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Potent Acetylcholinesterase Inhibitors: Design, Synthesis and Structure–Activity Relationships of Alkylene Linked Bis-Galanthamine and Galanthamine–Galanthaminium Salts†

Catherine Guillou,* Aude Mary, Dolor Zafiarisoa Renko, Emmanuel Gras and Claude Thal

Institut de Chimie des Substances Naturelles, C.N.R.S., 91198 Gif-sur-Yvette Cedex, France

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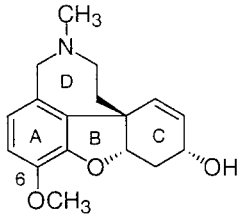
Abstract—The syntheses, the anticholinesterase activities and structure–activity relationships of homodimeric (**3a–c**) and heterodimeric (**6a–c**) alkylene linked bis-galanthamine are reported. Compounds **6b–c** were found to be more potent than galanthamine and tacrine in inhibiting AChE.

The cholinergic hypothesis postulates that memory impairments in patients with Alzheimer's disease (AD) result from a deficit of cholinergic function in the brain.[1] The most important changes observed in the brain of AD patients are a decrease in hippocampal and cortical levels of the neurotransmitter acetylcholine and associated enzyme choline transferase. One possible approach to treating this disease is to restore the level of acetylcholine by inhibiting acetylcholinesterase (AChE) with reversible inhibitors. Clinical trials indicate that AChE inhibitors such as tacrine and physostigmine effectively improve memory in some patients. Other cholinesterase inhibitors such as galanthamine **1**[2] have received recent attention. This compound is less potent, but also less toxic than tacrine and physostigmine. Galanthamine, a centrally-acting competitive and reversible inhibitor, has been shown to produce significant improvement of cognitive performances in AD patients.[3] Moreover, Nivalin® (galanthamine hydrobromide) has recently received its first approval for the treatment of AD in Austria and will soon be commercially available in Europe and the United States. A variety of synthetic galanthamine derivatives has been previously described including C-ring derivatives,[4] quaternary ammonium derivatives,[5] esters and carbamates of 6-*O*-demethylgalanthamine[5] and bis-interacting ligands.[6]



1 : (-)-Galanthamine

In continuation of our work in the galanthamine series,[6] we report in this paper the synthesis, the anticholinesterase activities and structure–activity relationships of homodimeric **3a–c** and heterodimeric **6a–c** alkylene linked bis-galanthamines. Our bis-ligand strategy is based on the crystallographic structure of AChE from *Torpedo californica*.[7] The AChE active site contains a catalytic triad (Ser 200, His 440, Glu 327) located at the bottom of a deep and narrow gorge, ~20 Å long, lined with aromatic residues and a subsite, including Trp 84, located near the bottom of the cavity. Trp 84 has been identified as the binding site of the quaternary groups of acetylcholine, decamethonium and edrophonium.[8] In addition, Trp 279 at the peripheral site, located at the opening of the gorge, is involved in the binding of the second quaternary group of decamethonium. The distance between these two tryptophan residues (84 and 279) is 12 Å. We thus hypothesized that the bis-ligands could simultaneously interact with the active and the peripheral sites (Trp 84 and 279) and therefore the potency of such ligands can be greatly improved. Recently, alkylene linked bis-tacrine[9] and

*Corresponding author. Tel.: +33-1-69-82-30-75; fax: +33-1-69-07-72-47; e-mail: guillou@icsn.cnrs-gif.fr

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heterodimeric huperzine A-tacrine[10] derivatives have been described to be highly potent inhibitors of acetylcholinesterase.

The homodimeric **3a–c** and the heterodimeric **6a–c** alkylene-linked bis-galanthamines were prepared from norgalanthamine **2**.[11] Alkylation of norgalanthamine (**2**) with the commercially available 1,*n*-dibromo alkane ($n = 6, 8, 10$) afforded the bis-galanthamines **3a–c** in low yields. The imine **4** was synthesized by oxidation of norgalanthamine (**2**) with *N*-bromosuccinimide. Subsequent alkylation of **4** with the 1,*n*-dibromo alkane ($n = 6, 8, 10$) afforded the iminium salts **5a–c**. The heterodimeric bis-functional derivatives **6a–c** were prepared by alkylation of norgalanthamine (**2**) with compounds **5a–c** (Scheme 1, Table 1).

Table 1.

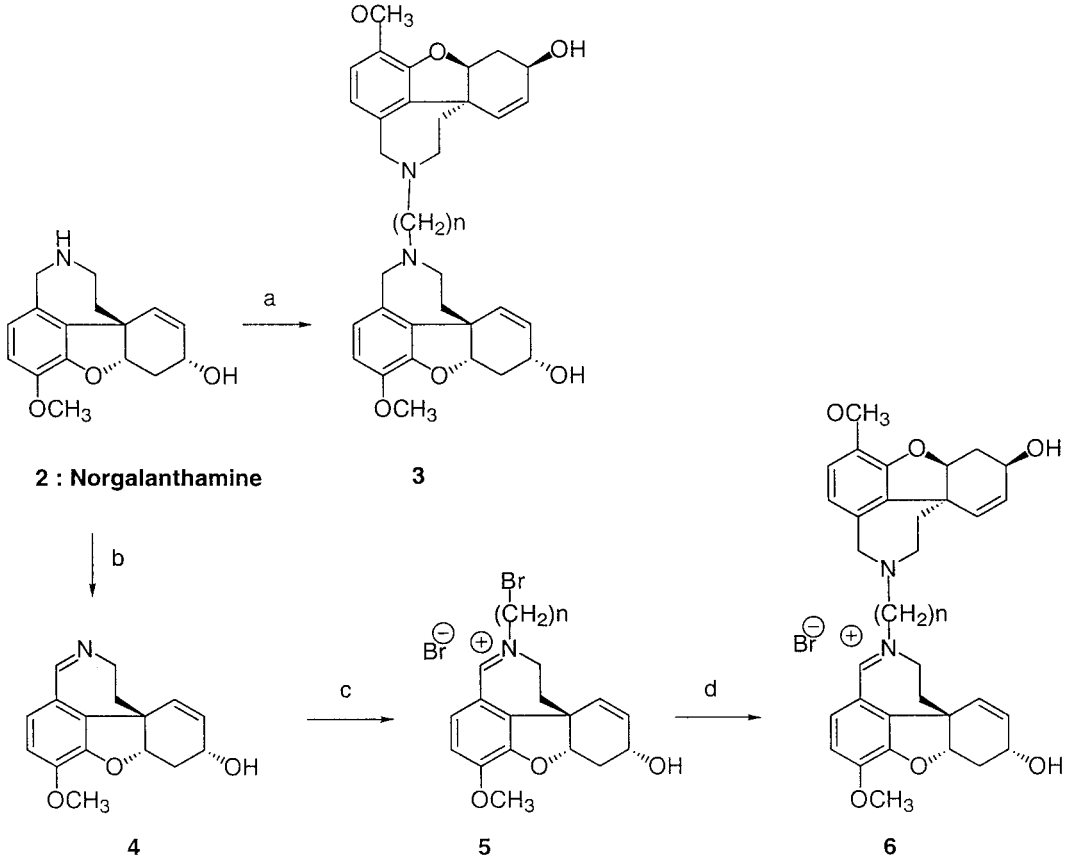
n	Products (yield %)					
6	**3a**	(7)	**5a**	(53)	**6a**	(76)
8	**3b**	(23)	**5b**	(66)	**6b**	(86)
10	**3c**	(14)	**5c**	(58)	**6c**	(60)

Biological Evaluation and Discussion

Newly synthesized compounds were tested for potency as acetylcholinesterase inhibitors. In vitro acetylcholinesterase inhibition was determined using the spectroscopic method of Ellman et al.[12] The results are summarized in Table 2.

As shown in Table 2, the potency of acetylcholinesterase inhibition depends on the length of the alkylene tether as well as the presence of the iminium function. Variation of the length of the tether should affect the possibility of an efficient interaction with the peripheral site. A length of eight methylenes (i.e. **3b**) is favoured in the bis-galanthamine series whereas this increases to ten methylenes in the heterodimeric galanthamine–galanthaminium series (i.e. **6c**). Furthermore, compounds in the heterodimeric series (i.e. **6a–c**) are more potent than those in the homodimeric series (i.e **3a–c**). These results confirm that the presence of the iminium function is a key feature for enhancing inhibitory potency as was previously shown in another heterodimeric galanthamine series.[6b]

In summary, new homodimeric alkylene-linked bis-galanthamines and heterodimeric galanthamine–galanthaminiums were prepared. Compounds **6b** and **6c** were more potent than galanthamine (16- and 30-fold, respectively) and tacrine (2- and 4-fold, respectively). These results confirm that acetylcholinesterase inhibitors which can simultaneously interact with the peripheral



Scheme 1. (a) $Br(CH_2)_nBr$, NEt_3, CH_3CN, 25 °C, 60 h. (b) NBS, CH_3CN, 25 °C, 1 (70%). (c) $Br(CH_2)_nBr$, CH_3CN, reflux, 20 h. (d) **2**, Na_2CO_3, CH_3CN, 25 °C, 7 d.

Table 2.

n	In vitro inhibition of AChE[a] IC_{50} (nM)			
6	**3a**	450 ± 20	**6a**	68 ± 7
8	**3b**	230 ± 20	**6b**	22 ± 1
10	**3c**	560 ± 20	**6c**	12 ± 1
Galanthamine		360 ± 10		
Tacrine		50 ± 4		

[a]The source of AChE was *Electrophorus electricus*.

and the catalytic sites demonstrate enhanced potencies compared to compounds which interact with a single site. Further studies on heterodimeric alkylene-linked galathaminium–tacrine and galanthamine–tacrine derivatives are in progress and will be reported in due course.

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