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Research Article

The Design and Evaluation of a Novel Monoamine Oxidase B Inhibitor Through in Silico Approach

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ABSTRACT
Parkinson's disease is an age related neurodegenerative disease. Pioglitazone is a Peroxisome proliferator-activated receptor gamma agonist that has been shown to display a neuroprotective effect in parkinsonian models (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treated mice). This effect was partially attributed to the ability of thiazolidinedione (TZD) moiety in Pioglitazone to selectively inhibit monoamine oxidase B (MAO-B) enzyme. In the current study, we screened several thiazolidine containing compounds against MAO-B enzyme both in silico and in vitro. Based on the resulted data and information from previous literatures, we were able to design a novel scaffold for MAO-B inhibitors. This scaffold (compound 5482440) was able to inhibit MAO-B enzyme with IC_{50} value of 1.447 µM. Structure-based virtual analysis showed that this compound was able to participate in water-bridge formation and obtain an extended conformation within MAO-B active site.

Keywords: Parkinson's disease; thiazolidine; scaffold; MAO-B; docking.

INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder that mainly affects geriatric population. It is characterized by a progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* region of the midbrain, this gradual damage is responsible for the emergence of motor related hallmarks including tremor, rigidity and bradykinesia[1]. Non-motor symptoms like depression may also appear as the disease progresses in course[2]. Although the exact cause of Parkinson's disease remains unknown, genetic factors and environmental triggers may play a role in increasing the susceptibility to develop the disease[3,4]. Current therapeutic strategy depends mainly on symptomatic mitigation through the use of dopamine replacement therapy, monoamine oxidase B (MAO-B) inhibitors and catechol-O-methyltransferase inhibitors[5]. However, there is essential need to introduce new therapeutic agents with neuroprotective and/ or disease modifying effect[6].

Monoamine oxidase B (MAO-B) is a flavin adenine dinucleotide (FAD) containing enzyme that is bound to the outer mitochondrial membrane. This enzyme plays an important role in the degradation of dopamine and therefore the inhibition of MAO-B enzyme will lead to an increase in dopamine level[7,8]. Several in vitro and in vivo studies have shown that some classes of MAO-B selective inhibitors are neuroprotective and/ or neurorestorative[9]. Pioglitazone is a thiazolidinedione (TZD) containing anti-diabetic medication which has been shown to be neuroprotective in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) parkinsonian mouse model. This neuroprotective effect has been attributed in part to the selective inhibition of MAO-B enzyme and this will halt the conversion of MPTP to the toxic metabolite MPP^{+}[10]. Previous docking studies have shown that TZD moiety is essential to MAO-B inhibitory effect of glitazone-type compounds[11].

In this paper, five thiazolidine containing compounds were selected and screened both in silico and in vitro for their ability to inhibit MAO-B enzyme. The obtained data were combined with information from previous literatures to generate a scaffold design for efficient thiazolidine containing MAO-B inhibitor for possible use in Parkinson's disease management.

MATERIAL AND METHODS

Prospective candidates

Five thiazolidine containing chemicals were selected randomly and purchased from Chembridge online chemical store (www.hit2lead.com) as possible MAO-B enzyme inhibitors. The chemical structure and store database identification for these compounds can be seen in table 1. Our novel scaffold design (compound 5482440) was also bought from Chembridge online chemical store. Zonisamide was used as a positive control for MAO-B inhibition assay and it was purchased from

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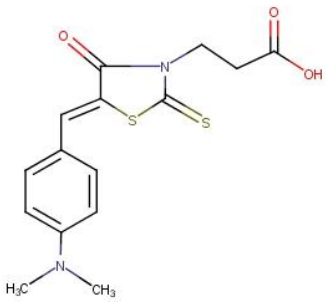
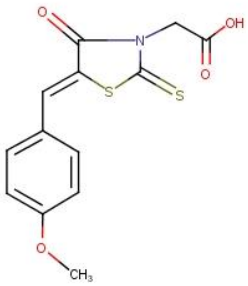
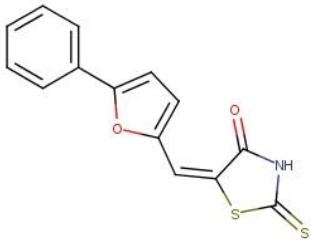
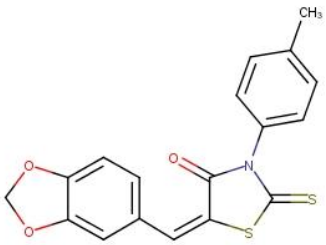
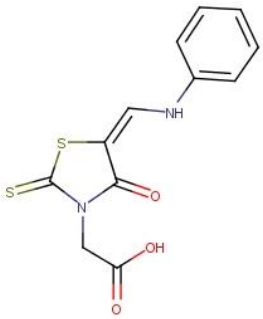
Table 1: Effect of the screened compounds on MAO-B enzyme activity.

	Compound ID	Structure	MAO-B IC_{50}, µM
1	5140311		9.708
2	5140321		104.5
3	5143886		2.637
4	5144779		84.50
5	5160602		90.51

Sigma-Aldrich (www.sigmaaldrich.com). Each compound was dissolved in an appropriate volume of dimethyl sulfoxide (DMSO) and a stock solution of 10mM was obtained.

Monoamine oxidase inhibition assay

A 96-well plate assay was employed as specified previously[12]. In principle, the ability of MAO enzyme to convert the non-fluorescent substrate (kynuramine) to the fluorescent product (4-hydroxyquinoline) can be used to assess MAO inhibitory potential of each compound[12,13]. In this assay, MAO-B enzyme (0.015 mg/ml) was

incubated for 20 minutes at 37 °C with the tested compound and in the presence of 20 µM kynuramine. DMSO was used as a vehicle for the prospective compounds and its final concentration in this test was less than 0.5%. Compounds under investigation were serially

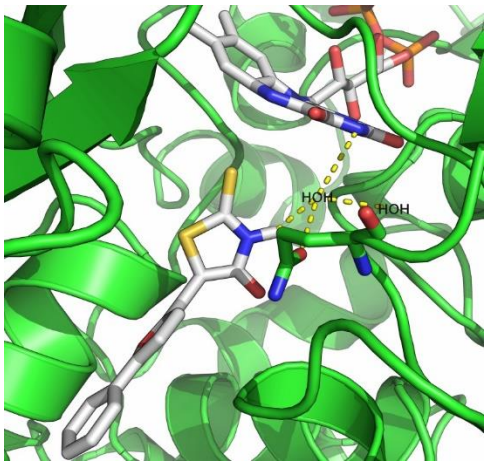


Figure 1: Docking of compound 5143886 into MAO-B crystal (2BK3). Hydrogen bond is represented by dashed yellow line.

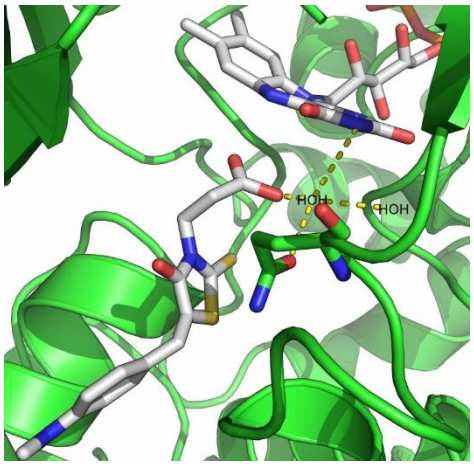


Figure 2: Docking of compound 5140311 into MAO-B crystal (2BK3). Hydrogen bond is represented by dashed yellow line.

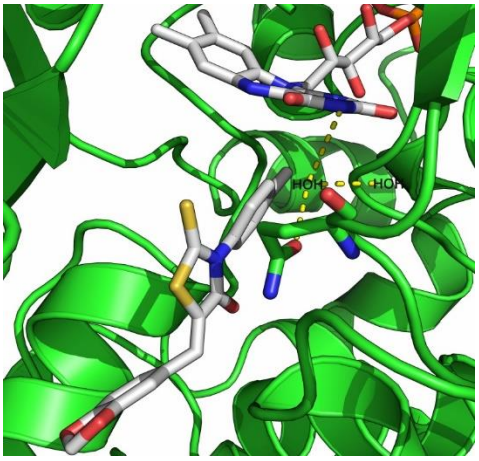


Figure 3: Docking of compound 5144779 into MAO-B crystal (2BK3). Hydrogen bond is represented by dashed yellow line.

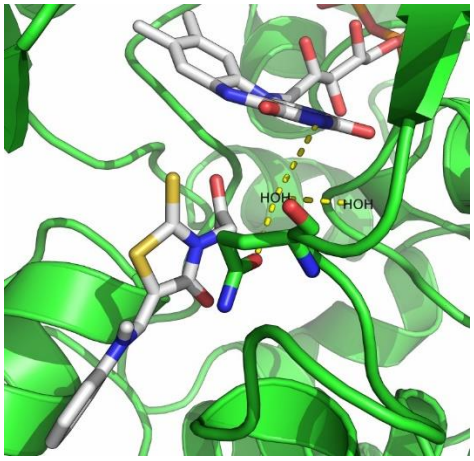


Figure 4: Docking of compound 5160602 into MAO-B crystal (2BK3). Hydrogen bond is represented by dashed yellow line.

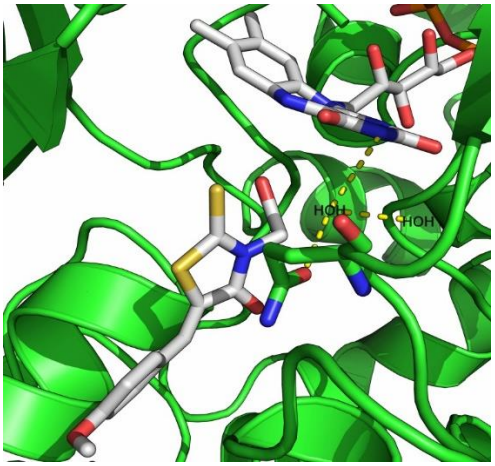


Figure 5: Docking of compound 5140321 into MAO-B crystal (2BK3). Hydrogen bond is represented by dashed yellow line.

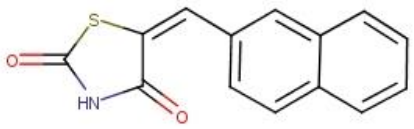


Figure 6: Chemical structure of novel scaffold (compound 5482440).

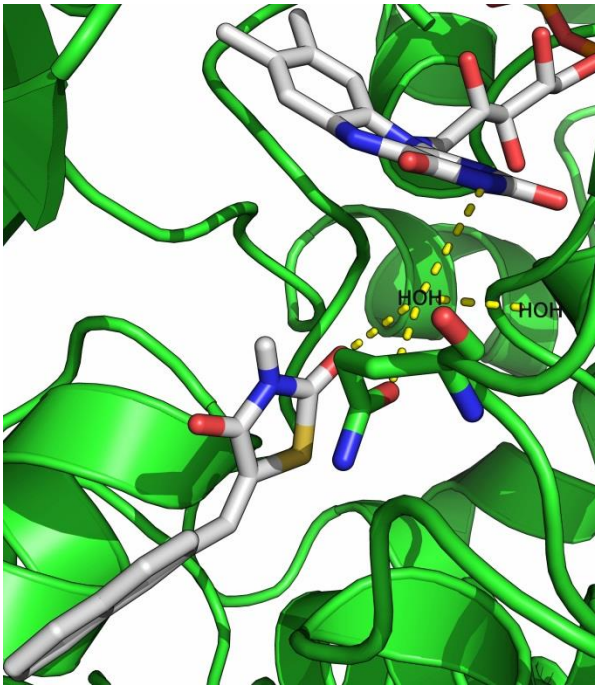


Figure 7 Docking of compound 5482440 into MAO-B crystal (2BK3). Hydrogen bond is represented by dashed yellow line.

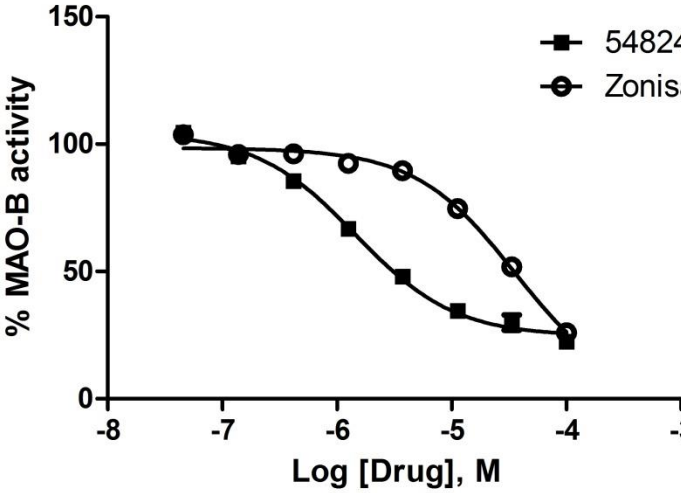


Figure 8 Dose-inhibition curve for compound 5482440 and Zonisamide.

diluted across the 96-well plate so that an eight points dose-inhibition curve was generated. The fluorescence level of 4-hydroxyquinoline was measured at 310/ 380 nm wavelength pair by using a Flourometer (top reader, BMG-FlouStar). The fluorescence level in the absence of any inhibitor was considered to reflect 100% enzyme activity.

Structure-based virtual screening study

For docking analysis, we used AutoDock Vina plugin for PyMOL[14,15]. Images processing was done by using PyMOL version 1.7.6.0 (www.schrodinger.com). The chemical structure of each prospective inhibitor was drawn and converted to three dimensional form by using MarvinSketch version 15.9.7.0 (www.chemaxon.com). Human MAO-B crystal with code 2BK3[16] was obtained from Protein Data Bank (www.rcsb.org) and it was used as a target for docking.

Statistical analysis

Dose-inhibition curve was fitted to a one site binding curve by using GraphPad Prism 5 (www.graphpad.com). This program was also used to calculate the half maximal inhibitory concentration (IC_{50}) for each dose-response curve.

RESULTS

The inhibition capacity of the screened compounds against MAO-B enzyme can be seen in table 1. By examining this table, we can clearly notice that the strongest inhibition was produced by compound 5143886 and compound 5140311 with IC50 values within early micro-molar range (2.637 µM and 9.708 µM respectively).

IC_{50} is the half maximal inhibitory concentration.

DISCUSSION

The difference in inhibition potency among the tested compounds can be attributed to the variation in binding behavior of these chemicals to the active site of MAO-B crystal. Docking images in figure 1 and figure 2 showed that compounds 5143886 and 5140311 are involved in water-bridge formation with Gln 206 residue and nitrogen atom at position three of the isoalloxazine ring of FAD molecule.

The presence of 4-methylphenyl moiety in compound 5144779 and acetic acid group in compound 5160602 attached to the nitrogen atom at position three of the thiazolidine ring will disrupt the ability of these compounds to participate in a similar water-bridge within MAO-B active site.as seen in figure 3 and figure 4. The replacement of propanoic acid group attached to thiazolidine ring in compound 5140311 with acetic acid group in compound 5140321 will prevent the later compound from taking part in water-bridge formation as can be seen in figure 5. The disruption of water-bridge formation may explain the significant reduction in MAO-B inhibition potential for compounds 5144779, 5160602 and 5140321.

It is worth to mention that previous analysis of MAO-B crystal has found that oxygen and nitrogen atoms in thiazolidinedione ring of pioglitazone are involved in hydrogen bonds formation with conserved active site water molecules[17].

An important structural feature of selective MAO-B inhibitors is that they should exhibit an extended conformation that traverses both the entrance cavity and substrate cavity of MAO-B active site. Such behavior will force Ile 199 residue (gating residue) to acquire an open configuration leading to the fusion of these two cavities[16].

It is also important to mention that ligand binding affinity is reduced by about 0.5 kcal for each two rotatable bonds introduced to ligand chemical structure as determined by previous in vitro studies[18].

Here we introduce our novel design for a new scaffold of MAO-B inhibitors (compound 5482440) by considering the previously mentioned structural criteria. We combined 1,3-thiazolidine-2,4-dione moiety with naphthalene group so that only one rotatable bond was introduced. The structural formula of compound 5482440 is shown in figure 6.

We believe that such design can participate in water-bridge within the active site of MAO-B enzyme and it can also force Ile 199 to obtain an open configuration leading to efficient inhibition of MAO-B enzyme as seen in figure 7.

Monoamine oxidase inhibition assay has shown that MAO-B IC_{50} value for compound 5482440 is 1.447 µM which is lower than that measured for Zonisamide with IC_{50} value of 35.59 µM. The dose-inhibition curve for both compound 5482440 and Zonisamide is shown in figure 8.

Future plans involve further manipulation of our novel scaffold design through the introduction of iron chelating groups or CNS targeting group to the naphthalene moiety of compound 5482440.

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