

Chapter 7

Conclusion

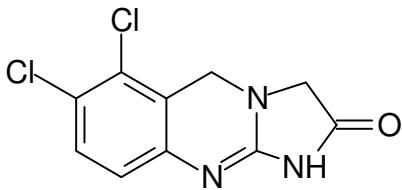
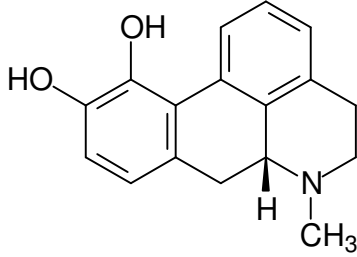
The present study attempted to discover inhibitors of the MAO type A and B enzymes by using a molecular modelling approach. The focus was on identifying MAO inhibitors among a database of FDA approved drugs. For this purpose a virtual database of approved drugs were screened for potential binding to the MAOs using structure-based pharmacophore models. These models were constructed by using the reported crystallographic structures of MAO-A and MAO-B. Drugs that are found to act as MAO inhibitors may be repurposed for the treatment of neuropsychiatric disorders such as depression and Parkinson's disease. Since approved drugs are already used in human subjects it is a relatively simple and more cost effective to re-register them as MAO inhibitors. For these drugs only clinical efficacy has to be proven and preclinical and clinical development is not required. From a toxicological point of view it is also of value to identify approved drugs that may act as inhibitors of MAO-A and MAO-B. MAO-A inhibitors in particular may lead to serious adverse effects such as the cheese reaction and serotonin toxicity. The finding that certain approved drugs may act as MAO-A inhibitors will alert to potentially serious adverse effects that may occur with the use of such drugs.

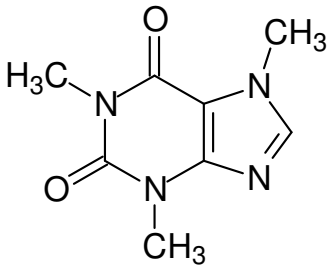
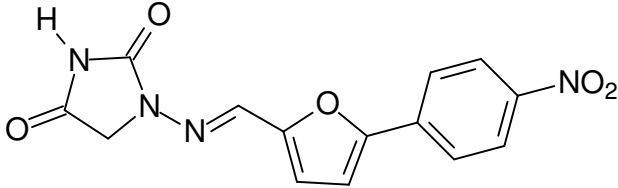
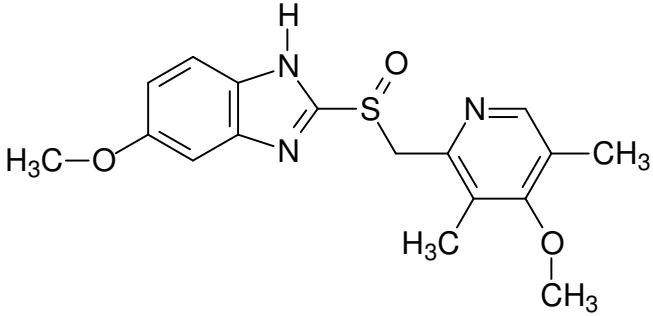
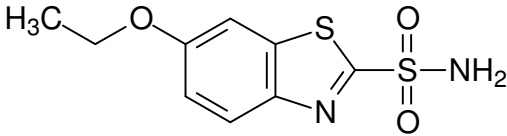
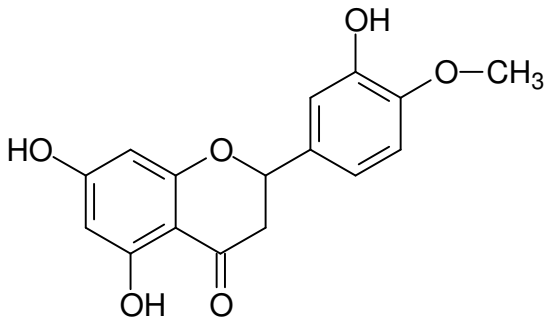
The pharmacophore models were constructed using the structure-based approach. For this purpose, the X-ray crystal structures of human MAO-A co-crystallized with harmine and human MAO-B co-crystallized with safinamide was used. All calculations were carried out with Discovery Studio[®]. The pharmacophore models are representative of potential interactions between the enzyme active site and an inhibitor. In addition, the general shapes of the active sites were approximated with a shape feature, which restricts the size of the compounds that map to the pharmacophore models. Test sets, consisting of known MAO inhibitors and compounds known not to inhibit the MAOs were used to determine the accuracy of the models. The results demonstrated that the pharmacophore models possessed reasonable abilities to distinguish between inhibitors and compounds which are not inhibitors.

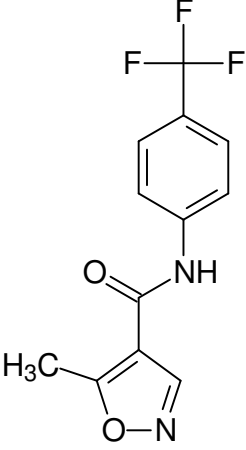
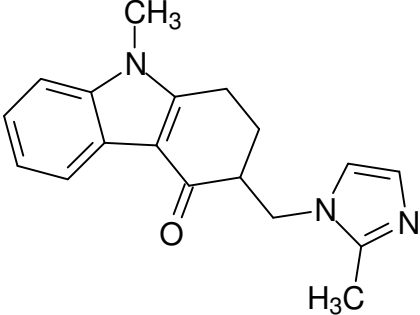
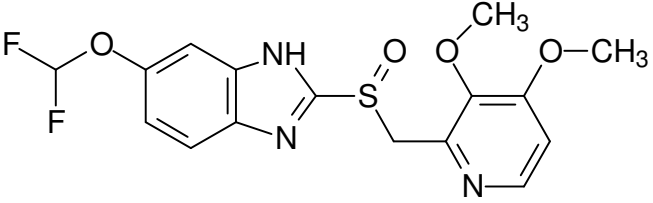
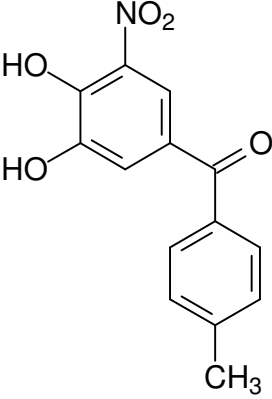
The MAO-A and MAO-B pharmacophore models were thus used to screen a library of FDA-approved drugs and 45 compounds were found to map to the MAO-A and MAO-B pharmacophore models. These hits represent the drugs which mapped to four pharmacophore features, of which the shape feature is one feature. Among the hits, 29 compounds were further investigated by determining their inhibitory activities towards MAO-A and MAO-B. For this purpose, IC_{50} values were determined for the test compounds.

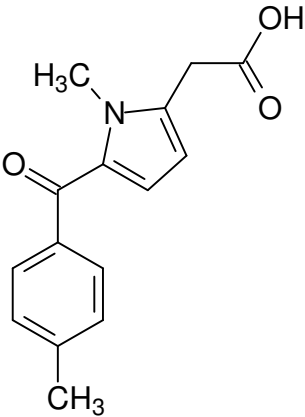
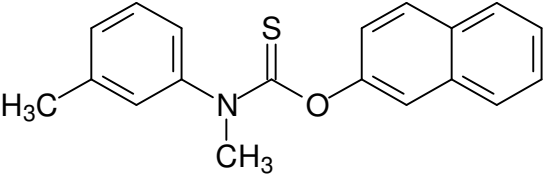
From the IC_{50} values it was evident that 13 of the 29 test compounds (which mapped to the pharmacophore model) possessed MAO inhibitory activities. The structures of these compounds are given in table 7.1. Although 16 of the test compounds did not possess significant MAO inhibitory properties, the approach followed in this dissertation may still be viewed as successful. When considering that the virtual library of FDA-approved drugs consists of approximately 1200 compounds, finding 13 active compounds among 29 selected hits may be deemed as a success. Employing structure-based pharmacophore models may thus be recommended as an approach to identify MAO-A and MAO-B inhibitors among a library of compounds.

Table 7.1. The structures of the compounds which possessed inhibitory activity towards MAO-A and/or MAO-B.

Anagrelide	
Apomorphine	

Caffeine	
Dantrolene	
Esomeprazole	
Ethoxzolamide	
Hesperetin	

Leflunomide	 <chem>CC1=CN(O1)C(=O)Nc2ccc(C(F)(F)F)cc2</chem>
Ondansetron	 <chem>CC1=CN(C1Cc2c(c3ccccc3n2C)C(=O)C4CCCCC4CN5C=CC(=C5)C</chem>
Pantoprazole	 <chem>COc1cc(OC)c(CS(=O)(=O)c2nc3cc(OC(F)F)ccc3[nH]2)cc1</chem>
Tolcapone	 <chem>CC1=CC=C(C=C1)C(=O)c2cc(O)c(O)c([N+](=O)[O-])c2</chem>

Tolmetin	
Tolnaftate	

Of the 13 compounds that showed *in vitro* inhibitory activity towards MAO-A and MAO-B, 3 compounds were selected for further investigation. These compounds are caffeine, esomeprazole and leflunomide. For this purpose, the reversibility of inhibition of MAO-A and MAO-B by caffeine and esomeprazole was tested. For caffeine, esomeprazole and leflunomide, possible binding modes within the MAO-A and MAO-B active sites were also examined using molecular docking.

Although caffeine is not a potent MAO-A and MAO-B inhibitor, it was selected for further investigation based on the fact that caffeine is of great dietary importance. The results documented that caffeine is an inhibitor of both MAO-A and MAO-B with IC_{50} values of 0.761 μ M and 5.08 μ M, respectively. The reversibility of MAO-A and MAO-B inhibition by caffeine was investigated by measuring the recoveries of enzyme activities after dialysis of enzyme-inhibitor mixtures. After dialysis, the enzyme recovered to levels of 97% for MAO-A and 96% for MAO-B. This behaviour is consistent with a reversible interaction between the MAO enzymes and caffeine. The modes of MAO-A and MAO-B inhibition by caffeine was investigated by constructing a set of Lineweaver–Burk plots

for the inhibition of MAO-A and MAO-B. The results show that the Lineweaver–Burk plots are linear and intersect at a single point on the y-axis. This suggests that caffeine most likely interacts competitively with both MAO isozymes. Modeling suggests that, in the MAO-A active site, caffeine undergoes several productive interactions such as π - π and hydrophobic interactions. Caffeine also forms a non-productive interaction with Asn181, which may explain the relatively low inhibition potency of caffeine towards MAO-A. In the MAO-B active site, caffeine undergoes a non-productive hydrogen bonding interaction with Tyr326. This interaction may explain the relatively low inhibition potency of caffeine towards MAO-B. An analysis of the MAO inhibitory data suggests that, at doses achieved by normal human consumption, the MAO inhibitory potencies of caffeine are unlikely to be of pharmacological relevance in humans.

Among the compounds tested, esomeprazole represents a particularly interesting MAO-A and MAO-B inhibitor. Esomeprazole is an inhibitor of both MAO-A and MAO-B with IC_{50} values of 23.2 μ M and 48.3 μ M, respectively. The reversibility of the interaction of esomeprazole with MAO-A and MAO-B was investigated by evaluating the recovery of the enzymatic activity after dilution of the enzyme-inhibitor complexes. The results showed that, after diluting the MAO-esomeprazole complexes to concentrations equal to $0.1 \times IC_{50}$, the MAO-A and MAO-B activities were recovered to levels of 94% and 87%, respectively. In addition, MAO-A and MAO-B inhibition by esomeprazole is almost completely reversed after dialysis, with the MAO-A and MAO-B activities recovering to levels of 93% and 88%, respectively. This behaviour is consistent with a reversible interaction of esomeprazole with MAO-A and MAO-B. Lineweaver–Burk plots for the inhibition of both MAO-A and MAO-B by esomeprazole were found to be linear and these plots intersect at a single point. It may be concluded that esomeprazole most likely interacts competitively and therefore reversibly with both MAO enzymes. The results from the molecular docking studies showed that esomeprazole undergoes several non-productive interactions with the active site of MAO-A. The experimental results, however, show that esomeprazole is a MAO-A inhibitor. This result suggests that molecular docking may not be appropriate to determine if compounds bind to MAO-

A and confirm that an alternative approach such as pharmacophore screening, used in this study, may be more effective. In the MAO-B active site, esomeprazole undergoes several productive interactions such as π - π and hydrophobic interactions, which is in accordance with the observations of this dissertation that esomeprazole is a MAO-B inhibitor. Considering the available pharmacokinetic data and typical therapeutic doses of esomeprazole, the inhibitory potencies towards MAO-A and MAO-B are, however, unlikely to be of pharmacological relevance in humans.

Leflunomide was also found to be a good inhibitor of both MAO-A and MAO-B with IC_{50} values of 19.1 μ M and 13.7 μ M, respectively. Although leflunomide is a relatively good MAO inhibitor, the interactions of leflunomide with the MAO-A and MAO-B enzymes were not further examined. Leflunomide is a prodrug and has a very short half-life in plasma. It is therefore not probable that leflunomide will be a significant inhibitor of MAO *in vivo* in humans. The potential interactions of leflunomide with the active sites of MAO-A and MAO-B were, however, examined. Molecular docking showed that in the MAO-A active site, leflunomide undergoes hydrogen bonding with Cys323 and Tyr444. The interaction with Tyr444 represents a productive interaction while the interaction with Cys323 is non-productive. Significant hydrophobic and π - π interactions also occur. In the MAO-B active site, leflunomide undergoes several significant hydrophobic interactions. Based on the relatively good MAO-A and MAO-B inhibitory potencies of leflunomide, this compound may serve as a lead for future inhibitor design.

The results found in this study suggest that there may be an opportunity to discover new drugs with MAO-inhibitory potencies by screening a virtual library of existing drugs with the use of a structure-based pharmacophore model. The inhibitory potencies of the compounds discovered in this study may not be significant enough to reappropriate them for the treatment of diseases such as Parkinson's disease, but as mentioned above, structures such as leflunomide may serve as leads for the design of MAO inhibitors.

In conclusion, the results of this study suggest that the use of structure-based pharmacophore models to screen a virtual library for potential MAO inhibitors may be an

effective approach. Although highly potent MAO inhibitors, which may be repurposed as clinically used MAO inhibitors have not been found, this study provides proof of concept that this approach may be useful for the identification of new MAO inhibitors. This study therefore recommends that the construction of structure-based pharmacophore models of MAO-A and MAO-B be pursued further. In this respect additional virtual libraries, such as those of suppliers of small organic chemicals, can be screened with the models. Such a study may yield many new promising MAO inhibitors and leads for the design of new MAO inhibitors.