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SYNTHESIS AND BIOLOGICAL ACTIVITY OF GALANTHAMINE DERIVATIVES AS ACETYLCHOLINESTERASE (AChE) INHIBITORS

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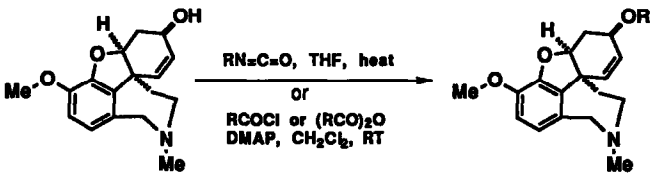
Abstract: The syntheses of several ester and carbamate derivatives of galanthamine are described. These compounds are potential therapeutic agents in the treatment of Alzheimer's disease (AD). The inhibition of cortical acetylcholinesterase (AChE) by these drug candidates with different side chains was investigated. Side chain length as well as branching affected the AChE inhibitory activity. Esters were generally less effective than carbamates.

Alzheimer's disease (AD) is the fourth leading cause of death among the elderly.[1] It is well-documented that mnemonic and cognitive impairments in patients suffering from AD are associated with acetylcholine deficiency in the brain. Cholinesterase inhibitors have been used to relieve this deficiency. Compounds such as physostigmine[2,3] and tetrahydroaminoacridine[4] have produced replicable improvements of cognitive functions, but are likely to cause undesirable side effects.[5,6] Galanthamine (**1**), a centrally-acting competitive AChE inhibitor,[7,8] has also been evaluated in the treatment of AD. Previous studies of **1**, an *Amaryllidaceae* alkaloid, supported the proposal that it might be effective in treating the central cholinergic deficits in AD.[9-11] A recent clinical study found that **1** was a well-tolerated drug during long term treatment.[12] Some patients showed improvement in almost all tests (short-term and long-term memory, speed-attention capacity, etc.) and in the clinical rating scales.

In continuation of our structural studies on galanthamine and its methiodide,[13] we have synthesized carbamates and esters of **1** to compare their acetylcholinesterase inhibitory activity. We have prepared and tested fifteen derivatives of galanthamine, using the Ellman assay to determine their effectiveness in acetylcholinesterase inhibition. Earlier investigations had shown that the free base of galanthamine was a potent inhibitor.[9-11] Two types of derivatives (carbamates and esters) were synthesized for pharmacological comparison. **Scheme I** shows an overview of the synthetic pathways employed. The carbamates were prepared by refluxing galanthamine as the free base[14] with the appropriate isocyanates in tetrahydrofuran (THF). The esters were synthesized by treating the free base with either an acid chloride or anhydride, and dimethylaminopyridine (DMAP) in methylene chloride (CH_2Cl_2) at room temperature.[15]

Scheme I



1 **2-16**

The compounds were tested using the biological assay known as the Ellman Method.[16,17] The data obtained are presented as follows: [Compound No., R, IC_{50} (mM)] **1**, -H, 1.03; **2**, -CONH*n*-propyl, 3.13; **3**, -CONHEt, 4.31; **4**, -CONH*n*-octyl, 8.82; **5**, -CONMeCONHMe, 9.67; **6**, -CONH*n*-butyl, 11.1; **7**, -CO-4-F-3-NO_2-C_6H_3, 11.3; **8**, -CO*iso*-butyl, 4.29; **9**, -CO*n*-pentyl, 9.36; **10**, -COEt, 9.46; **11**, -CO-2-F-C_6H_4, 14.2; **12**, -CO*n*-butyl, 14.8; **13**, -CO-*cyclo*-C_3H_5, 17.8; **14**, -CO*n*-nonyl, 20.7; **15**, -$COCH_2CH_2$-4-F-OC_6H_4, 48.3.

Longer chain carbamates and esters were less effective AChE inhibitors than the corresponding shorter chain derivatives. Branching increased the inhibitory activity. Generally, carbamates were more effective than esters. This observation suggests that hydrolysis is probably not taking place; otherwise, the IC_{50} values of the esters should be lower. In vivo studies of galanthamine *n*-butyl carbamate (**6**) showed that the optimal dose of this compound is considerably less (0.5mg/kg in BF-lesioned mice) than that of galanthamine (3mg/kg in BF-lesioned mice), thereby suporting the hypothesis that **6** is not first hydrolyzed to the parent compound.[18] In vitro, all derivatives were less effective AChE inhibitors than galanthamine.

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(13) Carroll, P.; Furst, G. T.; Han, S.-Y.; Joullié, M. M. *Bull. Soc. Chim. Fr.* **1990**, *127*, 769.
(14) Galanthamine free base (**1**) was obtained from galanthamine hydrobromide (Nivalin) by treatment with ammonium hydroxide. Nivalin was purchased from Pharmachim State Economic Association, 16 Iliensko, Chaussée, Sofia, Bulgaria.
(15) **Procedure A** - *General procedure for preparation of all carbamates* (**2-4**, and **6**). The appropriate isocyanate (3.14 mmol) was added to a stirred solution of galanthamine (**1**, 1.04 mmol) in THF (5 mL) at RT, under an argon atmosphere. The reaction mixture was heated to reflux for 24 h. After the solvent was evaporated, the crude material was purified by silica gel flash column chromatography using acetone at first, and then MeOH:acetone (1:9) as eluants to afford the product carbamate.
Procedure B/C - *General procedure for preparation of esters from anhydrides(B)/acid chlorides(C)* (**7-15**). To a stirred solution of galanthamine (**1**, 1.04 mmol) in dry CH_2Cl_2 (10 mL) was added anhydride/acid chloride (1.35 mmol) followed by dimethylaminopyridine (1.87 mmol) at 0 °C, under an argon atmosphere. The reaction was stirred for 10 min at 0 °C and at RT overnight. The solution was taken up in 25 mL CH_2Cl_2 and washed with brine (50 mL), followed by 10% sodium carbonate solution (50 mL), and then brine again. The organic layer was dried (Na_2SO_4), evaporated in vacuo, and the crude product purified by silica gel flash column chromatography using acetone, and then MeOH:acetone (1:9) as eluants.
Procedure D - Methyl isocyanate (1.14 mmol) was added to a stirred green suspension of galanthamine (**1**, 1.04 mmol) and copper(I) chloride(1.04 mmol) in DMF (5 mL) at RT, under an argon atmosphere. The reaction mixture was stirred for 2 h. The mixture was diluted with ether (20 mL), washed with water (10 mL) and brine (5 mL), dried (Na_2SO_4), and then the solvent evaporated in vacuo. The crude material was purified by silica gel flash column chromatography using acetone at first, and then MeOH:acetone (1:9) as eluants to afford compound **5**.
All new compounds were characterized by ^1H NMR, ^{13}C NMR, IR, elemental analysis, and/or HRMS.
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(17) The solution to be tested was prepared by making a mixture of homogenized rat brain tissue plus a drug candidate. Absorbances over different time intervals were obtained. Acetylthiocholine was the substrate that was acted upon by the enzyme, while dithio-bis(2-nitrobenzoic acid) was used to produce a yellow anion as a photo-indicator. The change in absorbance per minute was calculated by plotting the absorbance versus time and obtaining the slope. An average over 2-3 runs was the number taken for the Δ absorbance/min. (slope). The rate (R), in moles of substrate hydrolyzed per minute per gram tissue, was calculated by using the formula $R = \Delta A/1.36(10^4)*1/(400/\ 3120)C_o = 5.74(10^{-4})\Delta A/C_o$ (ΔA = change in absorbance/min [slope]; C_o = original concentration of tissue [mg/mL] = 20 mg/mL). At this point the percent inhibition was calculated by obtaining percent remaining ([rate of drug/rate of control]*100) of acetylcholinesterase enzyme and substracting this number from 100. Then log(%remaining/ %inhibition) was plotted versus log concentration of drug solution. When the inverse log of the y-intercept of this graph was taken, the IC_{50} value for that particular drug was obtained.
(18) The in vivo studies will be published elsewhere.