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SYNTHESIS OF DIHYDROXANTHONE DERIVATIVES AND EVALUATION OF THEIR INHIBITORY ACTIVITY AGAINST ACETYLCHOLINESTERASE: UNIQUE STRUCTURAL ANALOGS OF TACRINE BASED ON THE BCD-RING OF ARISUGACIN

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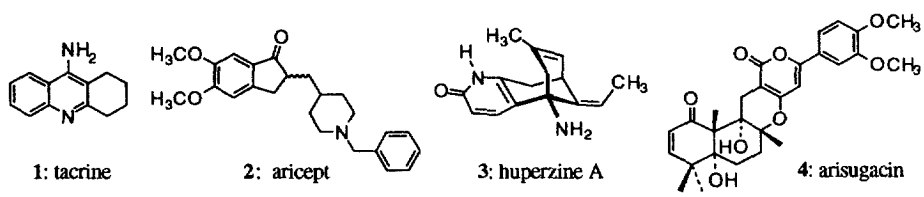
Abstract: A general approach to synthesis of dihydroxanthone derivatives is described here. *In vitro* evaluation of these dihydroxanthones demonstrated that some derivatives possess moderate anti-cholinesterase activities and better selectivities than tacrine for acetylcholinesterase over butyrylcholinesterase. Structural effects on anti-cholinesterase activities were also examined, and docking experiments were carried out to provide preliminary understandings of these experimental observations.

A rational and consistent approach for development of the therapeutic treatment of Alzheimer's Disease (AD) has been designed on the basis of the cholinergic deficiency hypothesis.[4,5] This hypothesis links AD to the loss of acetylcholine, a neurotransmitter responsible for memory and cognitive functions.[5] In the cerebral cortex and hippocampus of AD patients, the activity of choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine, is reduced dramatically by 60–90%, thereby causing severe shortage of acetylcholine. Acetylcholine esterase (AChE), on the other hand, serves to maintain the chemical equilibrium between choline and acetylcholine by catalyzing the hydrolysis of acetylcholine to choline. With loss of the ChAT activity in AD patients, the equilibrium would clearly be shifted towards choline. To maintain concentrations of available acetylcholine in AD patients, three types of treatment have been developed based on the cholinergic deficiency hypothesis.[4] The first one consists of administering acetylcholine equivalents in an attempt to enhance the acetylcholine level, thereby enhancing the central cholinergic activity. The second treatment involves the use of muscarinic receptor agonists selective for M_1 (and M_3) subtype receptor to enhance the acetylcholine uptake at the postsynaptic end. The third one involves the use of AChE inhibitors to increase the acetylcholine level at the synaptic stage by decreasing its rate of hydrolysis.

Figure 1



1: tacrine 2: aricept 3: huperzine A 4: arisugacin

The third treatment has received the most attention and the only therapeutic drugs for combating dementia diseases are inhibitors of AChE. One is tacrine[6] or Cognex (**1** in **Figure 1**) from Parke–Davis and the other is aricept[7] (**2**) from Eisai in Japan as well as Pfizer in the U.S. Another inhibitor that has become available

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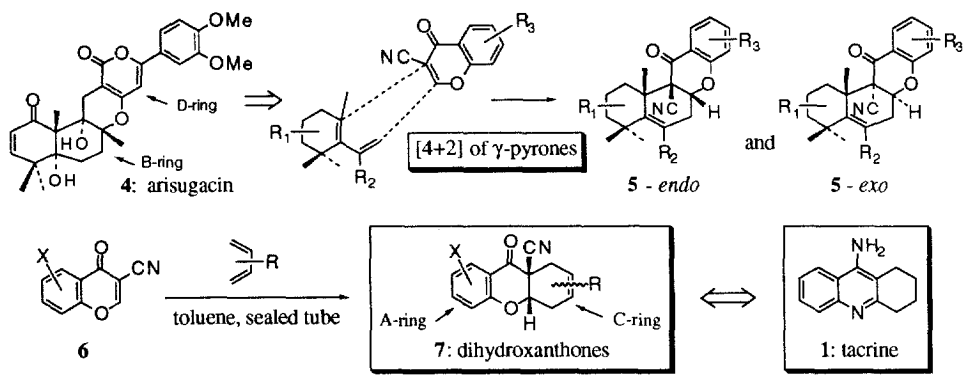
recently is Huperzine A[8] (**3**), a nootropic agent isolated from a Chinese folk medicine and used by Chinese for centuries to improve memory. Administrations of these therapeutic drugs have demonstrated improvement in memory and cognitive functions of AD patients at early stages by maintaining the concentration of available neurotransmitter acetylcholine. However, as the disease progresses, these drugs tend to lose their therapeutic effects since cholinergic neurons continue to be destroyed. Therefore, efforts in searching for more potent and selective inhibitors of AChE will remain highly significant in the therapeutic treatment of Alzheimer's disease.

We have been building our research program in the area of Alzheimer research by focusing on arisugacin[9,10] (**4** in **Figure 1**). Arisugacin (**4**) was first isolated from the culture broth of *penicillium* sp. FO-4259,[9] and has demonstrated impressive inhibitory potency against AChE with an IC_{50} value of 1 nM, compared to 200 nM for tacrine (**1**). The inhibitory activity of **4** is also highly selective for AChE since >18,000 nM is required to achieve the desired inhibition of butyrylcholinesterase (BuChE). In contrast, tacrine (**1**) is much more selective for BuChE with an IC_{50} value of 12 nM. The scarcity of arisugacin (**4**) from the natural source severely hinders meaningful investigations of its significant therapeutic potential in the treatment of Alzheimer's disease. Hence, development of strategies for synthesis of arisugacin and its analogs should be significant in therapeutic development of Alzheimer treatment. We report here our synthesis of some dihydroxanthone derivatives and evaluation of their inhibitory activities against cholinesterase. These dihydroxanthone derivatives can be considered as structural analogs of tacrine (**1**) but constructed based on the BCD-ring of arisugacin (**4**).

Scheme 1

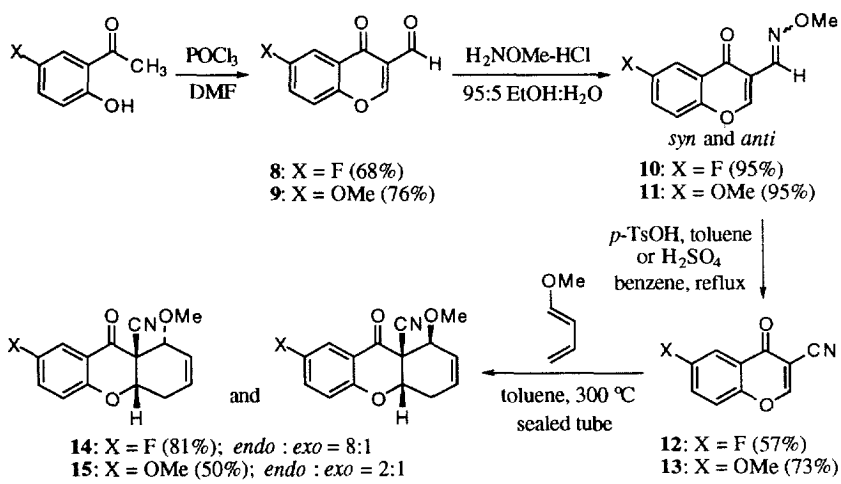


We recently developed a highly convergent approach to arisugacin (**4**) by employing [4+2] cycloaddition reactions of activated γ-benzopyrones (**Scheme 1**).[10a,11] In addition to constructing the tetracyclic core (compound **5**) of arisugacin using this strategy, our investigation also led to preparation of some dihydroxanthone derivatives **7**. Because dihydroxanthone derivatives **7** not only bear structural similarities with tacrine (**1**), but also serve as BCD-ring analogs of arisugacin (**4**),[12] we became interested in their potential inhibitory activities against AChE and BuChE. Furthermore, we were also interested in the potential structural effects of the aromatic A-ring and the saturated C-ring. Although some dihydroxanthones derivatives **7** could be prepared from commercially available substituted 3-cyano-γ-benzopyrones using our cycloaddition conditions, the only reported method[13] for preparation of the 3-cyano-γ-benzopyrone was neither synthetically useful nor general for synthesis of unavailable 3-cyano-γ-benzopyrones. Therefore, we first developed an

efficient method that would allow us to gain synthetic access to various 3-cyano-γ-benzopyrone derivatives, and some relevant examples are shown in **Scheme 2** for 6-fluoro- and 6-methoxy-3-cyano-γ-benzopyrones.[14]

Aldehydes **8** and **9** could be obtained readily from available 5-fluoro- and 5-methoxy-2-hydroxyacetophenones, respectively, using Vilsmeier conditions.[15] Formation of methoxy imines **10** and **11** from **8** and **9** could be accomplished in high yields by refluxing with methoxylamine hydrochloride salt in 95:5 ethanol : H_2O.[16] Both methoxy imines **10** and **11** were isolated as mixtures of *syn* and *anti* isomers. Without further separation, both isomers were subjected to elimination conditions by refluxing with either *p*-TsOH in toluene or H_2SO_4 in benzene. The desired 3-cyano-γ-benzopyrones **12** and **13** could be obtained in 57% and 73% yields, respectively. Cycloaddition reactions of **12** and **13** with 1-methoxy-1,3-butadiene were carried out in toluene at 300 °C in sealed tubes to provide the desired cycloadducts **14** and **15** in 81% and 50% yields, respectively. The adduct **14** was isolated with an *endo*:*exo* ratio of 8:1, whereas the ratio for **15** is 2:1. These ratios correlate well with our previous studies in which electron withdrawing groups at C-6 of 3-cyano-γ-benzopyrones provided higher diastereomeric ratios than those with electron donating groups.[10a,11] Preparation of derivatives **14** and **15** using this synthetic sequence allowed us to have complete access to a series of dihydroxanthones where substituents at C-6 position varied in electronic properties (**Table 1**).

Scheme 2

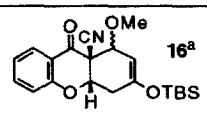
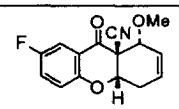
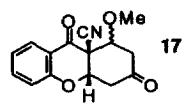
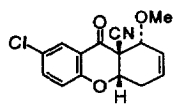
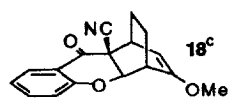
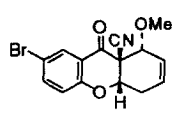
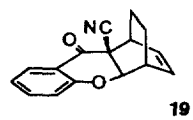
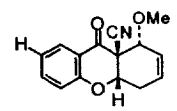
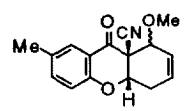
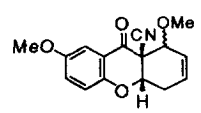


We employed an assay protocol based on Ömura's method,[9] but carried out the assays on larger scales. Solutions of enzymes and substrates (acetylcholine hydrochloride) were prepared in 0.1 *M* potassium phosphate buffers (pH ~7), and solutions of dihydroxanthones were prepared in 50% ethanol in distilled H_2O. Enzymatic activities were measured by calibrating rates of increasing absorption at 492 nm (due to the production of red quinone in the assay) using a single-beam UV/VIS spectrometer, and relative differences in rates (with or without dihydroxanthones) were used to calculate IC_{50} values. Control studies consistently provided an IC_{50} value of 0.18–0.21 μM for tacrine (**1**) (the literature value is 0.2 μM[9]). Results of our assay of a variety of dihydroxanthones against AChE and BuChE are summarized in **Table 1**.

Dihydroxanthone derivatives **16**–**19** vary structurally in the C-ring. Compound **16** has the most steric bulk, whereas **18** and **19** contain bridged bicyclic structures. As shown in **Table 1**, compound **17** (entry 2), which contains a flatter and less congested C-ring, yielded relatively the best IC_{50} value against AChE, albeit

none of these substrates appeared to be highly active against the enzyme. In entries 5-10, the structural variation specifically resides at the C-6 position in the aromatic A-ring. Two interesting observations can be made here. First, it appears that strong electron withdrawing groups such as F and Cl (entries 5 and 6) at the C-6 position lead to better inhibitory activity against AChE. Second, with the exception of entries 6 and 7, these dihydroxanthones demonstrate some selectivity for AChE over BuChE. It should be noted that some assays (entries 1–2, and 9–10) were carried out with mixtures of both *endo* and *exo* isomers due to difficulties in separations.

Table 1 Inhibitory Activities of Dihydroxanthones Against Cholinesterases.

Entry	Substrate	IC_{50} (μM) AChE	IC_{50} (μM) BuChE	Entry	Substrate	IC_{50} (μM) AChE	IC_{50} (μM) BuChE
1	 **16**[a]	≥ 2.0[b]	0.35	5	 **14**	0.28	≥ 2.0
2	 **17**	0.32	≥ 2.0	6	 **20**	0.24	0.27
3	 **18**[c]	≥ 2.0	≥ 2.0	7	 **21**	1.42	0.73
4	 **19**	0.58	≥ 2.0	8	 **22**	1.12	≥ 2.0
				9	 **23**	0.79	≥ 2.0
				10	 **15**	0.62	1.41

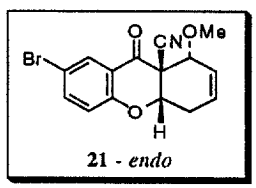
a. Mixtures of *endo* and *exo* isomers were used in entries 1-2 and 9-10.
b. The difference in activity is too small to assign meaningful IC_{50} numbers.
c. The *endo* isomer was the dominant isomer in entries 3-8.

Because of these observed inhibitory activities, we obtained the X-ray structure of a single crystal of **21**-*endo* (**Figure 2**). Given the conformation provided by the X-ray crystal structure, and assuming that **21**-*endo* has the same binding site as tacrine (**1**), we docked **21**-*endo* (using Insight II) at the active site of AChE. As shown in **Figure 3**, the dihydroxanthone **21** (green) fits well at the active site and avoids serious steric interaction with the surrounding amino acid residues. The cyano group is hydrogen bonded to a nearby H_2O, while the aromatic ring stacks well between Phe 330 and Trp 84. Both interactions may contribute to the binding potential of these dihydroxanthone derivatives to AChE, although they lack positively charged quaternary nitrogen atom found in tacrine (**1**).

The superimposed figure indicates that these derivatives could bind to AChE similarly to tacrine (**1**) (blue). This modeling study also supports the notion that more steric congestion in the C-ring could hinder the binding property of these dihydroxanthones, thereby diminishing the inhibitory activity as indicated by entries

1–4 in **Table 1**. Hence, tacrine (**1**) is much better than dihydroxanthones **16–19** because of its relatively less hindered C-ring. In addition, the docking experiment suggests a potential reason for which electron withdrawing groups at C-6 enhance the inhibitory activity of dihydroxanthones. Since carbonyl groups of dihydroxanthones are aligned with the amino group in tacrine (**1**) and face the π-electron density of the indole ring of Trp 84, an electron withdrawing group such as a halogen should diminish the electron density of the carbonyl oxygen (inductively from C-6), thereby alleviating the electrostatic repulsion with Trp 84 and enhancing the binding. By the same reasoning, tacrine (**1**) shows better inhibitory activity than these dihydroxanthones because of the ability of its amino group to become positively charged, thereby enhancing the binding through the electrostatic attraction with Trp 84.

Figure 2. X-Ray Crystal Structure of **21**-*endo*.



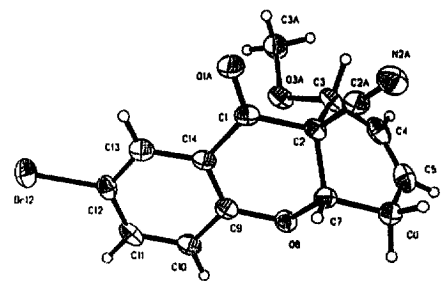
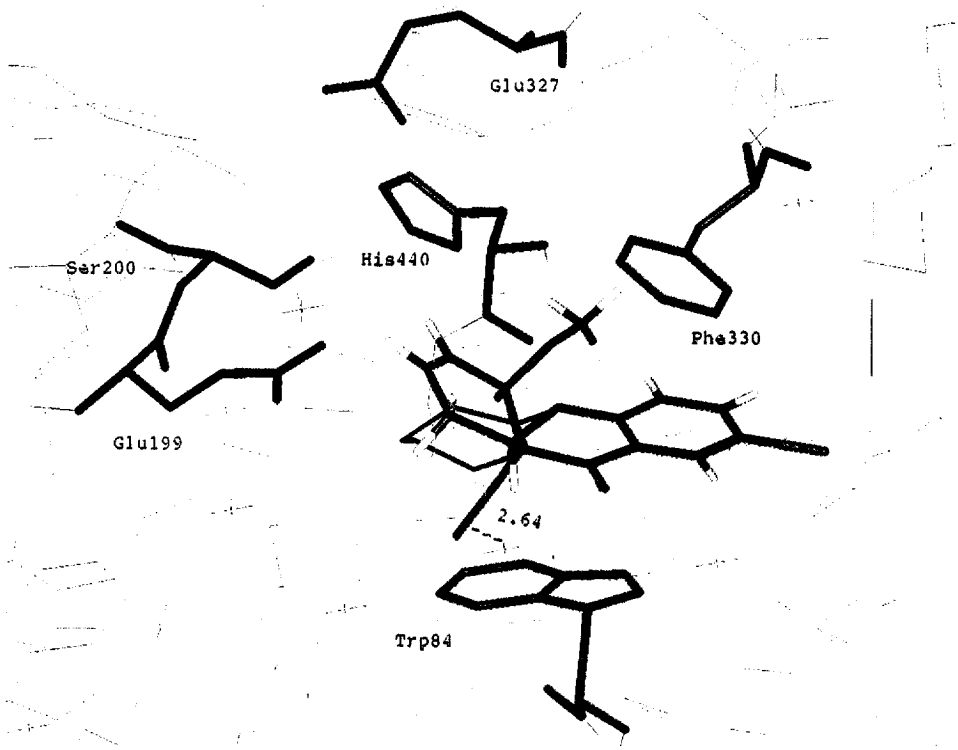


Figure 3. Docking of **21**-*endo* in AChE.



We have here prepared a new class of structural analogs of tacrine and identified their inhibitory potential as well as selectivity toward AChE. Their inhibitory activities appear to be dependent upon the C-6 substituents on the aromatic ring, and the steric congestion on the unsaturated ring. We are currently exploring other structure-activity relationships of this class of compounds in inhibition of Cholinesterases, and preparing new analogs that could improve their activities.

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