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Insights into the binding mode of new N-substituted pyrazoline derivatives to MAO-A: docking and quantum chemical calculations

Safiye Sağ Erdem · Seyhan Türkkan · Kemal Yelekçi · Nesrin Gökhan-Kelekçi

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Abstract The binding modes of four N-substituted pyrazoline derivatives as novel MAO-A inhibitory agents were investigated using docking and quantum chemical molecular modelling tools.

Keywords N-substituted pyrazolines · Docking · PM6

Introduction

Monoamine oxidase (MAO) is a flavoprotein located in the outer membrane of the mitochondria that contains a covalently bound flavin adenine dinucleotide (FAD) as a coenzyme and that has considerable physiological and pharmacological interest due to its central role in the metabolism of monoamine neurotransmitters. MAO exists in two isoforms, MAO-A and MAO-B, which share approximately 70 % sequence identity on the amino acid levels and differ in their substrate specificity, susceptibility to specific inhibitors, and three-dimensional structure (Binda et al. 2004; Edmondson et al. 2004). Since they metabolize the principal biogenic amines, MAO-A and MAO-B play an important role in the regulation of their concentrations mainly in the central nervous system, where abnormal values have been involved in psychiatric and neurodegenerative disorders such as, depression, Alzheimer's disease, and Parkinson's disease (Shih 2004; Riederer et al. 2004). Selective inhibition of MAO-A results in elevated noradrenaline and serotonin concentrations, thus gradually improving the symptoms of depression. On the other hand, inhibition of MAO-B is a crucial strategy for treatment of Parkinson disease (Li et al. 2006). Indeed, treatment of pre-Parkinson's patients with selective MAO-B inhibitors has been shown to be effective in reducing the development of this neurodegeneration. All these findings support the clinical importance of MAO inhibitors in the treatment of several neurological and psychiatric disorders. In the light of these knowledge and the previous findings (Jayaprakash et al. 2008; Gökhan-Kelekçi et al. 2007), we synthesized N-substituted pyrazoline derivatives (Fig. 1) as novel potential MAO inhibitory agents which were found to be selective to MAO-A. Continuing our efforts in computational studies (Erdem and Yelekçi 2001; Toprakçi and Yelekçi 2005; Erdem et al. 2006; Akyüz et al. 2007; Yelekçi et al. 2007; Erdem and Büyükmenekşe 2011), we aimed to present molecular insights into the binding modes of these compounds in the active site of MAO-A through the use of molecular modelling tools. Ultimate aim of this study is to contribute to the design and development of more effective and selective inhibitors than the ones presently involved into clinical studies such as rasagiline and selegiline.

Materials and methods

The MAO-A crystal structure was obtained from protein data bank, code 2Z5X (Son et al. 2008). The ADT (Auto Dock Tools) package (Michel and Saner 1999) was

S. S. Erdem (✉) · S. Türkkan
Department of Chemistry, Faculty of Arts and Sciences, Marmara University, 34722 Göztepe, Istanbul, Turkey
e-mail: erdem@marmara.edu.tr

K. Yelekçi
Bioinformatics and Genetics Department, Faculty of Engineering and Natural Sciences, Kadir Has University, 34083 Fatih, Istanbul, Turkey

N. Gökhan-Kelekçi
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100 Sıhhiye, Ankara, Turkey

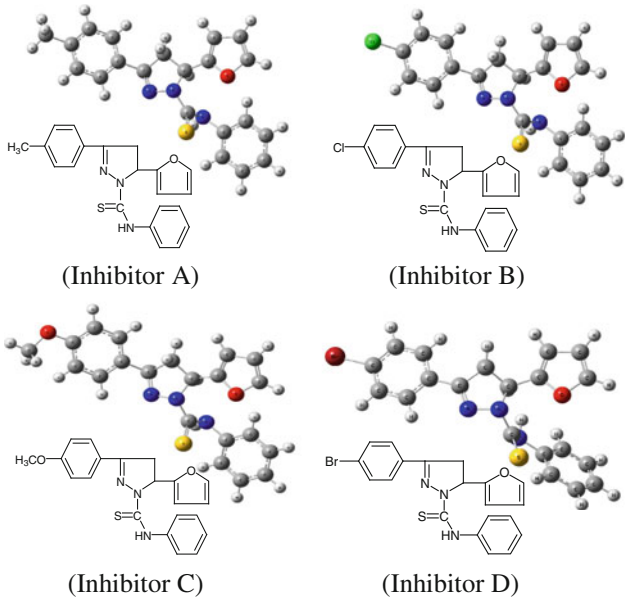

Fig. 1 Structures of the inhibitors studied

employed to generate the docking input files of the protein and four inhibitors. Hydrogen atoms were added to the crystal structure, and partial atomic charges were calculated via the Gasteiger–Marsili method (Gasteiger and Marsili 1980).

Prior to docking, the system was subjected to 10 ns of molecular dynamic simulation at 310 K using NAMD v2.6. (Phillips et al. 2005). The docking procedure utilized in previous studies (Toprakçi and Yelekçi 2005; Yelekçi et al. 2007) was used. However, some differences in the procedure are as follows: AutoDock 4.2 (Morris et al. 2009) was employed to perform the docking simulation using a Lamarckian genetic algorithm (Morris et al. 1998; Huey et al. 2007). A grid of 70, 70, 70 points in x, y, and z directions was built on the center of mass of the N5 atom of the flavin. The structure with the most favorable free energy of binding was selected and analyzed using the Accelrys Software 3.1 (Discovery Studio Modeling Environment 2011).

The resultant docked structures were analyzed using the Gaussview 5.0 program. 23 amino acids in the active site surrounding the inhibitor were selected and the remaining amino acids were removed from the structure. 14 water molecules were then inserted into the truncated structure with the same coordinates as in the X-ray structure. The resultant structure consisted of about 550 atoms. Three methyl carbons of FAD and two backbone carbons of side chains were frozen to prevent unnatural changes in the structure prior to optimization. Geometry optimization was performed employing semi-empirical PM6 method (Rezac et al. 2009) in Gaussian 09 (Frisch et al. 2009).

Results and discussion

The inhibition constants, K_i, calculated from docking are 6.9, 8.5, 5.4, and 4.3 nM for inhibitors A, B, C, and D, respectively, and are in good agreement with the experimental results (manuscript in preparation). Tight binding interactions with MAO-A enzyme are expected since these compounds have highly potent K_i values. All four compounds bind to the *re*-face of FAD in the active site of MAO-A (Fig. 2). A common feature of their binding mode is the packing of the furan moiety between the phenolic side chains of Tyr407 and Tyr444 so that binding can be enhanced through a favorable π–π stacking interaction. The distances between furan oxygen and Tyr444 are 4.00, 3.45, 3.87, and 3.66 Å for A, B, C, and D, respectively. Corresponding distances with Tyr407 are 4.02, 4.38, 4.14, and 3.73 Å for A, B, C, and D, respectively. The *p*-substituents of the phenyl groups are situated near Lys305, facilitating attractive interactions in C and D (2.14 and 2.73 Å, respectively). Another common feature is the extension of the phenyl substituent of the thiosemicarbazide moiety towards Ileu180 and Asn181. NH hydrogen in thiosemicarbazide moiety acts as a hydrogen bond donor to the nearby side chains Ile180 in B (2.27 Å) and Asn181 in D (2.07 Å).

Moreover, additional hydrogen-bonding interactions are observed between hydroxyl hydrogen of Tyr444 and thiosemicarbazide sulfur atoms for A and C (2.46 and 2.59 Å, respectively).

Docking theories are known to be less sensitive than quantum mechanical methods in predicting electronic interactions. As a quantum mechanical semi-empirical method, the recently developed PM6 method (Rezac et al. 2009) was employed here, since it achieved major improvements in accuracy for the interaction energies of biologically relevant, non-covalently bound systems, with empirical corrections for dispersion and hydrogen-bonding interactions (Stewart 2007). The superposition of PM6-optimized structures with those of docked orientations is shown in Fig. 2. A key advantage of PM6 calculations is that they allow us to observe the interactions between water and active site protein residues as well as water and each inhibitor. Such information cannot be gained by docking calculations since water molecules are excluded from the enzyme.

Several discrepancies are noticeable in the PM6-optimized structures, mainly as the result of the interactions of water molecules. All four inhibitors show H-bonding type attractions between the sulfur atom in thiosemicarbazide and the hydrogen of the nearby water molecule. The interaction distances are predicted to be 2.75, 2.63, 2.65, and 2.68 Å for inhibitors A, B, C, and D, respectively. In C, *p*-OCH_3 hydrogen atoms exhibit non-bonded interactions

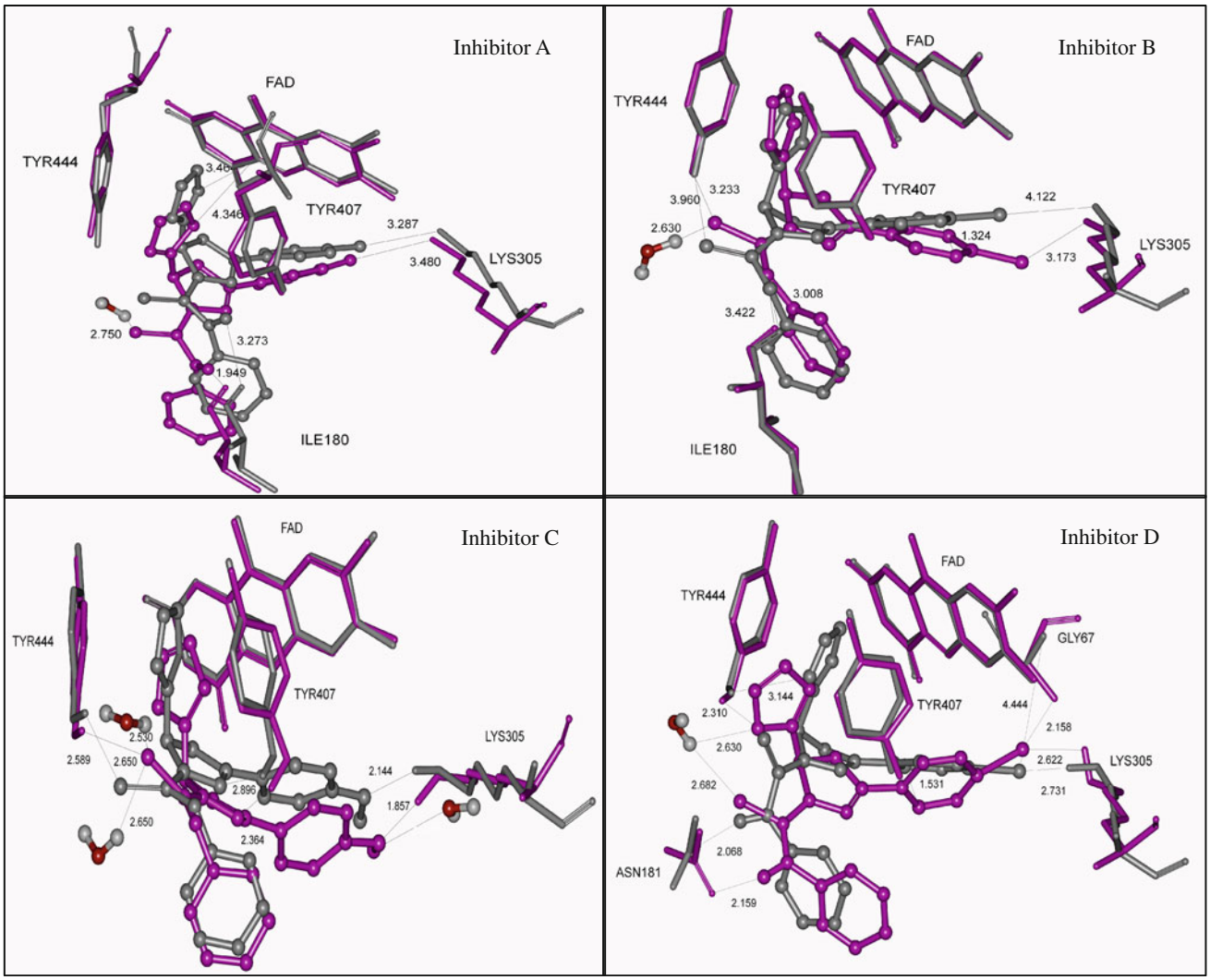

Fig. 2 Binding orientation of inhibitors in MAO-A (*grey*: docking, *purple*: PM6). Remaining side chains are not shown for clarity

with the oxygen of two different water molecules having distances of 2.53 and 2.15 Å.

The orientation of the furan moiety is slightly altered after PM6 optimization. In C, it adopts a nearly perpendicular conformation with respect to Tyr407 and Tyr4444, contrary to the alignment in A, B, and D in which it aligns in a parallel manner with the tyrosines. Only in inhibitor D does the furan oxygen point away from FAD and creates a H-bond with the nearby water molecule which is also H-bonded to the thiosemicarbazide sulfur atom. As a result, the furan ring is pushed slightly out of the aromatic cage facilitating an additional H-bond with Tyr444 hydroxyl hydrogen (2.31 Å), which is a very weak interaction in the docked structure (3.13 Å).

Similarly, some other binding modes appear to be stronger, which is evident from the shorter interaction distances in PM6-optimized structures. The H-bond distance to Lys305 shortens from 2.14 to 1.86 Å in C, and from 2.73 to 2.62 Å in D.

Conclusion

Several discrepancies were observed between the docked pose of the compounds and the optimized pose obtained from the quantum chemical PM6 method, which presents more realistic interactions. A general feature of the binding mode in both methods is that the furan moiety of the inhibitors aligns in between the aromatic cage tyrosine residues (Tyr407 and Tyr444) and interacts either in π–π stacking interactions or H-bonding interactions with the hydroxyl group of tyrosines. Interactions with active site water molecules provided additional insights into the binding modes of the inhibitors studied. Considering the

calculated K_i values (4.28–8.51 nM) and strong binding interactions, these compounds appear to be promising MAO-A inhibitors.

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