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ACETYLCHOLINESTERASE INHIBITION BY TACRINE ANALOGUES

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Abstract. Analogues of tacrine were synthesized and evaluated for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity. Compound **2a** was the most potent inhibitor of AChE with a higher selectivity than tacrine for AChE over BuChE. Tacrine, on the other hand, showed the opposite behaviour with a weaker inhibitory effect on AChE than on BuChe. The compounds displayed correlated activities in isolated enzymes as well as in rat brain homogenates. © 1997 Elsevier Science Ltd.

Alzheimer disease (AD) is a neurodegenerative disorder that is the most common cause of dementia among the elderly. AD appears to be closely associated with defects of the central cholinergic system. Cholinergic enhancement can be achieved by acetylcholine precursors, or by muscarinic agonists and AChE inbibitors, the latter being the most clinically successful to date.[1] Tacrine (**1**), one of the most extensively evaluated AChE inhibitors,[2] can significantly improve cognitive function in AD.[3] Other AChE inhibitors that have received attention include galanthamine,[4] huperzine[5] and heptylphysostigmine.[6]

Tacrine is a reversible, non-competitive[7] or mixed[8,9] AChE inhibitor, that binds near the esterasic site of the enzyme, but not directly to the catalytically active serine. While its most important interactions[10] are well known, the contribution of tacrine's saturated ring to AChE binding is not easy to assess.
Tacrine also potently inhibits BuChE,[11] which is found in both plasma and brain. Inhibition of BuChE may lead to adverse peripheral side effects.[11] In addition tacrine is known to cause hepatotoxic side effects in a significant number of patients.[12,13]

The lack of selectivity of **1** and its potential hepatotoxic side effects suggest that selective, non hepatotoxic AChE inhibitors may be of therapeutic utility in AD treatment. With this aim in mind we synthesized some derivatives (compounds **2a-d**) in which the saturated ring of **1** was modified, while maintaining the two aromatic rings (Scheme 1).
These compounds would be expected to retain the critical molecular interactions described above between **1** and AChE. We have also prepared and tested an open model of compounds **2a-d**, i.e. the 2-phenyl-4-aminoquinoline[14] (**3**) in order to evaluate the contribution of structural rigidity to bioactivity.

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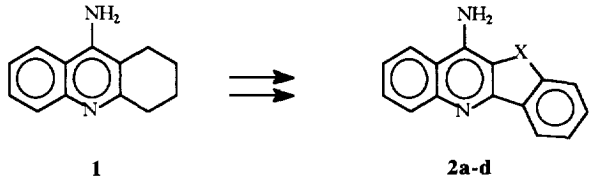
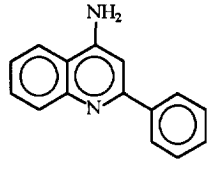
Compounds **2a-d**[15] were prepared as shown in the following scheme 2. *o*-Aminobenzonitrile and the selected ketone (indanone, α-tetralone, chromanone and thiochromanone, respectively) were heated in the presence of anhydrous $ZnCl_2$. After cooling, the reaction mixture was hydrolyzed and the products were purified by crystallization from a suitable solvent or by flash-chromatography.

Scheme 1



1 **2a-d**

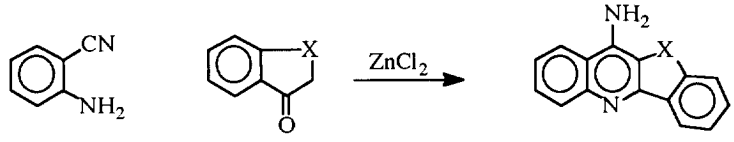


3

	X
2a	CH_2
2b	CH_2CH_2
2c	OCH_2
2d	SCH_2

AChE and BuChE inhibitory activities on isolated enzymes were measured as previously described.[16,17] Experiments for *in vitro* inhibition of AChE- activity in rat cortex were conducted using a radiometric assay as previously described.[18,19] The results are expressed as IC_{50} values (concentration required to inhibit enzyme activity by 50%).

Scheme 2



2a-d

Results and discussion

The IC_{50} values for AChE and BuChE inhibition are summarized in Table 1. Compound **2a**, bearing a methylene bridge between the quinoline moiety and the phenyl ring and possessing a rigid planar structure, was the most potent AChE inhibitor, showing a definitive preference for AChE over BuChE (about seven fold), with higher selectivity than tacrine.

The IC_{50} for AChE inhibition increased by one order of magnitude upon insertion of an ethylene bridge (**2b**) and by two orders of magnitude for **2d** (thiomethylene bridge) and **3** (open structure).The oxomethylene

derivative (compound **2c**) was the least active of all the compounds. The inhibitory effect of **2a-d** and **3** for BuChE was much lower than that of tacrine, being generally of the same order of magnitude. The coplanarity of the two aromatic moieties in **2a** might be responsible for its AChE selectivity, inducing positive interaction with the AChE aromatic amino acids of the esterasic site, some of which are absent in the BuChE.[20]

*TABLE 1. Comparison of in vitro inhibition of AChE and BuChE with compounds **2a-d**, 3 and tacrine (1).*

Comp.	IC_{50} (μM) for AChE Inhibition	IC_{50} (μM) for BuChE Inhibition	Selectivity for AChE	Selectivity for BuChE	Relative selectivity for AChE	IC_{50} (μM) for AChE Inhibition AChE in rat cortex
2a	0.676 ± 0.02	4.67 ± 0.09	6.91	0.145	32.14	0.48 ± 0.23
2b	2.98 ± 0.09	1.18 ± 0.035	0.394	2.52	1.83	4.0 ± 2.9
2c	245 ± 7.4	6.58 ± 0.164	0.027	37.23	0.13	> 100
2d	18.58 ± 0.46	4.90 ± 0.19	0.264	3.79	1.22	> 100
3	11.48 ± 0.46	12.0 ± 0.4	1.045	0.956	4.86	> 100
1	0.254 ± 0.008	0.0547 ± 0.0022	0.215	4.64	1	0.2 ± 0.16

The reversibility of enzyme inhibition was verified by injecting the compounds and tacrine into a HPLC system with immobilised AChE and BuChE stationary phases. All the compounds were eluted chemically unchanged, as judged by their UV spectra before and after chromatography. Moreover, the enzyme activity was found maintained for four months, by determining with a HPLC method[21] the amount of on-column hydrolyzed thiocholine after the injection of known amounts of acetylthiocholine. Compound **2a** was thus judged to be a reversible inhibitor of AChE.

The IC_{50} values obtained using rat cortex homogenate (Table 1) correlated with the values obtained for pure AChE, at least for the most active compounds, thus indicating that their inhibitory activity is maintained in the target biological system.

In conclusion, a series of novel AChE inhibitors related to tacrine was prepared, in which an aromatic ring was introduced and the structure of the C-ring was modified. Anti-AChE activity was strictly dependent on both the size and the introduction of heteroatoms in the C-ring, as well as on the presence of the ring itself. In contrast, BuChE inhibitory activity appeared 100 fold less for all derivatives with respect to their precursor tacrine, thus indicating an improvement of their pharmacological selectivity. The preference of compound **2a** for AChE over BuChE proposes that this molecule could be a starting point for further drug design endeavors.

Acknowledgments

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15. ^{1}H NMR ($DMSOd_6$) and MS data of the synthesized compounds: **2a** δ 3.95 (s, 2H, CH_2), 7.5-8.45 (m, 10H, Ar and NH_2); M^+ 232. **2b** δ 2.85 (m, 2H, CH_2), 2.95 (m, 2H, CH_2), 7.35-8.45 (m, 10H, Ar and NH_2); M^+ 246. **2c** δ 5.3 (s, 2H, CH_2), 6.9-7.8 (m, 8H, Ar); M^+ 248. **2d** δ 4.1 (s, 2H, CH_2), 7.3-8.45 (m, 8H, Ar); M^+ 264. The elemental compositions of the compounds agreed within ± 0.4% of the calculated value.
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