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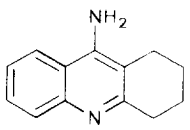
SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NEW ACETYLCHOLINESTERASE INHIBITORS: MORPHOLINOALKYLCARBAMOYLOXYESEROLINE DERIVATIVES

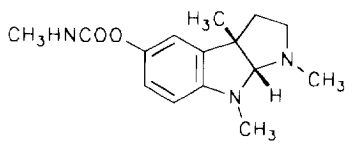
*Maria A. Alisi, Mario Brufani, Luigi Filocamo**, *Gianluca Gostoli*
Dipartimento di Scienze Biochimiche "A. Rossi Fanelli", Università "La Sapienza", Via degli Apuli 9, 00185 Roma, (Italy)
Emanuela Licandro
Dipartimento di Chimica Organica e Industriale dell'Università, Via Venezian 21, 20133 Milano, (Italy)
M. Candida Cesta, Sperandina Lappa, Donata Marchesini and P. Pagella
Mediolanum Farmaceutici S.p.a., Via S. G. Cottolengo 31, 20143 Milano (Italy)

Abstract: Several new potent acetylcholinesterase inhibitors have been synthesised as potential drugs for the treatment of Alzheimer's disease. Heptylphysostigmine (MF201) is a drug analogue of physostigmine under clinical evaluation. In order to obtain new physostigmine analogues, the methylcarbamoyloxy group was substituted with ω-morpholinoalkylcarbamoyloxy moieties of different chain lengths (C2-C12). Potent *in vitro* inhibition is seen when the chain length is composed of eight to twelve methylene groups. The inhibitory activity of the C10 and C11 is 7-fold greater with respect to heptylphysostigmine.

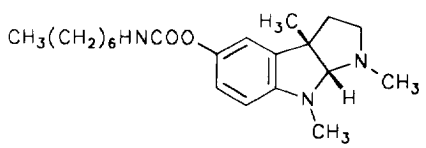
The cholinergic approach in the treatment of Alzheimer's Disease (AD) has so far been the most studied and exploited. Multiple cholinergic abnormalities in AD have been demonstrated. Clinical studies indicate that acetylcholinesterase (AChE) inhibitors, such as tetrahydro-9-aminoacridine (THA) and physostigmine, may be useful in enhancing memory in patients with AD[1,2]. However, THA induces a high incidence of liver toxicity[3], while physostigmine suffers from a short half-life, a variable bioavailability, and a narrow therapeutic index[4]. That could account for its inconsistent clinical efficacy. Heptylphysostigmine (MF201), a derivative of physostigmine, which is significantly less toxic and retains its *in vitro* potency as inhibitor of AChE[4] is currently undergoing clinical evaluation for the treatment of AD.



Tetrahydro-9-aminoacridine

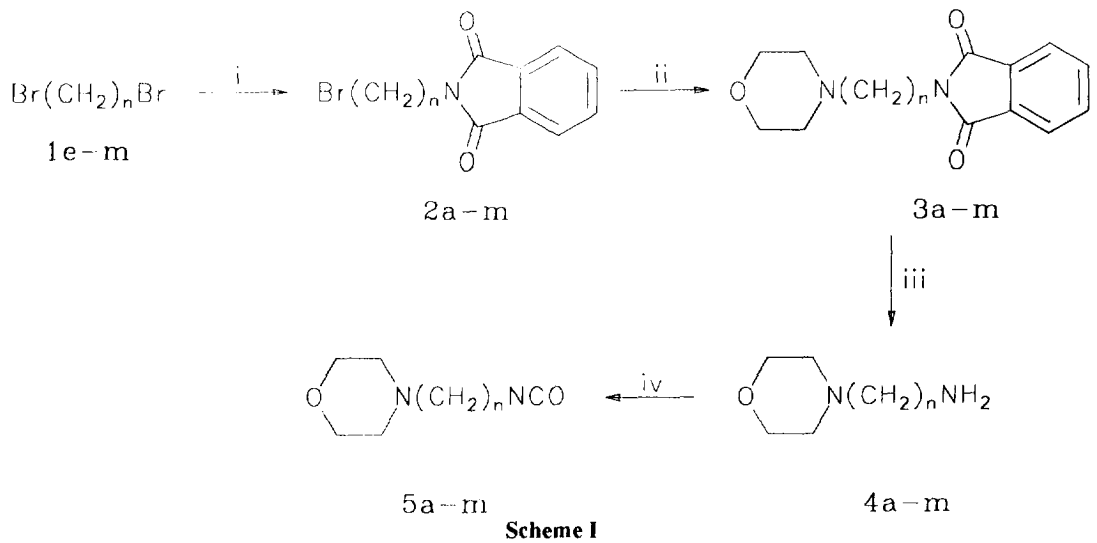


Physostigmine



Heptylphysostigmine (MF201)

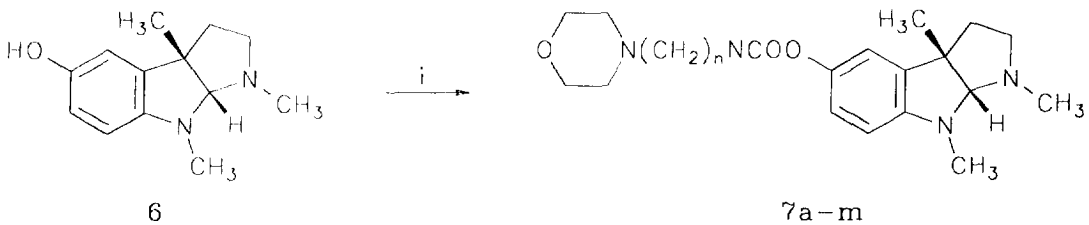
During our investigations of analogues of physostigmine as agents for the treatment of AD[5], we have prepared compounds which differ by their lipophilicity. The methyl group of physostigmine was substituted by aliphatic alkyl groups of increasing carbon chain length (from 2 to 12), which carries a terminal amino group. The



Scheme I

Reagents and conditions: i) Potassium phthalimide, DMF, reflux, 2 h; ii) Morpholine, CH_3CN, r.t., 18 h; iii) NH_2NH_2, MeOH, reflux, 1.5 h; iv) Phosgene (20%) in toluene, Et_3N, 0°→r.t., 3 h.

morpholino group was chosen since its pKa (8.33 in aqueous solution) was expected to maintain the basicity of the inhibitors low enough to allow a sufficient rate of oral adsorption and penetration of the blood-brain barrier. The new compounds were synthesised according to Schemes I and II. Phthalimidoalkyl bromides **2a-d** are commercially available, while compounds **2e-m** were synthesised by refluxing potassium phthalimide with three equivalents of alkyldibromides **1e-m** in DMF for 2 h. The reaction products (**2e-m**) were purified by flash chromatography on silica gel using gradient elution (hexane/diethyl ether 9:1 and then 8:2) (yields from 60 to 74%). Morpholine was alkylated with compounds **2a-m** in acetonitrile at r.t. for 18 h. After purification of the products **3a-m** by column chromatography on silica gel (elution with CH_2Cl_2/MeOH 95:5) (yields 70-76%), the phthalimido group was removed by hydrazinolysis in refluxing MeOH to give the morpholinoalkylamines **4a-m** yields of 75-91%. The synthesis of the carbamates **7a-m** was performed by reacting eseroline (**6**) (generated by hydrolysis of physostigmine with 10% NaOH in absolute ethanol[6] under N_2) with 1,1'-carbonyldiimidazole (2 equiv) in benzene at r.t. for 1 h, and then adding the amines **4a-m** (3 equiv) and stirring for 2 h at r.t.[7]. After workup, purification of the crude products by chromatography on two separate silica gel columns (the first eluted with $(Me)_2CO$/MeOH 8:2 and the second with $CHCl_3$/MeOH 9:1), gave compounds **7a-m** in yields ranging from 52 to 71%. With the aim of obtaining better yields of **7a-m**, the isocyanates **5a-m** were first synthesised by adding



Scheme II

Reagents and conditions: i) a. N,N'-carbonylimdidazole, benzene, r.t., 1 h; b. morpholinoalkylamine (**4a-m**), benzene, r.t., 2 h or morpholinoalkylisocyanide, Na, toluene, r.t., 2 h.

dropwise the amines **4a-m** (1 equiv) dissolved in toluene and triethylamine (5 equiv) to 1.5 equiv of phosgene (20% in toluene) at 0 °C and then stirring the mixture at r.t. for 2 h. After a further addition of 1.5 equiv of phosgene (20% in toluene), the mixture was stirred for 1 h at r.t. and then the solvents were evaporated under reduced pressure. The purification of the isocyanates **5a-m** by distillation at 0.6 mbar was rather difficult due to the large amounts of decomposition byproducts generated during this step, and it was successful only using large amounts of products; the following reaction with eseroline in toluene with catalytic amounts of Na proceeded at r.t. in 2 h to give good yields of the carbamates **7a-m** (from 91 to 96%) after purification by column chromatography (eluent $CHCl_3$/MeOH 9:1).

The L-tartrate salts of the new series of eseroline derivatives **7a-m** were prepared and tested for biological activity *in vitro* using AChE from human RBC. Esterase activity was determined according to the method of Ellman *et al.*[8]. The anti-ChE activity of these derivatives (expressed as IC_{50}, μM) are shown in Table I.

Table I - AChE inhibiting activity (IC_{50} μM) of compounds **7a-m**.

Comp.	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**	**7l**	**7m**	**Hep***	**Phy****
(CH$_2$)n	2	3	4	5	6	7	8	9	10	11	12	—	—
IC$_{50}$	0.7	3.8	21	7.6	0.93	0.38	0.08	0.06	0.04	0.04	0.07	0.29	0.03

* Heptylphysostigmine (MF201)

** Physostigmine

The inhibiting activity of the morpholinoalkyl derivatives decreases from **7a** to **7c**, However, when the alkyl chain is further elongated, the inhibitory activity increases again (Fig. 1). Compounds **7i** and **7l** show an inhibiting activity comparable to physostigmine.

The AChE active site contains a catalytic subsite and a so-called "anionic" subsite which binds the quaternary group of acetylcholine[9]. A second, "peripheral anionic site" is so named since it is distinct and away from the active site[10]. It is already known that some bisquaternary AChE inhibitors derive their enhanced potency, relative to homologous monoquaternary ligands[11], from their ability to span these two "anionic sites", which are *ca.* 14 Å apart. The crystallographic structure of *Torpedo californica* AChE has revealed that the enzyme displays a catalytic triade (S200, H440, E327) at the bottom of a deep and narrow cavity and the walls of this gorge are lined up with 14 aromatic residues ("aromatic gorge")[12]. The 3D structure of crystalline complexes, obtained by soaking the bisquaternary ligand decamethonium into native *Torpedo* AChE crystals, shows the van der Waals contact of both quaternary groups with tryptophan indole moieties, one with that of W84, at the base of the "aromatic gorge", and the other with W279 near the entrance of the gorge[13]. The distance of these two tryptophan molecules is *ca.* 12 Å. We presume that, under the assay conditions (pH 7.2), the protonated aminomorpholino group of compounds 7 is able to interact with the "peripheral anionic site" when the alkyl chain is of suitable length (from 8 to 12 CH_2). When the alkyl chain is shorter (compounds **7b-d**) the morpholino group probably hinders the optimal fitting of the molecule in the narrow gorge affecting the inhibitory activities of these compounds. However, the inhibiting activities of compounds **7a** (n = 2) and **7e** (n = 6) are only slightly decreased and compound **7f** (n = 7) is nearly equipotent with heptylphysostigmine. We presume that in these compounds AChE can accommodate the morpholinoalkyl chain in the gorge. However, when the length of the alkyl chain allows the interaction of the

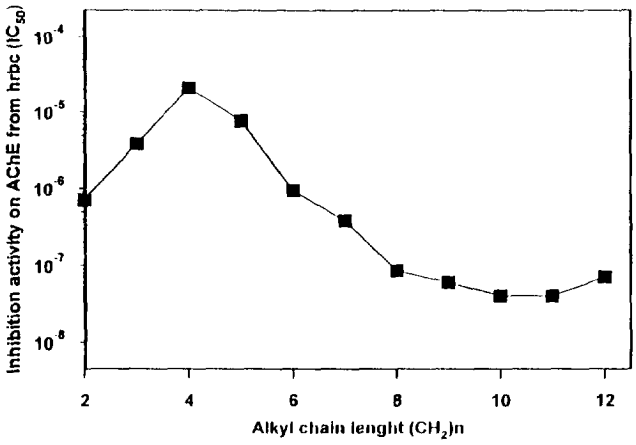


Fig. 1 - Chain length-inhibition activity relationship of the derivatives **7a-m**. The inhibitory activities are reported in molar IC_{50}. On the X-axis are reported the number of carbon atoms of the N-carbamic morpholinoalkyl chain.

protonated aminomorpholino moiety with the AChE "peripheral anionic site" (compounds **7g-m**, n = 8-12), the IC_{50} ranges from 0.08 to 0.04 μM, a gain of *ca.* one order of magnitude (fig. 1).

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