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Ultrasound promoted synthesis of 2-imidazolines in water: A greener approach toward monoamine oxidase inhibitors

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Abstract—A series of sixteen 2-substituted-2-imidazolines (where the substituent R = Ph, Me-4-Ph; MeO-4-Ph; $(MeO)_2$-3,4-Ph; $(MeO)_3$-3,4,5-Ph; Ph-4-O-C(O)-Ph; Cl-4-Ph; Cl-2-Ph; Cl_2-2,4-Ph; NO_2-4-Ph; NO_2-3-Ph; Naphth-2-yl; Fur-2-yl; Benzofur-2-yl; Pyridin-2-yl; Quinolin-2-yl) has been synthesized from the reaction of the substituted-aldehydes and ethylenediamine by ultrasound irradiation with NBS in an aqueous medium in high yields (80–99%). The 2-imidazoline ability to inhibit the activity of the A and B isoforms of monoamine oxidase (MAO) was investigated and some of them showed potent and selective MAO inhibitory activity especially for the MAO-B isoform and could become promising candidates for future development.

Monoamine oxidase (MAO), localized in the outer mitochondrial membrane, is a flavin adenine dinucleotide (FAD)-containing enzyme responsible for the oxidative deamination of amines in the brain and the peripheral tissues, regulating their level.[1] This reaction produces the corresponding aldehyde and free amine, with the generation of hydrogen peroxide.[1] MAO exists in two isoforms, namely, MAO-A and MAO-B, which differ according to their substrate specificity and their selectivity to the inhibitor.[2] MAO-A preferentially metabolizes serotonin and noradrenaline and is inhibited by low concentrations of clorgyline.[3] MAO-B acts preferentially on 2-phenylethylamine and benzylamine and is inhibited by selegiline (*L*-deprenyl).[4] Dopamine, tyramine, and tryptamine are the substrates for both isoforms of MAO.[4]

Due to their role in neurotransmitter metabolism, the regulation of MAO-A and MAO-B activity has been an important target for treating psychiatric and neurodegenerative disorders. Currently, compounds able to inhibit these enzymes have been used in the therapy of Parkinson's and Alzheimer's disease, depression syndrome and panic disorders.[5] In fact, Parkinson's and Alzheimer's disease have been associated with oxidative stress and increasing MAO-B activity in the CNS.[6]

Over the last 15 years, since the demonstration that I_2-imidazoline sites are associated with the mitochondrial fraction of membranes,[7] several studies have provided evidence that these sites represent regions on MAOs.[8] In fact, I_2-binding sites have been identified on both MAO-A and MAO-B isoforms as regulatory sites are able to modulate MAO activity through a not yet fully understood inhibitory mechanism.[8] Moreover, because of the different tissue sensitivities to amiloride, a guanidine able to discriminate between I_{2A}- and I_{2B}-subtypes, it has been proposed that I_{2A}-sites are located on MAO-A and I_{2B}-sites on MAO-B.[9] These I_2-binding sites do not appear to be located within the enzyme catalytic site, or the prosthetic group, or the binding domain of classical MAO inhibitors.[8a,10] However, most recent reports have demonstrated that the majority of amino acid residues identified as I_2-sites on MAO-B

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(149–222) are obtained within the active site or entrance cavity of the enzyme according to the crystallographic studies.[11] Recently, it was also demonstrated that imidazoline compounds are able to bind to the MAO-A active site.[12]

Therefore, some reports from the literature have demonstrated that imidazoline and guanidinium derivative compounds are able to inhibit MAO activities.[10a,13] This effect has been attributed to a high affinity I_2 binding site on MAO-B (I_{2B}) and a similar lower affinity site on MAO-A (I_{2A}).[14] In this context, the limited accessibility of the I_2 imidazoline binding domain on MAO-B in various tissues indicates the existence of a distinct subpopulation of the enzyme.[15] Thus, the use of imidazoline derivates such as MAO inhibitors may allow for the development of new therapeutic agents that target the enzyme in a cell-type selective manner.

We are particularly interested in the therapeutic role of MAO-B inhibitors in Parkinson's and Alzheimer's disease. In this paper, in an attempt to identify novel imidazoline derivatives endowed with MAO inhibitory activity and selectivity we have synthesized a series of 2-imidazolines combining a greener synthetic approach with assessment of their in vitro activities.

The energy provided by ultrasound has been utilized recently to accelerate a number of synthetically useful reactions.[17] Its effect observed on organic reactions is due to cavitation, a physical process that creates, enlarges, and implodes gaseous and vaporous cavities in an irradiated liquid, thus enhancing the mass transfer.[17a] Compared with traditional methods, this technique is more appropriate in the consideration of green chemistry concepts. Ultrasound application is considered a processing aid (or auxiliary) in terms of energy conservation and waste minimization.[18] Recently, we have demonstrated the use of this form of energy in heterocyclic preparations providing a remarkable rate of enhancement and a dramatic reduction in reaction time.[19]

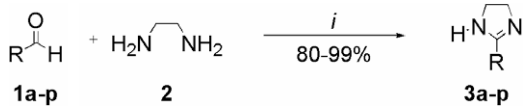
In recent decades, the use of water as solvent in organic reactions has been reinvestigated.[20] Water is a nontoxic solvent and readily available at low cost. It is also nonflammable and environmentally benign, providing opportunities for clean processing and pollution prevention. In addition, because organic reactions often display unique reactivity and selectivity, performing them in an aqueous medium at or slightly above room temperature has become of great interest in order to exploit, for example, their so-called hydrophobic effects.[20a]

The synthesis of 2-imidazolines (**3a**–**p**) was carried out by the previously reported method of condensation involving aldehydes (**1a**–**p**) and ethylenediamine (**2**) in the presence of *N*-bromosuccinimide.[16] The reaction was performed in water as solvent under ultrasonic irradiation furnishing the products (**3a**–**p**) in high yields and significantly shorter times (Scheme 1).

The method for forming 2-imidazolines under ultrasonic irradiation offers several advantages including faster reaction rates, higher purity, and higher yields. In comparison with conventional methods, the main goal of ultrasound application was the significant decrease of reaction time. While conventional methods require agitation overnight,[16] under ultrasonic irradiation the products were obtained in 12–18 min.[21,22] Moreover, this approach does not require the use of any halogenated solvent or an additional method for product purification. In contrast to other methods,[23] the synthesis of 2-imidazolines did not require the use of any catalyst.

All compounds **3** showed physical and spectrometric properties corresponding to the proposed structures and in accordance with the literature.[16,25]

The in vitro inhibitory activity of compounds **3a-p** against MAOs was determined as previously described[24] and the results are shown in Table 1. The compounds obtained were considered potent when K_i was less than 10 μM and were particularly selective when their selectivity index (K_i MAO-A/K_i MAO-B) was greater than 10. Among the synthesized compounds that inhibited preferentially MAO-A (entries **3c**–**e**, **3j**) only compound **3d** was found to be selective, presenting a K_i for MAO-A of approximately 73-fold lower than its K_i for MAO-B. Imidazoline **3p** also showed a potent inhibitory effect (K_i 4.86 μM) for this MAO isoform, however, this compound was not selective (0.91).



1a-p **2** **3a-p**

Entry	R	Time (min)	Yield (%)
a	Ph	12	99
b	Me-4-Ph	18	95
c	MeO-4-Ph	15	92
d	$(MeO)_2$-3,4-Ph	15	90
e	$(MeO)_3$-3,4,5-Ph	15	86
f	Ph-4-O-C(O)-Ph	15	84
g	Cl-4-Ph	18	98
h	Cl-2-Ph	15	92

Entry	R	Time (min)	Yield (%)
i	Cl_2-2,4-Ph	12	93
j	NO_2-4-Ph	18	82
k	NO_2-3-Ph	15	96
l	Naphth-2-yl	15	91
m	Fur-2-yl	18	80
n	Benzofur-2-yl	15	87
o	Pyridin-2-yl	15	95
p	Quinolin-2-yl	18	97

Scheme 1. Reagents and conditions: (i) H_2O, NBS,))), 65–70 °C, 12–18 min.

Table 1. Monoamine oxidase inhibitory activity of 2-imidazolines **3a–p**

Entry	K_i MAO-A[a] (μM)	K_i MAO-B[a] (μM)	SI[b]
3a	28.75 (21.0–39.4)	21.27 (14.7–30.7)	1.35
3b	102.10 (64.4–162)	13.09 (8.1–21.0)	7.79
3c	15.75 (10.2–24.2)	32.67 (20.9–51.1)	0.48
3d	13.57 (9.1–20.2)	~1000	0.013
3e	114.40 (75.0–174.4)	~1000	0.114
3f	12.84 (8.6–19.2)	1.38 (0.97–1.97)	9.27
3g	166.30 (102.3–270.5)	5.35 (3.4–8.3)	31.05
3h	>1000	24.57 (15.2–39.8)	>40.70
3i	28.75 (20.9–39.4)	3.98 (2.9–5.3)	10.52
3j	181.40 (115–286.2)	>1000	0.181
3k	>1000	19.27 (11.1–33.5)	>51.89
3l	13.89 (6.02–32.04)	1.49 (0.4–5.1)	9.32
3m	>1000	>1000	—
3n (2-BFI)	24.94 (14.3-43.6)	3.63 (0.3–4.0)	6.86
3o	>1000	25.28 (11.8–54.0)	>39.55
3p (BU224)	4.86 (3.6–6.6)	5.32 (3.8–7.4)	0.91

[a] Each value represents the mean (confidance interval) of three or four independent experiments.

[b] In vitro selectivity index = (K_i MAO-A/K_i MAO-B).

Among the compounds obtained that selectively inhibited MAO-B (entries **3g–i**, **3k**, **3o)**, only imidazolines **3g** and **3i** were shown to be potent with K_i values of 5.35 μM and 3.98 μM, respectively. One can conclude that the replacement of chlorine at the *ortho* position in the 2-imidazoline **3h** with the *para* position on compound **2g** seemed to decrease selectively for MAO-B and increase the inhibitory potency against this isoform. The dichloro-substituted compound **3i** lost selectivity with a slightly increasing inhibitory activity against MAO-B. An interesting finding was the inversion of selectivity of 2-imidazoline **3k** from the replacement of an electron-withdrawing group (NO_2) at the *meta* position of benzene with an electron-donating group (OMe) in compound **3d**. While the imidazoline **3k** was able to selectively inhibit MAO-B, compound **3d** selectively inhibited MAO-A. This suggests that electronic effects can govern the recognition process for 2-imidazolines on both MAOs.

Some of the synthesized compounds were shown to exert potent and selective binding at I_2-sites, such as **3l** (benazoline), **3n** (2-BFI), and **3p** (BU224).[25] In fact, these imidazolines showed good inhibitory activity especially against MAO-B. The 2-BFI and BU224 IC_{50} values reported in the literature range from 11 to 16.5 and 4.8 to 10.7 μM for MAO-A and from 23 to 27.9 and 44.8 to 51.4 μM for MAO-B, respectively.[13,29,30] Our obtained 2-BFI and BU224 IC_{50} values were 65 and 12.7 μM for MAO-A and 9.1 and 13.3 μM, respectively. Thus, in our conditions 2-BFI and BU224 seems to be less potent to inhibit MAO-A and more potent to inhibit MAO-B when compared with the literature values. However, when K_i values were compared, the 2-BFI potency on MAO-A obtained in our study (29.94 μM) are similar with the literature data (26 μM).[12] As K_i values are more appropriated for potency comparison among different studies, our results are consistent with the literature data. However, none of the compounds endowed with high affinity for the I_2-site were able to inhibit MAO with significant selectivity. Reports from the literature have affirmed that there are no significant correlations between the potencies of imidazoline derivatives at I_2-sites and their values for inhibition of MAO-A or MAO-B activities. In general, these compounds show two to three times less activity for MAO inhibition than their reported values of I_2-site affinity.[13] Thus, it has been suggested that I_2-sites are not directly related to the site of action of these drugs against MAO activity.

The nature of the interaction of imidazolines with MAO has been described as either noncompetitive[8b] and mixed,[10a] or competitive for MAO-A inhibition and mixed for MAO-B inhibition.[13] Our preliminary kinetics studies showed that compound **3g**, obtained as a lead-like compound, inhibited MAO-B in a competitive manner. However, additional studies are necessary to clarify this point.

In summary, we have reported the preparation of 2-imidazolines using a highly efficient and environmentally benign synthetic protocol to obtain novel compounds endowed with MAO inhibitory activity. The simplicity, the use of water instead of organic solvents as reaction medium, the high yields (80–99%), and the short reaction times (12–18 min) make this reaction highly attractive. In addition, the existence of a distinct subpopulation of MAO-B with a different accessibility to the I_2-site may allow access to inhibitors of this isoform in a tissue-specific manner, avoiding numerous side effects attributed to these classes of compounds. Our contribution toward this understanding was to obtain novel 2-imidazoline derivatives as active MAO inhibitors in the μM range with good selectivity. Studies on the use of 2-imidazoline compounds in models of Parkinson's and Alzheimer's disease are in progress and these data will be communicated hereafter.

Acknowledgments

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References and notes

1. Youdim, B. H.; Bakhle, Y. S. *Br. J. Pharmacol.* **2006**, *147*, 287.
2. Shih, J. C.; Chen, K.; Ridd, M. J. *Annu. Rev. Neurosci.* **1999**, *22*, 197.
3. Weyler, W.; Hsu, Y. P.; Breakefield, X. O. *Pharmacol. Ther.* **1990**, *47*, 391.
4. Kalgutkar, A. S.; Dalvie, D. K.; Castagnoli, N., Jr.; Taylor, T. J. *Chem. Res. Toxicol.* **2001**, *14*, 1139.
5. Youdim, B. H.; Edmondson, D.; Tipton, K. F. *Nat. Rev. Neurosci.* **2006**, *7*, 295.
6. (a) Volz, H. P.; Gleiter, C. H. *Drugs Aging* **1998**, *13*, 341; (b) Good, P. F.; Werner, P.; Hsu, A.; Olanow, C. W.; Perl,

D. P. *Am. J. Pathol.* **1996**, *149*, 21; (c) Drukarch, B.; Muiswinkel, F. L. *Biochem. Pharmacol.* **2000**, *59*, 1023.
7. Tesson, F.; Parini, A. *Eur. J. Pharmacol.* **1991**, *208*, 81.
8. (a) Raddatz, R.; Parini, A.; Lanier, S. *J. Biol. Chem.* **1995**, *270*, 27961; (b) Tesson, F.; Limon-Boulez, I.; Urban, P.; Puype, M.; Vardekerckhove, J.; Coupry, I.; Pompon, D.; Parini, A. *J. Biol. Chem.* **1995**, *270*, 9856; (c) Raddatz, R.; Parini, A.; Lanier, S. M. *Mol. Pharmacol.* **1997**, *52*, 549; (d) Raddatz, R.; Savic, S. L.; Bakthavachalam, V.; Lesnick, J.; Jasper, J. R.; Mcgrath, C. R.; Parini, A.; Lanier, S. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 1135.
9. Parini, A.; Moudanos, C. G.; Pizzinat, N.; Lanier, S. M. *Trends Pharmacol. Sci.* **1996**, *17*, 13.
10. (a) Carpéné, C.; Collon, P.; Remaury, A.; Cordi, A.; Hudson, A.; Nutt, D.; Lafontan, M. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 681; (b) Limon-Boulez, I.; Tesson, F.; Gargalidis-Moudanos, C.; Parini, A. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 359.
11. (a) Binda, C.; Newton-Vinson, P.; Hubalek, F.; Edmondoson, D. E.; Mattevi, A. *Nat. Struct. Biol.* **2002**, *9*, 22; (b) Ma, J.; Yoshimura, M.; Yamashita, E.; Nakagawa, A.; Ito, A.; Tsukihara, T. *J. Mol. Biol.* **2004**, *338*, 103.
12. Jones, T. Z. E.; Giurato, L.; Guccione, S.; Ramsay, R. R. *FEBS J.* **2007**, *274*, 1567.
13. Ozaita, A.; Olmos, G.; Boronat, M. A.; Lizcano, J. M.; Unzeta, M.; García-Sevilla, J. A. *Br. J. Pharmacol.* **1997**, *121*, 901.
14. Paterson, L. M.; Tyacke, R. J.; Robinson, E. S. J.; Nutt, D. J.; Hudson, A. L. *Neuropharmacology* **2007**, *52*, 395 (and references therein).
15. Raddatz, R.; Parini, A.; Lanier, S. M. *J. Biol. Chem.* **1995**, *270*, 27961.
16. (a) Fujioka, H.; Murai, K.; Ohba, Y.; Hiramatsu, A.; Kita, Y. *Tetrahedron Lett.* **2005**, *46*, 2197; (b) Fujioka, H.; Murai, K.; Kubo, O.; Ohba, Y.; Kita, Y. *Tetrahedron* **2007**, *63*, 638.
17. (a) Manson, T. J. *Chem. Soc. Rev.* **1997**, *26*, 443; (b) Cella, R.; Stefani, H. A. *Tetrahedron* **2006**, *62*, 5656; (c) Guzen, K. P.; Guarezemini, A. S.; Órfão, A. T. G.; Cella, R.; Pereira, C. M. P.; Stefani, H. A. *Tetrahedron Lett.* **2007**, *48*, 1845.
18. Cintas, P.; Luche, J.-L. *Green Chem.* **1999**, *1*, 115.
19. Martins, M. A. P.; Pereira, C. M. P.; Cunico, W.; Moura, S.; Rosa, F. A.; Peres, R. L.; Machado, P.; Zanatta, N.; Bonacorso, H. G. *Ultrason. Sonochem.* **2006**, *13*, 364.
20. (a) Dallinger, D.; Kappe, C. O. *Chem. Rev.* **2007**, *107*, 2563; (b) Lindström, U. M. *Chem. Rev.* **2002**, *102*, 2751.
21. Synthesis of 2-imidazolines **3a**–**p** (*General procedure*): a mixture of aldehyde **1** (1 mmol) and ethylenediamine **2** (0.072 g, 1.2 mmol) in water (12 mL) was added to a glass tube of 15 mL. With aid of adapter, a titanium microtip (Ø 6 mm) was attached to the reaction glass container and the mixture was sonicated by 2 min (Vibra Cell VC50, *Sonics & Material Inc.*). After this time, NBS (0.213 g, 1.2 mmol) was added to the mixture and the resulting solution was sonicated for the appropriate time (Scheme 1). The reaction temperature was raised to 65–70 °C after sonication for 8–10 min. After cooling to room temperature NaOH (20%) aq was added to the reaction mixture. Finally, the solution was extracted with ethyl acetate (3× 15 mL); after combined, the organics layers were washed with water (2× 10 mL) and dried with Na_2SO_4. The solvent was removed under reduced pressure to afford the 2-imidazolines **3** with excellent purity.
22. ^1H and ^{13}C NMR spectra were acquired on a Bruker DPX 200 or Bruker DPX 400 spectrometer (^1H at 200.13 MHz or 400.13 MHz and ^{13}C at 50.32 MHz or 100.63 MHz, respectively) at 300 K, in 5 mm sample tubes, and with a digital resolution of ±0.01 ppm. $CDCl_3$ was used as solvents containing TMS as internal standard. Mass spectra were registered in a HP 5973 MSD connected to a HP 6890 GC and interfaced by a Pentium PC. The GC was equipped with a split-splitless injector, autosampler cross-linked HP-5 capillary column (30 m, 0.32 mm of internal diameter), and helium was used as the carrier gas. Select data for compound **3f**: mp 116–118 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.80 (s, 4H, $2CH_2$), 3.98 (br, 1H, NH), 7.25–7.69 (m, 5H, Ph), 7.84–8.22 (m, 4H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 49.8 ($2CH_2$), 121.7, 127.5, 128.5, 128.6, 129.0, 130.0, 133.7, 152.8 (Ph), 164.1 (C2), 164.7 (C=O); MS EI (70 ev) *m/z* (%): 266.1 (M^+, 48), 237.1 (4), 132.1 (4), 105.1 (100), 77.1 (62), 51.0 (11); Anal. calcd for $C_{16}H_{14}N_2O_2$: C 72.16%, H 5.30%, N 10.52%; found: C 72.03%, H 5.17%, N 10.47%.
23. Mirkhani, V.; Moghadam, M.; Tangestaninejad, S.; Kargar, H. *Tetrahedron Lett.* **2006**, *47*, 2129.
24. *MAO inhibitions assay.* The synthesized compounds were tested for their inhibitory activity on MAO-A and -B in rat brain mitochondrial homogenates.[26] The protein concentration was determined according to Bradford.[27] The mitochondrial fractions were preincubated at 37 °C for 5 min with the irreversible and selective inhibitor selegiline (250 nM) or clorgyline (250 nM) to assay MAO-A or MAO-B activity, respectively. The imidazoline compounds were dissolved in dimethyl sulfoxide (DMSO) and added to the reaction mixture from 0 to 10^3 μM. After 5 min, kynuramine was added as a nonselective substrate at concentrations equal to the corresponding K_M value (90 μM for MAO-A and 60 μM for MAO-B). The solutions were incubated at 37 °C for 30 min. Addition of three chloroacetic acids ended the reaction and the samples were centrifuged at 16,000*g* for 5 min. The supernatant fractions were removed and the concentrations of the MAO generated product, 4-hydroxyquinoline (4-OHQ), which was measured spectrophotometrically at 314 nm. When there was interference of the compounds tested on the spectrophotometric methods, the fluorescent product of the reaction was measured by fluorimetric method, using 315 nm (excitation) and 380 nm (emission). Apparent K_i values were calculated using the equation for competitive inhibitors.[28]
25. Anastassiadou, M.; Danoun, S.; Crane, L.; Baziard-Mouysset, G.; Payard, M.; Caignard, D.-H.; Rettori, M.-C.; Renard, P. *Bioorg. Med. Chem.* **2001**, *9*, 585.
26. Soto-Otero, R.; Méndez-Alvarez, E.; Hermida-Ameijeiras, A.; Sánchez-Sellero, I.; Cruz-Landeira, A.; Lamas, M. L. *Life Sci.* **2001**, *69*, 879.
27. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
28. Cheng, W. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
29. Tesson, F.; Prippbuus, C.; Lemoine, A.; Pegorier, J. P.; Parini, A. *J. Biol. Chem.* **1991**, *266*, 155.
30. Lalies, M. D.; Hibell, A.; Hudson, A. L.; Nutt, D. J. *Ann. NY Acad. Sci.* **1999**, *881*, 114.