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**ARTICLE** *in* JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 2001

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# *Brief Articles*

## Coumarins Derivatives as Dual Inhibitors of Acetylcholinesterase and Monoamine Oxidase

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*Received April 4, 2001*

A set of 17 coumarin and 2 chromone derivatives with known inhibitory activity toward monoamine oxidase (MAO) A and B were tested as acetylcholinesterase (AChE) inhibitors. All compounds inhibited AChE with values in the micromolar range (3−100 $\mu$M). A kinetic study showed that most compounds acted as noncompetitive AChE inhibitors. This finding may be of interest in the context of Alzheimer's disease because recent observations suggest that MAO and AChE inhibition might decrease $\beta$-amyloid deposition.

### Introduction

Alzheimer's disease (AD), a neurodegenerative illness characterized by a progressive decline in cognitive function, is the fourth leading cause of death for people over 65 years of age in Western industrial countries.[1] AD is neuropathologically characterized by the presence of numerous plaques of amyloid $\beta$-peptide (A$\beta$) plaques, neurofibrillary tangles (NFT), and degeneration or atrophy of the basal forebrain cholinergic neurons.[2] The loss of basal forebrain cholinergic cells results in an important reduction in acetylcholine (ACh), which is believed to play an important role in the cognitive impairment associated with AD.[3−5] Accordingly, increasing the levels in ACh has been regarded as one of the most promising approaches for the symptomatic treatment of AD. To date, several acetylcholinesterase (AChE) inhibitors are commercially available, for example, galanthamine, donepezil, rivastigmine and tacrine (Chart 1), which was the first drug to be approved in the U.S. for the treatment of AD.

Recently, evidence has been presented that AChE could also play a key role in accelerating A$\beta$ plaques deposition.[6] This activity was affected by ligands of the peripheral anionic binding site (i.e., decamethonium and propidium) while it was not affected by active-site inhibitors (i.e., edrophonium), suggesting that the peripheral binding site of AChE may be involved in A$\beta$ deposition.

Among the other strategies under investigation,[7] monoamine oxidase B (MAO-B) inhibitors have also been proposed for the treatment of AD. Recent studies have shown that MAO-B activity can increase up to 3-fold in the temporal, parietal, and frontal cortex of AD patients compared with controls. This increase in MAO-B activity produces an elevation of brain levels of hydroxyl radicals, which has been correlated with the development of A$\beta$ plaques.[8] A$\beta$ is the main component of the senile plaques found in AD brains and any compound able to inhibit its aggregation might be regarded as potentially useful in the treatment of the disease.[9,10]

In a recent exploration of coumarin derivatives as MAO inhibitors,[11] we discovered that some compounds are also endowed with inhibitory activity toward AChE (EC 3.1.1.7) and cholinesterase (EC 3.1.1.8). A starting point was the observations that ensaculin (Chart 1) inhibits the activity of AChE in vitro ($IC_{50} = 0.36$ $\mu$M).[12] Furthermore, Fink et al.[13] showed that hybrids of an AChE inhibitor (i.e., physostigmine, Chart 1) and an irreversible MAO inhibitor such as L-deprenyl (Chart 1) resulted in dual AChE and irreversible MAO inhibitors. We therefore undertook an exploratory study of AChE inhibition using some of our coumarinic MAO-B inhibitors.[11]

### Materials and Methods

**Biological Assays.** AChE inhibitory activities were determined using the general method of Ellman[14] using AChE from electric eel (EC 3.1.1.7, C-2888 Sigma). The AChE activity was determined in a reaction mixture containing 15 $\mu$L of a solution of AChE (0.01 mg/mL in 0.01 M phosphate buffer, pH 7.4), 1.5 mL of a solution of 5,5′-dithiobis-2-nitrobenzoic acid (0.126 mM in 0.1 M phosphate-buffered solution pH 7.4), 50 $\mu$L of a water solution of the substrate, and 50 $\mu$L of a methanol solution of the inhibitor. Six final concentrations (0.039−0.62 mM) of acetylthiocholine (substrate) were examined at a single concentration of inhibitor corresponding approximately to its $IC_{50}$ value. The increase in absorbance at 412 nm was measured for 3.2 min at 37 °C with a Kontron Uvikon 941 spectrophotometer (Zürich Müllingen, CH).

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10.1021/jm010894d CCC: $20.00 © 2001 American Chemical Society
Published on Web 08/21/2001

**Chart 1**

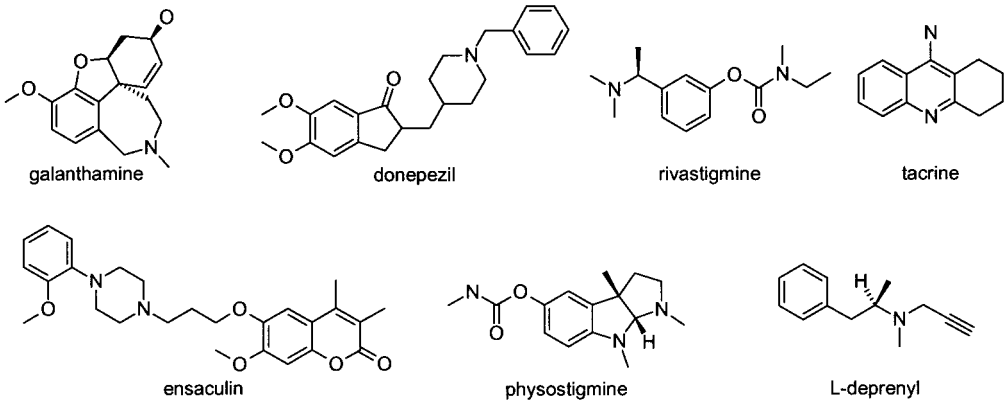
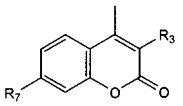



**Table 1.** Chemical Structures and MAO and AChE Inhibition Data of Some Coumarin Derivatives



| compd | $R_3$ | $R_7$ | p$K_{inc}$ [a] AChE | p$K_{ic}$ [b] AChE | p$IC_{50}$ [c] MAO-A | p$IC_{50}$ [c] MAO-B |
|---|---|---|---|---|---|---|
| **1** | $CH_3$ | $OCH_2C_6H_5$ | 5.39 | | 6.16 | 8.36 |
| **2** | $CH_3$ | $OCH_2C_6H_4$−2′-$CH_3$ | 4.68 | | 5.64 | 8.06 |
| **3** | $CH_3$ | $OCH_2C_6H_4$−3′-$CH_3$ | 5.03 | | 5.48 | 8.36 |
| **4** | $CH_3$ | $OCH_2C_6H_4$−3′-OH | 4.83 | | 6.38 | 8.01 |
| **5** | $CH_3$ | $OCH_2C_6H_4$−3′-$OCH_3$ | 4.75 | | 5.82 | 8.44 |
| **6** | $CH_3$ | $OCH_2C_6H_4$−3′-$NH_2$ | 4.74 | | 6.00 | 7.46 |
| **7** | $CH_3$ | $OCH_2C_6H_4$−3′-F | 5.11 | | 6.24 | 8.55 |
| **8** | $CH_3$ | $OCH_2C_6H_4$−3′-Cl | 5.47 | | 5.95 | 8.48 |
| **9** | $CH_3$ | $OCH_2C_6H_4$−4′-$CH_3$ | 5.17 | | 5.43 | 8.21 |
| **10** | $CH_3$ | $OCH_2C_6H_4$−4′-F | 4.90 | | 6.91 | 8.52 |
| **11** | $CH_3$ | $OCH_2C_6H_4$−4′-Cl | 5.14 | | 6.91 | 8.59 |
| **12** | $CH_3$ | $OCH_2C_6H_3$−3′,4′-$F_2$ | 5.06 | | 6.91 | 8.94 |
| **13** | $CH_3$ | $OCH_2C_6H_3$−3′,5′-$F_2$ | 4.73 | | 6.17 | 8.52 |
| **14** | H | $OCH_2C_6H_5$ | 4.51 | | 5.71 | 7.74 |
| **15** | $CH_3$ | $O(CH_2)_2C_6H_5$ | 4.80 | | 6.00 | 8.25 |
| **16** | $CH_3$ | OH | 4.00 | 4.54 ± 0.13 | 35% (30 $\mu$M) | 18% (30 $\mu$M) |
| **17** | $CH_3$ | $OCH_3$ | 4.56 | 4.80 ± 0.19 | not tested | not tested |

[a] Standard deviations on p$K_{inc}$ (noncompetitive inhibition) are less than 0.1. [b] p$K_i$ for competitive inhibition. Blank indicates pure noncompetitive inhibition. [c] MAO-A and MAO-B p$IC_{50}$ values taken from ref 11. Standard deviations on $IC_{50}$ values are less than 10%.

**Data Analysis.** To determine the type of inhibition, the Michaelis−Menten equation was fitted using two linear transformations: Lineweaver−Burk (1/*v* vs 1/[S]) and Hanes ([S]/*v* vs [S]) plots. Graphs were plotted using Prism V3.0 (GraphPad Software). Michaelis−Menten constants ($K_M$, $V_{max}$) were obtained by nonlinear regression of the reaction rate as a function of substrate concentration.

Apparent $K_i$ constants were then calculated using eq 1 for noncompetitive inhibition and eqs 1 and 2 for mixed inhibition.[15]

$$K_{inc} = \frac{V^*_{max}[I]}{V_{max} - V^*_{max}} \quad (1)$$

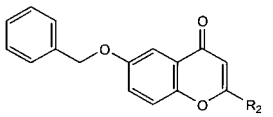
$$K_{ic} = \frac{V^*_{max}K_M[I]}{V_{max}K^*_M - V^*_{max}K_M} \quad (2)$$

where $K_M$ and $V_{max}$ were obtained in the absence of inhibitor I and $K^*_M$ and $V^*_{max}$ were obtained in the presence of inhibitor I. [I] is the concentration of inhibitor.

## Results and Discussion

**Chemistry.** The synthesis of the compounds (Tables 1 and 2) used in this study has been described elsewhere.[11]

**Table 2.** Chemical Structures and AChE and MAO Inhibition Data of Two Chromone Derivatives



| compd | $R_2$ | p$K_{inc}$ [a] AChE | p$K_{ic}$ [b] AChE | p$IC_{50}$ [c] MAO-A | p$IC_{50}$ [c] MAO-B |
|---|---|---|---|---|---|
| **18** | $CH_3$ | 4.53 | 4.78 ± 0.15 | 4.66 | 6.90 |
| **19** | H | 4.29 | | 5.17 | 6.25 |

[a] Standard deviations on p$K_{inc}$ (noncompetitive inhibition) are less than 0.1. [b] p$K_i$ for competitive inhibition. Blank indicates pure noncompetitive inhibition. [c] MAO-A and MAO-B p$IC_{50}$ values taken from ref 11. Standard deviations on $IC_{50}$ values are less than 10%.

**AChE Inhibition.** Among the 71 coumarin derivatives described earlier,[11] 19 compounds were tested for their inhibitory effect on AChE, most of which were analogues of 7-hydroxycoumarin. As reported, these compounds inhibited MAO-A and MAO-B in the micromolar to low-nanomolar range (Table 1),[11] with marked MAO-B selectivities. Two other coumarin analogues (**16**

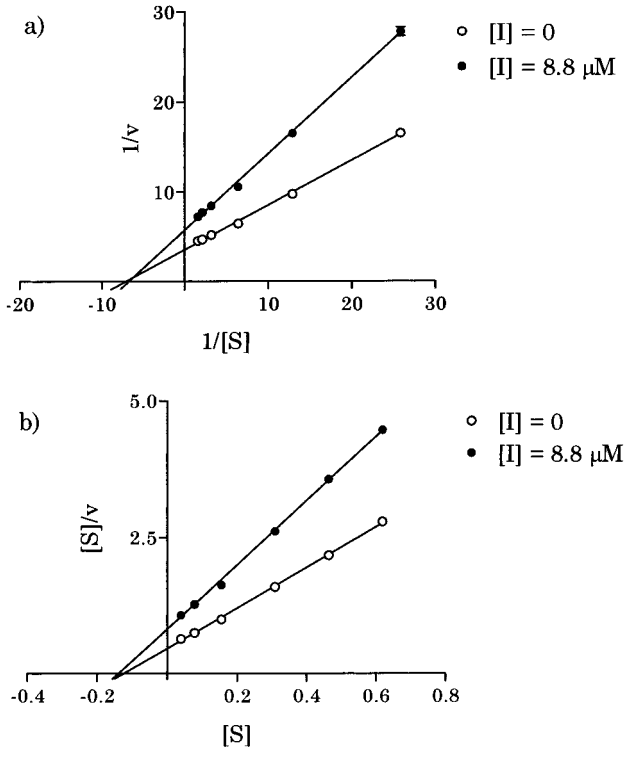



**Figure 1.** Noncompetitive inhibition of AChE by compound **4**: Lineweaver−Burk (a) and Hanes (b) plots.

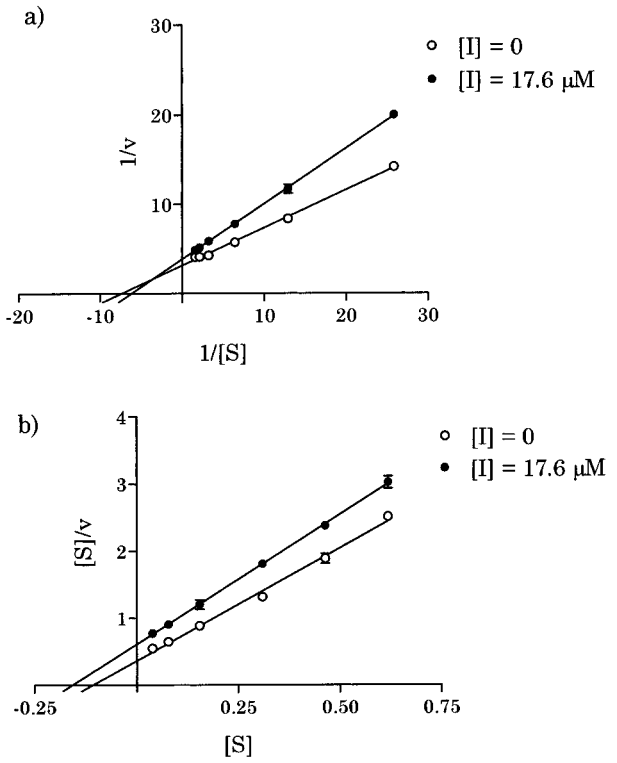



**Figure 2.** Mixed inhibition of AChE by compound **16**: Lineweaver−Burk (a) and Hanes (b) plots.

and **17**) were also studied to assess the influence of substituents in position 7 on AChE inhibition.

AChE inhibition data expressed as p$K_i$ values in Tables 1 and 2 show that all the compounds tested also inhibited AChE with $K_i$ values in the micromolar range (3−100 $\mu$M). The most active AChE inhibitor was 7-[3-(chlorobenzyl)oxy]-3,4,-dimethylcoumarin with a $K_i$ value of 3.40 $\mu$M (**8**; Table 1).

Coumarins substituted in position 7 by a benzyloxy group (**1**−**14**), compound **15** and the chromone **19**, were noncompetitive inhibitors of AChE (Tables 1 and 2). The nature of the inhibition of these compounds is illustrated by the Lineweaver−Burk and Hanes plots of compound **4** (Figure 1). These observations suggest that the compounds bind to the peripheral site of AChE as proposed for 3-chloro-7-hydroxy-4-methylcoumarin (CHMC).[16]

The two coumarins substituted with smaller groups in position 7 (i.e., hydroxy **16** and methoxy **17**) and the chromone **18** were mixed AChE inhibitors (Tables 1 and 2), as shown for **16** in Figure 2.

**Structure−Activity Relationships.** As illustrated in Figure 3, no clear relationship is observed between MAO and AChE inhibition of the tested compounds.

A comparison of Tables 1 and 2 shows that replacing the coumarin nucleus by chromone results in a loss of activity toward both enzymes. Moreover, this modification can lead to mixed inhibition of AChE (compound **18**).

Substitution in position 3 of the coumarin nucleus modulated AChE as well as MAO-B inhibitory activity. Substitution with methyl groups in positions 3 and 4 led to more active compounds toward both enzymes (compare **1** with **14**). Other monosubstituted analogues were not tested for AChE inhibition in this exploratory work because we demonstrated that monosubstitution

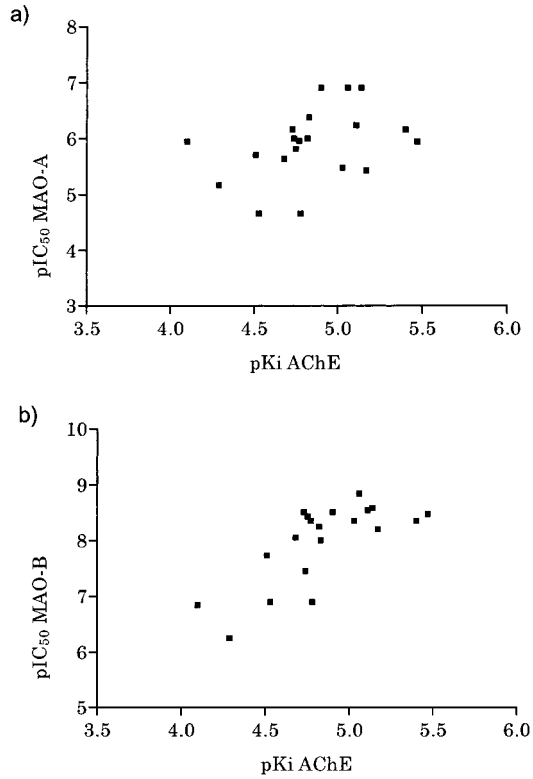



**Figure 3.** Relationships between MAO-A and AChE inhibition (a) and MAO-B and AChE inhibition (b).

in position 4 with phenyl, trifluoromethyl, and hydroxy groups strongly decreased MAO inhibitory activity.[11]

Because of its good activity toward MAO-A, MAO-B, and AChE, compound **1** was chosen to explore SARs by substitution in the 2′-, 3′-, and 4′-positions of its phenylbenzyloxy ring. Substitution of the phenyl ring by a

methyl group in ortho (**2**), meta (**3**), or para (**9**) positions slightly decreased AChE activity compared to **1**, ortho substitution being the least favorable. Substitution in the ortho position also produced a decrease in MAO inhibition. Introduction in the meta position of electron-donating substituents, i.e., OH (**4**), $OCH_3$ (**5**), or $NH_2$ (**6**), produced a decrease in AChE activity. Except for compound **8**, which was the best AChE inhibitor, substitution in the meta and/or para position by a halogen also produced a loss of activity toward AChE, while it led to a higher activity toward MAOs. Compound **8** is particularly interesting because it is among the best MAO-B inhibitors, with a good A/B selectivity.

As for other 7-substituents on the coumarin nucleus, a significant drop in AChE inhibition was observed for compounds bearing a small substituent, **16** and **17**. Increasing the length of the ether bridge with an additional methylene group also produced a drop of AChE activity but did not significantly change activity toward MAOs (**1** vs **15**).

## Outlook

The observation that some compounds can behave as inhibitors of both MAO (mainly MAO-B) and AChE suggests a therapeutic opportunity worth exploring. It is apparent from the data reported here that the activities toward MAO-B are about 3 orders of magnitude higher than toward AChE. However, a direct comparison of potencies is not possible because the present AChE inhibitors acted by a noncompetitive mechanism. Better assessment and optimization of AChE inhibitory activity without loss of MAO-B inhibition, followed by an in vivo proof of concept in animal models of AD, are the logical steps that come to mind in the continuation of this project.

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JM010894D