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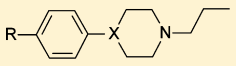
Synthesis and Evaluation of a Set of Para-Substituted 4-Phenylpiperidines and 4-Phenylpiperazines as Monoamine Oxidase (MAO) Inhibitors

Fredrik Pettersson,* Peder Svensson, Susanna Waters, Nicholas Waters, and Clas Sonesson

NeuroSearch Sweden AB, Arvid Wallgrens Backe 20, S-413 46 Göteborg, Sweden

Supporting Information

ABSTRACT: A series of para-substituted 4-phenylpiperidines/piperazines have been synthesized and their affinity to recombinant rat cerebral cortex monoamine oxidases A (MAO A) and B (MAO B) determined. Para-substituents with low dipole moment increased the affinity to MAO A, whereas groups with high dipole moment yielded compounds with no or weak affinity. In contrast, the properties affecting MAO B affinity were the polarity and bulk of the para-substituent, with large hydrophobic substituents producing compounds with high MAO B affinity. In addition, these compounds were tested in freely moving rats and the effect on the post-mortem neurochemistry was measured. A linear correlation was demonstrated between the affinity for MAO A, but not MAO B, and the levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT) in the striatum.



X = N, CH_2
R = H, Cl, CF_3, morpholine, OMe
O-nBu, CN, SO_2Me, SO_2CF_3

INTRODUCTION

Flavin containing monoamine oxidases (MAOs) constitute a heterogeneous family of enzymes that are present in mammals, plants, and microorganisms. In addition to metabolizing foods and pharmaceutical compounds, they are also responsible for the degradation of amine neurotransmitters. There are two distinct types of MAOs, MAO A and MAO B, which share 70% amino acid sequence homology.[1−5] Even though the two isomers have a number of structural similarities and are both bound to the outer mitochondrial membrane,[6] they have different functions in the brain. MAO A catalyzes the oxidative deamination of serotonin (5-hydroxytryptamine, 5-HT), and the therapeutic use of inhibitors of MAO A is primarily in the treatment of depression.[7,8] On the other hand, MAO B is responsible for the degradation of benzylamine and α-phenethylamine, and MAO B inhibitors are used to treat neurodegenerative disorders such as Parkinson's disease.[9] Dopamine (DA), adrenaline (A), and noradrenaline (NA) are metabolized by both isoforms, albeit more efficiently by MAO A.[10,11]

Both MAO A and MAO B are present in the rat brain.[5,12] MAO A is the isoform found primarily within dopaminergic nerve terminals,[13] whereas MAO B is found mainly in striatal neurons and glial cells.[14] Furthermore, it has been shown that MAO A has a predominant effect on dopamine catabolism, leading to production of the metabolite DOPAC (3,4-dihydroxyphenylacetic acid), and that MAO A inhibitors (e.g., clorgyline) therefore reduce striatal DOPAC levels.[6,15] In addition, since more synaptic DA is metabolized by COMT (catechol-*O*-methyltransferase) to 3-MT (3-methoxytyramine) and less 3-MT is metabolized to HVA (homovanillic acid) by MAO, a concomitant increase in 3-MT levels is observed. Because of the location of MAO B, specific inhibition of this isoform with, for example, selegeline has no effect or only mild effects on DOPAC and 3-MT concentrations.[16] However, in the presence of a MAO A inhibitor, MAO B inhibitors potentiate the effect of MAO A on DOPAC.[17] It is therefore crucial to take into consideration the plasma concentrations of different inhibitors when testing them in vivo, since most MAO inhibitors are only selective up to a certain concentration.

MAO inhibitors can bind either reversibly (e.g., moclobemide) or irreversibly (e.g., clorgyline and selegiline) (Figure 1).[18−20]

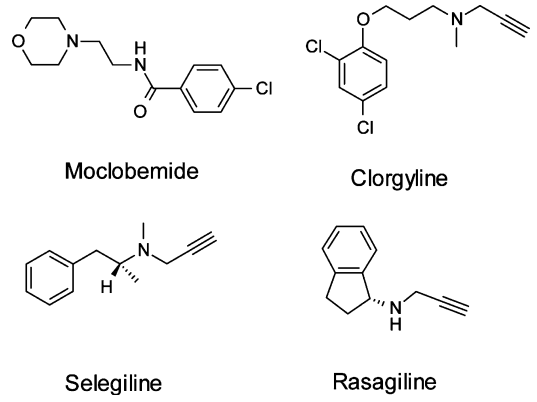


Figure 1. Examples of MAO A (moclobemide and clorgyline) and MAO B (selegiline and rasagiline) inhibitors.

It is believed that the newer reversible agents are less prone to induce tyramine potentiation, a common side effect of MAO inhibitors, than the irreversible inhibitors.[21−23]

Most of the known MAO A inhibitors have an aromatic moiety with basic nitrogen at two to four atoms distance from the ring. While the irreversible inhibitors have a functional group that enables covalent binding to the MAO enzyme (e.g.,

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alkyne), the reversible inhibitors lack such moiety.[24] Studies on para-substituted phenethylamines, benzylamines, and amphetamines have shown correlations between MAO affinity and different physiochemical properties of the para-substituent. Both size and electronic properties have been proposed to correlate with MAO A affinity, while mainly the hydrophobicity of the substituent seems to correlate with MAO B affinity.[25−29] CoMFA (comparative molecular field analysis) of substituted phenethylamines[25] and determination of the crystal structures of inhibitor-bound MAO A[30−32] have led to a greater understanding of the structure−activity relationships (SARs) of MAO A ligands.

Meta-substituted 4-phenylpiperidines have previously been reported to have dopaminergic effects, which are mediated primarily by the dopamine type 2 (DA D2) receptor. By modification of a partial DA D2 agonist, a compound with dopaminergic stabilizing properties was obtained (i.e., a compound with functional DA D2 antagonism and fast kinetic properties[33,34]). Neurochemical analysis of post-mortem brain tissue from freely moving rats shows that dopaminergic stabilizers induce an increase in the synthesis and release of dopamine in the basal ganglia (e.g., the striatum), a hallmark of DA D2 receptor antagonism. The effect can easily be followed by measuring changes in DOPAC levels in the striatum (i.e., an increase in levels).

In an attempt to explore further the SARs for substituted 4-phenylpiperidines/piperazines, we investigated the corresponding para-substituted analogues of the dopaminergic stabilizer pridopidine. Changing the aromatic position of the methylsulfone in pridopidine from meta to para (Figure 2) gave

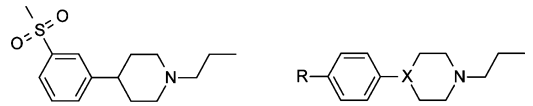


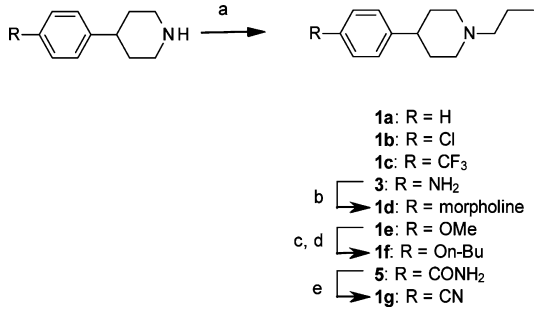
Figure 2. Pridopidine and the generic structure of para-substituted 4-phenylpiperidines/piperazines.

a compound devoid of effect on striatal DOPAC. However, by replacement of the electron-withdrawing methylsulfone with an electron-donating substituent (e.g., methoxy), a major decrease in DOPAC levels was observed. This was in sharp contrast to the effect of the ortho- or meta-substituted 4-phenylpiperidines/piperazines (Pettersson, manuscript in preparation). Additionally, the levels of 3-MT increased significantly in the striatum, as well as in limbic regions and in the prefrontal cortex. On the basis of these effects, we hypothesized that para-substituted 4-phenylpiperidines could act as MAO inhibitors. In order to test this hypothesis, these compounds were further characterized in vitro with respect to their affinity for MAO A and MAO B. Moreover, a series of new para-substituted 4-phenylpiperidines/piperazines (Figure 2) were synthesized and tested in vitro and in vivo to further investigate the SARs of these compounds. The results from these studies are presented in this article.

CHEMISTRY

Most of the para-substituted 4-phenylpiperidines/piperazines were obtained from readily available starting materials that were N-alkylated with iodopropane. A few compounds required further modification of the substituent to obtain the desired structure. The unsubstituted 4-phenylpiperidine (**1a**) along with the para-substituted chloro (**1b**), trifluoromethyl (**1c**), and methoxy (**1e**) analogues were obtained via N-alkylation of the corresponding 4-phenylpiperidines by reflux with iodopropane and potassium carbonate in acetonitrile. The same treatment of readily available starting materials generated the aniline (**3**) and benzamide (**5**) intermediates. The morpholine compound (**1d**) was synthesized from the 4-(1-propyl-4-piperidyl)aniline (**3**) by reaction with bis(2-chloroethyl) ether in dimethylformamide under microwave irradiation. The butoxy compound (**1f**) was formed by treatment of the corresponding methoxy analogue (**1e**) with 48% HBr, producing the phenol (**4**), followed by O-alkylation with 1-butyl bromide. Treating the 4-(1-propyl-4-piperidyl)benzamide (**5**) with phosphoryl chloride in dimethylformamide produced the desired nitrile (**1g**) in a good yield (Scheme 1).

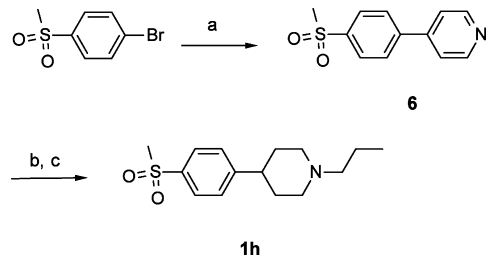
Scheme 1[a]



[a]Reagents and conditions: (a) PrI, K_2CO_3, CH_3CN, Δ; (b) bis(2-chloroethyl) ether, DMF, microwave; (c) HBr (48%), Δ; (d) 1-butyl bromide, K_2CO_3, CH_3CN, Δ; (e) $POCl_3$, DMF, Δ.

4-(4-Methylsulfonylphenyl)-1-propylpiperidine (**1h**) was synthesized from 1-bromo-4-methylsulfonylbenzene in three steps via an initial Suzuki coupling to give the 4-phenylpyridine **6**, followed by quaternization of the pyridine nitrogen by heating in neat iodopropane and finally reduction of the pyridine to the corresponding piperidine by catalytic hydrogenation (PtO_2) (Scheme 2).

Scheme 2[a]



[a]Reagents and conditions: (a) pyridine-4-boronic acid, Pd_2dba_3, PPh_3, K_3PO_4, toluene, EtOH, Δ; (b) PrI, Δ; (c) PtO_2, H_2, MeOH.

1-Phenyl-4-propylpiperazine (**2a**) and 1-(4-methoxyphenyl)-4-propylpiperazine (**2b**) were obtained by N-alkylation of the corresponding secondary amines. Treatment of **2b** with 48% HBr yielded 4-(4-propylpiperazin-1-yl)phenol which was further modified to the corresponding triflate (**2c**) by addition of triflic chloride in a suspension of dichloromethane and triethylamine (Scheme 3).

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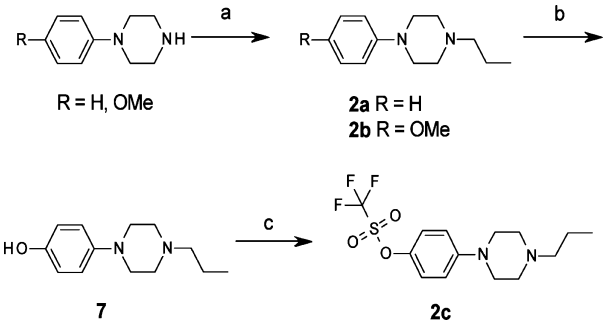
Scheme 3[a]

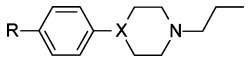


[a]Reagents and conditions: (a) PrI, K_2CO_3, CH_3CN, Δ; (b) HBr (48%), Δ; (c) TfCl, TEA, CH_2Cl_2, Δ

RESULTS AND DISCUSSION

In Vitro Pharmacology. The apparent K_i of the tested compounds to MAO A and MAO B was determined (Table 1 and Table S2, Supporting Information). There was a general preference for MAO A in the data set. Strong correlation was observed between the affinity to MAO A and two estimates of electronic properties of the aromatic para-substituent, namely, the group dipole moment μ_R and the Hammett's σ parameters (Figure 3 and Table S1, Supporting Information) (for μ_R, R^2 = 0.88 (see Figure 3); for σ_p, R^2 = 0.78). Compounds containing substituents with low dipole moment, such as methoxy, had a high affinity for MAO A, while groups with high dipole moment, such as cyano, yielded compounds with no or only weak MAO A affinity. Replacing the piperidine ring with a piperazine resulted in a slight decrease in affinity toward MAO A, both for the unsubstituted compounds (**1a** and **2a**) and the methoxy-substituted derivatives (**1e** and **2b**, Table 1).

Table 1. Generic Structures and Binding Affinities at MAO A and MAO B Enzymes of 1a−h, 2a−c, and Reference Compounds and the Dipole Moments of the Para-Susbtituents



compd	X	R	pK_i (MAO A)[a]	pK_i (MAO B)[a]	μ_R [c]
1a	CH	H	5.01	NT	0
1b	CH	Cl	5.82	4.42	−1.59
1c	CH	CF_3	5.16	4.89	−2.61
1d	CH	morpholine	5.92	4.89	−0.58
1e	CH	OMe	6.62	3.66[b]	−1.30
1f	CH	O-*n*-Bu	6.43	5.80	−1.19
1g	CH	CN	4.03[b]	3.23[b]	−4.08
1h	CH	SO_2Me	3.23[b]	3.23[b]	−4.75
2a	N	H	4.33	NT	0
2b	N	OMe	5.85	3.23[b]	−1.30
2c	N	OSO_2CF_3	4.77	7.48	−2.99
moclobemide			4.93[d]	<4.0[d]	
selegiline			5.92[e]	8.15[e]	

[a]Negative logarithm of binding affinities (apparent K_i) to MAO in rat cerebral cortex cells with [^{3}H]Ro 41-1049as ligand for MAO A and Ro 16-6491 for MAO B. NT: not tested. [b]IC_{50} higher than 1 mM is equivalent to K_i higher than 0.58 mM. [c]Group dipole moment for the aromatic para-substituent. [d]pK_i from Di Santo et al.[35] [e]pIC_{50} from Sterling et al.[36]

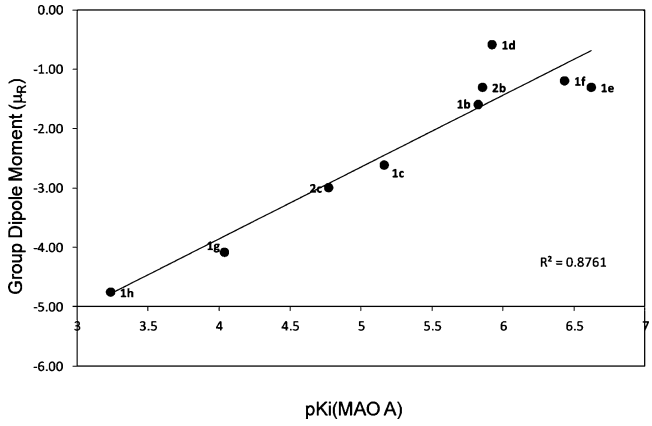


Figure 3. Relationship between negative logarithm of MAO A binding affinities (rat cerebral cortex cells with [^{3}H]Ro 41-1049 as ligand) (pK_i) and group dipole moment (μ_R) of the aromatic para-substituent. The compounds with no para-substituent (**1a** and **2a**) were excluded.

Scorza et al. have previously published the effect of different aromatic substituents on MAO affinity in the amphetamine-like compound series (Figure 5), and they also found that the most potent MAO A inhibitors were para-substituted with electron-donating groups like methoxy, ethoxy, or thioalkyls.[28] In addition, Nandigama et al. have published an extensive SAR analysis for the oxidation of para-substituted phenethylamines by MAO A. They showed that while there was a strong correlation between substrate propensity for the MAO A enzyme and steric effects of the para-substituent, it was mainly electronic parameters that determined binding affinity to MAO A (shown as K_d in Table 1 of ref 27).

Scorza et al. also showed that amphetamines para-substituted with thioalkyl groups containing more than two carbons gave a decrease in affinity to MAO A.[28] However, the three-carbon thioalkyl group in that series was *i*-PrS and Gallardo-Godoy et al.[25] later showed that the branched thioalkyls gave lower affinity to MAO A than unbranched *p*-thioalkylamphetamines which had affinity in the order *n*-PrS > *n*-BuS > EtS > MeS > *n*-PeS.[25] In comparison, the alkoxy compounds in our series had a slightly different order, where *n*-butoxy (**1f**) gave lower affinity than methoxy (**1e**) (pK_i of 6.43 and 6.62, respectively; Table 1). Molecular modeling, based on the X-ray crystal structure of MAO A irreversibly bound to the MAO A selective inhibitor clorgyline, described by Gallardo-Godoy et al.[25] showed a hydrophobic pocket at the location of the para-substituent as well as an interaction between CYS323 and the oxygen/sulfur of the alkoxy/thioalkyl groups in this position. We have adopted a similar approach, based on the more recent high resolution X-ray crystal complex of MAO A and the reversible inhibitor harmine (PDB entry 2Z5X),[37] and manually docked compound **1e**. Harmine and two crystal waters close to the pyridine was replaced by **1e** followed by relaxation of ligand, binding pocket amino acid side chains, and crystal waters close to the ligand, using the MMFF94x[38,39] force field and Born solvation[40] implemented in the MOE software.[41] The resulting binding pose of **1e** indicated a CYS323−oxygen interaction and a hydrophobic pocket perpendicular to the aromatic ring where the para-substituent is located (Figure 4). The flexibility of the substituent is therefore likely a determining factor for the fit. Thus, unbranched groups would give higher affinity than branched, which is a possible explanation for the lower than predicted affinity of the morpholine derivative **1d**

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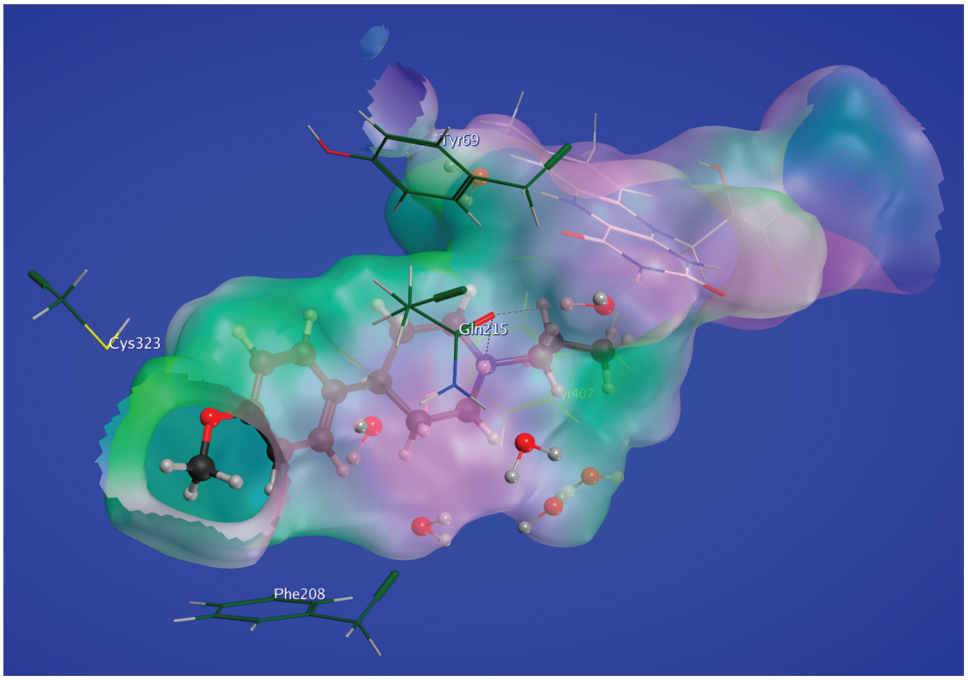

Figure 4. Binding pose of compound **1e** in the active site of human MAO A (2Z5X) is shown. The protein is represented by the Connolly surface[42,43] of the binding site amino acids color coded by lipophilic potential.[44] Lipophilic areas are coded as green and hydrophilic areas as purple. Side chains of the key residues from the Gallardo-Godoy et al. paper[25] are included. Part of FAD close to **1e** is shown with carbon atoms in white. Water molecules close to the ligand is included. The hydrogen bonds between the basic nitrogen in **1e** and Gln215 and one of the water molecules are shown. The hydrophobic pocket perpendicular to the aromatic ring in the area of the para-substituent is indicated by the opening in the molecular surface.

(pK_i = 5.92, Table 1 and Figure 3). Yet in the complete set of para-substituted phenylpiperidines/piperazines, no correlation between the size of the substituent and MAO A affinity was observed (R^2 = 0.01, Table S1, Supporting Information). Furthermore, Scorza et al. concluded that the amphetamine-like compounds in their series were reversible MAO A inhibitors.[28] The structural similarity to the amphetamines and a lack of functional groups that enable covalent binding indicate that the MAO A ligands in our series (Table 1) also bind reversibly (not tested). Another structure analogue to our compounds is MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Figure 5),

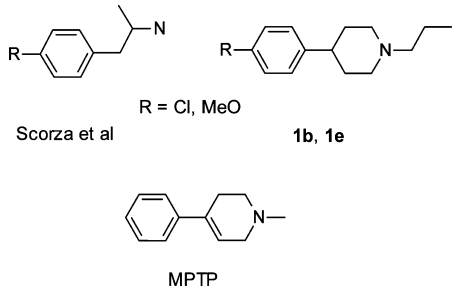

Figure 5. Para-substituted amphetamines/phenylpiperidines and the neurotoxin MPTP.

a neurotoxin that is known to be both a substrate and an inhibitor of the MAOs (preferably substrate to MAO B and inhibitor of MAO A).[45,46] However, specific modifications of the MPTP structure, such as saturation of the 1,2,3,6-tetrahydropyridine ring or increasing the length of the *N*-alkyl, generated compounds that were not MAO B substrates.[47,48] The unsaturated 1,2,3,6-tetrahydropyridine ring has been claimed to be essential for MPTP's substrate propensity to MAO,[49,50] and it is therefore unlikely that the compounds in Table 1 are substrates for the MAO enzymes (not tested).

In contrast to the properties yielding high MAO A affinity, the compound with the highest affinity for MAO B had a bulky and hydrophobic para-substituent (i.e., triflate). MAO B affinity was modeled against five physicochemical descriptors for the para-substituent and clogP for the whole molecule (Table S1, Supporting Information), using orthogonal partial least squares (OPLS) regression.[51] A (1 + 1) model with a R^2Y of 0.89 and a Q^2 of 0.70 was obtained in which all six descriptors correlated positively with the response. Hydrophobicity (π), clogP, and volume were the most important descriptors (Figure 6). This is

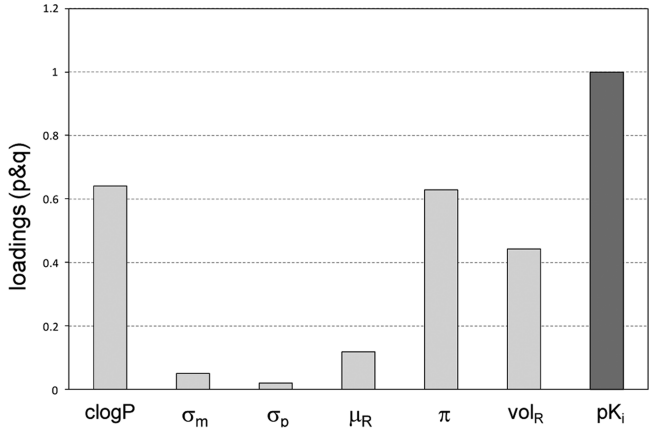

Figure 6. Loading plot of the predictive component of the OPLS model of pK_i (MAO B) versus physicochemical descriptors (values listed in Table S1 in Supporting Information) for compounds **1b–h** and **2b,c**.

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in line with previous studies, where docking of harmine and analogues showed a larger hydrophobic cavity at this specific location in the MAO B structure compared to MAO A.[52] The positive correlation with hydrophobicity is also in line with earlier work on para-substituted benzylamines reported by Walker et al.[29] However, in contrast to our observations, in the benzylamine series the volume of the para-substituent was negatively correlated with MAO B affinity.

In Vivo Pharmacology. The synthesized compounds in Table 1 and known MAO A and MAO B ligands (moclobemide and selegiline, respectively) were tested in freely moving rats. Post-mortem brain tissue neurochemistry was measured, and the levels of each monoamine and its respective metabolites were quantified (Table 2) using the method described in Supporting Information.

Table 2. Levels of DOPAC and 3-MT in Post-Mortem Neurochemistry

compd	DOPAC[a]	3-MT[a]
1a	130	100
1b	68	130
1c	86	100
1d	38	160
1e	22	260
1f	40	150
1g	105	115
1h	100	100
2a	180	100
2b	71	135
2c	121	120
moclobemide	18	400
selegeline low	83	110
selegeline high	37	145

[a]Levels of DOPAC and 3-MT in the striatum are expressed as percentage compared with saline control (n = 4 per dose). Doses are as follows: **1a–h** and **2a–c**, 100 μmol/kg; moclobemide, 37 μmol/kg; selegeline, 5.3 μmol/kg (low) and 53 μmol/kg (high).[1]

Moclobemide, a reversible and selective inhibitor of MAO A, produced a potent decrease in striatal DOPAC and an increase of 3-MT levels. Furthermore, the para-substituted 4-phenylpiperidines/piperazines displaying high affinity for MAO A had neurochemical profiles that were similar to that of moclobemide, whereas compounds with low or no affinity where devoid of these effects (a few of the compounds with low affinity to MAO A actually produced increases in striatal DOPAC, probably associated with weak DA D2 antagonism). Thus, we confirmed findings from previous studies showing that MAO A affinity is well correlated with the release and turnover of striatal dopamine (i.e., levels of DOPAC, Figure 7).[6,13,53] The same correlation, however, is not observed between the affinity for MAO B and DOPAC levels (R^2 = 0.05), supporting the general view that MAO B ligands have no or only a limited effect on striatal DOPAC levels.[6,54] We found that selegeline, an MAO B ligand with an approximately 100-fold selectivity for MAO B over MAO A, produced no significant effect on striatal DOPAC levels at the lower dose tested (1.1 mg/kg), while at the highest dose tested (10 mg/kg) a significant decrease in DOPAC was observed. This supports previous data suggesting that selegiline loses selectivity when administered in high doses and that MAO B affinity can potentiate the effect MAO A has on DOPAC levels.[17]

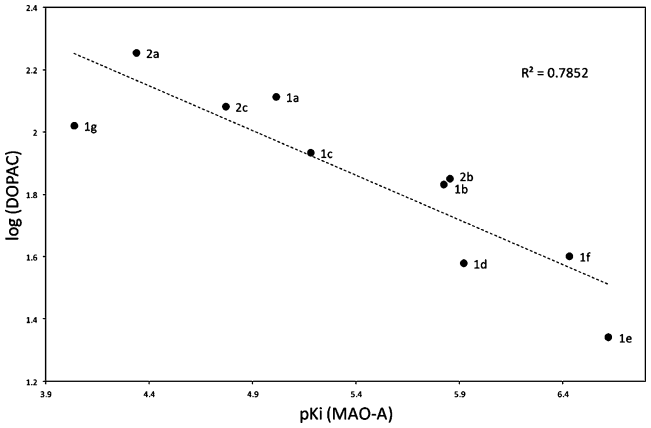

Figure 7. Relationship between DOPAC levels and pK_i (MAO A). Compound **1h**, which has K_i(MAO A) > 0.58 mM (IC_{50} > 1 mM), is excluded.

One of the most potent MAO A ligands within this series (**1f**) was administered to unrestrained rats, and the levels of striatal dopamine metabolites were subsequently measured using microdialysis. A sharp increase of 3-MT along with a significant decrease in both DOPAC and HVA was detected (Figure 8). These results are in line with previously published

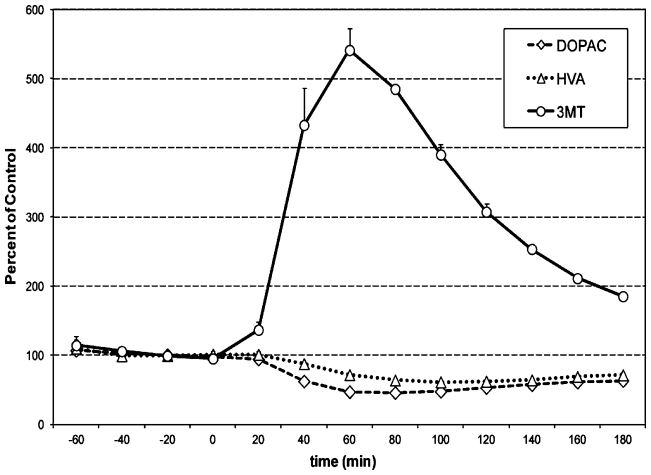

Figure 8. Striatal levels of 3-MT, DOPAC, and HVA, measured by microdialysis in freely moving rats (n = 2) and expressed as percentage of saline control, after administration of **1f** (50 μmol/kg) at 0 min.

microdialysis studies of known MAO A inhibitors,[15] indicating that the high-affinity MAO A compounds in Table 1 also act as inhibitors.

CONCLUSION

A set of para-substituted 4-phenylpiperidines/piperazines have been synthesized and evaluated both in vitro and in vivo. A clear correlation between the group dipole moment (i.e., μ_R) of the para-substituent and MAO A affinity was observed (R^2 = 0.88, Figure 3). Substituents with low dipole moment yielded compounds with a high MAO A affinity, whereas substituents with high dipole moment produced compounds that had no or only low affinity to MAO A. In contrast, the properties of para-substituents that correlated with MAO B affinity were mainly hydrophobicity and bulk. Large hydrophobic groups yielded compounds that had a high affinity to MAO B,

whereas compounds with small and/or polar groups were devoid of any affinity.

The effect of these compounds on rat post-mortem neurochemistry was also examined. Striatal DOPAC and 3-MT levels correlated well with MAO A affinity (R^2 of 0.79 (Figure 7) and 0.60 (data not shown), respectively) where high affinity compounds produced a decrease in DOPAC and an increase in 3-MT. The same profile was observed in microdialysis experiments, and these results, together with previous publications in the field, support our view that the high-affinity MAO A ligands in this class act as inhibitors. Furthermore, absence of functional groups required for irreversible bonding to MAO and structural similarities to known reversible inhibitors suggests that the inhibition is reversible. In contrast to the MAO A ligands, we found no correlation between affinity to MAO B and the levels of DOPAC or 3-MT, and the compound with the highest affinity to MAO B (**2c**) had only weak effects on post-mortem neurochemistry.

Within the structural class presented herein, we have determined correlations between the physicochemical properties of the para-substituent and affinity to MAO A and MAO B, respectively. In addition, we have shown that affinity to MAO A, but not MAO B, correlates well with the levels of striatal DOPAC and 3-MT in post-mortem neurochemistry and that the compounds displaying affinity to MAO A act as inhibitors.

EXPERIMENTAL SECTION

Chemistry. General. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD or $CDCl_3$ at 300 and 75 MHz, respectively, using a Varian XL 300 spectrometer, or at 400 and 100 MHz, respectively, using a Mercury Plus 400 spectrometer. Chemical shifts are reported as δ values (ppm) relative to an internal standard (tetramethylsilane). Low resolution mass spectra were recorded on a HP 5970A instrument operating at an ionization potential of 70 eV. The mass detector was interfaced with a HP5700 gas chromatograph equipped with a fused silica column (11 m, 0.22 mm i.d.) coated with cross-linked SE-54 (film thickness 0.3 mm, He gas, flow 40 cm/s). Elemental analysis was performed by MikroKemi AB (Uppsala, Sweden) or Mikroanalytisk Laboratorium (Copenhagen, Denmark). Melting points were determined with a point microscope (Reichert Thermovar) and are uncorrected. For flash chromatography, silica gel 60 (0.040−0.063 mm, VWR, no. 109385) was used. The amine products were converted to the corresponding salts by dissolving the free base in ethanol and adding 1−2 equiv of the acid (fumaric or oxalic) or ethanolic HCl solution. The solvent was removed and azeotroped with absolute ethanol in vacuo followed by recrystallization from appropriate solvents. Purity of all target compounds was assessed as greater than 95% by elemental analysis (C, H, N).

General Procedure for the Preparation of Compounds 1a−c,e and 2a,b. A solution of the phenylpiperidine/piperazine (1.0 equiv), 1-iodopropane (1.1 equiv), and potassium carbonate (3 equiv) in acetonitrile was refluxed for 15 h. The mixture was allowed to cool to ambient temperature. The solid was filtered off, and the solvent was evaporated. The crude product was purified by flash chromatography using ethyl acetate/methanol in appropriate ratios as eluent. The amines were converted to the corresponding salts and recrystallized from appropriate solvents.

4-Phenyl-1-propylpiperidine (1a). The compound was obtained in 81% yield. The amine was converted to the HCl salt and recrystallized from EtOH/diethyl ether: mp 234−235 °C; MS m/z (relative intensity, 70 eV) 203 (M^+, 6), 174 (bp), 103 (11), 91 (11), 70 (34). Anal. ($C_{14}H_{21}N\cdot HCl$) C, H, N. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.04 (t, J = 7.4 Hz, 3H), 1.77−1.85 (m, 2H), 2.00 (d, J = 12.7 Hz, 2H), 2.06−2.13 (m, 2H), 2.88−2.96 (m, 1H), 3.08−3.17 (m, 4H), 3.65 (d, J = 12.2 Hz, 2H), 7.22−7.33 (m, 5H).

4-(4-Chlorophenyl)-1-propylpiperidine (1b). The compound was obtained in 67% yield. The amine was converted to the HCl salt and recrystallized from ethanol/diethyl ether: mp 219−220 °C. Anal. ($C_{14}H_{20}ClN\cdot HCl\cdot 0.25H_2O$) C, H, N. MS m/z (relative intensity, 70 eV) 237 (M^+, 7), 210 (34), 209 (15), 208 (bp), 70 (34). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, J = 7.4 Hz, 3H), 1.43−1.66 (m, J = 15.3, 7.62, 7.5, 7.5 Hz, 2H), 1.68−1.90 (m, 4H), 2.01 (td, J = 11.4, 3.2 Hz, 2H), 2.31 (d, J = 8.1 Hz, 2H), 2.46 (td, J = 10.8, 5.9 Hz, 1H), 3.05 (d, J = 11.1 Hz, 2H), 7.11−7.20 (m, 2H), 7.25 (d, J = 8.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.0, 20.2, 33.5, 42.3, 54.3, 61.1, 128.2, 128.4, 131.6, 144.9.

1-Propyl-4-[4-(trifluoromethyl)phenyl]piperidine (1c). The compound was obtained in 77% yield. The amine was converted to the HCl salt and recrystallized from ethanol/diethyl ether: mp 236−237 °C. Anal. ($C_{15}H_{20}F_3N\cdot 1.5HCl$) C, H, N. MS m/z (relative intensity, 70 eV) 271 (M^+, 5), 243 (15), 242 (bp), 159 (8), 70 (30). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.4 Hz, 3H), 1.59 (dd, J = 15.7, 7.5 Hz, 2H), 1.78−1.94 (m, 4H), 1.99−2.16 (m, 2H), 2.38 (t, J = 2.7 Hz, 2H), 2.59 (t, J = 8.5 Hz, 1H), 3.11 (d, J = 11.9 Hz, 2H), 7.36 (d, J = 8.5 Hz, 2H), 7.57 (d, J = 8.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.1, 20.2, 33.2, 42.7, 54.2, 61.1, 122.8−123.2 (m, 1C) 125.3, 125.3, 125.4, 125.4, 127.2, 127.9, 128.2−128.6 (m, 1C) 128.9, 150.4.

4-(4-Methoxyphenyl)-1-propylpiperidine (1e). The compound was obtained in 89% yield. The amine was converted to the HCl salt and recrystallized from ethanol/diethyl ether: mp 197−198 °C. Anal. ($C_{15}H_{23}NO\cdot HCl$) C, H, N. MS m/z (relative intensity, 70 eV) 233 (M^+, 17), 205 (15), 204 (bp), 133 (16), 70 (20). ^{1}H NMR (300 MHz, CD_3OD) δ 0.91 (t, J = 7.5 Hz, 3H), 1.54 (dd, J = 16.1, 7.6 Hz, 2H), 1.73 (dd, J = 7.6, 3.1 Hz, 4H), 2.04 (td, J = 11.5, 3.5 Hz, 2H), 2.31 (t, J = 3.0 Hz, 2H), 2.42 (td, J = 10.7, 5.9 Hz, 1H), 3.01 (d, J = 11.9 Hz, 2H), 3.71 (s, 3H), 6.81 (q, J = 5.1 Hz, 2H), 7.11 (q, J = 5.1 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) δ 11.1 19.4, 33.0, 41.4, 54.0, 54.3, 60.7 113.5, 127.3, 138.0, 158.2.

1-Phenyl-4-propylpiperazine (2a). The compound was obtained in 21% yield. The amine was converted to the HCl salt and recrystallized from EtOH/diethyl ether: mp 210−211 °C. Anal. ($C_{13}H_{20}N_2\cdot HCl$) C, H, N. MS m/z (rel intensity, 70 eV) 204 (M^+, 10), 105 (31), 104 (32), 77 (58), 70 (bp). ^{1}H NMR (300 MHz, CD_3OD) δ d 1.02 (t, 3H, J = 7.3 Hz), 1.50−1.62 (m, 2H), 2.35−2.39 (m, 2H), 2.63 (t, 4H), 3.23 (t, 4H), 6.83−6.90 (m, 1H), 6.92−6.96 (m, 2H), 7.26−7.31 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 10.9, 16.1, 20.7, 44.1, 56.2, 59.7, 61.1, 117.6, 122.1, 130.3, 150.9.

1-(4-Methoxyphenyl)-4-propylpiperazine (2b). The compound was obtained in 61% yield. The amine was converted to the HCl salt and recrystallized from EtOH/diethyl ether: mp 215−216 °C. Anal. ($C_{14}H_{22}N_2O\cdot 2HCl$) C, H, N. MS m/z 235 (M^+ + 1, 16), 234 (M^+, bp), 205 (73), 135 (16), 70 (15). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95 (t, J = 7.4 Hz, 3H), 1.57 (dq, J = 15.3, 7.5 Hz, 2H), 2.38 (t, J = 2.3 Hz, 2H), 2.63 (d, J = 5.1 Hz, 4H), 3.12 (d, J = 5.1 Hz, 4H), 3.77 (s, 3H), 6.83−6.88 (m, 2H), 6.90−6.95 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 12.0, 20.1, 50.6, 53.4, 55.5, 60.7, 114.4, 118.1, 145.8, 153.7.

ASSOCIATED CONTENT

Supporting Information

Experimental details of the synthesis of **1a−1h** and **2a−c** as well as biological methods and physiochemical descriptors. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +(46) 31 7727710. Fax: +(46) 31 7720601. E-mail: fpe@neurosearch.se.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MAO, monoamine oxidase; 5-HT, 5-hydroxytryptamine (serotonin); DA, dopamine; NA, noradrenaline; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; COMT, catechol-*O*-methyltransferase; SAR, structure−activity relationship; CoMFA, comparative molecular field analysis; OPLS, orthogonal partial least-squares; σ_m, Hammett's σ meta; σ_p, Hammett's σ para; μ_R, group dipole moment; π, calculated hydrophobicity; Vol_R, calculated volume

■ REFERENCES

(1) Bach, A. W.; Lan, N. C.; Johnson, D. L.; Abell, C. W.; Bembenek, M. E.; Kwan, S. W.; Seeburg, P. H.; Shih, J. C. cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4934−4938.

(2) Ito, A.; Kuwahara, T.; Inadome, S.; Sagara, Y. Molecular cloning of a cDNA for rat liver monoamine oxidase B. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 970−976.

(3) Kuwahara, T.; Takamoto, S.; Ito, A. Primary structure of rat monoamine oxidase A deduced from cDNA and its expression in rat tissues. *Agric. Biol. Chem.* **1990**, *54*, 253−257.

(4) Kwan, S. W.; Abell, C. W. cDNA cloning and sequencing of rat monoamine oxidase A: comparison with the human and bovine enzymes. *Comp. Biochem. Physiol., Part B* **1992**, *102*, 143−147.

(5) Johnston, J. P. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* **1968**, *17*, 1285−1297.

(6) Waldmeier, P. C.; Delini-Stula, A.; Maitre, L. Preferential deamination of dopamine by an A type monoamine oxidase in rat brain. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1976**, *292*, 9−14.

(7) Pacher, P.; Kohegyi, E.; Kecskemeti, V.; Furst, S. Current trends in the development of new antidepressants. *Curr. Med. Chem.* **2001**, *8*, 89−100.

(8) Weyler, W.; Hsu, Y. P.; Breakefield, X. O. Biochemistry and genetics of monoamine oxidase. *Pharmacol. Ther.* **1990**, *47*, 391−417.

(9) Palhagen, S.; Heinonen, E.; Hagglund, J.; Kaugesaar, T.; Maki-Ikola, O.; Palm, R. Selegiline slows the progression of the symptoms of Parkinson disease. *Neurology* **2006**, *66*, 1200−1206.

(10) Green, A. R.; Mitchell, B. D.; Tordoff, A. F.; Youdim, M. B. Evidence for dopamine deamination by both type A and type B monoamine oxidase in rat brain in vivo and for the degree of inhibition of enzyme necessary for increased functional activity of dopamine and 5-hydroxytryptamine. *Br. J. Pharmacol.* **1977**, *60*, 343−349.

(11) Neff, N. H.; Yang, H. Y.; Fuentes, J. A. Proceedings: the use of selective MAO inhibitor drugs to modify amine metabolism in brain. *Psychopharmacol. Bull.* **1974**, *10*, 9−10.

(12) Houslay, M. D.; Tipton, K. F. Multiple forms of monoamine oxidase: fact and artefact. *Life Sci.* **1976**, *19*, 467−477.

(13) Demarest, K. T.; Smith, D. J.; Azzaro, A. J. The presence of the type A form of monoamine oxidase within nigrostriatal dopamine-containing neurons. *J. Pharmacol. Exp. Ther.* **1980**, *215*, 461−468.

(14) Francis, A.; Pearce, L. B.; Roth, J. A. Cellular localization of MAO A and B in brain: evidence from kainic acid lesions in striatum. *Brain Res.* **1985**, *334*, 59−64.

(15) Kato, T.; Dong, B.; Ishii, K.; Kinemuchi, H. Brain dialysis: in vivo metabolism of dopamine and serotonin by monoamine oxidase A but not B in the striatum of unrestrained rats. *J. Neurochem.* **1986**, *46*, 1277−1282.

(16) Arbuthnott, G. W.; Fairbrother, I. S.; Butcher, S. P. Dopamine release and metabolism in the rat striatum: an analysis by "in vivo" brain microdialysis. *Pharmacol. Ther.* **1990**, *48*, 281−293.

(17) Butcher, S. P.; Fairbrother, I. S.; Kelly, J. S.; Arbuthnott, G. W. Effects of selective monoamine oxidase inhibitors on the in vivo release and metabolism of dopamine in the rat striatum. *J. Neurochem.* **1990**, *55*, 981−988.

(18) Fulton, B.; Benfield, P. Moclobemide. An update of its pharmacological properties and therapeutic use. *Drugs* **1996**, *52*, 450−474.

(19) Livingston, M. G.; Livingston, H. M. Monoamine oxidase inhibitors. An update on drug interactions. *Drug Saf.* **1996**, *14*, 219−227.

(20) Mann, J. J.; Aarons, S. F.; Frances, A. J.; Brown, R. D. Studies of selective and reversible monoamine oxidase inhibitors. *J. Clin. Psychiatry* **1984**, *45*, 62−66.

(21) Anderson, M. C.; Hasan, F.; McCrodden, J. M.; Tipton, K. F. Monoamine oxidase inhibitors and the cheese effect. *Neurochem. Res.* **1993**, *18*, 1145−1149.

(22) Blackwell, B.; Marley, E.; Price, J.; Taylor, D. Hypertensive interactions between monoamine oxidase inhibitors and foodstuffs. *Br. J. Psychiatry* **1967**, *113*, 349−365.

(23) Lotufo-Neto, F.; Trivedi, M.; Thase, M. E. Meta-analysis of the reversible inhibitors of monoamine oxidase type A moclobemide and brofaromine for the treatment of depression. *Neuropsychopharmacology* **1999**, *20*, 226−247.

(24) Binda, C.; Hubalek, F.; Li, M.; Herzig, Y.; Sterling, J.; Edmondson, D. E.; Mattevi, A. Binding of rasagiline-related inhibitors to human monoamine oxidases: a kinetic and crystallographic analysis. *J. Med. Chem.* **2005**, *48*, 8148−8154.

(25) Gallardo-Godoy, A.; Fierro, A.; McLean, T. H.; Castillo, M.; Cassels, B. K.; Reyes-Parada, M.; Nichols, D. E. Sulfur-substituted alpha-alkyl phenethylamines as selective and reversible MAO-A inhibitors: biological activities, CoMFA analysis, and active site modeling. *J. Med. Chem.* **2005**, *48*, 2407−2419.

(26) Miller, J. R.; Edmondson, D. E. Structure−activity relationships in the oxidation of para-substituted benzylamine analogues by recombinant human liver monoamine oxidase A. *Biochemistry* **1999**, *38*, 13670−13683.

(27) Nandigama, R. K.; Edmondson, D. E. Structure−activity relations in the oxidation of phenethylamine analogues by recombinant human liver monoamine oxidase A. *Biochemistry* **2000**, *39*, 15258−15265.

(28) Scorza, M. C.; Carrau, C.; Silveira, R.; Zapata-Torres, G.; Cassels, B. K.; Reyes-Parada, M. Monoamine oxidase inhibitory properties of some methoxylated and alkylthio amphetamine derivatives: structure−activity relationships. *Biochem. Pharmacol.* **1997**, *54*, 1361−1369.

(29) Walker, M. C.; Edmondson, D. E. Structure−activity relationships in the oxidation of benzylamine analogues by bovine liver mitochondrial monoamine oxidase B. *Biochemistry* **1994**, *33*, 7088−7098.

(30) De Colibus, L.; Li, M.; Binda, C.; Lustig, A.; Edmondson, D. E.; Mattevi, A. Three-dimensional structure of human monoamine oxidase A (MAO A): relation to the structures of rat MAO A and human MAO B. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12684−12689.

(31) Edmondson, D. E.; DeColibus, L.; Binda, C.; Li, M.; Mattevi, A. New insights into the structures and functions of human monoamine oxidases A and B. *J. Neural Transm.* **2007**, *114*, 703−705.

(32) Ma, J.; Yoshimura, M.; Yamashita, E.; Nakagawa, A.; Ito, A.; Tsukihara, T. Structure of rat monoamine oxidase A and its specific recognitions for substrates and inhibitors. *J. Mol. Biol.* **2004**, *338*, 103−114.

(33) Dyhring, T.; Nielsen, E. O.; Sonesson, C.; Pettersson, F.; Karlsson, J.; Svensson, P.; Christophersen, P.; Waters, N. The dopaminergic stabilizers pridopidine (ACR16) and (−)-OSU6162 display dopamine D(2) receptor antagonism and fast receptor dissociation properties. *Eur. J. Pharmacol.* **2010**, *628*, 19−26.

(34) Pettersson, F.; Ponten, H.; Waters, N.; Waters, S.; Sonesson, C. Synthesis and evaluation of a set of 4-phenylpiperidines and 4-phenylpiperazines as D2 receptor ligands and the discovery of the dopaminergic stabilizer 4-[3-(methylsulfonyl)phenyl]-1-propylpiperidine (huntexil, pridopidine, ACR16). *J. Med. Chem.* **2010**, *53*, 2510−2520.
(35) Di Santo, R.; Costi, R.; Roux, A.; Artico, M.; Befani, O.; Meninno, T.; Agostinelli, E.; Palmegiani, P.; Turini, P.; Cirilli, R.; Ferretti, R.; Gallinella, B.; La Torre, F. Design, synthesis, and biological activities of pyrrolylethanoneamine derivatives, a novel class of monoamine oxidases inhibitors. *J. Med. Chem.* **2005**, *48*, 4220−4223.
(36) Sterling, J.; Herzig, Y.; Goren, T.; Finkelstein, N.; Lerner, D.; Goldenberg, W.; Miskolczi, I.; Molnar, S.; Rantal, F.; Tamas, T.; Toth, G.; Zagyva, A.; Zekany, A.; Finberg, J.; Lavian, G.; Gross, A.; Friedman, R.; Razin, M.; Huang, W.; Krais, B.; Chorev, M.; Youdim, M. B.; Weinstock, M. Novel dual inhibitors of AChE and MAO derived from hydroxy aminoindan and phenethylamine as potential treatment for Alzheimer's disease. *J. Med. Chem.* **2002**, *45*, 5260−5279.
(37) Son, S. Y.; Ma, J.; Kondou, Y.; Yoshimura, M.; Yamashita, E.; Tsukihara, T. Structure of human monoamine oxidase A at 2.2-Å resolution: the control of opening the entry for substrates/inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 5739−5744.
(38) Halgren, T. A. MMFF VII. Characterization of MMFF94, MMFF94s, and other widely available force fields for conformational energies and for intermolecular-interaction energies and geometries. *J. Comput. Chem.* **1999**, *20*, 730−748.
(39) Halgren, T. A. MMFF VI. MMFF94s option for energy minimization studies. *J. Comput. Chem.* **1999**, *20*, 720−729.
(40) Wojciechowski, M.; Lesyng, B. Generalized Born model:analysis, refinement, and applications to proteins. *J. Phys. Chem. B* **2004**, *108*, 18368−18376.
(41) *Molecular Operating Environment (MOE)*, version 2009.10; Chemical Computing Group Inc.: Montreal, Canada, 2009.
(42) Connolly, M. L. Solvent-accessible surfaces of proteins and nucleic acids. *Science* **1983**, *221*, 709−713.
(43) Sethian, J. A. A fast marching level set method for monotonically advancing fronts. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1591−1595.
(44) Wildman, S. A.; Crippen, G. M. Prediction of physicochemical parameters by atomic contributions. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 868−873.
(45) Heikkila, R. E.; Hess, A.; Duvoisin, R. C. Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) in the mouse: relationships between monoamine oxidase, MPTP metabolism and neurotoxicity. *Life Sci.* **1985**, *36*, 231−236.
(46) Kula, N. S.; Baldessarini, R. J.; Murphy, F.; Neumeyer, J. L. Substituted phenylpiperidines and phenylpyridines as reversible selective inhibitors of monoamine oxidase type A in rodent brain and liver. *Biochem. Pharmacol.* **1988**, *37*, 763−766.
(47) Heikkila, R. E.; Manzino, L.; Cabbat, F. S.; Duvoisin, R. C. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and several of its analogues on the dopaminergic nigrostriatal pathway in mice. *Neurosci. Lett.* **1985**, *58*, 133−137.
(48) Maret, G.; el Tayar, N.; Carrupt, P. A.; Testa, B.; Jenner, P.; Baird, M. Toxication of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and analogs by monoamine oxidase. A structure−reactivity relationship study. *Biochem. Pharmacol.* **1990**, *40*, 783−792.
(49) Langston, J. W.; Irwin, I.; Langston, E. B.; Forno, L. S. The importance of the "4−5" double bond for neurotoxicity in primates of the pyridine derivative MPTP. *Neurosci. Lett.* **1984**, *50*, 289−294.
(50) Youngster, S. K.; McKeown, K. A.; Jin, Y. Z.; Ramsay, R. R.; Heikkila, R. E.; Singer, T. P. Oxidation of analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by monoamine oxidases A and B and the inhibition of monoamine oxidases by the oxidation products. *J. Neurochem.* **1989**, *53*, 1837−1842.
(51) Trygg, J.; Wold, S. Orthogonal projections to latent structures (O-PLS). *J. Chemom.* **2002**, *16*, 119−128.
(52) Reniers, J.; Robert, S.; Frederick, R.; Masereel, B.; Vincent, S.; Wouters, J. Synthesis and evaluation of beta-carboline derivatives as potential monoamine oxidase inhibitors. *Bioorg. Med. Chem.* **2010**, *19*, 134−144.
(53) Campbell, I. C.; Robinson, D. S.; Lovenberg, W.; Murphy, D. L. The effects of chronic regimens of clorgyline and pargyline on monoamine metabolism in the rat brain. *J. Neurochem.* **1979**, *32*, 49−55.
(54) Braestrup, C.; Andersen, H.; Randrup, A. The monoamine oxidase B inhibitor deprenyl potentiates phenylethylamine behaviour in rats without inhibition of catecholamine metabolite formation. *Eur. J. Pharmacol.* **1975**, *34*, 181−187.