SYNTHESIS AND *IN VITRO* EVALUATION OF GALANTHAMINE DERIVATIVES FOR EXAMINATION OF NICOTINIC ACETYLCHOLINE RECEPTOR SYSTEM

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Summary: The syntheses and radioactive labeling of several galanthamine derivatives, 6-O-demethyl-6-O-fluoroethylgalanthamine, 10-N-demethyl-10-N-fluoroethylgalanthamine and N-methylgalanthaminium are reported. First *in vitro* evaluations were carried out to determine their properties as allosterically potentiating ligands of nicotinic receptors. N-methylgalanthaminium was found to be a promising candidate for further investigations.

Key Words: galanthamine, nicotinic acetylcholine receptors, fluorine-18, tritium

The most commonly applied therapeutic approach to balance nicotinic cholinergic deficits in Alzheimer's disease (AD) patients is the administration of acetylcholinesterase inhibitors (AChE-I) although they have been proven to be of limited therapeutic value [1]. A novel approach to drug treatment in AD is the application of allosterically potentiating ligands (APL) acting on nicotinic acetylcholine receptors (nAChR). APLs interact with the receptor via binding sites that are distinct from those for nicotinic agonists and antagonists. They also up-modulate the channel activity of nAChRs in response to acetylcholine (ACh) and other nicotinic agonists [2]. Representative members of this class of ligands are the plant alkaloids physostigmine, codeine and galanthamine.

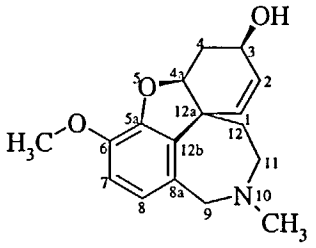

Fig.1: Structure of galanthamine (1)

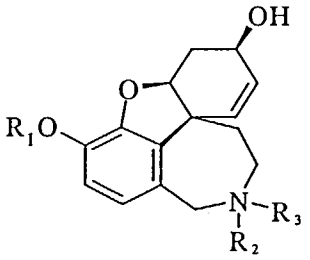

Fig.2: Structures of the galanthamine derivatives:
6-O-demethyl-6-O-fluoroethylgalanthamine (3):
$R_1=CH_2CH_2F$, $R_2=CH_3$;
10-N-demethyl-10-N-fluoroethylgalanthamine (6):
$R_1=CH_3$, $R_2=CH_2CH_2F$;
N-methylgalanthaminium (7):
$R_1=R_2=R_3=CH_3$

Galanthamine (Fig.1) acts as either an AChE-I or an APL (it also enforces receptor dependent neurotransmitter release on account of an unknown mechanism). The primary object of this work was to synthesize a galanthamine derivative which possesses only one of these properties, preferrably acting as APL. These derivatives might be employed in PET studies and psychopharmacological examinations. Fig.2 shows the derivatives that have been synthesized and tested *in vitro* up to now.

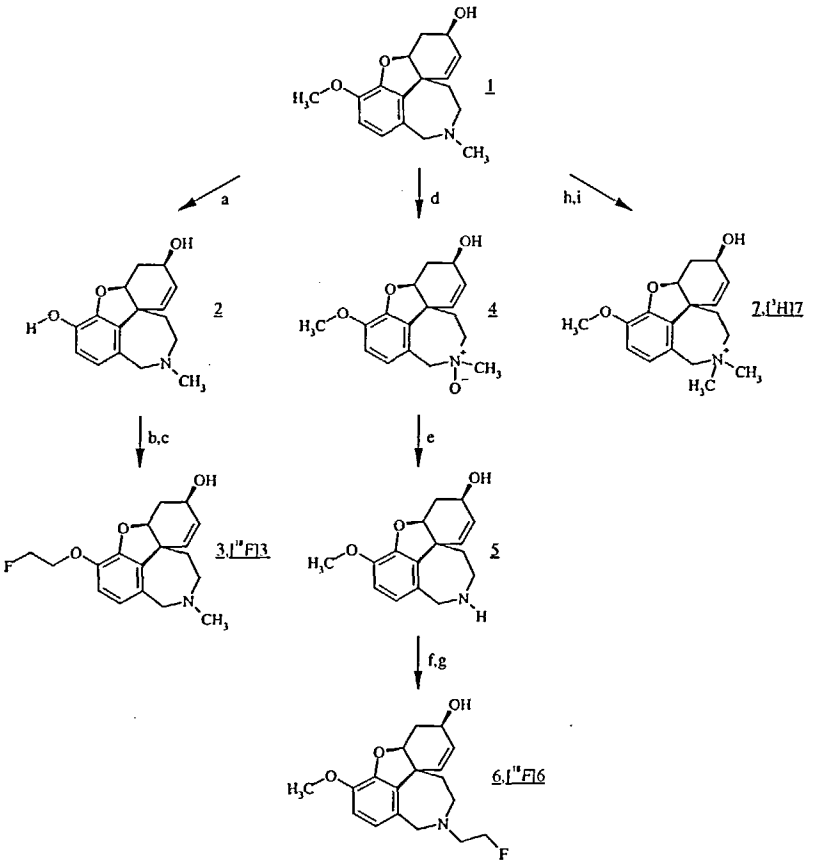

Fig. 3: Reaction conditions:
a) L-Selectride, THF [3]; b) FCH_2CH_2Br, Cs_2CO_3, DMF; c) [^{18}F]FCH_2CH_2OTos, NaOH, DMF; d) MCPBA, $CHCl_3$ [4]; e) $FeSO_4 \cdot 7\ H_2O$, CH_3OH [4]; f) FCH_2CH_2Br, NEt_3, CH_3CN; g) [^{18}F]FCH_2CH_2OTos, DMF; h) CH_3I; Et_2O [5]; i) [^{3}H]CH_3I, toluene

Radioactive labeling of precursors 2 and 5 could easily be achieved by fluoroalkylation with 2-[^{18}F]fluoroethyltosylate in DMF (2: 5 mg, 0.95 eq. 1 N NaOH, 20 min, 100°C; 5: 5 mg, 30 min, 140°C) in mean radiochemical yields of 92% ([^{18}F]3) and 31% ([^{18}F]6) (referred to 2-[^{18}F]fluoroethyltosylate, decay corrected). [^{3}H]7 was synthesized by reaction of 1 with [^{3}H]CH_3I (10 mCi, s.a.

80 Ci/mmol) in toluene (reaction conditions: r.t., 1 day) with a radiochemical yield of 62% (s.a. 54 Ci/mmol).

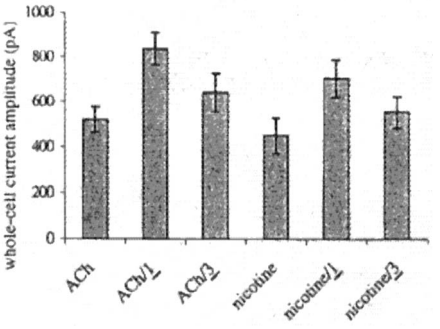

Fig.4: Whole-cell current measurements for the effect of 3 on human α4β2 nAChR subtype-expressing HEK-293 cells (the holding potential was set to –70 mV and 50 μM ACh or nicotine were co-applied with 1 and 3)

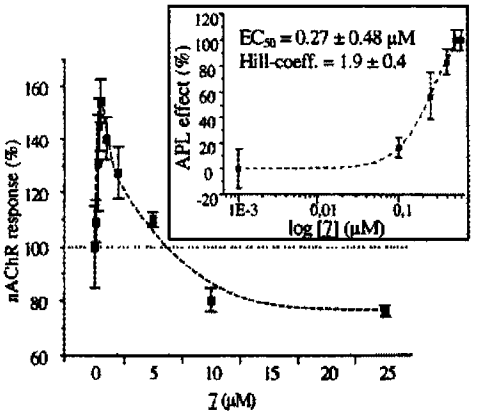

Fig.5: Dose-response curve for the effect of 7 on nicotine-activated Ca^{2+} influx into the human α4β2 nAChR subtype-expressing HEK-293 cells (after initial stimulations with 50 μM nicotine 0-25 μM 7 were co-applied); small figure: APL-effect of 7 under same conditions

In vitro evaluation was performed using the fura-2-calcium imaging method and whole-cell measurements on HEK-293 cells stably transfected with human α4β2 nAChR. The latter method showed 3 and 6 to have only a weak allosteric potentiating effect. The increased nicotinic response for those compounds was only 50% of that observed in the case of 1 (Fig.4). The current amplitudes induced by ACh or nicotine and further enhanced by 3 and 6 could be suppressed by the nicotinic antagonist mecamylamine. These findings suggest a nAChR-related mechanism. On the other hand, the calcium imaging method showed no increase of the α4β2 nAChR dependent Ca^{2+} influx for both fluoroalkylated derivatives (3, 6). However, under the same conditions 7 produced a 54% increase of the Ca^{2+} influx evoked by nicotine (Fig. 5).

Conclusion: The galanthamine derivatives 3 and 6 are no likely candidates for the intended studies while 7 as a more potent modulator than galanthamine itself will be further developed. These results prompted us to tritiate 7 in order to conduct a binding assay which is currently carried out.

References:

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