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Synthesis of 6β-D-glucosyl and 6-nitroxy (–)-galanthamine derivatives as acetylcholinesterase inhibitors

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Galanthamine is an alkaloid approved for the treatment of Alzheimer’s disease. In this paper the syntheses and the anticholinesterase activities of new glucosyl and nitroxy derivatives substituted on position 6 are reported. Compounds **2**, **3** and **5** presented a percentage of inhibition of 35.22%, 47.48% and 67.89 % respectively.

1. Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder of the central nervous system (CNS), associated with cholinergic insufficiency, resulting in dementia that is characterized by profound memory impairments, emotional disturbance and personality changes (Bartus et al. 1982; Dunnet et al. 1993; Weinstock 1997). Currently, the most effective way of improving the reduced acetylcholine levels is treatment with reversible competitive inhibitors of acetylcholinesterase. Galanthamine is an alkaloid found in the bulbs and flowers of the common snowdrop (*Galanthus nivalis* L.) and of several other members of the Amaryllidaceae family. It is approved for the treatment of AD as hydrobromide salt under the name Nivalin® or Reminyl®. Limited supplies and high costs of Galanthamine isolation from natural sources (Shieh et al. 1994; Kuenburg et al. 1999) led to the development of several synthetic routes for the preparation of (±)-galanthamine, epigalanthamine and narwedine (Kametani et al. 1971a, b; Barton et al. 1962; Kametani et al. 1969; Shimizu et al. 1977; Vlakow et al. 1989; Marco-Contelles, J. et al. 2006). Moreover a variety of synthetic galanthamine derivatives have been previously described including C-ring derivatives, quaternary ammonium derivatives (Han et al. 1992) or esters and carbamates of 3-*O*-dimethyl-galanthamine (Bores et al. 1996) as well as some galanthamine analogues (Lewin et al. 2005). Most of these compounds are characterized by the unsubstituted hydroxyl group in position 6.

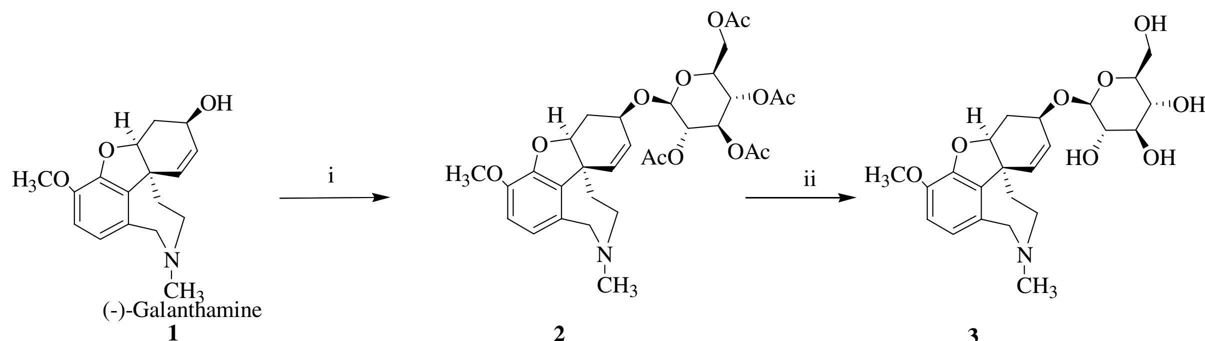
In order to better evaluate the role of the hydroxyl group in position 6, we synthesized new glucosyl and nitroxy galanthamine analogues. These substituents were selected starting from two different considerations: firstly, these groups represents important biological scaffolds both as transporting carriers substrates (glucosyl derivatives) (Bell et al. 1993; Barret et al. 1999) than as useful NO donors in CNS (Emson et al. 1995). On the other hand, their different sterical hindrance could allow us to better define the impact of the substituent’s encumbrance in position 6 on the galanthamine binding mode to the AchE active site (Greenblatt et al. 1999).

2. Investigations, results and discussion

(−)-Galanthamine was been synthesized according to a procedure reported in the literature (Czollner et al. 1998). The syntheses of tetra-acetyl derivative **2** and desacetyl derivative **3** were carried out following the synthetic procedure reported in Scheme 1. (−)-Galanthamine (**1**) was treated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide affording the 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl derivative **2**. Acetyl groups were removed in dry methanol with catalytic amounts of 0.1 M sodium methanolate affording the desired 6β-D-glucosyl(−)-galanthamine (**3**), without methyl ether as by-product. The nitroxy derivative **5** was prepared as reported in Scheme 2, starting from (−)-Galanthamine (**1**) which was treated with $SOCl_2$ to give the corresponding 6α-chloride derivative **4**. Treatment with $AgNO_3$ in CH_3CN converted compound **4** into 6β-nitroxy-(−)-galanthamine **5**.

These new products were tested *in vitro* on rat brain homogenate and the results are reported in the Table as percentage of inhibition at highest tested concentration (10^{-4} M). Even if lower in comparison with galanthamine (inhibition 89.88 %; IC_{50} 5.86 μM, the results supported the hypothesis of Greenblatt et al. (1999, 2004). In agreement with their reports, our derivatives showed that the increasing of the steric hindrance in position 6 provides a lower interaction of galanthamine in the enzyme active site; in fact, the percentage of inhibition of our compounds increased when 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl derivative (**2**) was converted into 6β-D-gluco-

Scheme 1 Reagents and conditions: (i) 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide, AgOTfl, anhydrous CH_2Cl_2, −10 °C. (ii) 0.1 M sodium methanolate, rt, 3 h



Scheme 2 Reagents and conditions: (i) $SOCl_2$, toluene, 55 °C. (ii) $AgNO_3$, CH_3CN, rt, 48 h

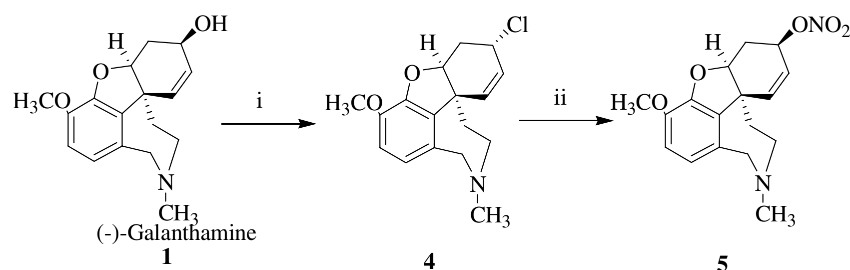


Table: *In vitro* AChE inhibition of compounds 1, 2, 3 and 5

Compd.	% Inhibition[a] ($\bar{M}$ ± s.d.)	IC_{50} Values
1	89.88 ± 1.20	5.86 ± 0.345
2	35.22 ± 0.94	–
3	47.48 ± 1.33	–
5	67.89 ± 0.57	45.09 ± 2.596

[a] % inhibition at highest tested concentration (10^{-4} M)

syl(−)-galanthamine (**3**), while the best result was obtained with the introduction of the nitroxyl group. Compound **5**, characterized by lower hindrance on the enzyme catalytic site, showed, in fact, a percentage of inhibition of 67.89% with a IC_{50} value of 45.09 μM.
In conclusion, the new derivatives, even if characterized by a lower enzymatic inhibition, could represent a new valuable strategy in order to improve the pharmacokinetic and pharmacological properties of the bioactive isomer of galanthamine. In fact we could hypothesize that, by means of glucose carriers, the novel glucosyl derivatives could have a better bioavailability in the CNS, as well as for the nitroxyl derivative we could hypothesize a release of NO, an important neuromediator over BBB. These will be investigated in further *in vivo* studies.

3. Experimental

3.1. Synthesis

3.1.1. General procedures

All reagents were commercial products purchased from Aldrich. Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AMX-500 spectrometer in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in ppm using Me_4Si as internal standard. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet). Mass spectra of the final products were performed on a LCQ Thermoquest-Ion trap mass spectrometer. Results obtained were within ± 0.4% of the theoretical values. All reactions were followed by thin-layer chromatography (TLC), carried out on Merck silica gel 60 F_{254} plates with fluorescent indicator and the plates were visualized with UV light (254 nm). Preparative chromatographic purifications were performed using silica gel column (Kieselgel 60). Organic solutions were dried over anhydrous Na_2SO_4 and concentrated with Büchi rotary evaporator at low pressure while, in case of aqueous solution, the final product was recovered by lyophilization.
For the pharmacological assays all the used solvents and powders were for analysis and were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. The measurements were carried out with a Beckman DU-650 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA).

3.1.2 (−)-(4a α,6β)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6 H-benzofuro [3a,3,2-e,f] [2] benzazepin-6-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**2**)

To a solution of (−)-galanthamine **1** (0.39 g, 1.36 mmol) and triethylamine (0.185 mL, 1.36 mmol) in anhydrous dichloromethane (10 mL) was added AgOTfl (0.35 g, 1.36 mmol). The mixture was stirred under nitrogen atmosphere and cooled to −10 °C. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.56 g, 1.36 mmol) was added and then the mixture was stirred for 2 h. After 12 h at room temperature the suspension was neutralized with 2,3,5-collidine and centrifuged. The supernatant was concentrated, redissolved in acetonitrile and purified by preparative HPLC (eluent: A, 0.1% trifluoroacetic acid in acetonitrile, B, 0.1% trifluoroacetic acid in water; linear gradient from 0 to 70% over 25 min, flow rate: 1 ml/min) and lyophilized affording the pure product **2** (yield: 60%). M. p: 96–98 °C. $[\alpha]_D^{25}$: −132.5° (c = 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.68 (q, 2 H), 6.02 (m, 2 H), 5.27–5.14 (m, 3 H), 5.08 (d, 1 H), 4.63 (bs, 1 H), 4.45 (bs, 1 H), 4.26 (dd, 1 H), 4.15 (dd, 1 H), 4.02 (d, 1 H), 3.83 (s, 3 H), 3.79 (m, 1 H), 3.71 (d, 1 H), 3.28 (t, 1 H), 3.06 (d, 1 H), 2.69 (dd, 1 H), 2.40 (s, 3 H), 2.10 (m, 2 H), 2.06, 2.04, 2.03 and 2.02 (4s, 12 H, acetyl), 1.47 (dd, 1 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ (ppm): 169.1, 169.2, 170.1, 170.4, 145.9, 144.2, 133.3, 129.5, 127.8, 127.1, 122.2, 111.5, 99.1, 88.8, 72.7, 71.3, 68.2, 62.1, 61.9, 60.6, 56.1, 54.1, 48.4, 42.3, 33.9, 30.1, 20.5. ESI-MS: 618.8 $[M + H]^+$.
$C_{31}H_{39}NO_{12}$

3.1.3. (−)-(4a α,6β)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6 H-benzofuro [3a,3,2-e,f] [2] benzazepin-6-β-D-glucopyranoside (**3**)

A solution of **2** (0.50 g, 0.81 mmol) in anhydrous methanol (15 mL) was treated with catalytic amounts of 0.1 M sodium methanolate (1 mL) and stirred at room temperature. After 3 h the solution was neutralized by ion-

exchange resin (Amberlite IR-120 H^+, purchased from Aldrich), the resin was filtered off and the filtrate was concentrated *in vacuo*. The residue was directly purified by preparative HPLC (eluent: A, 0.1% trifluoroacetic acid in acetonitrile, B, 0.1% trifluoroacetic acid in water; linear gradient from 0 to 60% over 25 min, flow rate: 1 mL/min) and lyophilized affording the pure product **3** (yield: 80%). M. p.: 88–90 °C. $[\alpha]_D^{25}$: −123.6° (c = 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.68 (q, 2 H), 6.02 (m, 2 H), 5.27–5.14 (m, 3 H), 5.08 (d, 1 H), 4.63 (bs, 1 H), 4.45 (bs, 1 H), 4.26 (dd, 1 H), 4.15 (dd, 1 H), 4.02 (d, 1 H), 3.83 (s, 3 H), 3.79 (m, 1 H), 3.71 (d, 1 H), 3.28 (t, 1 H), 3.06 (d, 1 H), 2.69 (dd, 1 H), 2.40 (s, 3 H), 2.10 (m, 2 H), 1.98 (bs, 4 H), 1.47 (dd, 1 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ (ppm): 145.9, 144.2, 133.3, 129.5, 127.8, 127.1, 122.2, 111.5, 99.1, 88.8, 72.7, 71.3, 68.2, 62.1, 61.9, 60.6, 56.1, 54.1, 48.4, 42.3, 33.9, 30.1. ESI-MS: 450.3 $[M + H]^+$.
$C_{23}H_{31}NO_8$

3.1.4. (−)-(4a α,6α)-6-Chloro-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6 H-benzofuro [3a,3,2-e,f] [2] benzazepin (**4**)

A mixture of (−)-galanthamine (0.08 g, 0.27 mmol) was dissolved in toluene (10 mL). Thionyl chloride (0.065g, 0.55 mmol) was added in portions over a period of 30 min and the solution was stirred for 2 h at 55 °C; the solvent was then evaporated obtaining **4** (yield: 100%) as a pure brown oil. $[\alpha]_D^{25}$: −282.8° (c = 0.61, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.69 (q, 2 H), 6.1 (m, 2 H), 4.63 (bs, 1H), 4.1 6 (bs, 1H), 4.1 1 (d, 1 H), 3.84 (s, 3 H), 3.72 (d, 1 H), 3.28 (t, 1 H), 3.08 (d, 1 H), 2.71 (dd, 1 H), 2.41 (s, 3 H), 2.07 (m, 2 H), 1.48 (dd, 1 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ (ppm): 145.9, 144.3, 133.5, 129.5, 127.8, 127.0, 122.2, 111.5, 88.9, 62.2, 60.8, 56.1, 54.0, 48.4, 42.3, 33.9, 30.2. ESI-MS: 306.1 $[M + H]^+$.
$C_{17}H_{20}ClNO_2$

3.1.5. (−)-(4a α,6β)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6-nitroxy-6 H-benzofuro[3a,3,2-e,f] [2] benzazepin (**5**)

A mixture of **4** (0.07 g, 0.23 mmol) and $AgNO_3$ (0.0714 g, 0.42 mmol) in acetonitrile (10 mL) was stirred under light exclusion for 48 h. The mixture was filtered on Celite and the solvent was evaporated under vacuum; the residue was then dissolved in ethyl acetate, washed with H_2O and brine and then the organic phase was separated, dried with anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified on silica gel column with a mixture dichloromethane/methanol (8 : 2, v/v) as eluent obtaining (−)-galanthamine-6β-yl-6-nitrate **5** as a brown oil (yield: 11%). $[\alpha]_D^{25}$: −261.4° (c = 0.6, $CHCl_3$) ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.92 (q, 2 H), 6.4 (m, 2 H), 4.88 (bs, 1 H), 4.47 (bs, 1 H), 4.23 (d, 1 H), 4.01 (s, 3 H), 3.91 (d, 1 H), 3.72 (t, 1 H), 3.18 (d, 1 H), 2.80 (dd, 1 H), 2.51 (s, 3 H), 2.22 (m, 2 H), 1.98 (dd, 1 H); ^{13}C NMR ($CDCl_3$, 500 MHz) δ (ppm): 146.8, 144.9, 133.9, 131.0, 128.5, 127.9, 122.9, 111.9, 89.6, 62.9, 61.5, 56.9, 54.9, 49.0, 42.9, 34.6, 30.8. ESI-MS: 333.0 $[M + H]^+$.
$C_{17}H_{20}N_2O_5$

3.2. In vitro pharmacological assays

The method of Ellman et al. (1961) was used with some modifications. Rat brain was homogenized (approximately 20 mg of tissue per mL of 0.1 M phosphate buffer, pH 8.0) with a Polytron® Apparatus – Kinematica GmbH, Littau, Switzerland. The preparation of brain homogenate was carried out on male Sprague-Dawley rats (Harlan Italy s.r.l., Correzzana, Milan) that were sacrificed by decapitation. A 0.2 mL aliquot of this homogenate was added to a cuvette containing 0.58 mL of phosphate buffer. 0.1 mL of test compound dissolved in 5% DMSO or 0.1 mL of solvent alone were added too (final concentrations of tested compounds were: 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} M, each performed in sextuple). The mixture was incubated at room temperature for 5 min and then at 37 °C for additional 5 min. At the end of this procedure 0.1 mL of 0.0033 M DTNB reagent and 0.020 mL of 0.075 M acetylthiocholine iodide were added to the photocell. The absorbance was measured at 412 nm at 37 °C for 5 min. Galanthamine was employed as reference compound. The percent of inhibition was calculated as reported in the literature (Han et al. 1991). The results are shown in the Table.

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