Research Article

# *In Silico* Approach for Validation of Maltol Derivatives as Acetylcholinesterase Inhibitors

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**ABSTRACT**

*In silico* molecular docking approach, through screening of chemical libraries to the active site of the target protein is playing a vital role in identification of lead molecules. Prediction of drug properties and calculation of adverse effects for the lead molecules computationally simplify the crucial and cost-effective aspect in drug discovery. In this study, we performed the docking simulations on AChE active site with maltol and its derivatives from ZINC database in autodock vina in PyRx software. Further, the lead molecules identified in the docking process were subjected to molinpiration and Osiris servers to get their ADME and adverse effect properties respectively. Moreover, biological targets and functions of lead structures were predicted in PASS, an online server. The results demonstrated that the derivatives of maltol viz. ZINC01655221, ZINC02545409, ZINC40891630, ZINC1674584 and ZINC00161033 were found to have a good binding affinity with the active site of the AChE in docking studies. Further, these compounds along with standard compound maltol have shown the hydrogen bond interactions with key amino acid residues of AChE. All lead ZINC compounds have qualified the drug properties and adverse effect calculations. PASS prediction also supported the inhibitory effect of lead compounds on neurological disease viz. Alzheimer's disease. Based on docking, drug property prediction, adverse effect calculations and PASS prediction results, it was inferred that all the identified lead molecules have the ability to inhibit the AChE target protein and thus can be suggested as the most desired compounds to treat Alzeihmer's disease.

**Keywords:** Alzheimer's disease, Acetylcholinesterase, Maltol, Docking, Drug properties.

## INTRODUCTION

Alzheimer's disease (AD) or Senile Dementia of the Alzheimer Type (SDAT) is an irreversible but a progressive neurodegenerative disorder caused by the loss of cholinergic neurons and synapses in the cerebral cortex, also in certain sub-cortical regions. Cholinesterase's (ChEs), in vertebrates have been classified into two types, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), on the basis of distinct substrate specificities and inhibitor sensitivities which serves as enzyme targets for AD[1]. The cholinergic system is impaired in the Alzeihmers diseased brain due to low concentration of the neurotransmitter, acetylcholine (ACh), deficiency of cholineacetyltransferase and AChE enzymes. Hence, either alteration of the cholinergic transmission or development of AChE inhibitors are the best approaches for treating AD[2].

Maltol (2-methyl-3-hydroxy-1,4-pyrone) is a naturally occurring substance that is widely used as a flavoring agent. It is formed through thermal degradation of starch or sucrose pyrolysis. It is found in baked products as well as Korean ginseng root, coffee, chicory, soybeans, bread crusts, and caramelized foods. Maltol is not only used in breads, cakes, malt beverages, and chocolate milk as a flavour enhancer[3], but is also used in medications such as vanadylmaltolate for the treatment of diabetes, and ferric trimaltol for the treatment of iron deficiency anemia[4-5]. It has been reported that maltol has a neuroprotective effect through its antioxidant properties[6-7] and anti-apoptotic effects[8]. Few studies have shown that maltol attenuates neurotoxicity and prevents oxidative damage, but there has been no comprehensive study of the protective effects of maltol on neuro degenerated cells in Alzheimer's disease.

On the basis of these previous reports, in the present *in silico* study, we aimed to perform docking studies on AChE therapeutic target using the flavouring agent maltol and its derivatives as inhibitors and subsequently also look into maltol interactions with amino acid residues of the target protein through good binding affinity.

Finally, our study also includes application of the Lipinski rule of five and calculation of the adverse effects of the screened lead molecules to improve the quality of maltol and also to recommend it for treatment of AD.

## MATERIALS AND METHODS

### Preparation of Protein and Ligands for Docking Studies

The crystallographic macromolecular structure of target protein was found in PDB (PDB ID-5HF8: Crystal structure of human acetyl cholinesterase in complex with paraoxon (alternative acyl loop conformation))[9].

Macromolecule as PDB format was used in a docking studies, preferably protein structure for docking was made by removing hetero atoms and paraoxon inhibitor using arguslab software, and finally it was energy minimized in the swisspdb viewer software tool.

For the docking, micro molecules such as maltol and its derivatives were downloaded from pubchem database

and ZINC database respectively in the form of structure definition file (SDF) format[10].

**Active Site Prediction**

Proteins have specific sites viz. active sites, where the amino acid residue side chains form an active cavity or cleft to which the ligands or atoms or other proteins are capable to bind. The active site of the AChE protein was done using a CAST p server,[11] which identifies and characterizes protein active sites, binding sites and functional residues located on protein surface, and voids buried in the interior of proteins by measuring concave surface regions on the three dimensional structures of protein.

**Molecular Docking Studies**

Molecular docking studies on maltol and their derivatives against the AChE active site were done in auto dock vina in PyRx software, a freely accessible one and designed for molecular docking studies and virtual screening of libraries of compounds[12]. Moreover, PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for Rational Drug Design (http://pyrx.sourceforge.net/). The Prepared macromolecule was uploaded into auto dock vina, made the protein ready for docking. Later, the Micromolecules such as Maltol and its derivatives were subjected to energy minimization and converted them into a pdbqt format for docking. Genetic algorithm was used to perform docking in between macro and micro molecules. After completion of docking studies, the best maltol and its derivatives were selected based on the binding affinity values in the active site of AChE.

**Visualization of Molecular Interactions**

Docking study generated pair of protein and ligands were saved in pdb format, and visualized in the PyMOL graphical visualization tool[13]. PyMOL, a Python-enhanced molecular graphics tool, excels at 3D visualization of proteins, small molecules, density, surfaces, and trajectories. It also includes molecular editing, ray tracing, and movies.

The ligand binding site in AChE active site was visualized and molecular interactions in the form of hydrogen bonds between protein and ligands were characterized, and also the distance of hydrogen bonds were calculated**.**

**Prediction of Drug Properties and Adverse Effects**

Finally, the predicted lead molecules were subjected to calculation of drug properties in Molinspiration, a chemo informatics tool. It calculates Lipinski rule of five[14], a method it evaluates drug likeness or determine the chemical compound's pharmacological and biological properties. The lead molecules were also searched for their adverse effects in OSIRIS server[15] which determines the Drug likeness, mutagenic, tumorigenic, reproductive effects and irritation etc. for any selected compounds.

**PASS Prediction**

We predicted the activity and inactivity ratios of the lead molecules on their biological targets in PASS **(**Prediction of Activity Spectra for Substances), a free online software tool, provides simultaneous predictions of many types of biological activity based on the structure of organic compounds[16]. It predicts more than 300 pharmacological effects and biochemical mechanisms based on structural information of interested compound and also predict their biological target.

## RESULTS AND DISCUSSION

Structural genomics, though provided a number of structures, but it does not give enough functional annotations of proteins. The computational tools are successful in order to obtain the definite function of proteins based on their structure and not from sequence. It investigates and identifies the functional sites of protein based on structure. In case of non enzymes, these sites are called functionally importance regions, whereas in enzymes, they are called as active sites.

In our present study, we predicted the active site of a key enzyme, AChE involved in manifestation of a serious disease i.e. Alzheimer' Disease and represented it in Figure 1.

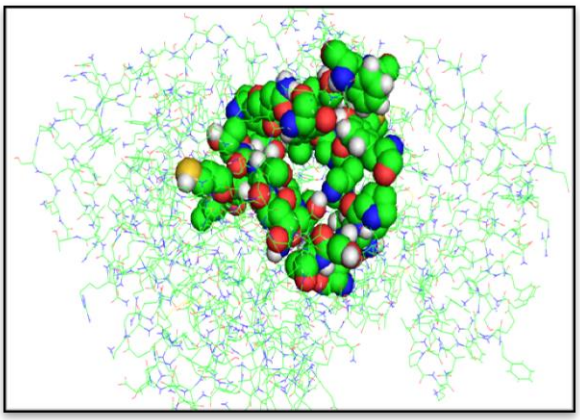

**Figure 1:** AChE protein represented in stick model, whereas active site amino acids are shown in balls.

In this study, we performed docking studies in between maltol derivatives against cholinergic enzyme AChE active site. The least binding affinity (more negative value) of the compounds is considered as the best drugs in pharmaceutics. Based on these criteria, we selected docking poses of top five lead molecules. Binding affinity and interactions of lead molecules with the active site of target protein were represented in Table. 1. From our observations, it was obvious that the First ranked ZINC01655221 has shown three hydrogen bonds interactions with the amino acids viz. TYR72, ASN87 and TYR341 when it was fit into the active site by -11.8 kcal/mol binding affinity. Oxygen atom of the two amino acids, TYR72 and ASN87 have bonded with an oxygen atom of lead molecule with bond length 3.3 Å and 3.5 Å, whereas the oxygen atom of TYR 341 has formed a bond

with a hydrogen atom of lead molecule with bond distance 2.6 Å. Similarly, the second lead molecule viz.ZINC02545409 has shown two hydrogen bond interactions with the amino acids, TRP86 and TYR337 with -10.2 kcal/mol binding affinity through bond distance 3.5 Å and 2.0 Å respectively.

In line with the second lead molecule, the third one viz. ZINC40891630 molecule also has shown two hydrogen bond interactions with the amino acids, SER125 and TYR337 with -10.2 kcal/mol binding affinity, where bond distances were 2.3 Å and 2.1 Å respectively.

Contrary to these three lead molecules, the fourth lead molecule, ZINC1674584 has shown only one hydrogen bond interaction with TYR337 amino acid with -10kcal/mol binding affinity through bond distance 2.4 Å.

The Fifth ranked lead molecule ZINC00161033 has shown two hydrogen bond interactions with TRP86 and TYR337 amino acid with -10.2 kcal/mol binding affinity containing respective bond distances 2.0 Å and 2.6 Å.

The most interesting observation was that the standard maltol has -5.6 kcal/mol binding affinity with the active site of AChE through six hydrogen bond interactions with 6 key amino acid residues viz. GLY121,GLY122, SER203, GLU202, HIS447 and TYR337.

Molecular visualizations of these interactions of lead molecules with AChE were represented in Figure 2.

Identification of a proper lead compound for a given molecular target is a critical step in the process of drug discovery.

Traditionally, high-throughput screening (HTS) of large chemical libraries has been a primary source of identification of novel lead compounds.

The docking process involves the prediction of ligand conformation and orientation (or posing) within a targeted binding site[17].

The recent docking report evidenced that the drug glimepiride has bounded at the active site of the AChE, where its substrate also AChI (acetylcholine iodide) bind.

That drug has shown interaction with key amino acids such as Gln71, Tyr72, Val73, Asp74, Trp86, Asn87, Tyr124, Ser125, Trp286, Phe295, phe297, Tyr337, Phe338 and Tyr341[18].

Our docking results also shared common amino acids (Tyr72, Asn87, Trp86, Tyr341, Tyr337, Ser125) of active site of AChE as that of glimepiride.

Based on these common interactions, it may be inferred that the identified lead molecules in the present study may act like glimepiride to inhibit AChE function.

On the whole, observation on the docking interactions of lead molecules revealed that all compounds have common bonding with TYR 337, which is a key amino acid of active site interacted by maltol, a standard drug.

In drug discovery, drugs interactive atoms with specific amino acid residues of therapeutic target have played key role in functional characterization of proteins using mutational studies.

*In silco* understanding of drug interactions with active site amino acid residues of target protein has given a solution to scientists for inhibition of targets[19].

Docking results of the present study revealed that, key amino acid residues such as TYR72, TRP86, ASN87, GLY121, GLY122, SER125, GLU202, SER203, TYR337, TYR34 and HIS 447 were in active site of AChE and are involved in molecular interactions.

**Table 1**: Binding Interactions of the Lead Molecules with Active Site of the AChE.

| S. No. | Lead molecules | Maltol and its derivatives binding interactions with AChE | | | |
|---|---|---|---|---|---|
| | | Protein -Ligand | Amino acid | Distance in Å | Binding energy (Kcal/mol) |
| 1 | ZINC01655221 | O----------O<br>O----------O<br>O----------H | TYR72<br>ASN87<br>TYR341 | 3.3<br>3.5<br>2.6 | -11.8 |
| 2 | ZINC02545409 | O----------O<br>H----------O | TRP86<br>TYR337 | 3.5<br>2.0 | -10.2 |
| 3 | ZINC40891630 | O----------H<br>H----------O | SER125<br>TYR337 | 2.3<br>2.1 | -10.2 |
| 4 | ZINC1674584 | H----------O | TYR337 | 2.4 | -10 |
| 5 | ZINC00161033 | O----------H<br>H----------O | TRP86<br>TYR337 | 2.0<br>2.6 | -9.4 |
| 6 | MALTOL | H----------O<br>H----------O<br>H----------O<br>O----------H<br>N----------H<br>H----------O | GLY121<br>GLY122<br>SER203<br>GLU202<br>HIS447<br>TYR337 | 2.3<br>2.6<br>2.1<br>2.5<br>3.0<br>2.2 | -5.6 |

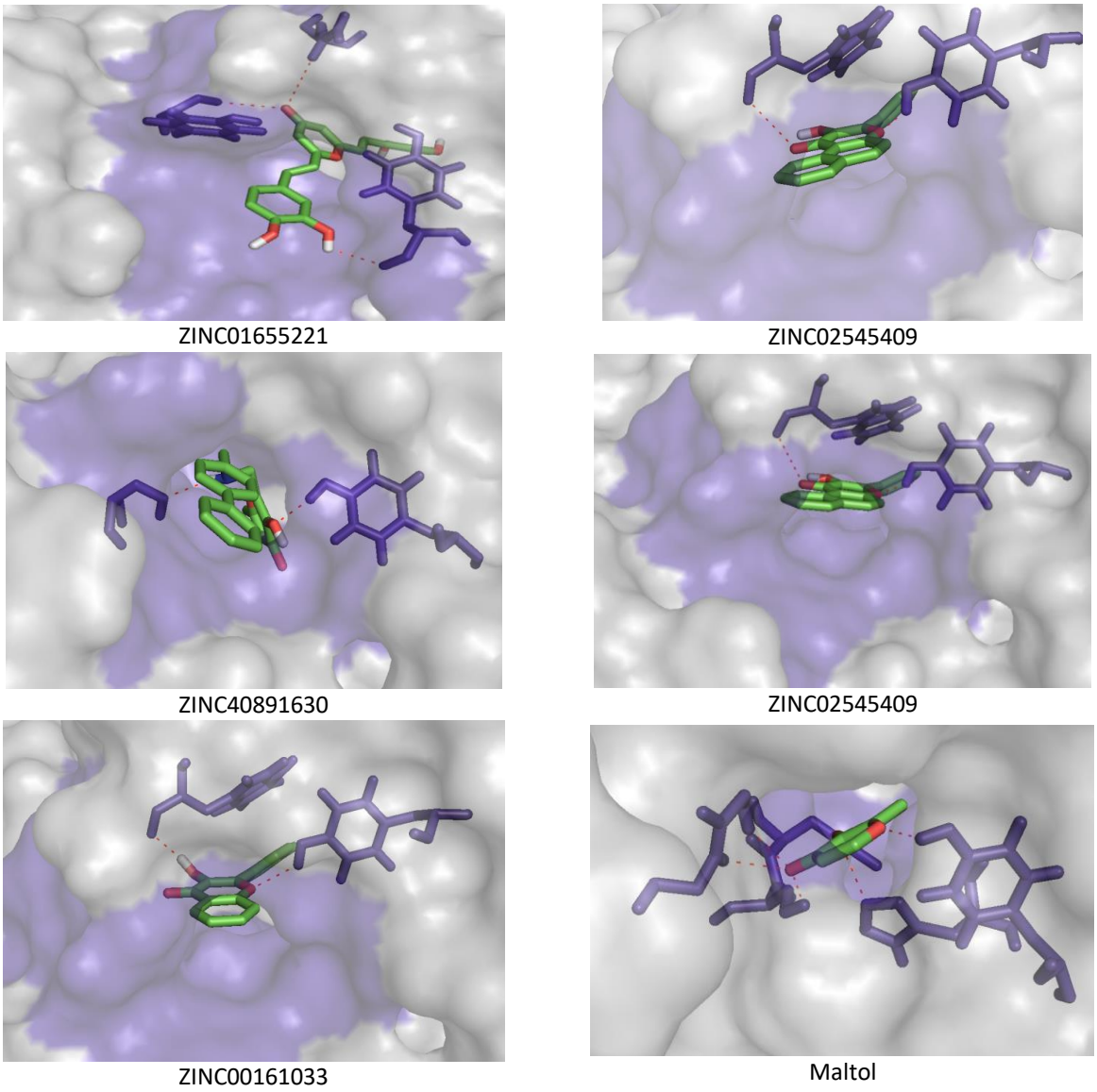


**Figure 2**: Binding poses of lead molecules in active site of the AChE. Lead molecules and their interacted amino acids are shown in stick model, where the lead molecules are coloured in green and amino acids in purple blue.

**Table 2:** Predicted Lead Chemical Compound 2D Structures

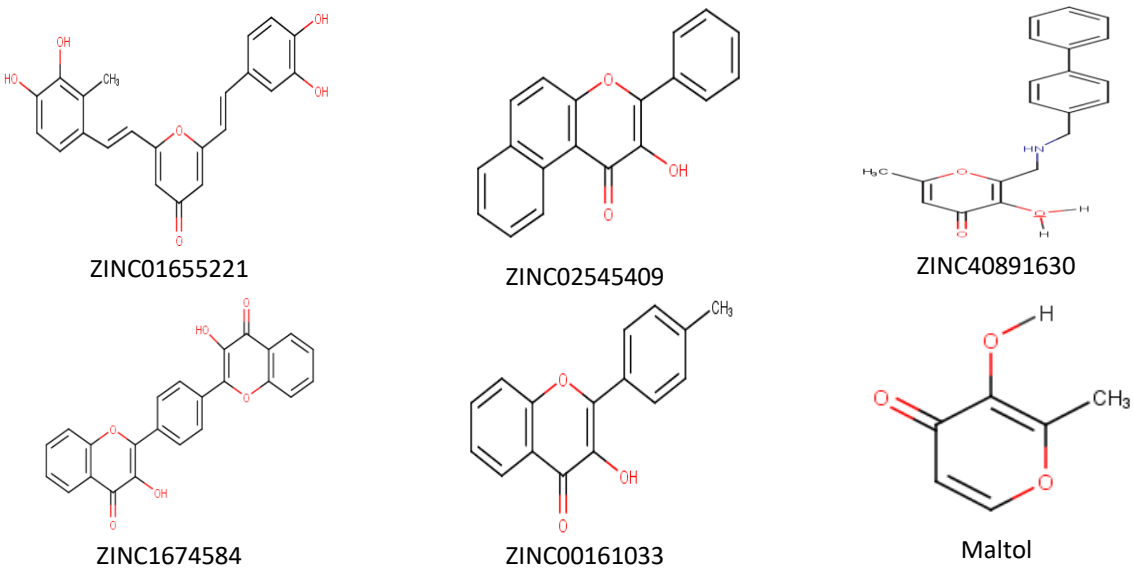

**Table 3:** Prediction of adverse effects and Lipinski rule of five for Maltol and its derivatives

| S. No | Compound name | Adverse Effect Calculations | | | | Lipinski Rule of Five | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mutagenic | Tumorigenic | Reproductive effective | Irritation | Mi LogP | MW | Non | nOHNH | TPSA |
| 1 | ZINC01655221 | None | None | None | None | 3.81 | 364.35 | 6 | 4 | 111.12 |
| 2 | ZINC02545409 | High | High | None | None | 4.61 | 288.30 | 3 | 1 | 4.61 |
| 3 | ZINC40891630 | None | None | None | None | 0.62 | 321.38 | 4 | 2 | 69.87 |
| 4 | ZINC1674584 | None | None | None | None | 4.95 | 398.37 | 6 | 2 | 100.88 |
| 5 | ZINC00161033 | None | None | None | None | 3.89 | 252.27 | 3 | 1 | 50.44 |
| 6 | Maltol | High | None | None | High | -0.24 | 126.11 | 3 | 1 | 50.44 |

**Table 4:** Prediction of Biological Activity of the Lead Molecules using PASS Prediction Tool

| Lead Molecule | ZINC01655221 | ZINC02545409 | ZINC40891630 | ZINC1674584 | ZINC00161033 | Maltol |
|---|---|---|---|---|---|---|
| **Function** | Free radical scavenger.<br>Antioxidant. | Acute neurologic disorders treatment. | Acute neurologic disorders treatment.<br>Neuropathy treatment. | Acute neurologic disorders treatment | Acute neurologic disorders treatment.<br>Oxygen scavenger.<br>Free radical scavenger. | Alzheimer's disease treatment.<br>Neurodegenerative diseases treatment.<br>Oxygen scavenger.<br>Antioxidant. |

Lipinski rule of five is a thumb to evaluate drug likeness, or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it an orally active drug in humans.

The rule describes molecular properties, important for a drug's pharmacokinetics in the human body, including its ADME.

In the present investigation, the drug property prediction for lead molecules with Lipinski rule of five and ADME were presented in the Table.3. Bioinformatics tools are very robust in the field of pharmacology because they are able to predict the side effects of the chemical compounds based on their structural features.

All lead molecules, identified as AChE inhibitors were not having any adverse effects except ZINC02545409, which has mutagenic and tumorigenic properties.

From the data represented in Table 4 regarding the biological activity of the lead molecules, it was evident that the compounds, ZINC02545409, ZINC02545409, ZINC1674584 and ZINC00161033 have abilities to treat AD, whereas, ZINC01655221 has neuroprotective effect by virtue of having antioxidant and scavenging properties to protect the neurons from free radicals.

Neuronal biochemical composition is mainly susceptible to free radicals since it involves pool of unsaturated lipids those are labile to peroxidation and oxidative modification.

Double bonds of unsaturated fatty acids are hot spots for attack by free radicals those initiate cascade or chain reactions to damage neighbouring unsaturated fatty acids[20].

## CONCLUSION

In view of the results obtained in the present study, it may be concluded that all the identified lead molecules (ZINC01655221, ZINC02545409, ZINC40891630, ZINC1674584 and ZINC00161033) have shown good binding affinity than the standard maltol, with active site amino acids of the AChE.

Except ZINC02545409, the remaining lead molecules have free from adverse effects such as mutagenic, tumarogenic, reproductive effect and irritant. Hence, the Maltol derivatives viz. ZINC01655221, ZINC40891630, ZINC1674584 and ZINC00161033 may act as potential AChE inhibitors to treat Alzeihmers disease.

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