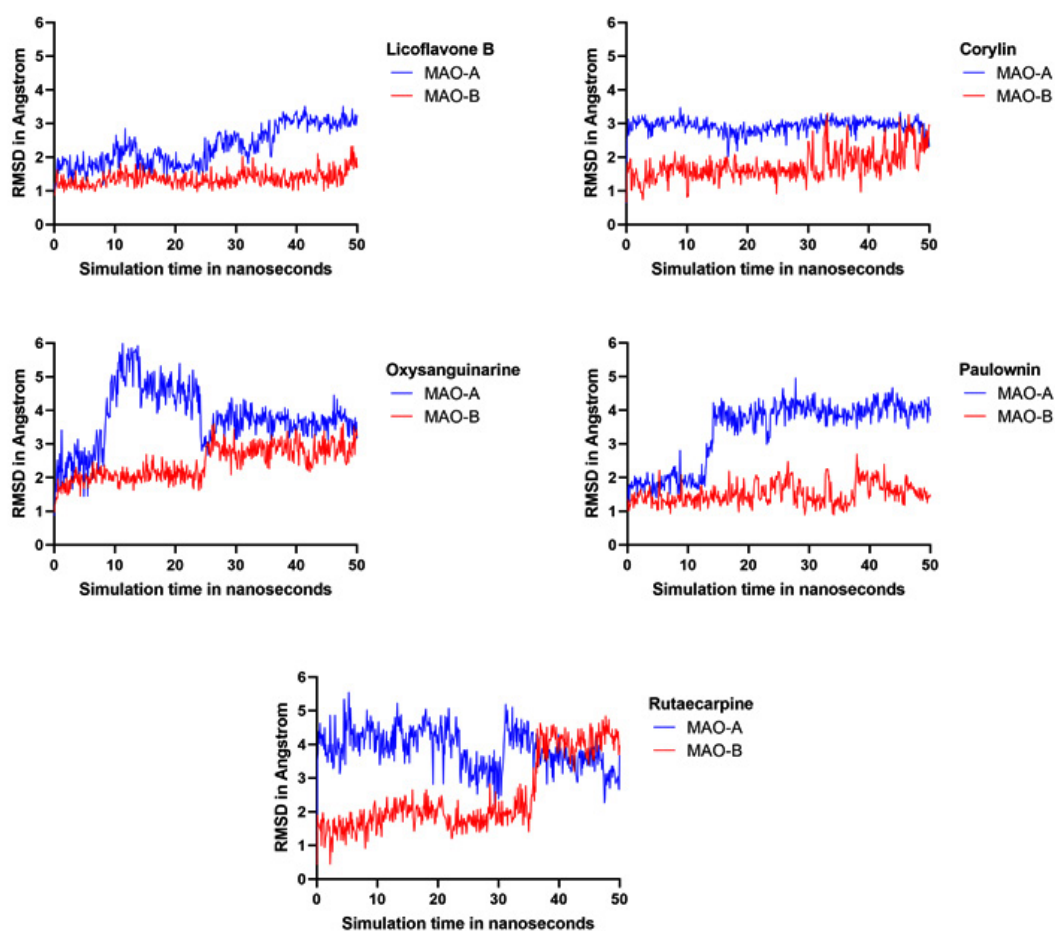
No.	hit ID	Drug- likeness	Pharmacokinetics				Toxicity	
			Solubility in water (mg/ml)	% of GI absorption	VDss (L/Kg)	Log BB	Ames toxicity	LD ₅₀ (mg/ Kg)
1	Licoflavone B	0.51	9.96e-06 (Poor)	92.15	1.17	0.21	Yes	2,570
2	Corylin	0.15	6.46e-03 (Moderate)	96.86	2.69	0.18	Yes	3,850
3	Oxysanguinarine	-1.35	1.26e-02 (Moderate)	100.00	0.54	-0.46	Yes	1,000
4	Paulownin	-0.65	5.60e-01 (Soluble)	97.12	0.55	-0.57	No	1,500
5	Rutaecarpine	0.05	5.00e-02 (Soluble)	97.29	0.87	0.67	Yes	1,400

GI: gastrointestinal; VDss: volume of distribution in the steady state; Log BB: logarithm of brain to blood concentration ratio; LD₅₀: median value of lethal dose.

Table 5. A summary analysis of the MD simulation study for the best TCM phytocompounds acquired by the virtual screening

No.	Phytomedicine name	RMSD for mean ligand movement (Å)		Average value for MM-PBSA binding energy (Kcal/ mol)	
		MAO-B	MAO-A	MAO-B	MAO-A
1	Licoflavone B	1.36	2.31	19.91	17.16
2	Corylin	1.76	2.93	13.30	12.11
3	Oxysanguinarine	2.43	3.79	13.48	2.06
4	Paulownin	1.52	3.39	18.53	6.27
5	Rutaecarpine	2.46	3.87	11.91	4.82
6	Farnesol	3.10	-	28.69	-
7	Clorgyline	-	2.50	-	24.00

MD: the molecular dynamics; Å: angstrom; RMSD: the root mean squared deviation; MM-PBSA: the molecular mechanic Poisson Boltzmann surface area.

**Fig. 2.** A detailed presentations for the closeness of the hit compounds to MAO-B and MAO-A active sites throughout simulation interval.

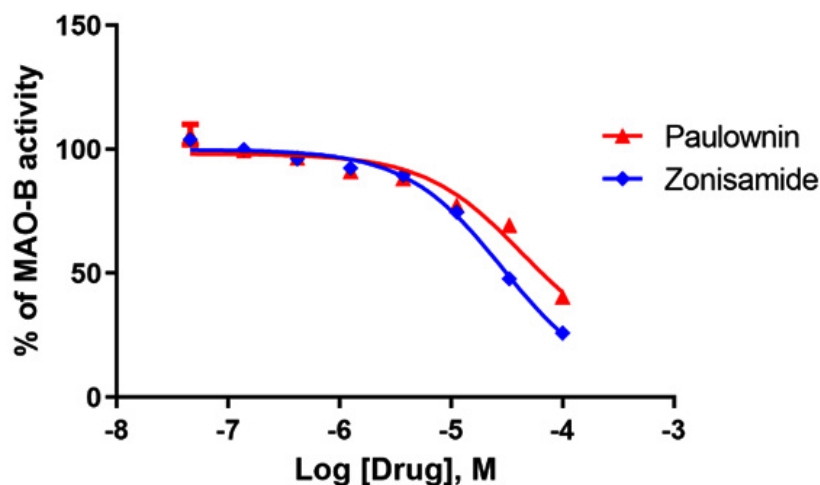


Fig. 3. The dose-inhibition curve for both Paulownin and Zonisamide against MAO-B.

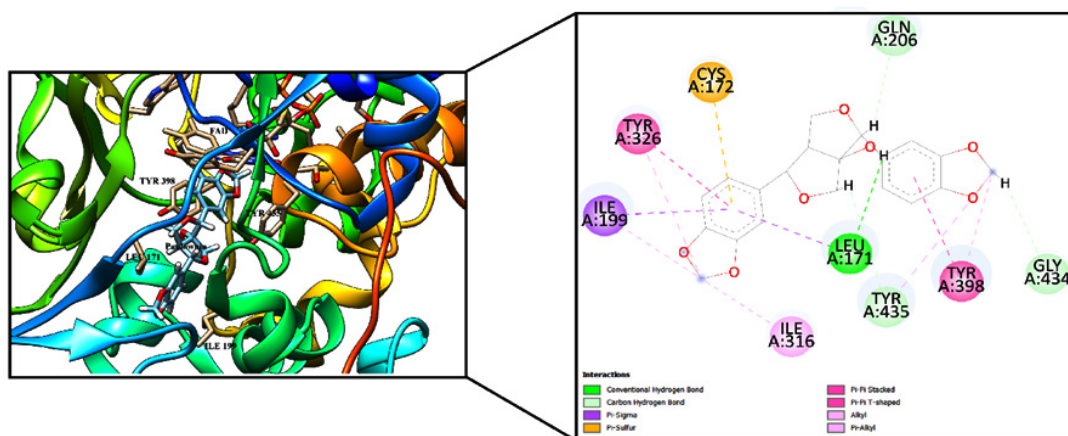


Fig. 4. Visualization of docking interactions between Paulownin and MAO-B active site.

Based on the redocking outputs in Figure 1, the conformational change RMSD was only 0.8 Å between the naive and docked Farnesol when considering the MAO-B crystal. Also, a low RMSD for the variation in conformation of 0.2 Å was computed by aligning the docked and co-crystallized Clorgyline in the case of MAO-A target. As such, it is predicted that the applied docking approach in this study is accurate and reliable due to the computed low conformational change RMSD.⁴⁸

After finishing the virtual screening of the compounds in TCM database against MAO-B,

the output hits were organized according to their energy of binding. As indicated in Table 1, only the best five hits with the least energy of binding were listed. And to consider the selectivity of each hit, the MAO-A docking energy was also included in Table 1. It can be noticed from this table that the energy of binding is slightly higher for MAO-B enzyme as compared to MAO-A. Therefore, these findings in Table 1 can refer to the possible slight selectivity of these five hits against MAO-B. However, further verification may be necessary by using MD simulation to assess the selectivity of these selected hits.

Then, these five hit compounds were reviewed in Table 2 for their natural source and documented biological effects. Depending on this table, these listed hits are supposed to possess anticancer, anti-inflammatory or antimicrobial activities. Additionally, several chemical characteristics were anticipated for these five hits as seen in Table 3. And by considering the chemical formula in Table 3, then it can be observed that these compounds are either flavonoids or alkaloids with the exclusion of the lignan Paulownin. Also, it is predicted that most of these hits do have a good oral absorption due to the alignment of their chemical features in Table 3 with the Lipinski's rule of five (RO5) conditions.⁴⁴ The single violation for RO5 conditions can be noted for the first hit Licoflavone B, which has a Log P value of more than 5. In the same direction, it is believed that these listed compounds do have a reduced molecular flexibility with less than 10 rotatable bonds. Moreover, the topological polar surface area (PSA) for these compounds in Table 3 is expected to be lower than 120 Å². Consequently, the reduced molecular flexibility and polar surface area can suggest a good oral bioavailability for these candidates.⁴⁹

In the next stage of this in-silico study, the drug-likeness degree was calculated to each hit as shown in Table 4. Based on this table, all the listed hits except Licoflavone B were predicted to have a low drug-likeness degree. This finding refers to the fact that these compounds may have chemical and physical properties that are not similar to that of the already approved drugs. Also, in Table 4, the pharmacokinetics profile for these hits was evaluated by predicting the water solubility, intestinal absorption and volume of distribution inside body. In this regard, it is expected that the listed compounds are hydrophilic with a good or moderate water solubility. The only exclusion is the first compound Licoflavone B, which is anticipated to have a poor water solubility. This falls in agreement with the predicted Log P value for the Licoflavone B that was slightly higher than 5 as mentioned before in Table 3. Also, all the presented hits in Table 4 are believed to have a high degree of gastrointestinal absorption but the steady state volume of distribution (VDss) was moderate for both Oxysanguinarine and Paulownin. It is well-known that compounds with a moderate VDss

can distribute equally through body tissues and blood.⁵⁰ In addition, it is expected that these best five hits are CNS permeable as the theoretical log BB values for these compounds were higher than -1. It is believed that a log BB value of more than -1 can refer to a moderate or high permeability of the tested compounds according to previously established BBB permeability models.⁵¹ Finally, the assessment of toxicity potential in Table 4 indicated that these hits may be slightly toxic with an LD₅₀ value of 1000 mg/ Kg or more.⁵² On the contrast, all the listed hits but Paulownin are anticipated to be mutagenic and have the capacity to interact and inhibit multiple variants of CYP450 enzyme.

And to gain a dynamic understanding for the evolution of each hit-target complex, the MD simulation stage was performed with the aid of multiple forcefields for 50 ns duration. In this study, both mean hit movement and average simulation binding energy were reported in Table 5 for both MAO-A and MAO-B enzymes. When considering the mean hit movement RMSD value in Table 5, it is obvious that this parameter was lower for MAO-B as compared to MAO-A. This can point to the possibility of closer hit nearness to MAO-B active site, consequently a stronger binding can be inferred in this case. In addition, a higher average MM-PBSA binding energy was calculated for the interaction of each hit with MAO-B enzyme. As such, it is expected that these five hits may have a higher preference toward MAO-B enzyme as indicated by the closer hit proximity to enzyme active site and the higher binding energy. However, the binding of these five hits to MAO-B may be weaker than Farnesol as the computed average binding energy for this control compound in Table 5 was higher. Interestingly, it seems that the compound Paulownin is the most MAO-B selective candidate. Depending on Table 5, this compound is anticipated to have more than 2.2 and 2.9 times of MAO-B selectivity when considering the ligand closeness to enzyme active site and the simulation binding energy respectively. This summary analysis of MD study in Table 5 can be more confirmed by inspecting the detailed plot of the ligand motion as a function of the simulation time duration as seen in Figure 2 for MAO-B and MAO-A.

It is well-known that the application of molecular docking is limited by multiple

constraints like inaccurate scoring algorithm and possible flaws in predicting the solvation and target flexibility effects.⁵³ Moreover, the dynamics simulation tools are known to rely on empirical force fields to estimate atomic interactions and this may lead to some inaccuracy in the calculations of binding affinity.⁵⁴ Also, the use of predictive models may lead to flawed outputs in some instances due to overfitting as these models were built on limited experimental datasets with a confined application area. As such, the current theoretical findings must be considered as preliminary findings to be further validated by appropriate in-vitro laboratory testing.

Consequently, the hit compound Paulownin was more validated inside laboratory by the monoamine oxidase inhibition assay. In this in-vitro assay, different titrations of the compound were evaluated for its capacity to inhibit MAO-B activity. Zonisamide, a well-known antiepileptic and antiparkinsonian medication, was included as a positive control in this in-vitro assay. Based on the results of this assay, the inhibition capacity of Paulownin against MAO-B was inferior when compared to Zonisamide with a computed IC_{50} value of 42.6 and 28.9 μM respectively.

Furthermore, a careful examination of the docking complex for the most MAO-B selective candidate revealed that the ligand Paulownin may be involved in interactions with several essential residues in MAO-B active site. As noted by Figure 4, the compound Paulownin is engaged in hydrophobic interactions with the gating residues Ile 199, Tyr 326 and the aromatic cage residues Tyr 398, Tyr 435.^{55,56} These interactions suggest that the compound Paulownin may occupy a location within MAO-B active site that ensure enzyme inhibition.

CONCLUSION

MAO-B enzyme inhibitors are usually used as additive options in Parkinson's disease (PD) treatment to reduce the daily dose of L-dopa therapy and thereby minimizing the associated motor complications. It is believed that the inhibition of MAO-B can decrease the metabolism of CNS dopamine while lowering the release of H_2O_2 as a side product. Thus, the selective block of MAO-B enzyme can have a neuroprotective

effect by hindering the production of the oxidizing agent H_2O_2 . Therefore, in this in-silico study, both dynamics simulation and docking were utilized to recognize a selective inhibitor of MAO-B enzyme among phytocompounds in the Traditional Chinese Medicine (TCM) database. As a result, it is believed that the lignan compound Paulownin may be the most selective inhibitor of MAO-B. The compound appears to have a close proximity to MAO-B active site with a high binding energy during simulation analysis. Besides, the compound Paulownin is believed to be implicated in several interactions with MAO-B active site key residues as observed by the docking study. Moreover, the prediction of chemical features revealed that Paulownin may have good oral absorption as it not violating any condition in the rule of five (RO5). Also, this hit is supposed to be water soluble, safe, non-mutagenic and it may have a moderate volume of distribution and CNS permeability. Lastly, the in-vitro monoamine oxidase inhibition assay had shown that the compound Paulownin was able to record an IC_{50} value of 42.6 μM against MAO-B.

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Author Contributions

Hasanain Abdulhameed Odhar: Conceptualization, Methodology, Data collection, Analysis, Writing – Original Draft; Suhad Sami Humadi: Writing – Review & Editing, Supervision; Azher Abdulmutaleb Ibrahim: Methodology, Review & Editing.

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