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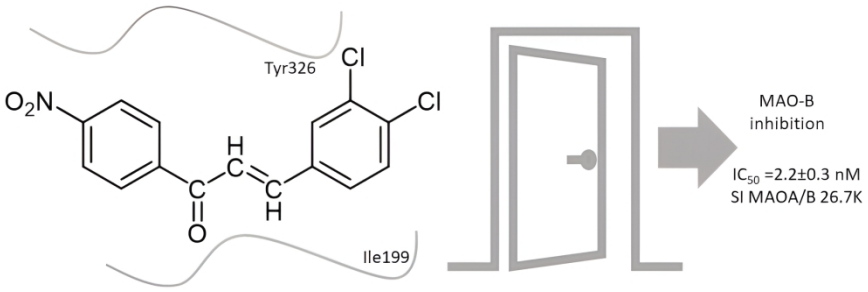

Graphical Abstract

216x121mm (300 x 300 DPI)

Exploring the 1-(4-nitrophenyl) -3-arylprop-2-en-1-one scaffold for the selective inhibition of monoamine oxidase B

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ABSTRACT A small library of 1-(4-nitrophenyl)-3-arylprop-2-en-1-one derivatives was synthesized to identify new human monoamine oxidase B selective inhibitors. Their inhibitory activity toward MAO-A and MAO-B isoforms was evaluated to determine potency and selectivity. All newly synthesized compounds were nanomolar inhibitors of the B isoform with IC_{50} concentrations ranging from 120 to 2.2 nM. Conversely, their activity towards the A isozyme was only observed at micromolar concentrations. Our results bear out the hypothesis that the 1,3-diarylpropenone scaffold could represent a valuable starting point for designing efficient and selective MAO B inhibitors.

KEYWORDS MAO-B selective inhibitors; 1,3-diarylpropenone; oxidative stress; neurodegeneration.

Monoamine oxidases (MAOs) are ubiquitous enzymes that catalyze the oxidative deamination of endogenous and exogenous amines [1, 2]. Dopamine (DA), norepinephrine (NE), serotonin (5HT), and phenylethylamine are common substrates. Hence, MAOs play an essential role in the metabolism of monoamine neurotransmitters and have been extensively investigated for their role in several neurological disorders [3-10]. The mammalian family of MAO comprises two isozymes, MAO-A and MAO-B. These two isoforms differ in substrates and inhibitors selectivity, being 5HT and NE preferential substrates of MAO-A, while MAO-B preferentially deaminates PEA and benzylamine. Moreover, although MAO-A and MAO-B exhibit a high sequence similarity, they present substantial differences in the shape and volume of the catalytic site, allowing for the design of selective inhibitors [11, 12]. MAO-A presents a single cavity with a volume of about ≈550 Å^3, while MAO-B has three functional domains named an "entrance cavity", "substrate pocket", and "aromatic cage"(Y398 and Y435). Altogether, these form the active site with a volume of approximately ≈700 Å^3 [13-15]. Not surprisingly, according to this structural information and to selectively target one or the other isoform, several selective inhibitors have been identified and investigated for their therapeutic applications for the treatment of depression and neurological diseases [5-7, 16, 17]. Noteworthy, the oxidative deamination catalyzed by MAOs produces hydrogen peroxide and other reactive species that are involved in neurodegeneration, such as in Alzheimer's disease (AD), Parkinson's disease (PD) and other neurodegenerative diseases (NDDs) [18]. Therefore, MAO inhibition could exert an indirect *in vivo* antioxidant activity at neuronal level[19-21]. In addition, overexpression of MAO-B and DA metabolism, as well as the formation of α-synuclein (α-Syn) aggregates, are observed in PD [22]. It has also been reported that α-Syn selectively binds MAO-B and increases its catalytic activity [23]. More recently, the key role of MAO-B in PD was further evidenced in animal models by Nakamura and co-workers [24]. Indeed, they observed that either inhibition or knock-down of MAO-B can reduce α-Syn intracellular accumulation. In addition, they observed that selegiline delayed the striatal formation of α-Syn aggregates, and mitigated loss of nigrostriatal dopaminergic neurons [24]. According to these findings, MAO-B selective inhibitors might not only increase the level of DA in nigrostriatal dopaminergic neurons but also exert a neuroprotective effect by reducing α-Syn aggregation and oxidative stress. Therefore, MAO-B inhibitors might be useful for ameliorating motor and non-motor symptoms, reducing "OFF" time, and preventing or delaying disease progression [25]. Our research group has already focused on the design of selective MAO-B inhibitors, and we have identified potent and selective agents through scaffold optimization from 3,5-diaryl dihydropyrazoles to 3,5-diaryl dihydroisoxazoles [26-38]. Based on our previous findings (Figure 1) and with the aim to further explore the structural requirements for the selective inhibition of MAO-B, we have synthesized a small library of 1-(4-nitrophenyl)-3-arylprop-2-en-1-one derivatives **3 a-g** and evaluated their activity and selectivity toward the two isozymes human MAO-A and MAO-B (*h*MAO-A and *h*MAO-B).

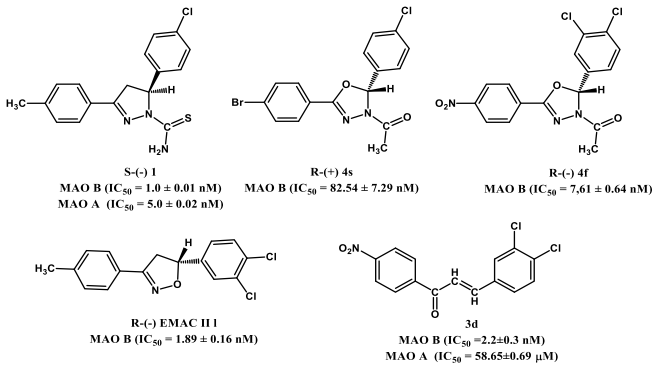
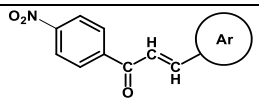


Figure 1. Comparison of previously reported MAO B inhibitors (S-(-) 1, R-(+) 4s, R-(-) 4f, and R-(-) EMAC II l), named as in the original paper, and compound 3d.[26, 27, 31, 38]

3 a-g derivatives could be considered as structurally simplified bioisosteres of previously investigated 3-acetyl-2,5-diaryl-2,3-dihydro-1,3,4-oxadiazoles [27, 31] and dihydro-isoxazole nucleus [26]. Our previous findings evidenced that the construction of an appropriately substituted five terms heterocycle, bearing two aryl substituents, could efficiently and selectively inhibit MAO-B isozyme. In this respect, and to further explore scaffolds and ligand geometries we have replaced the heterocyclic cores with a propenone moiety in which the two aryl substituents bind the position 1 and 3. As in the previously studied derivatives, the presence of a 4-nitrophenyl moiety remained a constant structural feature throughout the new derivatives series, to allow an easier comparison. Substituted phenyl moieties differing for steric hindrance and electronic distribution were introduced in position 3 of the propenone linker. Nevertheless, as a future development, replacements of the potentially toxic nitrophenyl group with bioisoteric moieties such as phenylcarboxylic acid, benzo-oxadiazole, and dihydro-benzofuranone will be explored.

Thus, compounds **3a-g** were synthesized, characterized, and submitted to biochemical evaluation to assess their activity and selectivity towards the MAO-A and MAO-B isozymes. All derivatives are MAO-B isozyme nanomolar inhibitors with IC_{50} values ranging from 120 to 2.2 nM. Contrarywise, their activity toward the MAO-A isoform is only observed at micromolar concentrations with IC_{50} values varying from 89 to 2.7 μM (Table 1).

Table 1. Inhibition data towards *h*MAO-A and -B of compounds 3 a-g



Comp.	Ar	*h*MAO-A (IC_{50} μM)	*h*MAO-B (IC_{50} nM)	S.I.*
3a	4-Cl-Ph	52±0.6[a]	10±0.02[a]	5,200
3b	2-CH_3-Ph	89±0.9[b]	120±7.9[b]	740
3c	4-F-Ph	7.8±0.12[c]	48±3.8[c]	160
3d	3,4-Cl-Ph	59±0.7[d]	2.2±0.3[a]	27,000
3e	4-OCH_3-Ph	27±4.7[e]	93±5.5[d]	290
3f	2-Naph	13±2.6[c,f]	21±3.4[a,e]	620
3g	4-CH_3-Ph	2.7±1.9[c]	13±2.5[a,f]	210
Selegiline		47±1.1[a]	30±1.8[e]	1,600

* Selectivity index (S.I.) = IC_{50}(*h*MAO-A)/IC_{50}(*h*MAO-B). Each value is the mean ± SD of three independent measurements (n =3). Different letters within the same column indicate statistically significant differences between compounds ($p < 0.0001$); one–way ANOVA followed by Tukey's Multiple Comparisons Test).

According to these results, we can affirm that all derivatives are selective MAO-B inhibitors with selectivity indexes (S.I. [IC_{50} (*h*MAO-A)] / [IC_{50} (*h*MAO-B)] ranging from 160, in the case of compound **3c**, to 27,000 for compound **3d**. The latter compound is the most potent and selective among all the tested derivatives indicating that the presence of a 3,4-dichlorophenyl moiety is highly beneficial for the potency similarly to what was observed for the heterocyclic core based analogues[27]. Conversely, the introduction of a 4-methoxyphenyl group led to a 42 folds reduction of the activity compared with the most potent derivative **3d**. Thus, the introduction of a strong electron donating group is not well tolerated in position 4 of the phenyl ring. By introducing a single chlorine atom in the same position, as for compound **3a**, only a moderate reduction of the potency (less than 5 folds) compared with **3d** was observed, indicating that this substitution is well tolerated. Moreover, the isosteric substitution of the chlorine atom with a methyl group in position 4 of the phenyl ring (**3g**) is also well tolerated, leading to an IC_{50} value statistically equal to that of the 4-chlorophenyl substituted derivative. According to this information, we can assume that the electronic density in the phenyl ring is most likely not the main actor for the activity and selectivity. On the other hand, the introduction of a non-polar substituent of an appropriate volume (Cl or CH_3), regardless of its electronic effect, in position 4 appeared as more determinant for the activity. Most probably, these substituents are optimal to interact with specific lipophilic residues of the enzyme. Moreover, we observed that the introduction of a proximal second non-polar substituent, as in the case of the 3,4-dichloro substituted compound **3d**, was able to maximize the ligand efficacy. This hypothesis is further corroborated by the fact that when a 4-fluorophenyl (**3c**) moiety is introduced a reduction of 22 folds of the potency was observed, with respect to **3d**. Probably, the fluorine atom is not sufficiently bulky to correctly interact with the enzyme lipophilic region, resulting in a decrease of ligand potency. The introduction of a methyl moiety in the position 2 of the phenyl ring (**3b**) leads to a reduction of the potency, with respect to the most potent compound of about 55 folds, probably due to its intramolecular steric effect that might limit the conformational freedom of the ligand. Surprisingly, the introduction of the bulky 2-naphtyl only led to a moderate reduction of the activity (less than 10 folds). All together this information indicates that both size and polarity of the substituents on the 3-aryl moiety can play a role in determining the activity of the compound. Indeed, the orientation of the aromatic moiety appeared to be relevant in determining the activity of these compounds, indicating that this portion must retain the freedom to correctly orientate within the binding pocket. However, considering the potential toxicity associated with the nitro group, QikProp software was employed to perform in silico ADMET [39] predictions to evaluate the drug-likeness of compound **3d**. The predicted values are provided in the Supplementary Information (SI, Table S3 and S4). All values fall within acceptable ranges, except for the electron affinity, which is borderline. Overall, the compound exhibits a favorable predicted drug-like profile. The predicted heart toxicity warrants further investigation but given the compound's IC_{50} in the nanomolar range, this is not likely to create a significant issue. The compound also shows limited predicted ability to cross the blood-brain barrier (BBB). This should be considered during the optimization process, while an appropriate delivery formulation could mitigate this limitation.

Therefore, to further rationalize these structure activity observations, molecular docking and post-docking energy optimization experiments were carried out considering the most promising compound **3d**.

The docking protocol has been validated by running re-docking and cross-docking studies considering the following reversible ligand-MAO-B complexes available in the PDB[40]: 1OJ9[13], 2C67[41], 1OJA[42], 2BK3[43], 2V5Z[44], 2V60[44], 2V61[44], 6FW0[45].

Among all the examined protocols (see the methods part) the best was the QPDL-SP[46], likely due to the incorporation of QM treatment which helped achieve better RMSD. Indeed, the described

settings could reproduce the experimental binding modes of considered co-crystallized ligands with an RMSD value below 2.5 Å.

The QPLD[46] protocol is a very accurate docking method that combines the quantum mechanical (QM) approach in the binding region of the ligand and in the pocket binding, while the rest is treated according to the molecular mechanic's laws. The docking protocol validation allows relying qualitatively on the docking results and to analyze the ligand putative binding mode. After the molecular docking, the obtained complexes have been energy minimized allowing the residues of the cavity to adapt to the ligand simulating the induced fit effect. The resulting best binding mode is depicted in Figure 2.

It can be observed that the ligand shape is optimal to occupy both the entrance and the catalytic cavity. Ile199 and Tyr326 are considered "gatekeepers" of the MAO-B catalytic cavity", while the MAO-A isoform does not have an entrance cavity. Therefore, this could explain the preference for MAO-B binding affinity and the selectivity. Concerning the complex **3d-MAO-B** stabilizing interactions, a number of van der Walls contacts can be observed with the aromatic cage residues Tyr435, Tyr398, Phe343, and Tyr188, as well as several hydrophobic residues in the entrance cavity Ile198, Ile199, Tyr326, Leu164, Leu167, Leu171, Phe168. The MAO B binding site was analyzed using SiteMap, [47] which not only assesses the suitability of a site to host a ligand but also identifies the areas where specific moieties preferentially interact. The SiteMap study highlighted a large hydrophobic area occupied by the diaryl-propenone derivative (Figure 3). A further molecular interaction fields (MIFs) analysis was considered. After removing the inhibitor, the interaction capabilities of the MAO-B active site were investigated by means of Cl probe as implemented in the program GRID [31, 48]. The resulting isocontour maps were inspected taking into account the docking reported pose of the **3d** inhibitor into the hMAO-B binding. MIFs indicated strong interaction between the target and organic chlorine (Figure S1). Nevertheless, the presence of well-defined areas where hydrogen bonding could be established with the ligand was evidenced. Thus, the introduction of hydrogen bond acceptors and donors in the ligand structure, pointing to these specific areas, may result in increased activity and specificity toward the target MAO-B isozyme. All together these data indicate that 1-(4-nitrophenyl)-3-arylprop-2-en-1-ones may represent a valid structural simplification of previously reported heterocyclic MAO-B inhibitors. Indeed, all the synthesized compounds were selective nanomolar inhibitors of the MAO-B enzyme, while their potency on the MAO-A isozyme was significantly lower. This selectivity can be attributed to differences in the binding sites, including shape, size[13, 49] and calculated energetically favored maps (Figure S2). Consequently, the binding energy contributions reflect these differences and explain the observed activity. For instance, we present the docking data for compound **3d** with both isoenzymes A and B (Table S5).

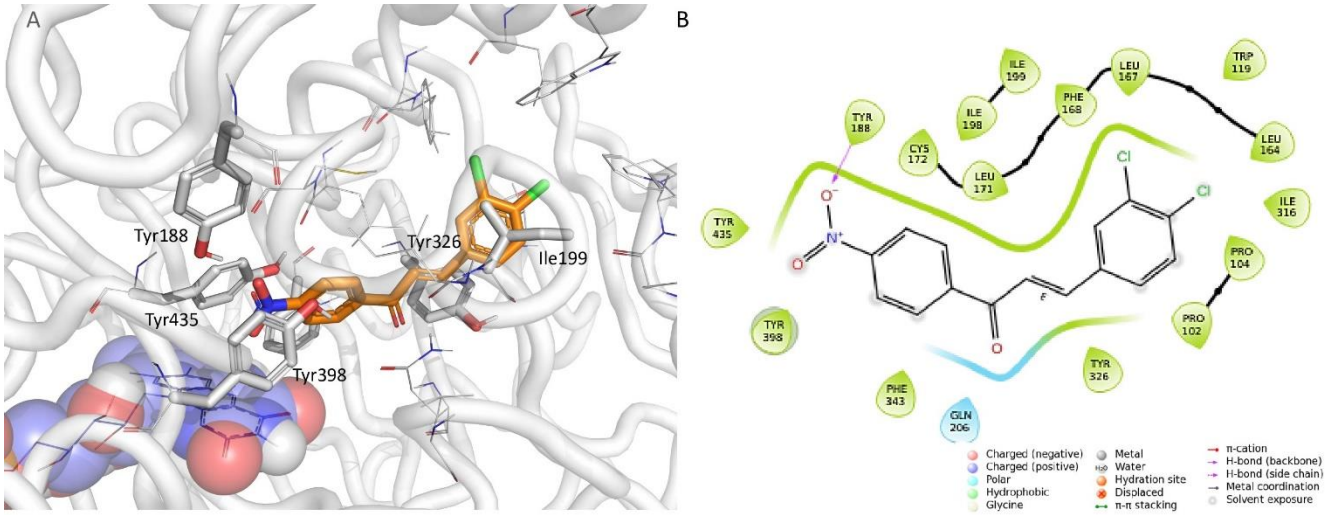


Figure 2. A) Representation of the molecular docking result of **3d** after subsequent complex energy minimization and B) 2D representation of the compound and interacting residues: in light green the hydrophobic, in blue the polar ones. In magenta arrow the hydrogen bond acceptor.

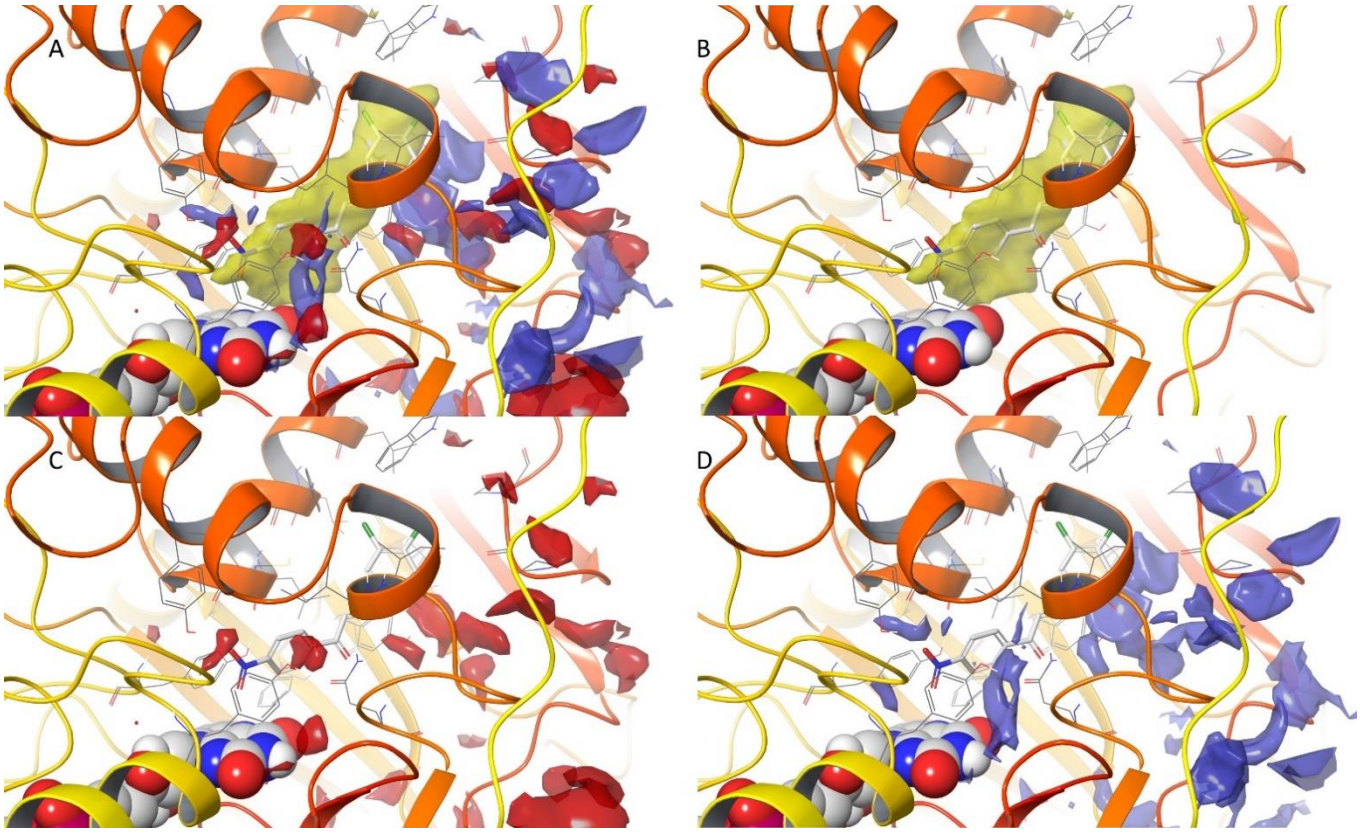


Figure 3. A) Visualization of binding site cavity favorable maps obtained with SiteMap: energetically favored maps are displayed all at once, then split in B, C, D. In yellow are shown the areas where the hydrophobic moieties are favored, in blue the hydrogen bond donors and in red the acceptors.

Both SARs and molecular modelling provided clear indications for further optimization of these derivatives that may positively influence the ability to inhibit the monoamine oxidase enzyme's B isoform selectively. Noteworthy, the new derivatives exhibited a structure-activity profile similar to the previously investigated dihydropyrazoles, dihydrooxadiazoles, and dihydroisoxazoles, suggesting potential further structural options regarding the spacer between the two aromatic moieties. Interestingly, although all compounds were potent MAO-B inhibitors, the relevant role of a 3,4-dichlorophenyl moiety at the 3-position of the propenone for inhibitory potency was pointed out. These data prompt us to further explore the structural requirements for the selective and potent inhibition of MAO-B. Concerning molecular modeling studies, the presence and nature of favorable energy maps will be considered for future structural modifications of this set of molecules.

ASSOCIATED CONTENT

Supporting Information

Methods Chemical physical properties of compounds, ^{1}H NMR, ^{13}C NMR, HRMS spectra, HPLC chromatograms, biological assay and molecular modeling details, and drug like properties prediction. The Supporting Information is available free of charge on the ACS Publications website.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally. Conceptualization: R.M., A.F., and S.D.; Formal analysis: R.M, A.F., B.E., P.C. Investigation: R.M., S.D. B.E., S.F., A.L., F.C., A.O., E.S., D.S., G.A., L.D., D.V., and P.C; Methodology: R.M., A.F., and S.D.; Project administration: E.M., and A.F.; Resources: E.M. and A.F.; Supervision: R.M., S.D., F.C., and A.L.; Original draft preparation: R.M., S.D., and E.M.

Notes

All experiments involving hazardous materials were conducted in compliance with relevant safety protocols and guidelines. Appropriate safety measures, including the use of protective equipment and fume hoods, were employed for both synthetic and biochemical studies. The authors declare no competing financial interest.

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ABBREVIATIONS

MAOs, Monoamine oxidases; DA, dopamine; NE, norepinephrine; 5HT, serotonin; AD, Alzheimer's disease; PD, Parkinson's disease; NDDs, neurodegenerative diseases; *h*MAO-A, human monoamine oxidase A; *h*MAO-B, human monoamine oxidase B, ADMET, absorption, distribution, metabolism, excretion, and toxicity; BBB, blood brain barrier; RMSD, root mean square deviation; MIFs, molecular interaction fields.

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