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Identification of novel monoamine oxidase B inhibitors by structure-based virtual screening

Werner J. Geldenhuys [a], Altaf S. Darvesh [a], Max O. Funk [b], Cornelis J. Van der Schyf [a], Richard T. Carroll [a,*]

[a] Department of Pharmaceutical Sciences, Northeastern Ohio Universities Colleges of Medicine and Pharmacy, 4209 State Route 44, Rootstown, OH 44272, USA
[b] Department of Chemistry, University of Toledo, Toledo, OH 43606, USA

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ABSTRACT

Parkinson's disease is a severe debilitating neurodegenerative disorder. Recently, it was shown that the peroxisome proliferating-activator receptor-γ agonist pioglitazone protected mice from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity due to its ability to inhibit monoamine oxidase B (MAO-B). Docking studies were initiated to investigate pioglitazone's interactions within the substrate cavity of MAO-B. Modeling studies indicated that the thiazolidinedione (TZD) moiety was a likely candidate for its specificity to MAO-B. To explore this potential novel MAO-B scaffold, we performed a structure-based virtual screen to identify additional MAO-B inhibitors. Our search identified eight novel compounds containing the TZD-moiety that allowed for a limited study to identify structural requirements for binding to MAO-B. Inhibition assays identified two TZDs (A6355 and L136662) which were found to inhibit recombinant human MAO-B with IC_{50} values of 82 and 195 nM, respectively.

Parkinson's disease is a common neurodegenerative disorder which affects around 3% of the population in the United States,[1,2] and is diagnosed or characterized by symptoms of bradykinesia, rigidity, and tremors.[3] Although seen largely in geriatric patients, probably due to environmental factors,[4,5] genetic manifestations do occur at younger ages of onset.[6] These symptoms have been correlated with a significant loss of dopaminergic neurons in the substantia nigra leading to decreased levels of dopamine (DA) in the striatum.[1,2] In addition to the motor deficits seen with these patients, they also can display symptoms of depression and cognitive impairment as the disease progresses.[3] Quality of life decreases significantly with disease progression. This places a large burden on family members as well as the health care system. It has been shown that monoamine oxidase inhibitors which selectively inhibit the B isoform (MAO-B) may have neuroprotective and/or neurorestorative properties as demonstrated in vivo by rasagiline.[7–10]

Recently, it was shown that the anti-diabetic drugs pioglitazone and rosiglitazone (Table 1) were protective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) parkinsonian mouse model.[11,12] These two glitazones are Type II diabetic drugs that act as peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists. Pioglitazone protects against MPTP-induced DA depletion in the striatum of mice. The neuroprotective mechanism of pioglitazone was shown to be, at least in part, through its inhibition of MAO-B which prevented the conversion of MPTP to its toxic metabolite MPP^+.[11] In addition, these TZDs have been shown to reduce inflammation mediated events and also to be protective in preclinical models such as cerebral re-perfusion injury where inflammation results in a secondary insult leading to additional neuronal death.[13] Similar activities may also be contributing to protection in the MPTP mouse model.

Taking into consideration the protective activity demonstrated by pioglitazone, **1**, in the MPTP model, we modeled this drug into the MAO-B enzyme to determine important binding attributes of the molecule needed for binding. We identified the thiazolidinedione moiety (TZD) as the relevant scaffold to search for therapeutic leads towards the treatment of Parkinson's disease. This decision was based on initial docking studies which indicated the TZD-moiety was contributing largely towards the activity of pioglitazone in MAO-B (Fig. 1; 2V5Z.pdb). During the docking process, the bonds were allowed to rotate freely, without regard to any specific enantiomer which may exist. The ZINC database (http://zinc5.docking.org), which allows the downloading of structures from a variety of vendors as SDF files, was used in this screen. Using

* Corresponding author. Tel.: +1 3303256657; fax: +1 3303255936.
E-mail address: rcarroll@neoucom.edu (R.T. Carroll).

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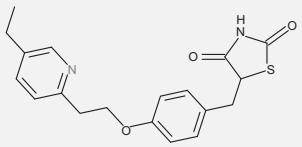
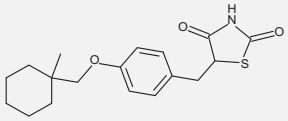
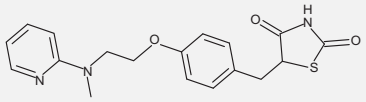
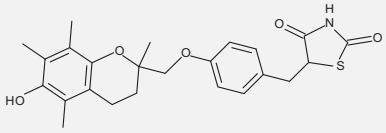
Table 1
Inhibition of MAO-B by selected PPAR-γ inhibitors

Compound	Name	Structure	MAO-B inhibition IC_{50} (μM)
1	Pioglitazone		0.298
2	Ciglitazone		0.225
3	Rosiglitazone		0.832
4	Troglitazone		2.07

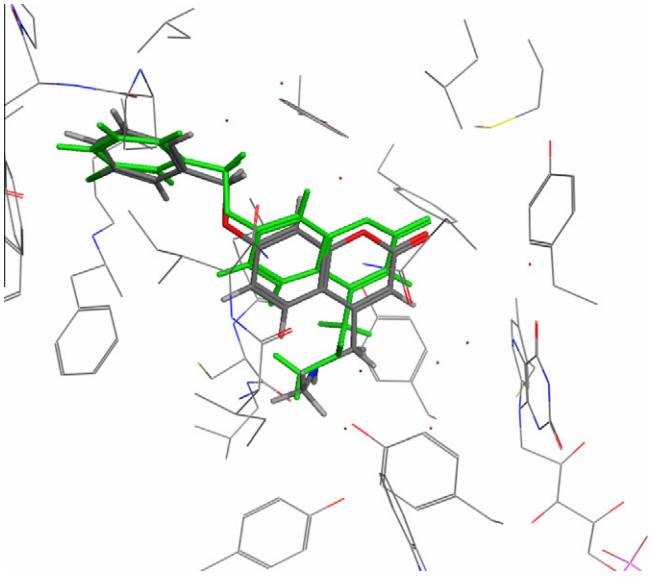


Figure 3. Redocking of the MAO-B inhibitor in the protein crystal structure 2V61.pdb, 7-(3-chlorobenzyloxy)-4-(methylamino)methyl-coumarin showing a low RMSD overlay.

MOE 2009.10 (www.chemcomp.com), the databases were washed/desalted, and the 3D structure of each compound was built using the MMFF94x force field. These steps were done using the MOE nodes (Fig. 2) in the pipeline program KNIME 2.1.1 (http://www.knime.org/). As a first step, we extracted all TZD-based compounds from the databases using 2D SMARTS strings. Those compounds

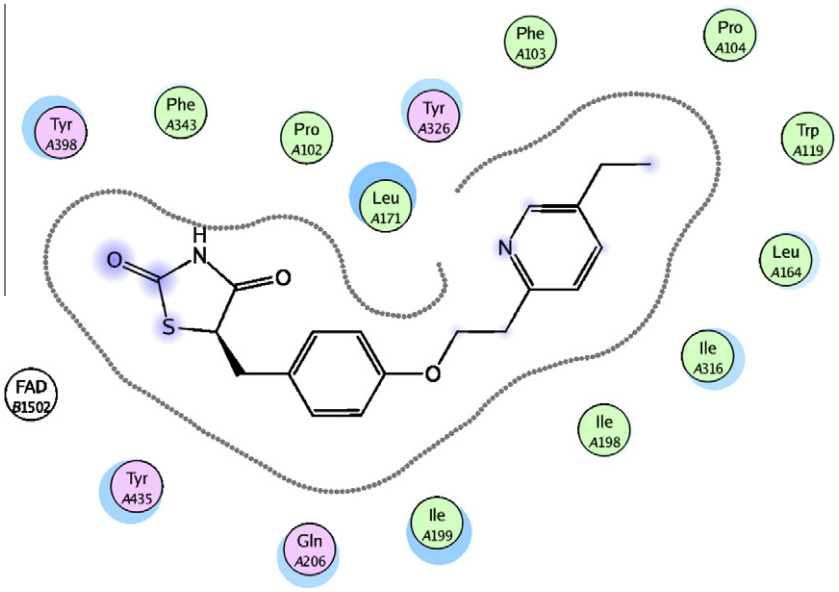


Figure 1. Docking of R-pioglitazone in the substrate cavity of MAO-B (2V57.pdb).

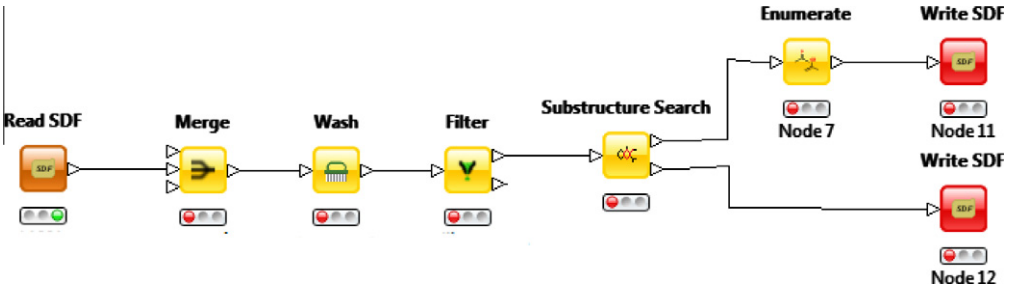
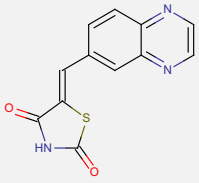
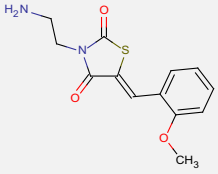
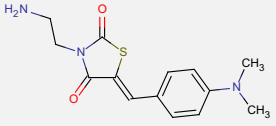
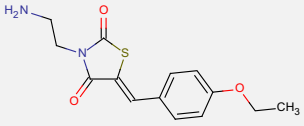
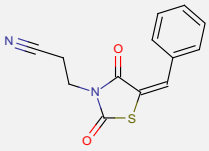
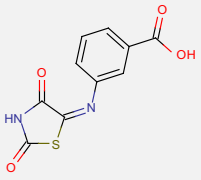
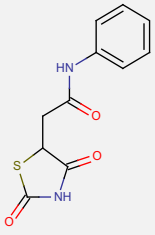
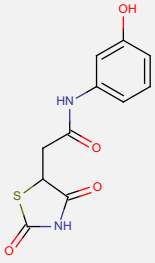


Figure 2. Pipeline of MOE nodes used in KNIME for database preparation for the virtual screening.

Table 2
Compounds identified by virtual screening

Compound	Name	Structure	MAO-B inhibition IC_{50} (μM)
5	AS605240		13
6	L136468		27
7	L136662		0.195
8	A6355		0.082
9	R598119		2.29
10	R598127		56
11	6163851		22
12	6164872		11

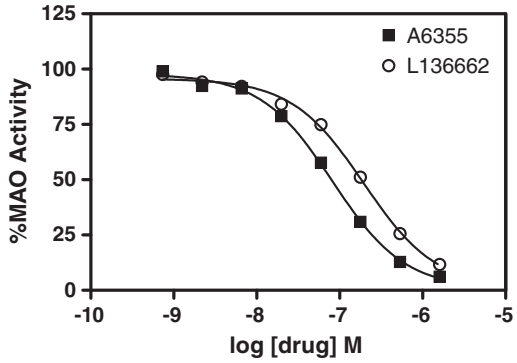


Figure 4. Recombinant human MAO-B inhibition by different TZD compounds.

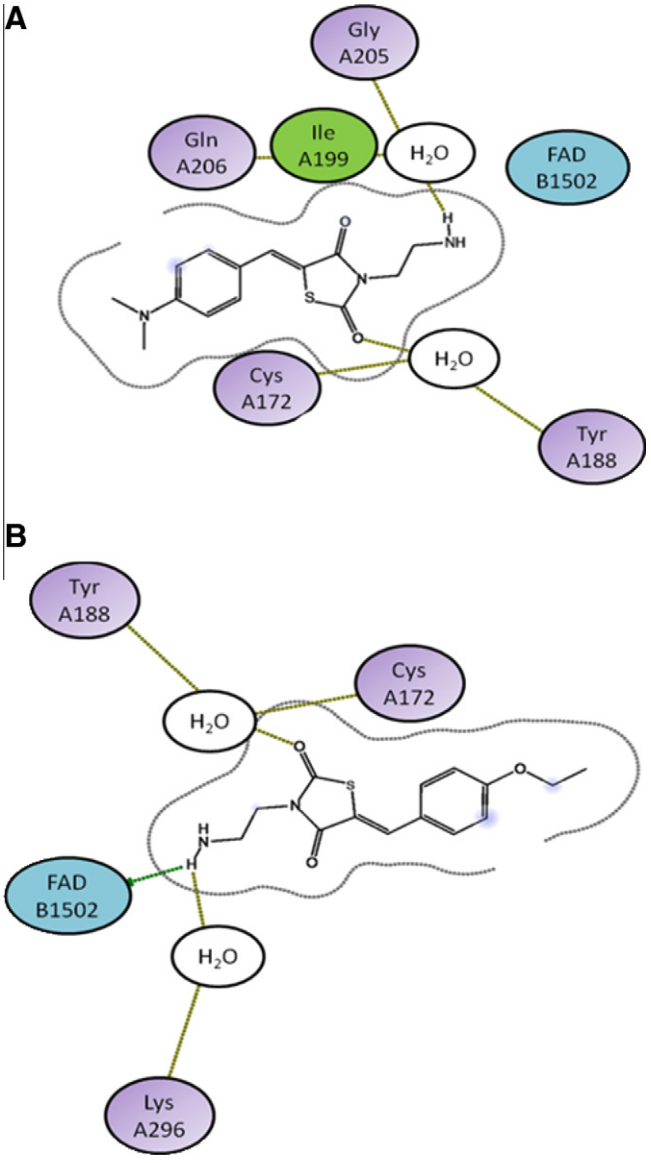


Figure 5. Binding modes of compounds **7** and **8** identified from the virtual screening workflow. Compound **7** (5.A; 2D map of amino acid interactions) and compound **8** (5.B; 2D map of amino acid interactions) are shown interacting with a water molecule in the binding cavity of MAO-B.

meeting these requirements were then used in the structure-based virtual screening.

For the final structure-based docking studies, we evaluated several available X-ray crystal structures of MAO-B from the Protein Data Bank website (1OJ9; 1GOS; 2V60; 2V61; 2V5Z). To determine which crystal structure to use for the virtual screen, we evaluated the ability of MOE-Dock implemented in MOE 2009.10 to re-dock the crystal structure with the co-crystallized ligand for each MAO-B structure. Each protein structure was protonated at pH 7.0 and the water molecules inside the substrate cavity of the enzyme were retained. The best docking poses returned from these were superimposed on the original crystal structure co-ligands and the root-mean-square deviation (RMSD) was calculated for each docking run. We found 2V61 to have the lowest RMSD value (RMSD <1 Å) and therefore utilized its characteristics for the docking studies (Fig. 3).

A second test to validate the virtual screening workflow was performed using a set of active compounds taken from the literature as well as a set of decoy compounds obtained from Schrodinger (www.schrodinger.com). The ability of the virtual screening workflow to separate active compounds from decoys was evaluated. MOE-Dock was able to select for the active compounds compared to the decoy set as demonstrated by its ability to score active compounds higher than non-active compounds analyzed by the docking experiment. When the number of active compounds found were expressed as % of total database screened (i.e., active + inactive), the active compounds could be found in the top 10% of the list. Table 2 shows the compounds which were scored near the top of the list using virtual screening. Compounds **5**–**10** were purchased from Sigma–Aldrich, while **11**–**12** were purchased from ChemBridge (www.hit2lead.com). To test the compounds' ability to inhibit MAO-B, we used the Invitrogen fluorescent Amplex Red detection kit as described by the manufacturer (www.Invitrogen.com), while using the recombinant human MAO-B enzyme (www.bdbiosciences.com) in a 96-well plate format (final concentration of MAO-B was 0.02 mg/ml), after 15 min incubation. The IC_{50} of pioglitazone was 0.298 μM (Fig. 4 and Table 1), which was similar to that reported by Quinn et al.[11] The inhibition of MAO-B activity by **5** was found to have an IC_{50} of 13 μM. This compound is also a known potent and selective PI3Kγ inhibitor.[14] The most potent compound screened was **8** which had an IC_{50} value of 82 nM (Fig. 4). Interestingly, **8** competes with ATP binding to ERK kinase with an IC_{50} of 25 μM in HeLa cells.[15] In addition, a second nanomolar inhibitor **7**, had an IC_{50} of 195 nM (Fig. 4).

Docking studies on compounds **7** and **8** revealed that these compounds tend to bind with the TZD-moiety oriented towards the FAD, similar to what was seen with pioglitazone (Fig. 1). However, the potency of this scaffold was increased over 100-fold when an ethylamine group was attached to the TZD-nitrogen. Fig. 5 shows the binding modes for compounds **7** and **8**, using the 2D interaction plot of the ligand with specific amino acids. As illustrated in Fig. 5, the preferred docked binding pose of **7** shows H-bond interactions with the TZD-*N*-ethylamine group forming a bridge to GLN-206 through a water molecule. Similarly, the preferred docked binding pose of **8** (Fig. 5) also formed a bridge with the TZD-N-ethylamine group to LYS-296 and the FAD cofactor through a water molecule. The phenyl ring also appears to favor substitutions in the *para*-position (cf. **8** to **6**). When the *p*-ethoxy substituent on **8** (82 nM) was compared to the *m*-methoxy substituent on **6** (27 μM), a significant loss of potency was observed. This illustrates a possible restrictiveness of the binding pocket at this location.

In conclusion, we have identified a novel scaffold which can be used for the inhibition of the human MAO-B enzyme. The compounds analyzed here could serve as lead compounds for medicinal chemistry programs whereby further structure–activity relationships can be evaluated. As already observed with the TZD Type II diabetes drugs, it is anticipated that these compounds will also be active as neuroprotective agents.

Acknowledgments

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