Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1825–1827

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis, Anticholinesterase Activity and Structure–Activity Relationships of *m*-Aminobenzoic Acid Derivatives

José Trujillo-Ferrara,* Leticia Montoya Cano and Michel Espinoza-Fonseca

Sección de graduados y Departamento de Bioquímica, Escuela Superior de Medicina del Instituto Politécnico Nacional, Apartado Postal 42-161, C.P. 11340, Mexico City, Mexico

Received 19 July 2002; accepted 18 December 2002

Abstract—The synthesis, acetylcholinesterase inhibitory capacity and structure–activity relationships of simple-structured *m*-Aminobenzoic acid derivatives are reported. Compound **1b** was found to be more potent than galanthamine and tacrine in inhibiting acetylcholinesterase.

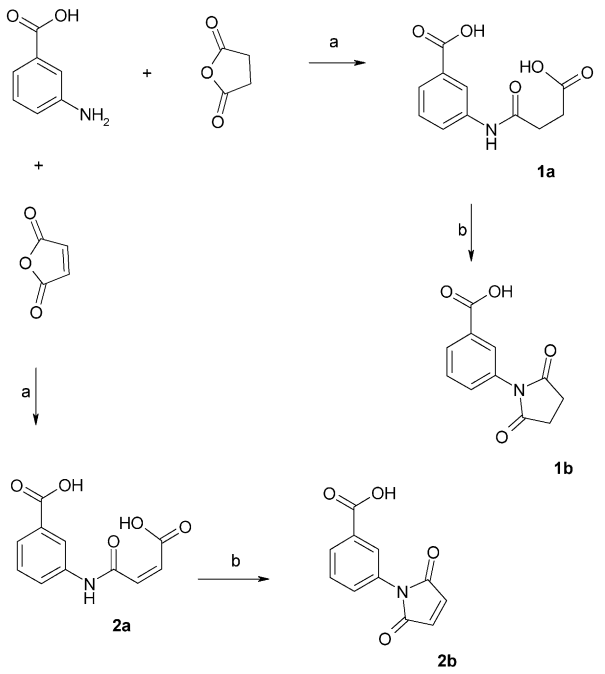
Over two decades ago, several autopsy studies inside hippocampus revealed that the levels of the neurotransmitter acetylcholine in patients with Alzheimer's disease are importantly decreased.[1,2] Thereby, the cholinergic hypothesis has been purposed, postulating that memory impairments in patients with Alzheimer's disease result from a deficit of the function in the brain.[3] One possible approach to treating this disease is to restore the acetylcholine levels by inhibiting acetylcholinesterase (AChE) with highly-selective inhibitors. Unfortunately, most common inhibitors are highly toxic,[4] possess a fixed charge in nitrogen nuclei, which renders them incapable of crossing the blood brain barrier[5] or well the synthesis pathway results, in many of cases, complicated. In previous papers we reported the synthesis and spectrometric characterization of relatively-easy synthesized and simple-structured arylamides and arylimides of pharmacological interest in the treatment of Alzheimer's disease.[6,7] In connection with our initial interest in the design of arylamides and arylimides structurally related to acetylcholine, we report the synthesis, anticholinesterase activities and structure–activity relationships of arylamides (**1a**, **2a**) and arylimides (**1b**, **2b**) derived from *m*-Aminobenzoic (*m*-AB) acid. In principle, our strategy is based on the crystallographic structure of the active-site gorge of AChE from *Torpedo californica*.[8] The active-centre contains a catalytic triad (Ser200, His440, Glu327), located at the bottom of the narrow gorge. Near to the active site, is located Trp84, a residue of the anionic site. Trp84 has been identified as the binding site of quaternary nitrogen of acetylcholine, decamethonium and edrophonium.[9] Previous studies made by our workgroup revealed that related compounds could interact with Trp84. It make us suppose that the aromatic ring of these compounds may interact, through π–π stacking, with Trp84, and the amidic or imidic fragment will be able to interact with the catalytic triad of the active site. Two of the compounds here reported (**2a** and **2b**) possess an α-β-unsaturation, allowing them to react through 1,4 Michael's addition with nucleophiles present in the active site gorge (imidazoles, alcohols, thiols); in this way, it is possible, moreover, that compounds **2a** and **2b** behave themselves as active-site selective irreversible inhibitors.

The synthesis of arylamides and arylimides was achieved by slight modifications to a previously reported method,[6] and consists briefly as follows. The reaction between *m*-AB acid and succinic and maleic anhydride, in the presence of tetrahydrofurane at room temperature, was carried out, obtaining the respective arylamides, **1a** and **2a**. These products were subsequently transformed to the corresponding arylimides, **1b** and **2b**, by heating them in acetic anhydride with an equimolecular amount of sodium acetate (Scheme 1, Table 1). Synthesized compounds were characterized employing ^{1}H–^{13}C NMR, IR and UV spectroscopy.

Synthesized compounds were tested for potency as acetylcholinesterase inhibitors. In vitro enzyme inhibition was determined using the modified Bonting and

*Corresponding author. Tel./Fax: +52-555-7296300; e-mail: jtrujillo@ipn.mx

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00198-7



Scheme 1. (a) THF, 25 °C, 1 h; (b) Ac_2O, AcONa, 80 °C, 3 h.

Table 1.

Products (Yield,%; mp*, °C)			
1a	(95, 236–238)	**1b**	(75, 136–138)
2a	(92, 218–220)	**2b**	(73, 228–230)

*mp are uncorrected.

Featherstone's colorimetric method.[10] The confirmation of irreversible inhibition of **2a** and **2b** was carried out through dialysis kinetics by several hours, observing the lost of hydrolytic activity of the enzyme. Results are summarized in Table 2.

Four compounds presented here turned out to be acetylcholinesterase inhibitors. As originally considered,[7] these molecules contain a high number of sp^2 carbons, and, therefore, the increasing of π orbitals; this implies that molecular recognition by the enzyme will be importantly increased. About inhibitory potency, we consider that possibly three lead factors are involved in the enzyme–ligand interactions, in the case of our compounds: (a) the assay pH, (b) the structural analysis, fundamentally based on the degrees of freedom in many torsion angles and the presence of the double bond in molecules **2a** and **2b** in comparison to molecules **1a** and **1b**, and (c) the electronic contribution of π orbitals from each molecule. First, we know that the inhibitory potency is related to the lipophilicity of compounds, and the recognition by acetylcholinesterase is preferably given on apolar surfaces. Higher inhibitory potency of arylimides **1b** and **2b**, compared with arylamides **1a** and **2a**, was observed. This behavior can be related with their molecular structure properties: arylimides possess one ionizable proton, and at the assay pH 8, they have a −1 net charge. On the other hand, arylamides possess two ionizable protons, and in the same assay conditions, the net charge of these molecules is equal to −2. Therefore, the increase in the number of charges in the arylamides, compared with arylimides, diminishes their lipophilicity and, consequently, the inhibitory potency. In addition, molecules **2a** and **2b** possess a double bond in the amidic and imidic fragment, respectively; this fact makes possible the electron delocalization, the facile polarization of these molecules and, as consequence, the decreasing in the lipophilicity of them, and of course, the inhibitory potency. To support the affirmations made above, we obtained the calculated log *P* (Crippen) value as lipophilicity indicator of four molecules, employing Vega software version 1.4.3.[11] Also, we obtained the Hansch's π value for each sustituent (imide or amide), using the benzoic acid as pattern. The correlation between these parameters and the IC_{50} let us know that lipophilicity of these compounds is tightly related with the inhibitory activity. Log *P* and Hansch's π sustituent values are summarized in Table 3.

Table 2.

Compd	IC_{50} (nM)	Inhibition
1b	33.4±2	Reversible
1a	92.5±5	Reversible
2b[a]	256±13	Irreversible
2a[b]	357±10	Irreversible
Aminophenol derivatives	100–4000	Mixed
Tacrine	50±4	Reversible
Galanthamine	360±10	Reversible

[a] $k_{+2} = 8.5\times10^{-1}$ min^{-1}.
[b] $k_{+2} = 5.03\times10^{-1}$ min^{-1}.

On the other hand, based on the structural analysis, arylamides **1a** and **2a** in comparison to arylimides **1b** and **2b**, possess more degrees of freedom in torsion angles, and therefore, more possibilities in the structure-conformation changes. Moreover, the assay media is highly polar, giving molecules the possibility of intra- and intermolecular interactions, and, as outcome, the important increasing in conformational changes. To simulate the possible conformational changes for each molecule in only-water media, we applied molecular dynamics to the four molecules in a box water cluster, at $T = 310$ K, integration time step of 1 ps, step size equal to 0.001 ps, no heating and one time step in data collection period, employing HyperChem software.[12] At the end of simulations, we observed, for molecule **1a**, that conformer populations may vary between *anti* and *gauche* forms, starting from the saturated carbons from amidic fragment, being *gauche* the must stable conformation. On the other hand, molecule **2a** presented fixed conformations in relation to vinilic fragment. Arylimides **1b** and **2b** did not present important structural changes in the imidic fragment, in base to the original energetic state.

Table 3.

Compd	Log *P* (Crippen)	π (log P_x/P_H)
1b	1.8092	0.2182
1a	1.4131	−0.5697
2b	1.4839	−0.1071
2a	1.0939	−0.4971

Starting with this evidence, we presuppose the existence of more arylamide conformers than arylimide conformers at the assay conditions. This fact make us suppose the increase in the steric and electronic interactions between arylamides here presented and the residues located in the enzyme's gorge. These interactions could make difficult the entrance and displacement of arylamides **1a** and **2a** through the gorge, and consequently diminish their inhibitory potency in comparison to arylimides **1b** and **2b**.

We analyzed molecules by means of theoretical calculations for four compounds. Energy minimization and nuclei charge of each molecule was carried out at semiempirical AM1 level using Mopac 6.0 included in Vega package;[11] optimized molecules were treated to obtain their respective π orbitals. As observed, compounds **2a** and **2b** possess more π orbitals distributed along the molecule than compounds **1a** and **1b** and, therefore, more electron density. This fact makes us suppose that compounds **1a** and **1b** have an advantage compared to compounds **2a** and **2b** because we know that the acetylcholinesterase's gorge has, at least, 14 aromatic residues[8] which may interact with electron-enriched compounds through π–π stacking, principally; this way, the diffusion through the gorge of compounds **2a** and **2b** is lower than compounds **1a** and **1b**. Low diffusion through the active site has as consequence the limitation in inhibitory potency for compounds **2a** and **2b**, as observed experimentally.

Also, it is possible that molecules **1b** and **2b** may be hydrolyzed to molecules **1a** and **2a** either in solution or in the enzyme active site, and the behavior of the molecules may be more complicated than presented above, but currently we are working on elucidating all possible mechanisms; but experimentally we know that, at pH 9, compounds **1b** and **2b** keep their cyclic structure, whereas at pH >9 and room temperature compounds are transformed to molecules **1a** and **2a**, respectively; this effect is spectroscopically observed, since at pH >9 A_4 and A_2 systems in the ^{1}H NMR spectra from imidic fragment of molecules **1b** and **2b**, respectively, changes to A_2B_2 and AB systems in the ^{1}H NMR spectra, corresponding to the amidic **1a** and **2a** forms. Therefore, imides here presented are stable at assay pH, being much more stable than arylimide **1b**. On the other hand, the possibilities of ring closing in arylamides are low, because of this reaction to be made requires high-energy conditions and the presence of a catalyzst; therefore, arylamides at the assay conditions (pH 8, $T=37\,^{\circ}C$, phosphates buffer) are only able to be solvated, but not cycled.

In summary, arylimide **2a** was more potent than tacrine and galanthamine (1.5- and 10.8-fold, respectively), while compound **2b** was less potent than tacrine (1.9-fold) but more potent than galanthamine (3.9-fold). Four compounds were more potent than aminophenol derivatives reported by our workgroup.[7] Furthermore, preliminary toxicity probes showed that compounds **1a**, **2a** and **1b** are relatively low toxicity ($LD_{50} > 1000$ mg/kg).

Further studies on improved arylamides and arylimides are in progress and will be reported in due course.

Acknowledgements

The authors would like to thank Dr. Hiram I. Beltrán for the suggestions made to the discussion and CONACYT and SEGEPI-IPN for financial support.

References and Notes

1. Bowen, D. M.; Smith, C. B.; White, P.; Davidson, A. N. *Brain* **1976**, *99*, 459.
2. Davies, K. L.; Thal, L. *J. Lancet*. **1976**, *2*, 1403.
3. Grundman, M.; Thal, L. *J. Neurol. Clin*. **2000**, *18*, 807.
4. Berman, H. A.; Decker, M. M. *Biochim. Biophys. Acta* **1986**, *872*, 125.
5. Berman, H. A.; Leonard, K. *Mol. Pharmacol*. **1991**, *41*, 412.
6. Trujillo-Ferrara, J.; Santillán, R.; Beltrán, H. I.; Farfán, N.; Höpfl, H. *Magn. Reson. Chem*. **1999**, *37*, 682.
7. Trujillo-Ferrara, J.; Vázquez, I.; Espinosa, J.; Santillán, R.; Farfán, N.; Höpfl, H. *Eur. J. Pharm. Sci*. in press.
8. Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
9. Harel, M.; Schald, I.; Ehret-Sabatier, L.; Bouet, F.; Goeldner, M.; Hirth, C.; Axelsen, P. H.; Silman, I.; Sussman, J. L. *Proc. Natl. Acad. Sci. U.S.A*. **1993**, *90*, 9031.
10. Bonting, S. L.; Featherstone, R. M. *Arch. Biochem. Biophys*. **1956**, *61*, 89.
11. Pedretti, A.; Villa, L.; Vistoli, G. *J. Mol. Graph*. **2002**, *21*, 47.
12. HyperChem(TM), Hypercube, Inc., 1115 NW 4th Street, Gainesville, FL 32601, USA.