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ACETYLCHOLINESTERASE INHIBITION BY FUSED DIHYDROQUINAZOLINE COMPOUNDS

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Abstract: A new type of dihydroquinazoline-based inhibitor of acetylcholinesterase (AChE) is described. These compounds were designed to interact with the catalytic site of AChE in a manner similar to the known inhibitor tacrine. In a manner analogous to the potency enhancement obtained by addition of chlorine atoms to the tacrine molecule, a 3-chloro derivative of the parent hexahydroazepino[2,1-*b*]quinazoline structure was found to be about 8 times more potent as an AChE inhibitor than the unsubstituted compound.

One of the earliest and most consistent biochemical changes in the brains of Alzheimer's disease patients is a decrease in hippocampal and cortical levels of the neurotransmitter acetylcholine and associated enzymes (acetylcholinesterase (AChE) and choline acetyltransferase).[1] This decrease correlates well with the loss of cholinergic neurons projecting from the basal forebrain to the neocortex and hippocampus.[2] In animal studies, lesioning of basal forebrain cholinergic neurons or treatment with muscarinic antagonists results in impaired cognitive performance,[3] while treatment with cholinomimetic agents reverses such impairment.[4] Collectively, these observations have given rise to the "cholinergic hypothesis of Alzheimer's disease", which proposes that loss of cholinergic innervation to the neocortex and hippocampus is responsible for the cognitive decline in Alzheimer's disease.[5]

The two types of cholinomimetic agents most widely studied clinically are muscarinic receptor agonists and AChE inhibitors.[6] Muscarinic agonists mimic the effects of acetylcholine at postsynaptic muscarinic receptors, while AChE inhibitors increase synaptic levels of acetylcholine by preventing its degradation. Recent clinical trials indicate that AChE inhibitors show promise in improving the condition of Alzheimer's patients.[7] One of the most extensively evaluated AChE inhibitors is tacrine (Cognex®), which has demonstrated significant improvement of cognitive function in AD.[8] Other AChE inhibitors that have received recent attention include galanthamine,[9] huperzine-A,[10] and heptylphysostigmine.[11]

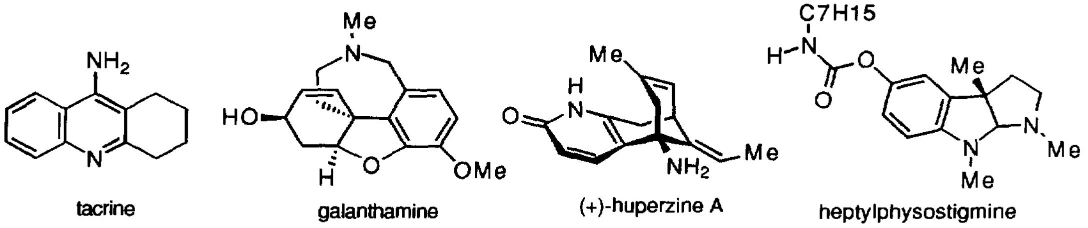


Figure 1. Some AChE inhibitors of current interest as antidementia drugs.

Tacrine is a reversible AChE inhibitor that binds near the enzyme's esteratic site but not directly to the catalytically active serine. A recent X-ray structure determination of tacrine soaked into crystals of *Torpedo Californica* AChE has provided detailed information on the binding mode of this inhibitor.[12] Its most important interactions include the following: (i) a hydrogen bond between the protonated acridine nitrogen and the backbone carbonyl oxygen of histidine-440 (a component of the catalytic Ser200-His440-Glu327 triad), and (ii) a π-π stacking of the two aromatic rings of tacrine between the indole ring of Trp-84 and the phenyl ring of Phe-330. While other, structurally diverse, AChE inhibitors are also thought to interact with Trp-84, the additional interaction with Phe-330 has only been documented for tacrine.[12] The contribution of tacrine's saturated ring to its binding with AChE is not easily ascertained from the X-ray structure. The 9-amino group does not interact with the enzyme structure; instead, it hydrogen-bonds with crystal water-32 and its influence on the affinity of tacrine for AChE may simply be to increase the basicity of the acridine nitrogen.

In search of novel AChE inhibitors, our group set out to design a new type of compound, structurally different from the aminoacridines, that would retain both critical molecular interactions described above between tacrine and AChE. As indicated in Figure 2, the aminopyridine moiety of tacrine can be viewed as a vinylogous amidine. According to this analysis, transposition of the 9-amino group to the vinylogous position within the ring system suggests dihydroquinazoline compounds of general structure **I**. Interestingly, a compound of this type, derived from natural sources (desoxypeganine; **I**, X = H; Y = H_2; n = 1) has been described as a weak AChE inhibitor.[13] This report describes the synthesis and AChE inhibitory activity of a series of compounds of type **I**.

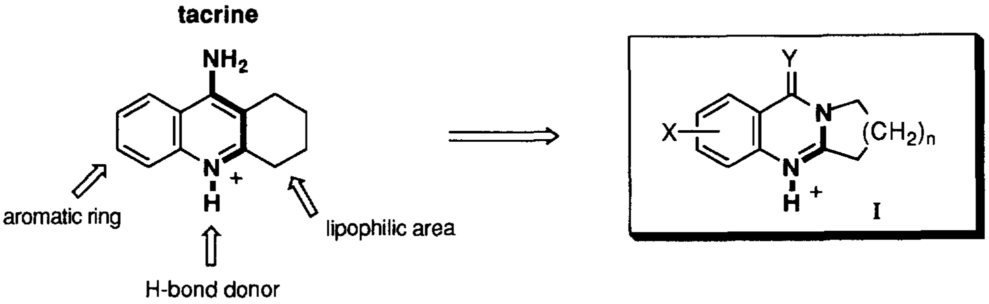


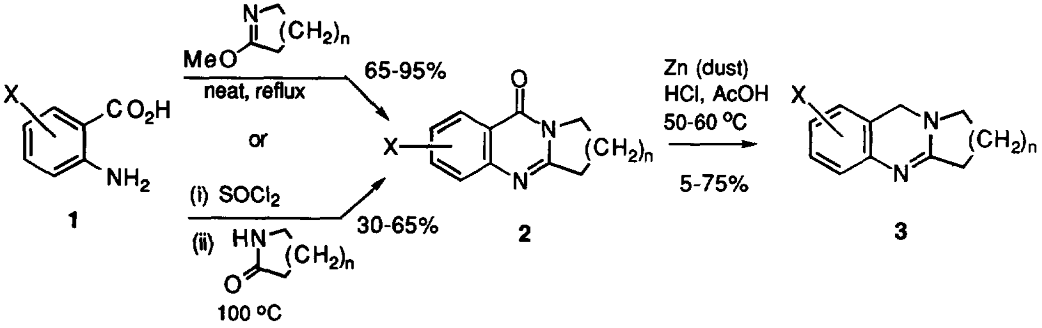
Figure 2. Structural elements important for tacrine binding to AChE and relationship to vinylogous compounds.

Chemistry

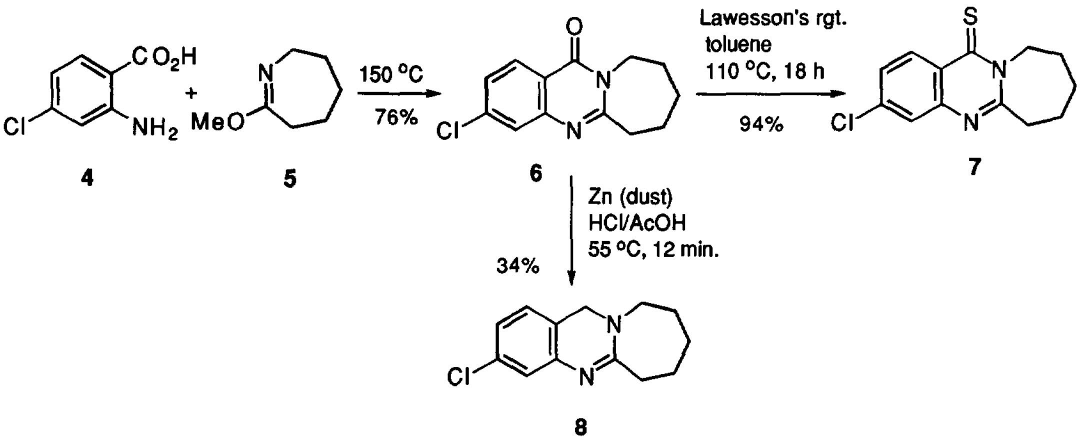
Fused dihydroquinazolines of formula **2** were prepared from the corresponding anthranilic acids **1** by heating directly with the desired iminium ether[14] or by previous reaction of **1** with $SOCl_2$, followed by reaction with the desired lactam[15] (Scheme 1). Several reagents were tried for the difficult reduction of **2** to **3**, including borane, alane, $NaBH_4$, $LiAlH_4$, and others. Usually, side reactions included incomplete reduction of the carbonyl group, over-reduction of the amidino group of **3**, and cleavage of the amidino C-N single bond to produce ring-expanded cyclic diamines. In general, reduction with a large excess of zinc dust in AcOH/HCl proved to be the best method. Careful monitoring of reaction progression was required to prevent chlorine removal in the chlorinated analogues (about 15-30 min at 50-60 °C). Yields are not optimized, and no obvious relationship was noted between ring size and yield for either the cyclization or reduction steps.

As shown in Scheme 2, reaction of 3-chloroquinazolinone **6** with Lawesson's reagent produced the corresponding thiolactam **7** in 94% yield. The imino derivative **10** was prepared in 70% yield by reaction of 5-chloro-anthranilonitrile **9** with neat iminoether **5** at 150 °C (Scheme 3).

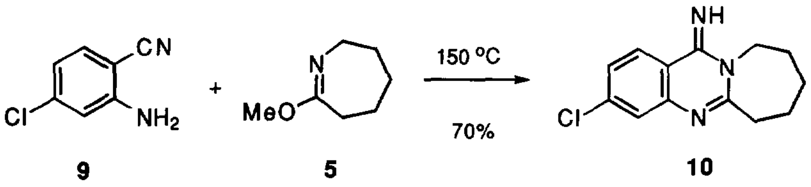
Scheme 1



Scheme 2



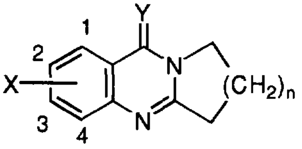
Scheme 3



Pharmacology

The inhibition of human red blood cell (RBC) AChE (Sigma, St. Louis, MO) by target compounds was determined by a previously described radiometric assay with [^{3}H]-acetylcholine as substrate.[16] Rat brain AchE activity was determined with a modified colorimetric Ellman assay.[17]

Table 1. Inhibition of acetylcholinesterase by fused dihydroquinazolines.



Compound[a]	X	Y	n	rat brain[b,c]	human RBC[b,c]
11	H	H_2	1	18,000	3720
12	H	H_2	2	>100,000	3660
13	H	H_2	3	15,000	3760
14	H	H_2	4	100,000	9200
15	H	H_2	5	>100,000	13510
16	1-Cl	H_2	3	4750	1,700
17	2-Cl	H_2	3	9300	500
8	3-Cl	H_2	3	1,900	158
18	4-Cl	H_2	3	16,400	1,500
6	3-Cl	O	3	>100,000	>100,000
7	3-Cl	S	3	>100,000	>100,000
10	3-Cl	NH	3	35900	1,260
tacrine				200	30
galanthamine				1760	100
huperzine A				94	150
heptylPhy[d]				600	125

[a] Satisfactory spectral and elemental analysis data were obtained for all new compounds. [b] IC50 values (nM). [c] Values shown are the mean of three independent determinations of IC_{50} values having a standard deviation of less than 10% of the mean. [d] Heptylphysostigmine.

Results and Discussion

As shown in Table 1, several unsubstituted fused dihydroquinazolines of various ring C sizes were evaluated against rat brain and human RBC AChE. Five to seven-membered analogues (**11-13**) were better against human RBC AchE than larger ring size analogues (**14-15**). A seven-member ring (cf. **13**) was found to be optimal against rat brain AChE. The five-member analogue (**11**) was slightly weaker, while the corresponding six-member compound (**12**) was practically inactive. Interestingly, this ring-size effect on rat brain AChE inhibition is exactly opposite to that observed for 1,2,3,4-tetrahydroacridines and homologous 2,3-polymethylenequinolines: tacrine and 9-alkyl-1,2,3,4-tetrahydroacridines are generally more potent AChE inhibitors than the corresponding 2,3-trimethylenequinoline compounds (five-member ring C analogues), which as a group are about equipotent to the corresponding 2,3-pentamethylenequinoline compounds (seven-member ring C analogues).[18] Neither the potency rank order of the tacrine-like inhibitors nor that of the fused dihydroquinazolines can be easily explained by the effect of the fused ring size on the pK_a of the molecules: for tacrine-like inhibitors, five-, six-, and seven-member ring analogues possess pK_a values of 8.55, 9.41, and 9.25, respectively.[19] Thus, while the effect of ring size on basicity rank order is 6>7>5, the effect on AChE inhibition can be ranked as 6>5≈7. For fused dihydroquinazoline compounds, the ring size effect on basicity is also expected to be 6>7>5, based on published data for similar compounds,[20] while the ring size effect on AChE inhibition is 7>5>6. It is possible that a combination of factors (ionic, steric, lipophilicity) account for the effects of ring-C size on the potency of both types of AChE inhibitors.

Several substituted tacrine derivatives have been shown to possess enhanced potency relative to the parent structure.[20] For example, chlorine substitution on the benzene ring, particularly at the 6-position, leads to a 3 to 19-fold increase in potency as an AChE inhibitor, relative to the unsubstituted compound (the actual increase depends on the source of enzyme used).[21] This potency enhancement has been rationalized in terms of a more favorable charge distribution on the aromatic rings of the protonated tacrine molecule, which results in an increased electrostatic interaction between these rings and the quadrupole and induced-dipole moments of the Trp-84 and Phe-330 aromatic rings.[22] As seen in Table 1, a similar potency enhancement was obtained by chlorine substitution on the benzene ring of dihydroquinazoline AChE inhibitors, particularly in the position equivalent to the 6-position of tacrine (see compound **8**). Thus, this 3-chlorosubstituted analogue (**8**) is about 8 times more potent than the unsubstituted parent structure **13**. This is an enhancement of potency similar to that seen going from tacrine to 6-chlorotacrine.[18] This observation is consistent with the hypothesis that the dihydroquinazoline and the aminoacridine types of inhibitors interact with AChE in a similar manner.

The AChE inhibitory activity of analogues of **8**, derivatized at the 11-position, was also evaluated. Of the three compounds prepared (**6**, **7**, and **10**), only the aza-derivative **10** displayed any activity against hRBC AChE. The lack of activity of compounds **6** and **7** may result from the expected decrease in basicity of these compounds relative to **8**. On the other hand, compound **10** has an IC_{50} (1260 nM) comparable to that of the parent compound **13** or the 1- and 4-chloro derivatives **16** and **18**. The 11-imino group of **10** may be H-bonded to crystal water 32 in a manner similar to the 9-amino group of tacrine.[12] It is possible that the reduced affinity associated with the decreased basicity of **10** might be offset by this additional ionic interaction.

In summary, a novel type of chloro-substituted dihydroquinazoline-based inhibitor of AChE has been discovered. The parallel SAR between the tetrahydroaminoacridine inhibitors and these novel compounds suggests that they may interact in a similar manner with the catalytic site of AChE. These results also confirm that while the basic acridine ring nitrogen is essential for AChE inhibition, the 9-amino group of tacrine is not necessary, as long as the presence of a basic ring nitrogen is maintained. A full report on the complete SAR of dihydroquinazoline-based AChE inhibitors is in preparation and will appear in due course.

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