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THE SYNTHESIS AND *IN VITRO* ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY OF TACRINE (COGNEX®) DERIVATIVES

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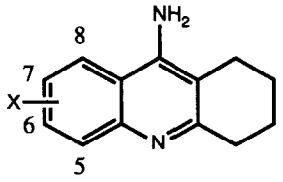
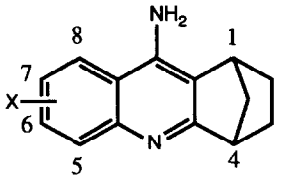
Abstract: Chlorosubstituted derivatives of tacrine **(1)**, 1,4-methylenetacrine **(2)**, and their *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities are described. The most potent analogues are 6-chlorotacrine **(1b)** in AChE and 7-chlorotacrine **(1c)** in BChE inhibition.

The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitor tacrine[1] (9-amino-1,2,3,4-tetrahydroacridine, THA) **(1)** and several of its analogs have recently been of considerable interest for the treatment of cognitive deficits associated with Alzheimer's disease.[2] The presence of side effects limit the dose that can be administered without affecting the patient's quality of life.[3] Even though a considerable amount of information on tacrine and its analogues exists, no comprehensive SAR analysis of 9-amino-1,2,3,4-tetrahydroacridine derivatives has yet been published.[2,4] To improve upon tacrine it is desirable to obtain compounds with increased potency as AChE inhibitors, increased metabolic stability, better penetration of the blood-brain barrier and diminished side effects. In an effort to identify compounds having these properties, we evaluated a number of substituted 4-aminoquinoline and 9-aminoacridine derivatives as inhibitors of AChE and BChE. We have noted that compounds having chlorosubstituents on the benzene ring, in particular those corresponding to the 6-position of tacrine, were more potent than other analogues. Based on this finding, we synthesized an additional group of benzene-ring monochlorosubstituted THA derivatives. Here we report the initial results from two series: tacrine **(1)**, and racemic 1,4-methylenetacrine **(2)** derivatives.



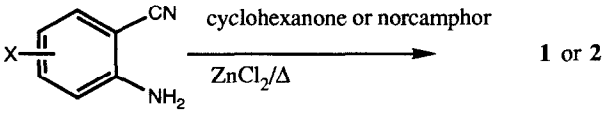
1 Tacrine (X=H)



2 1,4-methylenetacrine (X=H)

The synthesis of these analogues was performed by a modification of reported methodology.[5] Condensation of the appropriately substituted 2-aminobenzonitriles[6] **3** with excess of the corresponding cyclic ketone in the presence of excess of anhydrous zinc chloride gave the desired compounds. These were isolated, purified by column chromatography and converted to the corresponding hydrochlorides in 20-60% yields.[4]

Scheme I



3

The physical data and biochemical results from the *in vitro* evaluation of compounds **1** and **2** as inhibitors of AChE and BChE are presented in Table I. Several known AChE inhibitors are shown for comparison.

Table I.

***In vitro* biochemical data:**

Compounds	M.P.(°C) HCl salt	IC_{50} (nM) AChE Human red blood cell[8]	IC_{50} (nM) AChE Electric eel[8]	IC_{50} (nM) BChE Horse serum[8]
1 X=H (Tacrine)	-	33.5 ± 0.87	9.33 ± 0.17	5.17 ± 0.44
1a X=5-Cl	>304(dec)	68.9 ± 0.21	36.0 ± 2.0	45.0 ± 2.89
1b X=6-Cl	262-264(dec)	1.8 ± 0.27	2.87 ± 0.43	7.93 ± 1.09
1c X=7-Cl	>230(dec)base	743 ± 43.3	40.0 ± 2.89	0.7 ± 0.15
1d X=8-Cl	261-262(dec)	9.4 ± 0.13	4.43 ± 0.35	12.7 ± 1.45
2 X=H	183-184base	31.5 ± 2.47	11.67 ± 0.6	1.3 ± 0.17
2a X=5-Cl	>215(dec)	84.3 ± 2.19	493.3 ± 58.1	206.7 ± 52.1
2b X=6-Cl	291-293(dec)	4.5 ± 0.0	2.33 ± 0.08	21.7 ± 3.33
2c X=7-Cl	>292(dec)	1100 ± 0.23	77.7 ± 12.9	17.0 ± 4.36
2d X=8-Cl	256-258(dec)	42.2 ± 3.42	6.52 ± 0.39	15.3 ± 3.71
Velnacrine	-	164 ± 1.51	59.83 ± 3.77	53.3 ± 8.82
SM 10888	-	28.67 ± 1.42	6.88 ± 1.26	17.3 ± 2.33
E-2020	-	2.33 ± 0.38	9.2 ± 0.52	4166.7 ± 333.3
Physostigmine	-	61.0 ± 4.3	275 ± 78.5	4666.7 ± 333.3

The compounds were assayed for their inhibition of human red blood cell (Type XIII) AChE and electric eel (*Electrophorus electricus*) (Type V-S) AChE activity. The modified radiometric AChE assay of Johnson and Russell,[9] as described by Emmerling and Sobkowicz,[10] was used for these assays.[12] For BChE inhibitory activity, the compounds were tested using horse serum BChE by the microplate colorimetric Ellman assay.[11,12]

As seen in Table I, all of the tacrine derivatives exhibit significant AChE inhibitory activity. The 6 -

chlorosubstituted THA derivatives are considerably more potent than the other isomers in both human and electric eel AChE inhibition. This could be due to the increased lipophilicity, induction of a dipole moment with favorable orientation, and an effect on the compound's pKa, created by the chlorine substituent in this particular position. Perhaps these effects can contribute to more efficient π-π orbital interaction between the THA derivative and an electron-rich aromatic amino acid moiety (presumably tryptophan) of the AChE enzyme.[13] Thus, the most active compound of the series, 6-chlorotacrine (**1b**), is among the most potent reversible AChE inhibitors known with an IC_{50}=1.8nM, against human red blood cell AChE. In this assay it was equipotent with the benzylpiperidine derivative E-2020 and considerably more potent than THA (19x), SM 10888 (16x), velnacrine (91x) or physostigmine (34x). The racemic bridged 6-chloroderivative (**2b**) is also highly potent (IC_{50}=4.5nM). While tacrine itself is a more potent inhibitor of BChE than of AChE,[1] the 6-chlorosubstituted compound (**1b**) is significantly more active as an inhibitor of AChE (both human and electric eel) than of BChE. The 8-chlorosubstitution has a similar, although smaller effect, except in the racemic methylene bridged derivative (**2d**) where the human red blood cell AChE inhibition is about half that of the unsubstituted compound (**2**). However, chlorosubstitution in the 7-position of both **1** and **2**, produced highly potent and selective BChE inhibitors, **1c** (IC_{50}=0.7nM) being more than 1000 times more potent an inhibitor of BChE than of human red blood cell AChE. Analogously, this may be due to the selectively increased π-π orbital interaction between the BChE enzyme π-electron-rich moiety such as tryptophan, in addition to effects on the pKa, increased lipophilicity, dipole moment and its orientation, provided by the 7-chlorosubstitution on the THA derivatives.

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6. (a) 2-amino-5-chlorobenzonitrile and 2-amino-6-chlorobenzonitrile were purchased from Aldrich Chemical Co. (b) 2-amino-4-chlorobenzonitrile was prepared from 2-nitro-4-chloroaniline by diazotization, reaction with cuprous cyanide (Atkinson, C. M. and Simpson, J. C. E., *J. Chem. Soc.*, **1947**, 232) followed by reduction of the resulting 2-nitro-4-chlorobenzonitrile with stannous chloride (McKee, R. L.; McKee, M. K. and Bost, R. W., *J. Am. Chem. Soc.*, **1947**, *69*, 940). (c) 2-amino-3-chlorobenzonitrile was prepared from 3-chloro-2-nitrobenzoic acid (Aldrich), which was converted to its amide ($SOCl_2/NH_3$), dehydrated with phosphorus oxychloride (Rickborn, B. and Jensen, F., *J. Org. Chem.*, **1962**, *27*, 4608) to 3-chloro-2-nitrobenzonitrile, which was reduced with stannous chloride by the same procedure as described above. (d) General conditions for the condensation of **3** to **1** or **2**: One equivalent of anthranilonitrile **3** was combined with 5-10 equivalents of the ketone (cyclohexanone or norcamphor) and 2.5 equivalents of zinc chloride (exothermic). The mixture was then heated at 90-100°C, under nitrogen, for 4-24 hours. The excess of ketone was then removed by distillation *in vacuo*, the residue was basified with 10% aqueous sodium hydroxide, extracted with chloroform and chromatographed using 20:1 chloroform/methanol. The pure free base was dissolved in absolute ethyl alcohol, an equivalent amount of aqueous 4N HCl was added, concentrated in vacuo and the hydrochloride of **1** or **2** was recrystallized from absolute ethyl alcohol/ethyl acetate. The yields were typically between 20-60% of analytically pure materials.[7]

7. The compounds show physical properties (^1H NMR, MS, IR and elemental analyses (C,H,N, Cl) in accordance with their assigned structures.

8. The concentration of inhibitor producing 50% inhibition of AChE or BChE activity (IC_{50}) was determined graphically using data derived from triplicate determinations of enzyme inhibition by at least six different inhibitor concentrations, ranging from 1 nM to 100 μM.

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12. All assay materials were obtained from Sigma Chemical Co.

13. Suggested by preliminary modeling experiments with the *Torpedo californica* AChE structure, which was recently reported: Sussman, J. L; Harel, M.; Frolow, F.; Oefner, C; Goldman, A.; Toker, L.; Silman, I., *Science*, **1991**, *253*, 872.