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Monoamine Oxidase Inhibitory Properties of Some Benzazoles: Structure-; Activity Relationships

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ABSTRACT Benzazoles containing two or three nitrogen atoms were screened for their inhibitory activity toward monoamine oxidases MAO-A and MAO-B. In order to clarify the mechanism of interaction of these compounds with the enzyme, their electronic structure was calculated at the ab initio level and the influence of lipophilicity on activity was investigated. The mode of binding of benzazoles to MAO-B appears different from that of previously investigated heterocycles.

INTRODUCTION

Monoamine oxidase (MAO) is a FAD-containing enzyme of the outer mitochondrial membrane (1) existing as two isoenzymes (MAO-A and MAO-B) that differ in their substrate specificity and sensitivity to inhibitors (2).

Recently, 7-nitroindazole (7-NI), a potent inhibitor of nitric oxide synthase, was also found to be an MAO-B inhibitor (3). Taking into account the structural features of 7-NI and the extensive literature showing that a wide variety of planar, heterocyclic systems are competitive inhibitors of MAO (4), the reversible MAO-A and MAO-B inhibitory activity of benzazoles was investigated Table 1. Lipophilicity was measured in two solvent systems (*n*-octanol/water and 1,2-dichloroethane/water). Hydrogen bonding properties were deduced and are discussed in relation to biological activity. Ab initio molecular orbital calculations and molecular electrostatic potentials (MEP) were used to interpret and rationalize the data.

MATERIALS AND METHODS

The substituted benzotriazoles, benzimidazoles, and indazoles were purchased from Aldrich (Buchs, Switzerland) or prepared according to known procedures (5,6). The identity and purity of all synthesized compounds were checked by ^{1}H-NMR, IR and elemental analyses. Kynuramine was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

The in vitro MAO inhibitory activities were studied using a crude rat brain mitochondrial suspension (7). This suspension, which was set to a final concentration of 1.0 mg/ml, was preincubated at 37°C for 5 minutes with either clorgyline (irreversible MAO-A inhibitor) or (-)-deprenyl (irreversible MAO-B inhibitor) at a concentration of 250 nM. The potential inhibitor under study was then added and further incubated for 5 minutes. Finally the nonselective substrate kynuramine was added to a concentration equal to its Km (90 µM for MAO-A and 60 µM for MAO-B). The formation of its metabolite (4-hydroxyquinoline) was monitored at 314 nm using a Kontron UVIKON 941 spectrophotometer. IC_{50} values were calculated from a hyperbolic equation as reported previously (8).

Potentiometric titrations of ionizable compounds were performed with the PCA 101 Apparatus (Sirius Analytical Instruments, Forrest Row, East Sussex, UK) equipped with a semi-micro Ross-type double junction combination pH electrode (Orion 8103SC), a temperature probe, an overhead stirrer, a precision dispenser, and a six-way valve for distributing reagents and titrants (0.5 M HCl, 0.5 M KOH, 0.15 M KCl, and MeOH). Shake-flask measurements were performed according to the method described in (9). Calculated log P (ClogP) were obtained with the MedChem program (10). Quantum mechanical calculations were performed using the software Spartan 5.0 running on a Silicon Graphics Origin 2000 workstation. The geometries were fully optimized using the 3-21G basis set for ab initio calculations and standard convergence criteria.

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RESULTS AND DISCUSSION

Partitioning and intramolecular interactions

Lipophilicity was measured in two biphasic isotropic systems, namely *n*-octanol/water and 1,2-dichloroethane (1,2-dce)/water. The correlation between the log P_{oct} and the ClogP values was good (n =18, r^2 = 0.94, s = 0.15, F = 252) indicating that the intramolecular effects operating in *n*-octanol/water were similar for the three series of compounds. However, the behavior of the three series was different in 1,2-dce/water as illustrated by their $\Delta\log P_{oct\text{-}dce}$ values. Because $\Delta\log P_{oct\text{-}dce}$ measures the H-bonding capacity of a solute (mainly of its H-bonding donor capacity) (11,12), the results suggest that the benzimidazoles **2a, 2b,** and **2d-f** ($\Delta\log P_{oct\text{-}dce}$ about 1.5) and the benzotriazoles **3a, 3b,** and **3d-g** ($\Delta\log P_{oct\text{-}dce}$ about 1.2) are better H-bond donors than the corresponding indazoles **1a-f** ($\Delta\log P_{oct\text{-}dce}$ about 0.5). However, variations in other properties (dipole moment, polarizability) may also operate.

The comparison between partition coefficients also reveals the existence of a strong intramolecular H-bond between the nitro substituent and the N_1-H function, responsible for the small $\Delta\log P_{oct\text{-}dce}$ of **1d**, **2c**, and **3c**. As already described for *o*-nitrophenols (12), this intramolecular H-bond is stronger in 1,2-dce/water.

Structure-;activity relationships

The IC_{50} values of the tested compounds are reported in Table 1. Inspection of the table clearly reveals that the compounds were moderate or weak inhibitors of MAO-B, with little or no activity toward MAO-A. The values are in the range from 2.8 µM to >100 µM for MAO-B, and from 40 µM to >100 µM for MAO-A. The inhibitors seem to act in a reversible and time-independent manner, with a moderate selectivity toward MAO-B for the most active compounds.

Because MAO-A inhibition was low and presented insufficient variation, structure-;activity relationships could only be examined for MAO-B inhibition. As discussed below, only qualitative trends are apparent due to the limited structural variation of the compounds examined in this exploratory work.

Table 1. Lipophilicity and MAO inhibitory activities of some benzazoles

	Lipophilicity parameters				Inhibitory activity IC_{50} [µM]		Positive MEPg)	Negative MEP(e) g)	
	CLOGP[a)]	log P_{oct}[b)]	log P_{dce}[c)]	Δlog P[d)]	MAO-A	MAO-B	near N1	near N2	near N3
1a	1.63	1.80[e)]	1.23[e)]	0.57	› 100	72 ± 1.9	65.1	-50.1	-
1b	1.73	1.97[e)]	1.42[e)]	0.55	44 ± 2.1	3.3 ± 0.18	77.7	-39.0	-
1c	1.73	1.65[e)]	1.25[e)]	0.40	46 ± 1.5	2.8 ± 0.20	75.3	-40.0	-
1d	1.73	1.63[e)]	2.05[e)]	-0.42	70 ± 1.8	41 ± 1.1	58.1	-44.0	-
1e	2.51	2.42[e)]	1.98[e)]	0.44	38 ± 2.1	9.5 ± 0.66	75.3	-40.0	-
1f	0.40	0.47	-0.25[e)]	0.72	› 100	› 100	61.3	-53.5	-
2a	1.50	1.32	-0.37	1.69	› 100	› 100	71.2	-	-60.2
2b	1.45	1.50	0.30	1.20	› 100	35% (50µM)[f)]	84.2	-	-49.3
2c	1.45	1.62	1.16	0.46	› 100	64 ± 1.0	65.6	-	-49.7
2d	2.35	2.30	0.80	1.50	› 100	› 100	76.2	-	-55.3
2e	2.06	1.85	0.50	1.35	› 100	› 100	70.0	-	-61.5
2f	2.57	2.65	1.00	1.65	› 100	› 100	79.3	-	-53.2
3a	1.45	1.44	0.35	1.09	› 100	› 100	75.1	-46.8	-54.6
3b	1.19	1.20	0.26	0.94	› 100	38 ± 2.0	87.7	-36.0	-44.1
3c	1.19	1.00	0.91	0.09	› 100	› 100	69.8	-39.8	-44.8
3d	2.16	2.20	0.90	1.30	› 100	30 ± 1.5	79.5	-42.3	-50.3
3e	1.95	1.80	0.45	1.35	› 100	40 ± 1.2	74.2	-47.9	-56.0
3f	2.33	2.55	0.87	1.68	› 100	› 100	83.1	-39.7	-47.5
3g	2.66	2.47	1.12[e)]	1.35	24% (50µM)[f)]	35% (50µM)[f)]	78.3	-43.2	-51.0

a) Calculated octanol/water partition coefficients obtained from the Pomona Database (10).
b) Octanol/water partition coefficients measured by potentiometry except when otherwise indicated (SD = 0.05).
c) 1,2-Dichloroethane/water partition coefficients measured by potentiometry except when otherwise indicated (SD = 0.05)
d) log P_{oct} minus log P_{dce}.
e) Measured by the shake-flask method (SD < 0.05).
f) Percent inhibition at the concentration indicated.
g) For compounds 2 and 3, mean values (in kcal/mol) calculated for both tautomeric forms having a similar energy, except for 2c and 3c, where the most stable tautomers (possessing an internal H-bond between the N(1)-H and the NO_2 in position 7) were used.

No trends and no relations were found between lipophilicity descriptors (log P_{oct} or log P_{dce}) and activity. In contrast with other results (13), lipophilicity was not a discriminant factor in these series, suggesting that the stereoelectronic characteristics of the heterocycles must be the major modulator of activity.

As already described (14), charge transfer interactions between the FAD cofactor and inhibitors may contribute to MAO inhibition. Electronic properties (the energy and shape of the frontier molecular orbitals) were examined by ab initio calculations (results not shown). No clear relation between MAO-B inhibition, differences in topology,

and the energy of the frontier orbitals was found, suggesting that charge transfer interactions, if they exist, cannot explain variations in activity (Figure 1).

Indazoles were the most active compounds; benzotriazoles retained some inhibition potency; and benzimidazoles were mostly inactive. Thus, activity appears linked to the endocyclic topology in which nitrogens in position 1, 2, and 3 may act as proton donor (N_1) or acceptor (N_2 and N_3).

5-Nitro- and 6-nitroindazoles (IC_{50} about 3 μM for **1b** and **1c**) emerge as the most active MAO-B inhibitors. Moreover, the activity of the 7-nitro-substituted derivatives **1d**, **2c**, and **3c** having a strong intramolecular H-bond was lowered, suggesting that an H-bond between the NH function and the enzyme is required for inhibition.

Recently, Wouters et al. (13) presented a common pharmacophore for the binding of heterocyclic MAO-B inhibitors. They proposed a primary binding site characterized by three H-bond acceptor anchor points and a secondary lipophilic binding site. The heterocyclic compounds examined here do not fit this general pharmacophore. Indeed, they differ in the distance between the lipophilic and heterocylic moieties and in the presence of an H-bond donor N-H function. However, this function cannot be the only factor determining activity since the benzimidazoles and the triazoles were less active despite their higher H-bonding donor capacity.

The three nitrogen atoms elicit favorable or unfavorable interaction with the active site of the enzyme. Benzimidazole derivatives were completely inactive, except compounds **2b** and **2c,** in which the nitro group modifies the electronic features of the heterocycle leading to a weak activity toward MAO-B. This implies that the absence of N_2 and/or the presence of N_3 as H-bond acceptors are detrimental to activity. Indeed, benzotriazoles retained some activity, suggesting that N_2 is in interaction with the enzyme and counterbalances the unfavorable presence of N_3.

The three series of heterocycles display a different distribution of MEP. Only the regions around the heterocyclic nitrogens were found to be informative. They are represented in Figure 2 for the unsubstituted derivatives **1a**, **2a,** and **3a**. Close to the N_1-H function, a positive region is generated by the H-atom, which can be related to the H-bond donating capacity of this function. In the indazoles (**1a-;1f**), the positive potential was smaller than that in the corresponding benzimidazoles (**2a-;2f**) and benzotriazoles (**3a-;3g**), a result in line with the smaller $\Delta\log P_{oct\text{-}dce}$ values.

An electron withdrawing substituent (NO_2 or Cl) in the 5 or 6 position enhanced the positive electrostatic potential leading to more active compounds, whereas the formation of an intramolecular H-bond with the 7-NO_2 substituent gave a lower potential and less active compounds.

The lone pair of the N_2 and N_3 atoms produced a negative potential whose intensity changed with the nature of the heterocycle and its substituents. The present results thus suggest that, the negative zone of N_2 in indazoles and benzotriazoles allowed an additional interaction with an H-bond donor group in the enzyme, the lower activity of benzimidazoles and benzotriazoles was caused by the presence of a strongly negative region near the N_3 atom.

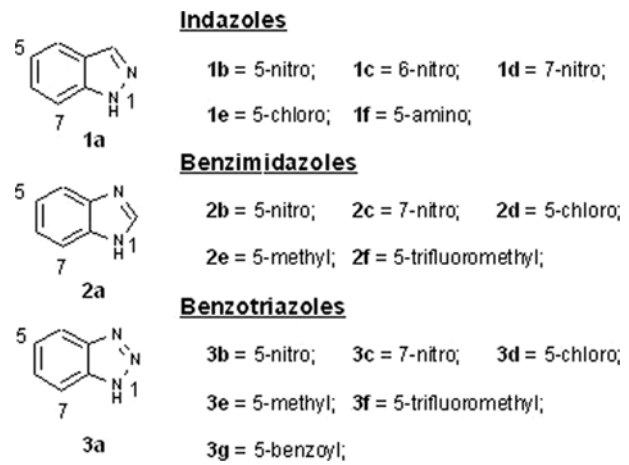

Figure 1: Chemical structures of investigated compounds.

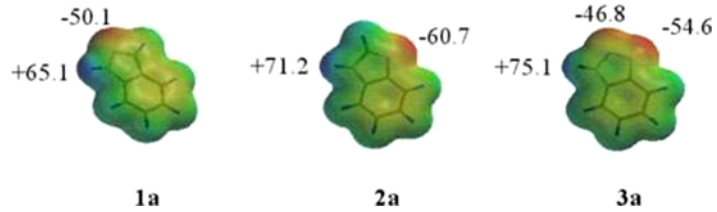

Figure 2: 3D-MEP (ab initio 3-21G calculations).

CONCLUSIONS

In conclusion, three features appear to influence MAO-B inhibition. These features may be related to an optimal binding mode allowing a good interaction between the ligand and the FAD cofactor in MAO-B.

First, the H-bond donor capacity near N_1-H influenced activity. When the H-bond donor capacity was lower, activity was lower (e.g., for **1d**, **2c,** and **3c)**. The presence of this anchor point is original with respect to previously investigated heterocycles, suggesting that these compounds may adopt a different mode of binding in the catalytic pocket of MAO-B.

The second feature is the presence of a negative region near the N_2 atom that offers an additional anchor point. This second binding site may be the one proposed by Wouters et al. in their pharmacophore (13).

The presence of a negative region near the N_3 atom lowered activity, suggesting that a high polarity in this region had a repulsive influence.

These relations lead us to propose an original pharmacophore for MAO-B inhibition (Figure 3), with the ring substituents modulating the electrostatic potential of both the positive and the negative region, and hence activity. Such results offer a promising starting point to design more potent MAO-B inhibitors with a binding pattern different from that of known inhibitors.

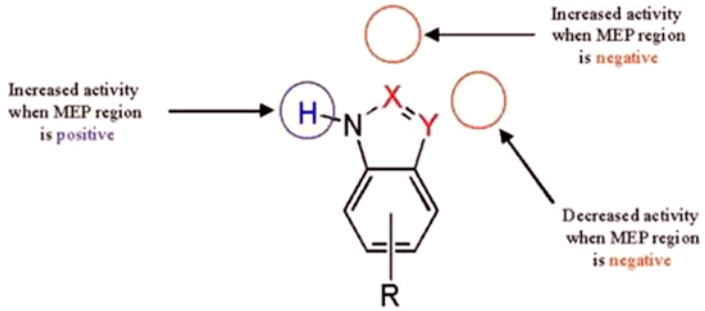

Figure 3: Pharmacophore for MAO-B inhibition.

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REFERENCES

1. Shih JC. Molecular basis of human MAO A and B. Neuropsychopharm. 1991;4:1-7.

2. Gargalidis-Moudanos C, Remaury A, Parini A. Predominant expression of monoamine oxidase B isoform in rabbit renal proximal tube: regulation by I2 imidazoline ligands in intact cells. Mol Pharmacol. 1997;51:637-643.

3. Castagnoli K, Palmer S, Anderson A, Bueters T, Castagnoli Jr. N. The neuronal nitric oxide synthase inhibitor 7-nitroindazole also inhibits the monoamine oxidase-B-catalyzed oxidation of 1-methyl-4-phenyl-1 , 2 ,3 , 6-tetrahydropyridine. Chem Res Toxicol. 1997;10:364-368.

4. Thull U, Testa B. Screening of unsubstituted cyclic compounds as inhibitors of monoamine oxidases. Biochem Pharmacol. 1994;47:2307-2310.

5. Paglietti G, Sparatore F. Preparazione di acidi b-benzotriazolilbutirrici variamente sostituiti quali potenziali coleretici. Farmaco. 1972;27:380-396.

6. Crowther AF, Curd FHS, Davey DG, Stacey GJ. Synthetic antimalarials. Part XXXIX. Sialkylaminoalkylaminoquinolines. J Chem Soc. 1949;1260-1271.

7. Weissbach H, Smith TE, Daly JW, Witkop B, Udenfriend S. A rapid spectrophotometric assay of monoamine oxidase based on the rate of disappearance of kynuramine. J Biol Chem. 1960;235:1160-1163.

8. Thull U, Kneubühler S, Testa B, Borges MFM, Pinto MMM. Substituted xanthones as selective and reversible monoamine oxidase A (MAO-A) inhibitors. Pharm Res. 1993;10:1187-1190.

9. Dearden JC, Bresnen GM. The measurement of partition coefficients. Quant. Struct-Act Relat. 1988;7:133-144.

10. DAYLIGHT Software 4.41, Daylight Chemical Information System, Inc., Irvine, California: 1995.

11. Wouters J, Ooms F, Jegham S, Koenig JJ, George P, Durant F. Reversible inhibition of type B monoamine oxidase. Theoretical study of model diazo heterocyclic compounds. Eur J Med Chem. 1997;32:721-730.

12. Steyaert G, Lisa G, Gaillard P, Boss G, Reymond F, Girault HH, Carrupt PA, Testa B. Intermolecular forces expressed in 1,2-dichloroethane/water partition coefficient: a solvatochromic analysis. J Chem Soc. 1997;93:401-406.

13. Chopineaux-Courtois V, Reymond F, Bouchard G, Carrupt PA, Testa B, Girault HH. Effects of charge and of intramolecular structure on the lipophilicity of nitrophenols. J Am Chem Soc. 1999;121:1743-1747.

14. Wouters J, Durant F, Champagne B, André JM. Electronic properties of flavins: implications on the reactivity and absorption properties of flavoproteins. Int J Quant Chem. 1997;64:721-733.