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Discovery of D-amino acid oxidase inhibitors based on virtual screening against the lid-open enzyme conformation

Bence Szilágyi [a], Žiga Skok [b], Anita Rácz [c], Rok Frlan [b], György G. Ferenczy [a], Janez Ilaš [b], György M. Keserű [a,*]

[a] Medicinal Chemistry Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok krt. 2. H-1117 Budapest, Hungary
[b] Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia
[c] Plasma Chemistry Research Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Magyar tudósok krt. 2, H-1117 Budapest, Hungary

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ABSTRACT

D-Amino acid oxidase (DAAO) inhibitors are typically small polar compounds with often suboptimal pharmacokinetic properties. Features of the native binding site limit the operational freedom of further medicinal chemistry efforts. We therefore initiated a structure based virtual screening campaign based on the X-ray structures of DAAO complexes where larger ligands shifted the loop (lid opening) covering the native binding site. The virtual screening of our in-house collection followed by the in vitro test of the best ranked compounds led to the identification of a new scaffold with micromolar IC_{50}. Subsequent SAR explorations enabled us to identify submicromolar inhibitors. Docking studies supported by in vitro activity measurements suggest that compounds bind to the active site with a salt-bridge characteristic to DAAO inhibitor binding. In addition, displacement of and interaction with the loop covering the active site contributes significantly to the activity of the most potent compounds.

Introduction

D-Amino acid oxidase (DAAO) metabolizes D-serine, a co-agonist at the glycine site on the *N*-methyl D-aspartate (NMDA) receptor.[1] Several pieces of evidence suggest that increased DAAO activity[2] and decreased D-serine level[3,4] are associated with schizophrenia. Therefore, it has been suggested that the inhibition of DAAO may result in an increase of brain D-serine level and may have beneficial effect on the positive, negative and cognitive symptoms of schizophrenia.[5]

Several DAAO inhibitors have been reported in the literature. Representative examples are shown in Fig. 1. Most of the known inhibitors are small and polar (Fig. 1a) similarly to the endogenous ligands, D-amino acids. It is notable that DAAO works optimally around pH 8[6] and the bound amino acids are deprotonated and bear a negative charge rather than being zwitter ionic.[7] In line with this finding, DAAO inhibitors are deprotonable and a significant amount of negatively charged microspecies is present around pH = 8.[8]

The important binding motifs of DAAO inhibitors include a negatively charged moiety where two heteroatoms of the ligand form a salt bridge with Arg283, and a planar, electron rich moiety positioned parallel with the isoalloxazine ring of the FAD cofactor. Further interactions contributing to the binding of some of the ligands are hydrogen bonds to the backbone of Gly313 and to the sidechain of Tyr228 and stacking interactions with Tyr224. It is worth noting that the binding pocket is highly polar at the Arg283 side while it is predominantly hydrophobic at the opposite side (Fig. 2*a*).

The small inhibitors bound to DAAO (Fig. 1a) are completely buried in the protein binding site with no access to the bulk water. This is thought to be the consequence of the switch of loop 216–228 between an open conformation that allows the ligand to enter and to leave the binding site, and a closed conformation adopted in the complex.[9]

X-ray structures with a lid-open conformation were reported[10] with extended compounds **6** and **7** (Fig. 1b). The polar head of these compounds (carboxylate in **6** and the hydroxy-pyridazinone in **7**) binds to Arg283, just as it was seen for small inhibitors. However, the annelated rings form additional van der Waals and polar interactions and the connected aromatic ring opens the lid forming non-polar interactions with the backbone and the hydrophobic residues of the loop (Fig. 2b).

As it has been demonstrated above the size of DAAO inhibitors spans a large range. They include fragment sized polar compounds most resemble to the endogenous ligands and larger lead-like compounds whose binding is possible owing to the plasticity of loop 216–228. This loop serves as a lid that is closed when the bound ligand is small and adopts an open conformation when the binding

* Corresponding author.
E-mail address: keseru.gyorgy@ttk.mta.hu (G.M. Keserű).

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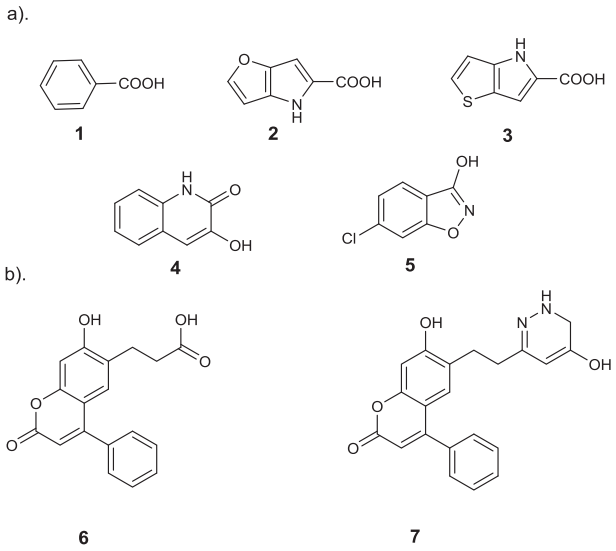

Fig. 1. Representative set of small (a) and extended (b) DAAO inhibitors.

site accommodates large ligands. The ability of large compounds to bind and inhibit DAAO offers an opportunity to drug discovery efforts to identify inhibitors with balanced properties. The polar head group that provides strong binding primarily by interacting with Arg283 and with some other polar groups (*cf.* Fig. 2) can be grown to include various chemical groups allowing the fine-tuning of the overall molecular properties. This recognition motivated our work to search for lead-like DAAO inhibitors that bind to the lid-open conformation of the enzyme. Our work started with structure-based virtual screening of our in-house compound library against the lid-open conformation of DAAO.

To find novel structural classes in-house compound library[11–13] of over 2000 unique compounds at the Faculty of Pharmacy, University of Ljubljana was used for virtual screening. Compounds were docked into three DAAO structures. Two were taken from complexes of open-lid proteins (PDB: 4QFC[10] and 4QFD[10]). These two structures have different sequences with missing residues and they were used to create a complete structure that were used as the third target for docking. Protein and ligand preparations were performed with Schrödinger's tools[14] with standard settings and Glide[14] was used for docking and scoring.

Hits were selected using Glide's docking score. Docked poses of top scored compounds were visually inspected and the best 16 compounds were subject to in vitro testing with D-kynurenine assay.[15] This procedure led to the identification of compound **8** (Table 1) as a micromolar DAAO inhibitor. The docked pose of **8** and its interactions are shown in Fig. 3. Docking suggests that the deprotonated carboxylic acid interacts with Arg283 forming a salt-bridge as it is observed in the X-ray structure of several complexes (see above). There is not enough space for the pyrrole-carboxamide moiety to be in the plane of the carboxylate group, parallel with the isoalloxazine ring of FAD, and it leans towards loop 216–228. An interesting feature of the docked compounds is that their carboxamide moiety between the pyrrole rings adopts cis conformation.

We performed an SAR study based on the newly identified DAAO inhibitor, compound **8**, with two objectives; first, to understand the structural requirements of lid opening, and second, to explore the interaction network around the inhibitor scaffold.

Compounds **20**–**28** were synthesized to explore how the interaction with the lid contributes to the activity. These compounds either have reduced size presumably not interacting with the lid, or they have varied substitution pattern on the terminal pyrrole ring. As shown in Scheme 1, compounds **20**–**28** were obtained from **9**.[16] Reduction of **9** followed by acylation with the corresponding acids or acid chlorides resulted **11**–**19**. These compounds were hydrolyzed in a mixture of dioxane and 1 M sodium hydroxide solution resulting **20**–**27**.

In order to explore the impact of the polar interaction network, specifically the role of the NH groups, methyl groups were added to the two amide N-atoms and also to the middle pyrrole ring N-atom. N-methylation affects the H-bonding ability of the compounds, the space they fill and also their preferred conformation. For the preparation (Scheme 2) of these N-methylated compounds (**36**, **39**, **40**), **29**[17] was converted to **31** through hydrogenation, followed by acylation with 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one. To get **36**, compound **31** was hydrolyzed to carboxylic acid **32** than **34** was formed with sarcosine methyl ester hydrochloride and HATU. Compound **36** was obtained from **34**

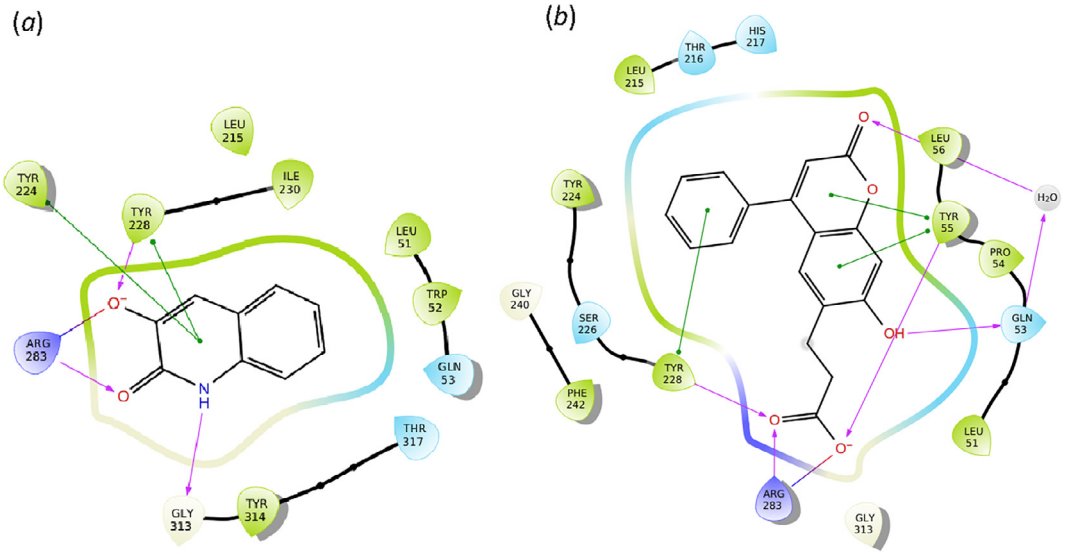

Fig. 2. Key protein-ligand interactions identified for DAAO inhibitors. Small (*a*) and extended (*b*) inhibitors are exemplified by compounds **4** (PDB 3G3E) and **6** (PDB 4QFC), respectively. Ligand carbon atoms are in black, protein amino-acids are shown in colored drops. Interactions are indicated by lines: magenta H-bond, green aromatic-aromatic interaction.