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Original article

New developments in redox chemical delivery systems by means of 1,4-dihydroquinoline-based targetor: Application to galantamine delivery to the brain

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ABSTRACT

The therapeutic efficiency of palliative treatments of AD, mostly based on acetylcholinesterase (AChE) inhibitors, is marred by serious adverse effects due to peripheral activity of these AChE inhibitors. In the literature, a redox-based chemical delivery system (CDS) has been developed to enhance drugs distribution to the brain while reducing peripheral side effects. Herein, we disclose two new synthetic strategies for the preparation of 1,4-dihydroquinoline/quinolinium salt redox-based systems particularly well designed for brain delivery of drugs sensitive to alkylation reactions. These strategies have been applied in the present case to the AChE inhibitor galantamine with the aim of alleviating adverse effects observed with cholinergic AD treatment. The first strategy is based on an intramolecular alkylation reaction as key step, whilst the second strategy relies on a useful coupling between galantamine and quinolinium salt key intermediate. In the course of this work, polymer-supported reagents and a solid-phase synthesis approach revealed to be highly helpful to develop this redox-based galantamine CDS.

1. Introduction

Alzheimer's disease (AD) is a multi-factorial disorder clinically characterized by several bio-chemical and pathological events: low concentrations of the neurotransmitter acetylcholine (ACh) in the central nervous system (CNS) [1], interneuronal deposits of aberrant proteins like β-amyloid plaques [2,3] and intraneuronal deposits of *tau*-proteins (neurofibrillary tangles) [4], oxidative stress [5] and dys-homeostasis of biometals [6]. Although different strategies are explored to prevent AD progression, only palliative treatments based on increasing ACh levels in the CNS by means of acetylcholinesterase (AChE) inhibitors are presently available along with memantine; a NMDA (*N*-methyl-D-aspartate) receptor antagonist [7]. Although AChE inhibitors have clearly demonstrated improved cognitive function in Alzheimer's patients, many side effects (*eg.* gastrointestinal events, nausea, vomiting, diarrhoea, dizziness) mainly associated with their peripheral AChE inhibitory activity, strongly impact on the patient's quality of life and may lead to treatment discontinuation [8]. Among the various strategies investigated to improve the transport and distribution of drugs into the brain, the redox-based chemical delivery system (CDS) developed by N. Bodor [9] appears as an appealing chemical tool to reach this goal. However, the use of lipophilic carriers derived from 1,4-dihydropyridines is prone to electrophilic attack on the enamine 5,6-double bond [10] and may severely impede the development of this CDS strategy. To circumvent this issue, our group has recently developed new lipophilic carriers in 1,4-dihydroquinoline series, in which the enamine character of the double bond is masked [11]. In this context, we sought to develop a new brain-targeting galantamine CDS based on 1,4-dihydroquinoline/quinolinium salt redox carrier. The delivery system for galantamine is depicted in Fig. 1.

After crossing the lipoidal blood brain barrier (BBB), the galantamine covalently-bound to 1,4-dihydroquinoline undergoes enzymatic oxidation to give rise to the corresponding quinolinium salt which is sequestered in the CNS. Sustained release of galantamine and elimination of the quinolinium salt take place through enzymatic hydrolysis in the CNS. According to this scenario, this

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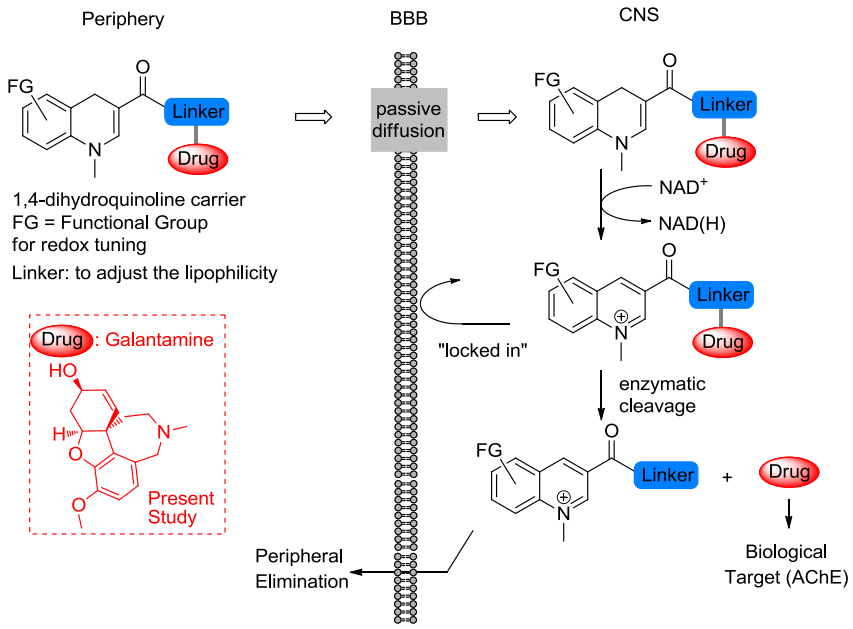


Fig. 1. Brain-targeting galantamine CDS by means of 1,4-dihydroquinoline carriers.

CDS strategy is intended to reduce peripheral levels of galantamine while improving its CNS delivery and should therefore attenuate side-effects associated with current AChE inhibitor treatments. It is worth mentioning that annelation of the 1,4-dihydropyridine ring is not only intended to protect the enamine 5,6-double bond towards electrophilic attack, but can also provide an easy way to tune the redox properties of the carrier by introducing various electron-donating and -withdrawing functional groups (FG) at the phenyl moiety to reach a good compromise between peripheral stability of the carrier and its oxidation in the CNS.

2. Results and discussion

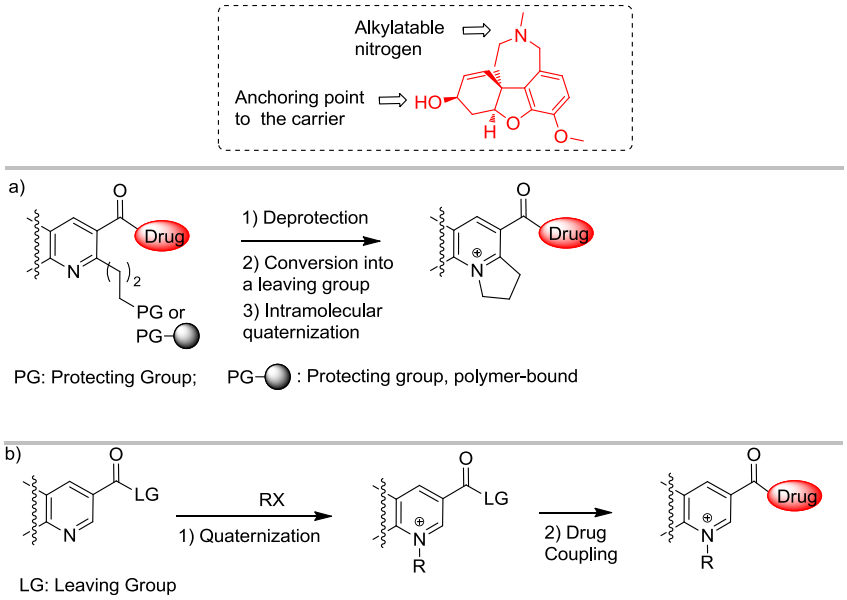
2.1. Chemistry

The design of a brain-targeting galantamine CDS based on 1,4-dihydroquinoline is not as straightforward as it might appear, due to the presence of a tertiary nitrogen atom in galantamine which may compete during the required quaternization step of the quinoline moiety. Two main approaches have been considered to address this issue while providing general solution for targeting drugs sensitive to alkylation reactions by these redox CDSs. The first approach implements an intramolecular quaternization, as key step, ensured by a properly functionalized alkyl chain at C-2 of the quinoline ring. The originality of this strategy consists in the use of a suitable protecting group which, after galantamine coupling with the carrier, would be easily converted into a leaving group able to promote smooth cyclization to form the desired quinolinium salt (Scheme 1a). A solid-phase variant of this intramolecular quaternization strategy is also reported (Scheme 1a). Last but not least, the second approach will consider the formation of the quinolinium salt prior to drug introduction, thus avoiding any problem of quaternization of the drug. To this end, it will be necessary to implement a challenging coupling between galantamine and an activated carboxylic acid at C-3 of the quinolinium salt (Scheme 1b).

First, we focused on the development of the intramolecular quaternization strategy. The required quinoline precursor **3a** was prepared through a Borsche modification of the Friedländer methodology [12] from imine **1** [13] and β-ketoester **2**. A brief screening of the conditions revealed that the reaction could be conducted under neat conditions in the presence of piperidine leading to the quinoline **3a** in 85% isolated yield. On the other hand, compound **2** was synthesized by treatment of γ-butyrolactone with lithio ethyl acetate, following a literature procedure previously reported with δ-valerolactone [14]. Hydroxy β-ketoester **2** was obtained in equilibrium with its cyclic hemiketal form (ratio 1:2) in 94% overall yield. With quinoline **3a** in hand, we then undertook the protection of the alcohol chain at C-2 with 3,4-dihydro-2*H*-pyran under acidic conditions leading to THP ether derivative **3b** in 90% yield. Among the plethora of alcohol protecting groups available in the literature, THP protecting group was selected not only because of its high stability under basic conditions that will be further required for ester hydrolysis at C-3, but also because of numerous literature reports that describe its straightforward one-step conversion into various leaving groups [15–17]. As laid down, hydrolysis of quinoline ester **3b** could be performed under alkaline conditions, without altering THP ether at C-2, affording quinoline carboxylic acid **3c** in 70% yield (Scheme 2).

At this stage and before undertaking the tricky coupling between the resulting quinoline carboxylic acid **3c** and galantamine, we sought to validate our intermolecular quaternization approach from quinoline **3b**. Thus, according to a procedure reported by Sonnet [16], quinoline **3b** was reacted with PPh_3/Br_2 in CH_2Cl_2 to give the expected bromide derivative. We actually determined by ^1H NMR analysis that, in the present case, the quinoline was *N*-protonated and upon addition of DIEA, quaternized quinoline **4a** could be obtained within 5 min with 82% overall yield from quinoline **3b**. We also obtained quinolinium salt **4a** in the same yield following the method described by Miokowski [17] which suggests an alternative procedure using a mixture of PPh_3 and CBr_4.

Having validated the crucial cyclization step from quinoline **3b**, we then investigated the coupling reaction between quinoline **3c** and galantamine by screening various coupling agents such as DCC/DMAP, FEP/DIEA and PyBOP/DIEA. However, ^1H NMR analysis of the crude reactions could not be conclusive regarding the formation of the desired compound **3d**. Tedious purifications resulting from

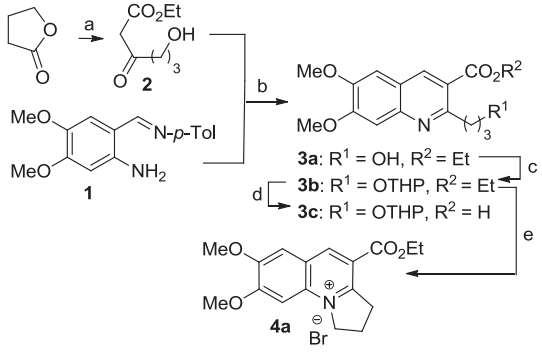


Scheme 1. Strategies developed to prepare redox CDS systems with drugs sensitive to alkylation reactions. a) intramolecular quaternization. b) quaternization prior to drug introduction.

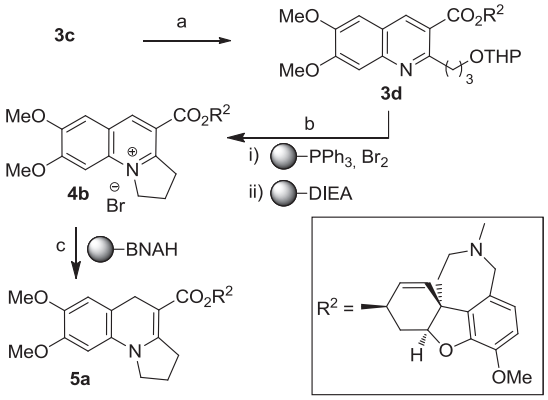
these reactions failed to isolate derivative **3d**. We then successfully turned our attention to HATU/DMAP-DIEA as coupling agent. Thereby, quinoline derivative **3d** having galantamine moiety at C-3 was successfully obtained in 85% yield (Scheme 3). Next, we applied the intramolecular cyclization procedure (developed above with quinoline **3b**) to quinoline **3d** having the AChE inhibitor galantamine. Unfortunately, although complete conversion could be reached, various attempts to isolate quinolinium salt **4b** with a sufficient purity, that may meet our requirements to continue the synthesis, failed. We therefore turned to the use of resin-supported reagents in order to overcome purification issues. Thus, bromination of quinoline **3d** was achieved by using a resin-supported triphenylphosphine. After simple filtration of the resin-bound triphenylphosphine oxide, the crude product was treated with a resin-supported DIEA to give rise to quinolinium salt **4b** (70%) without the need for further purification. The resulting quinolinium salt **4b** was easily reduced in presence of a resin-supported *N*-benzyl-1,4-dihydronicotinamide (PS-BNAH) [18] affording the desired redox CDS–galantamine **5a** (100%) with a satisfactory purity.

With the aim of improving access to this redox CDS by avoiding tricky workup procedures and time-consuming purification steps, we also sought to develop a solid-supported synthesis from the commercially available Ellman's dihydropyran resin (Scheme 4). To this end, quinoline **3a** was treated with Ellman's resin (0.94 mmol/g) in the presence of PPTS according to a known procedure [19] to afford resin-bound quinoline-3-carboxylic acid ethyl ester **6a** which was characterized by means of ^{13}C-gel phase NMR and FT-IR analyses (see Supplementary content). The yield was determined by nitrogen elemental analysis (1.09% N; loading 0.78 mmol/g). After ester hydrolysis, the resulting resin-bound quinoline 3-carboxylic acid **6b** (1.05% N; loading 0.75 mmol/g) was subsequently coupled with galantamine in the presence of DCC–DMAP to furnish the desired resin-bound quinoline-galantamine **6c** (1.18% N; loading 0.44 mmol/g). In contrast with homogeneous conditions reported above, the large excess of DCC–DMAP used in solid-phase synthesis enables the reaction to be driven to completion in 48 h.

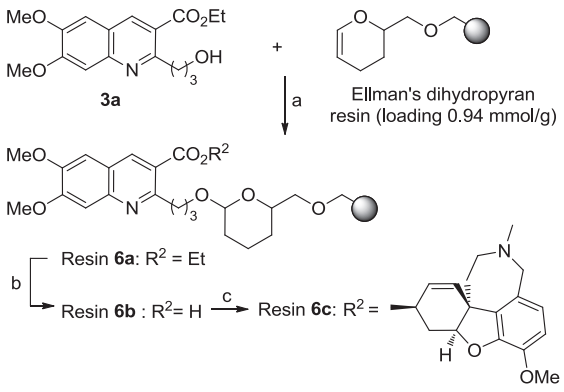
Treatment of resin **6c** with PPTS (Scheme 5) resulted in the smooth release of the quinoline-galantamine alcohol **3e** (80%) which was then reacted with methanesulfonylchloride in the



Scheme 2. *Reagents and conditions*: a) Ethyl acetate, LDA, THF, −78 °C, 1 h then γ-butyrolactone, −78 °C, 3 h (94%); b) piperidine, 80 °C, 6 h (85%); c) 3,4-dihydro-2*H*-pyrane, PTSA, CH_2Cl_2, reflux, 8 h (90%); d) 2 M LiOH, THF/water, 70 °C, 16 h (70%); e) PPh_3, Br_2, CH_2Cl_2, 25 °C, 5 h then DIEA, 25 °C, 5 min (82%) or PPh_3, CBr_4, CH_2Cl_2, 25 °C, 16 h then DIEA, 25 °C, 5 min (82%).



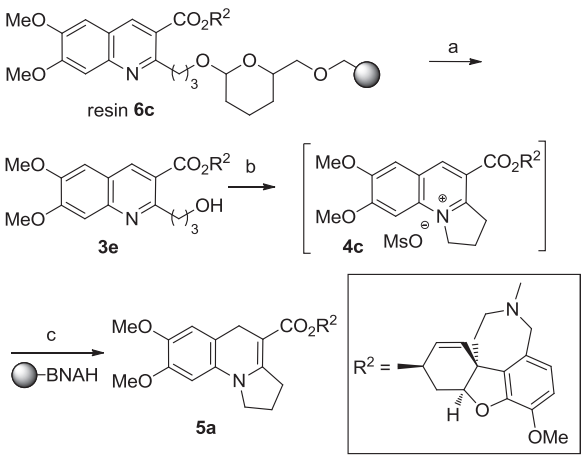
Scheme 3. *Reagents and conditions*: a) Galantamine, HATU, DIEA, DMAP, CH_2Cl_2, 25 °C, 16 h (85%); b) (i) PS-PPh_3, Br_2, CH_2Cl_2, 36 h, (ii) PS-DIEA, 48 h (70%); c) PS-BNAH, CH_2Cl_2, 25 °C, 6 h, (100%).



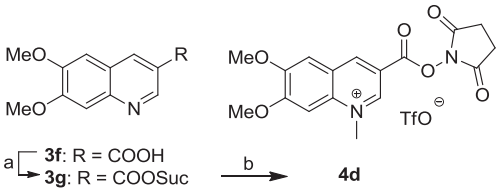
Scheme 4. *Reagents and conditions*: a) PPTS, dichloroethane, 80 °C, 16 h (0.78 mmol/g); b) LiOH, THF/H_2O (1/1), 60 °C, 50 h (0.75 mmol/g); c) Galantamine, DCC, DMAP, CH_2Cl_2, 25 °C, 48 h (0.44 mmol/g).

presence of K_2CO_3 to afford the corresponding mesylate derivative, which in turn cyclized spontaneously. The resulting quinolinium salt **4c** was not isolated but directly converted into the target 1,4-dihydroquinoline-galantamine **5a** following the same reduction procedure to that used for quinolinium-galantamine bromide **4b**; i.e. by means of PS-BNAH.

In parallel to this "intramolecular quaternization" approach, we developed another efficient strategy to prepare redox CDSs having a drug sensitive to the alkylation reaction. Hitherto, the preparation of such CDSs usually required the alkylation of the quinoline bearing the drug prior to the final reduction step to give the desired dihydroquinoline. In the strategy proposed herein, the drug is directly coupled with the quinolinium salt (Scheme 1b). Thus, the required key intermediate **4d** was prepared from carboxylic acid **3f** [11a] by a Steglich-type reaction with *N*-hydrosuccinimide (NHS) affording the desired compound **3g** in 80% yield (Scheme 6). NHS esters are widely used as coupling agents for peptide bond formation in a variety of bioconjugation processes [20]. They offer the advantage to be reactive towards various nucleophiles while being stable enough to be purified and stored without difficulty. The so-obtained quinoline *N*-succinimidate derivative **3g** was then readily reacted with methyl triflate to provide quantitatively the redox carrier precursor **4d** which could be stored for several weeks without any degradation.

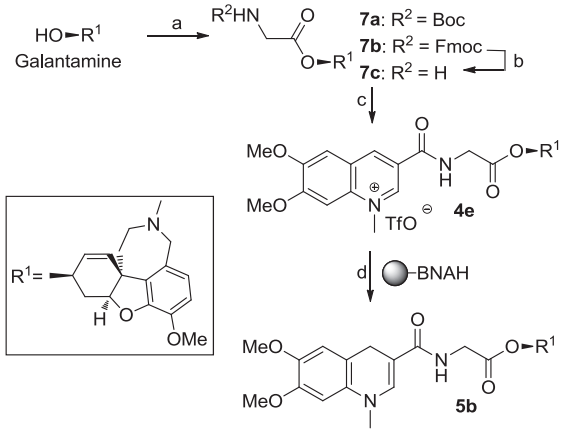


Scheme 5. *Reagents and conditions*: a) PPTS, 1,2-dichloroethane/*n*-butanol, 60 °C, 16 h; b) MsCl, acetone, K_2CO_3, −30 °C → 20 °C, 1h30; c) PS-BNAH, CH_2Cl_2, 25 °C, 6 h.



Scheme 6. *Reagents and conditions*: a) NHS, DCC, THF, 25 °C, 16 h (80%); b) MeOTf, CH_2Cl_2, 25 °C, 1 h (100%).

We then examined the possibility to introduce a glycine linker to connect galantamine to the quinolinium salt **4d** (Scheme 7). To this end, we first attempted a coupling reaction with galantamine and *N*-Boc-glycine in the presence of EDC/DMAP in DMF. However, after 12 h or 48 h of reaction, no formation of *N*-Boc-Gly-galantamine **7a** could be detected. We then moved to DCC as coupling agent under various conditions (DMAP or DMAP/NEt_3, CH_2Cl_2 or CH_2Cl_2/THF, 12h-4 days). The examination of the ^1H NMR spectra revealed the formation of the expected *N*-Boc-Gly-galantamine **7a**, but with a conversion rate that did not exceed 50%. Finally, HATU/DIEA-DMAP gave an excellent conversion yield and the resulting crude reaction mixture was easier to purify by flash-chromatography leading to *N*-Boc-Gly-galantamine **7a** in 76% yield. The latter was subjected to *N*-Boc deprotection under classical acidic conditions (TFA, CH_2Cl_2, 25 °C, 2 h). As confirmed by LC–MS analysis of the reaction mixture, the deprotection took place in solution, but all attempts to isolate Gly-galantamine **7c** led to the recovery of the starting galantamine. In view of this failure, we decided to investigate the coupling reaction from *N*-Fmoc-glycine. The best results were obtained in the presence of HBTU/DMAP giving rise to *N*-Fmoc-Gly-galantamine **7b** in 74% yield. Pleasingly, Gly-galantamine **7c** could be isolated in 88% yield using a standard *N*-Fmoc deprotection procedure. The key step of the strategy lay in the reaction of the modified galantamine **7c** with succinimidate quinolinium salt **4d**. To our delight, the reaction occurred efficiently to give compound **4e** in 89% yield. While initial attempts to reduce quinolinium salt **4e** in the presence of free BNAH did not yield satisfactory results (mainly due to separation issues), polymer-bound BNAH provided the required dihydroquinoline **5b** in 95% yield with a sufficient purity to perform preliminary *in vitro* binding assays.



Scheme 7. *Reagents and conditions*: a) *N*-Boc-Gly, HATU, DIEA, CH_2Cl_2, 25 °C (76%) for **7a** or *N*-Fmoc-Gly, HBTU, DMAP, CH_2Cl_2, 25 °C, 16 h (74%) for **7b**; b) piperidine, CH_2Cl_2, 25 °C, 2 h (88%); c) (i) **4d**, CH_2Cl_2, 25 °C, 30 min, (ii) Si-carbonate, 1 h (89%); d) PS-BNAH, CH_2Cl_2, 25 °C, 3 h, 95%.

2.2. In vitro assay

At this stage, since this CDS approach was predicted to prevent *in vivo* peripheral AChE inhibitory activity, it is worthwhile comparing the *in vitro* inhibitory potency (IC_{50}) of this brain-targeting galantamine CDS against AChE with that of the parent galantamine. Dihydroquinoline-galantamine derivatives **5a,b** were assessed *in vitro* against human AChE using Ellman's test [21]. While compound **5a** was not soluble and could not be assessed, compound **5b** exhibited an IC_{50} value (*hu* AChE) > 10 μM much lower than the parent galantamine (IC_{50} = 240 nM). This preliminary result confirms the inactivity of our brain-targeting galantamine CDS, this being a necessary condition to prevent peripheral side effects encountered with current AChE inhibitor treatments. The second crucial task of this CDS consists in crossing the BBB to deliver galantamine in the brain. *In vivo* studies are ongoing in our laboratory to validate this critical task by investigating the central and peripheral cholinergic activity profile in mice after administering our brain-targeting galantamine CDS. Results of this *in vivo* biological evaluation will be reported in due course.

3. Conclusion

Up till now, the synthesis of redox CDSs reported in the literature faced with a limitation; namely a quaternization step of the carrier poorly compatible with a large variety of drugs containing alkylatable groups. To overcome this limitation, we have developed two new strategies to prepare 1,4-dihydroquinoline-based redox CDS compatible with drugs sensitive to alkylation reactions. Both strategies were developed with galantamine with the aim at providing an efficient brain-targeting galantamine CDS to prevent adverse effects associated with current AChE inhibitor treatments for AD patients. Whereas the first strategy makes use of an intramolecular quaternization of the quinoline ring as key step, the second approach implements a challenging drug coupling with a quinolinium salt. It is worth noting that both strategies could be successfully developed thanks to the use of polymer-supported reagents and a solid-phase synthesis approach facilitating work-up procedures and limiting tricky purification steps. Both approaches are being investigated in our laboratory to exploit the potential of 1,4-dihydroquinoline derivatives as new drug-carriers to target other relevant neuroactive drugs.

4. Experiment procedure

4.1. Chemistry

Chromatographic purification of compounds was achieved with Silica gel 60 (40–63 μm). Thin layer chromatographies were carried out on plates of silica gel 60 F_{254} (1.1 mm) with spot detection under UV light or phosphomolybdic acid or ninhydrin or $KMnO_4$ oxidation. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AVANCE 300 using $CDCl_3$, CD_3CN or DMSO-d_6 as solvents and with the residual solvent signals as internal standards unless otherwise indicated. Data appear in the following order: chemical shifts in ppm, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broaded), coupling constant *J* in Hz and number of protons. ^{13}C NMR spectra were acquired at 75 MHz operating with broad band ^{1}H decoupling. IR spectra were obtained with Perkin Elmer Spectrum 100 FT-IR spectrometer or Perkin Elmer FT-IR 1650 spectrometer. The melting points of powder compounds were measured on a STUART-Advanced apparatus. Microanalyses were carried out at the analytical laboratory of our Department (IRCOF) on a Thermo Fisher FLASH 2000 series analyser and found within ±0.4% of the theoretical values. High resolution mass spectra were performed by the Mass Spectrometry Laboratory of the University of Rouen on a Waters LCT Premiere ESI-TOF spectrometer. All solid phase chemistry was carried out on a Quest 210 Parallel Synthesizer from Argonaut. *In vitro* AChE inhibitory activities were measured by the Centre d'Etudes et de Recherche sur le Médicament de Normandie (CERMN). PPh_3–polymer bound (1.6 mmol/g, 2% DVB crosslinked) and DIEA–polymer-bound (4.6 mmol/g, 2% DVB crosslinked) were acquired from Sigma–Aldrich; Si-Carbonate (0.15 mmol/g) was purchased from Biotage. The following compounds were prepared according to literature procedures: Imine **1** [14], BNAH–polymer-bound [18], and quinoline **3f** [11a].

4.1.1. Ethyl 6-hydroxy-3-oxohexanoate (2)

A solution of lithium diisopropylamide, generated at −78 °C from diisopropylamine (1.62 mL, 11.6 mmol) and *n*-butyllithium (5 mL, 11.6 mmol, 2.3 M in *n*-hexane) was stirred at 0 °C for 20 min and then cooled at −78 °C. Ethyl acetate (1.13 mL, 11.6 mmol) was added dropwise and the reaction mixture was maintained at −78 °C for 1 h. Then γ-butyrolactone (1.0 g, 11.6 mmol) was added and the solution was stirred at −78 °C for an additional 3 h and quenched with ethanol and warmed to 20 °C. The products were partitioned between diethyl ether and water. After neutralization of the aqueous layer using HCl 1 M, the ether phase was collected and dried over $MgSO_4$. The solvent was then removed to afford β-ketoester and its hemiketal form (ratio 1:2) (1.90 g, 94%) as an yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.63 (s, 1H), 4.34–4.08 (m, 3H), 4.07–3.94 (m, 1H), 3.94–3.78 (m, 1H), 3.60 (t, *J* = 6.0, 1H), 3.34 (s, 1H), 2.90–2.56 (m, 3H), 2.24 (s, 0.5H), 2.22–1.60 (m, 5H), 1.33–1.31 (m, 4.5H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.6, 172.4, 104.1, 68.2, 62.0, 61.8, 61.4, 49.7, 43.7, 39.9, 37.7, 26.6, 24.6, 14.4. IR (KBr) ν 3443, 2939, 1740, 1714, 1650, 1637, 1319, 1028 cm^{-1}. Anal. calcd for $C_8H_{14}O_4$: C 55.16, H 8.10, found C 54.98, H 8.23. MS (ESI^+) *m/z* = 175.3 $[M+H]^+$.

4.1.2. Ethyl 2-(3-hydroxypropyl)-6,7-dimethoxyquinoline-3-carboxylate (3a)

Imine 1 [14] (1.88 g, 6.95 mmol) and β-ketoester **2** (1.21 g, 6.95 mmol) were heated at 80 °C in presence of a catalytic amount of piperidine (0.3 mL, 50% mol) under solvent-free conditions for 6 h. Alcohol **3a** (1.56 g, 85%) was obtained as a beige powder (mp 117 °C) after washing with cold ethanol. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.60 (s, 1H), 7.32 (s, 1H), 7.09 (s, 1H), 4.42 (q, *J* = 7.2, 2H), 4.18 (t, *J* = 5.6, 1H), 4.05 (s, 3H), 4.01 (s, 3H), 3.70 (q, *J* = 5.6, 2H), 3.47 (t, *J* = 6.8, 2H), 2.14–2.10 (m, 2H), 1.44 (t, *J* = 7.2, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.7, 159.5, 154.7, 150.1, 148.8, 138.5, 122.0, 121.3, 107.0, 105.6, 62.4, 61.5, 56.5, 56.3, 34.0, 31.6, 14.4. IR (KBr) ν 3450, 3305, 1713, 1622, 1501, 1220, 1162, 1005, 835 cm^{-1}. Anal. calcd for $C_{17}H_{21}NO_5$: C, 63.94; H, 6.63; N, 4.39, found: C, 63.88; H, 6.83; N, 4.34. MS (ESI^+) *m/z* = 320.2 $[M+H]^+$.

4.1.3. Ethyl 6,7-dimethoxy-2-(3-tetrahydropyranyloxypropyl) quinoline-3-carboxylate (3b)

A solution of alcohol **3a** (0.30 g, 0.94 mmol) and 3,4-dihydro-2*H*-pyran (0.44 mL, 4.79 mmol) in CH_2Cl_2 containing PTSA·H_2O (0.22 g, 1.15 mmol) was heated under reflux for 8 h and then washed with brine (3 × 30 mL). The organic layer was dried over $MgSO_4$ and concentrated to afford a brown oil which was then purified on silica gel chromatography using CH_2Cl_2:ether (8:2) as eluent to afford the desired tetrahydropyranylether **3b** (0.348 g, 90%) as an oil which slowly crystallized to give yellow crystals (mp 89 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.56 (s, 1H), 7.36 (s, 1H), 7.07 (s, 1H), 4.62 (t, *J* = 2.6, 1H), 4.41 (q, *J* = 7.2, 2H), 4.04 (s, 3H), 4.00 (s, 3H), 3.91–3.86 (m, 2H), 3.54–3.46 (m, 2H), 3.39–3.32 (m, 2H), 2.15–2.05 (m, 2H), 1.86–1.50 (m, 6H), 1.43 (t, *J* = 7.2, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.9, 159.8, 154.2, 149.9, 146.1, 138.3, 122.0, 121.3, 107.6, 105.6,

98.7, 67.5, 62.2, 61.3, 56.5, 56.2, 34.6, 30.8, 30.1, 25.7, 19.6, 14.5. IR (KBr) ν 2941, 1709, 1621, 1498, 1430, 1213, 1029 cm^{-1}. Anal. calcd for $C_{22}H_{29}NO_6$: C, 65.49; H, 7.24; N, 3.47, found: C, 65.45; H, 7.21; N, 3.41. MS (ESI^+) m/z = 404.1 $[M+H]^+$.

4.1.4. 6,7-Dimethoxy-2-(3-tetrahydropyranyloxypropyl) quinoline-3-carboxylic acid (3c)

To a solution of ester **3b** (0.10 g, 0.25 mmol) in THF (23 mL), were added water (9.6 mL) and 2 M LiOH (0.37 mL, 0.74 mmol). The mixture was stirred at 70 °C for 16 h. THF was removed under vacuum and CH_2Cl_2 was added before adjusting the pH of the aqueous layer to 5 by means of diluted acetic acid. The organic layer was washed with water (3 × 20 mL), dried over $MgSO_4$ and concentrated until the desired product precipitated. The latter was then filtered, washed with ether and dried to yield the acid **3c** (655 mg, 70%) as a white powder (mp > 290 °C). ^1H NMR (DMSO, 300 MHz) δ 8.64 (s, 1H), 7.45 (s, 1H), 7.34 (s, 1H), 4.54 (s_{br}, 1H), 3.94 (s, 3H), 3.89 (s, 3H), 3.70–3.37 (m, 2H), 3.37–3.25 (m, 4H), 2.03–1.93 (m, 2H), 1.68–1.41 (m, 6H). ^{13}C NMR (DMSO, 75 MHz) δ 168.1, 158.5, 154.0, 149.5, 145.4, 137.7, 122.0, 120.9, 107.0, 106.2, 97.8, 66.5, 61.2, 55.9, 55.8, 33.4, 30.4, 29.2, 25.1, 19.2. IR (KBr) ν 2941, 2870, 1707, 1622, 1506, 1234, 1033, 1008 cm^{-1}. Anal. calcd. for $C_{20}H_{25}NO_6$: C, 63.99; H, 6.71; N, 3.73, found: C, 63.77; H, 6.84; N, 3.75. MS (ESI^+) m/z = 376.1 $[M+H]^+$.

4.1.5. 3-(O)-[6,7-dimethoxy-2-(3-tetrahydropyranyloxypropyl) quinolin-3-carbonyl] galantamine ester (3d)

To a solution of quinoline carboxylic acid **3c** (287 mg, 0.76 mmol), galantamine (200 mg, 0.69 mmol), HATU (317 mg, 0.83 mmol) and DMAP (101 mg, 0.83 mmol) in dry CH_2Cl_2 (10 mL) was added fresh distilled DIEA (137 μL, 0.83 mmol) under nitrogen atmosphere. The reaction was stirred for 16 h at 20 °C. The crude mixture was washed with saturated $NaHCO_3$ solution and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated to afford a yellow oil which was purified on silica gel chromatography using a mixture $CHCl_3$:MeOH 98:2 as eluent to afford the desired ester **3d** as white crystals (380 mg, mp 82 °C, 85%). ^1H NMR ($CDCl_3$, 300 MHz) δ 8.63 (s, 1H), 7.35 (s, 1H), 7.10 (s, 1H), 6.70 (d, J = 8.2, 1H), 6.61 (d, J = 8.2, 1H), 6.39 (d, J = 10.2, 1H), 6.06 (dd, J = 10.2, 4.7, 1H), 5.61 (t, J = 4.7, 1H), 4.66 (s, 1H), 4.55 (d, J = 3.1, 1H), 4.15 (d, J = 15.3, 1H), 4.04 (s, 3H), 4.01 (s, 3H), 3.90–3.63 (m, 6H), 3.52–3.24 (m, 5H), 3.09 (d, J = 14.6, 1H), 2.85 (d, J = 16.6, 1H), 2.42 (s, 3H), 2.29–2.12 (m, 2H), 2.12–1.97 (m, 2H), 1.90–1.40 (m, 7H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.3, 160.2, 154.3, 149.7, 147.0, 146.3, 144.1, 139.3, 132.4, 131.3, 129.6, 122.9, 121.7, 121.6, 121.4, 112.3, 107.5, 106.0, 98.7, 86.6, 67.4, 64.0, 62.1, 60.5, 60.4, 56.6, 56.4, 56.2, 53.7, 48.2, 41.8, 34.3, 30.8, 30.0, 27.9, 25.6, 19.6. IR (KBr) ν 3418, 2938, 1711, 1621, 1594, 1498, 1434, 1266, 1232, 1208, 1158, 1026 cm^{-1}. HRMS (ESI^+) calcd. for $[M+H]^+$ $C_{37}H_{45}N_2O_8$ m/z 645.3175, found 645.3159.

4.1.6. 3-(O)-[(3-hydroxypropyl)-6,7-dimethoxyquinolin-3-carbonyl] galantamine (3e)

Resin **6c** (100 mg, 0.44 mmol/g, 0.044 mmol) was first swelled in a 5 mL QUEST reaction vessel containing 1,2-dichloroethane (2 mL) and n-butanol (2 mL). After addition of PPTS (40 mg, 0.16 mmol), the resin was stirred at 60 °C for 16 h before being filtered. Then, it was washed with DCE (4 × 5 mL). The filtrate was then washed with H_2O (3 × 5 mL) and the organic layer was dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified on a silica gel TLC plate using a mixture of CH_2Cl_2 and ethanol (85:15) as eluent to give the desired product **3e** (20 mg) in 80% yield as colourless oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 8.65 (s, 1H), 7.31 (s, 1H), 7.11 (s, 1H), 6.69 (d, 1H, J = 8.3 Hz), 6.62 (d, 1H, J = 8.1 Hz), 6.40 (d, 1H, J = 10.3 Hz), 6.06 (m, 1H), 5.62 (t, 1H, J = 5.0 Hz), 4.66 (s_{br}, 1H), 4.15 (d, 1H), 4.04 (s, 3H), 4.01 (s, 3H), 3.82 (s, 3H), 3.73–3.31 (m, 6H), 3.13–2.85 (m, 2H), 2.42 (s, 3H), 2.22 (m, 2H), 2.07 (m, 3H), 1.68 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.5; 159.6, 154.4, 149.8, 145.7, 146.7, 144.2, 139.4, 132.2, 131.1, 129.5, 122.7, 121.7, 121.3, 112.0, 105.8, 102.5, 86.3, 64.2, 62.32, 58.7, 56.7, 56.3, 53.6, 48.0, 42.0, 34.0, 31.9, 29.7, 27.7. MS (ESI^+) m/z 561.6 $[M+H]^+$.

4.1.7. N-(3-carbonyloxy-6,7-dimethoxyquinoline)-pyrolidine-2,5-dione (3g)

To a solution of quinoline carboxylic acid **3f** (219 mg, 1 mmol) in THF (6 mL) was added under nitrogen, N-hydroxysuccinimide (119 mg, 1.1 mmol). The mixture was cooled at −5 °C and a solution of DCC (213 mg, 1.1 mmol) in tetrahydrofuran (4 mL) was slowly added. The resulting suspension was stirred at 25 °C for 16 h. The suspension was filtered, and the filtrate was evaporated under reduced pressure. The residue was washed with saturated solution of $NaHCO_3$ and extracted with chloroform (4 × 10 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification on silica gel (ethyl acetate:pentane 4:1) afforded **3g** (250 mg, 80%) as a white solid (mp 100 °C). ^1H NMR ($CDCl_3$, 300 MHz) δ 9.23 (d, J = 1.8, 1H), 8.73 (d, J = 1.8, 1H), 7.42 (s, 1H), 7.10 (s, 1H), 4.05 (s, 3H), 3.97 (s, 3H), 2.90 (s, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.3, 161.3, 155.4, 150.8, 148.3, 147.7, 137.8, 122.3, 116.4, 108.1, 106.1, 56.5, 56.3, 25.6. IR (neat) ν 3047, 1721, 1264, 1218, 1202, 1184, 1168, 1145, 1074, 738, 636 cm^{-1}. MS (ESI^+) m/z 331 $[M+H]^+$.

4.1.8. 4-Ethoxycarbonyl-7,8-dimethoxy-pyrrolo[1,2-a] quinolinium bromide (4a)

To a solution of PPh_3/Br_2 in dry CH_2Cl_2, generated at 0 °C by addition of Br_2 (17 μL, 0.34 mmol) to a solution of PPh_3 (80 mg, 0.34 mmol) in CH_2Cl_2, was added dropwise a solution of tetrahydropyranylether **3b** (60 mg, 0.15 mmol) in CH_2Cl_2. The reaction mixture was stirred at 20 °C for 5 h before addition DIEA (0.11 mL, 0.64 mmol). The resulting solution was stirred for an additional 5 min. Heptane or ether was added and the precipitate was then filtered, washed with heptane or ether and dried to yield the desired quinolinium salt **4a** (50 mg, 82%) as a white solid (mp 222 °C). ^1H NMR (DMSO, 300 MHz) δ 9.43 (s, 1H), 8.09 (s, 1H), 7.64 (s, 1H), 5.07 (s, 2H), 4.45 (m, 2H), 4.17 (s, 3H), 3.90 (s, 2H), 1.41 (t, J = 7.1, 3H). ^{13}C NMR (DMSO, 75 MHz) δ 162.9, 159.6, 158.2, 151.1, 144.3, 135.8, 123.7, 119.4, 108.7, 99.4, 62.2, 57.7, 56.6, 56.5, 34.9, 19.9, 14.1. IR (KBr) ν 3470, 3402, 3008, 1720, 1617, 1518, 1499, 1245, 1209 cm^{-1}. Anal. calcd. for $C_{17}H_{20}BrNO_4$: C, 53.42; H, 5.27; N, 3.66, found: C, 53.65; H, 5.21; N, 3.51. MS (ESI^+) m/z 302.1 $[M-Br^-]^+$.

4.1.9. 3-(O)-[7,8-dimethoxy-pyrrolo[1,2a]-quinolinium-3-carbonyl] galantamine ester bromide (4b)

To a gel solution of PS-PPh_3/Br_2 in dry CH_2Cl_2 [generated at 0 °C by addition of Br_2 (19 μL, 0.376 mmol) to a suspension of PS-triphenylphosphine (240 mg, 1.6 mmol/g, 0.376 mmol) in dry CH_2Cl_2 (5 mL)] was added dropwise a solution of tetrahydropyranylether **3d** (97 mg, 0.15 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at 20 °C for 36 h before adding PS-diisopropylamine (200 mg, 4.6 mmol/g, 0.903 mmol). The resulting mixture was stirred for an additional 24 h, then additional CH_2Cl_2 (10 mL) is added and the solids are filtered off, washed with CH_2Cl_2 (2 × 5 mL) and discarded. The solvent is evaporated under pressure up to ¼ volume. Heptane or ether (~10 mL) was added and the white precipitate formed was centrifuged, filtered, washed with ether and dried to yield the desired quinolinium salt **4b** (66 mg, 70%). ^1H NMR (CD_3CN + D_2O, 300 MHz) δ 9.19 (s, 1H), 7.67 (s, 1H), 7.37 (s, 1H), 6.91–6.81 (m, 2H), 6.43–6.37 (m, 1H), 6.17–6.09 (m, 1H), 5.68 (t, J = 4.4, 1H), 4.99–4.89 (m, 2H), 4.82–4.56 (m, 2H), 4.31–3.64 (m, 8H), 4.14 (s, 3H), 4.02 (s, 3H), 2.83 (s, 3H), 2.80–2.72

(m, 1H), 2.53 (dt, J = 15.4, 7.6, 2H), 2.41–2.31 (m, 2H), 2.12–2.03 (m, 1H). IR (KBr) ν 3402, 2932, 2698, 1718, 1623, 1518, 1502, 1459, 1438, 1408, 1383, 1285, 1260, 1204, 1170, 1106, 1010 cm^{-1}. HRMS (ESI^+) calcd. for $[M-Br]^+$ $C_{32}H_{35}N_2O_6^+$ *m/z* 543.2495, found 543.2491.

4.1.10. N-(3-carbonyloxy-(1-methyl-6,7-dimethoxyquinolinium)) pyrolidine-2,5-dione triflate (**4d**)

Quinoline **3g** (63.2 mg, 0.2 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and methyl trifluoromethane sulfonate (30 μL, 0.24 mmol) was added to the solution. The mixture was stirred at 25 °C for 1 h, then dry diethyl ether (10 mL) was added and after 1 h the precipitate formed was filtered, washed with diethyl ether and dried to give compound **4d** (98 mg, 100%) as a light yellow solid (mp 200 °C). ^1H NMR (DMSO, 300 MHz) δ 9.87 (d, J = 1.2, 1H), 9.68 (d, J = 1.2, 1H), 8.16 (s, 1H), 7.77 (s, 1H), 4.65 (s, 3H), 4.23 (s, 3H), 4.04 (s, 3H), 2.98 (s, 4H). ^{13}C NMR (DMSO, 75 MHz) δ 171.3, 162.2, 160.3, 154.4, 147.2, 145.8, 140.7, 127.4, 118.4, 109.9, 99.7, 58.4, 57.5, 46.0, 26.1. IR (neat) ν 3040, 1721, 1638, 1505, 1264, 1218, 1202, 1184, 1168, 1074, 1031, 1017, 738, 636 cm^{-1}. HRMS (ESI^+) calcd for $[M-TfO^-]^+$ $C_{17}H_{17}N_2O_6^+$ *m/z* 345.1087, found 345.1083.

4.1.11. 3-(O)-[(N-methyl-6,7-dimethoxy-quinolinium-3-carboxamid) glycinyl] galantamine ester triflate (**4e**)

A solution of 3-(*O*)-glycinyl galantamine **7c** (40 mg, 0.12 mmol) and quinolinium salt **4d** (57 mg, 0.12 mmol) into dry acetonitrile (2 mL) was stirred for 30 min under nitrogen atmosphere. Thereafter, Si-Carbonate (1 g) and acetonitrile (10 mL) were added and the suspension was stirred for an additional 1 h to scavenge NHS released during the coupling reaction. The solution was filtered and the solid was washed with acetonitrile. The solvent was removed under reduced pressure. The so-obtained orange oil was taken up in dry diethyl ether and subjected to ultrasound affording a deep orange precipitate. The solid was filtered an dried to give **4e** (76 mg, mp 141 °C, 89%). ^1H NMR (CD_3CN, 300 MHz) δ 9.24 (s, 1H), 9.17 (s, 1H), 8.23 (t, J = 5.6, 1H), 7.66 (s, 1H), 7.48 (s, 1H), 6.62 (d, J = 8.1, 1H), 6.54 (d, J = 8.1, 1H), 6.33 (d, J = 10.3, 1H), 5.85 (dd, J = 10.3, 4.2, 1H), 5.35 (t, J = 5.2, 1H), 4.51 (s, 3H), 4.18 (s, 3H), 4.14–4.04 (m, 3H), 4.03 (s, 3H), 3.67 (s, 3H), 3.60 (d, J = 15.2, 1H), 3.21 (t, J = 12.9, 1H), 2.96 (d, J = 14.5, 1H), 2.60–2.50 (m, 1H), 2.29 (s, 3H), 2.24–2.00 (m, 2H), 1.54 (dd, J = 13.8, 2.0, 1H). ^{13}C NMR (CD_3CN, 75 MHz) δ 170.0, 163.6, 160.0, 153.5, 147.5, 145.7, 144.6, 142.5, 138.7, 133.4, 132.3, 131.0, 126.7, 126.3, 123.0, 122.2, 112.9, 109.2, 99.3, 86.8, 65.7, 60.7, 58.5, 57.5, 56.7, 54.3, 48.8, 46.8, 42.8, 42.2, 34.8, 28.3. IR (KBr) ν 1030, 1167, 1203, 1225, 1272, 1438, 1512, 1672, 1738, 2942 cm^{-1}. HRMS (ESI^+) calcd for $[M+H]^+$ $C_{32}H_{37}N_3O_7$ *m/z* 574.2553, found 574.2561.

4.1.12. 3-(O)-[7,8-dimethoxy-pyrrolo[1,2a]-dihydroquinolin-3-carbonyl] galantamine ester (**5a**)

Procedure A: To a solution of quinolinium salt **4b** (20 mg, 0.032 mmol) in dry CH_2Cl_2 (5 mL) was added at once PS-BNAH (350 mg, 1.0 mmol/g, 0.350 mmol). The reaction was monitored by ^1H NMR until the starting material had disappeared (*ca.* 6 h). The reaction was filtered and the beads washed several times with CH_2Cl_2. The resulting solution was concentrated in *vacuo* to afford the desired dihydroquinoline **5a** (16 mg, 100%) as a yellow oil. **Procedure B**: Mesyl chloride (8.3 μL, 0.108 mmol) was added dropwise to a cooled solution (−30 °C) of compound **3e** (20 mg, 0.036 mmol) in acetone (1 mL) containing K_2CO_3 (10 mg, 0.072 mmol). Once the addition was completed, the reaction mixture was allowed to reach room temperature before being stirred for an additional 1h30. After adding CH_2Cl_2 (1 mL), the solution was filtered to remove K_2CO_3 and concentrated in *vacuo*. The resulting residue was dissolved in CH_2Cl_2 (10 mL) before adding at once polymer-supported BNAH (360 mg, 1.0 mmol/g, 0.36 mmol). The reaction was monitored by ^1H NMR until the starting material had disappeared (*ca.* 6 h). The reaction was filtered and the beads washed several times with CH_2Cl_2. The resulting solution was concentrated in *vacuo* to afford the desired dihydroquinoline **5a** (16 mg, 65%) as a yellow oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 6.63–6.52 (m, 3H), 6.20–6.18 (m, 2H), 5.92 (dd, J = 10.2, 4.8, 1H), 5.39 (t, J = 4.7, 1H), 4.55 (s, 1H), 4.08 (d, J = 15.0, 1H), 3.80–3.73 (m 10H), 3.65 (s, 2H), 3.53 (t, J = 7.2, 1H), 3.31–2.94 (m, 4H), 2.63 (d, J = 16.2, 1H), 2.42–2.27 (m, 4H), 2.14–1.96 (m, 4H), 1.56 (d, J = 4.7, 1H). IR (KBr) ν 3404, 2924, 2853, 1718, 1655, 1623, 1582, 1519, 1459, 1439, 1385, 1284, 1260, 1205, 1170, 1089, 1023 cm^{-1}. HRMS (ESI^+) calcd. for $[M+H]^+$ $C_{32}H_{37}N_2O_6$ *m/z* 545.2652, found 545.2667.

4.1.13. 3-(O)-[(N-methyl-6,7-dimethoxy-dihydroquinolin-3-carboxamid) glycinyl] galantamine ester (**5b**)

To a solution of quinolinium salt **4e** (33 mg, 0.045 mmol) in dry and degassed CH_2Cl_2, was added at once polymer-supported BNAH (750 mg, 1.0 mmol/g, 0.75 mmol) at 25 °C. The reaction was monitored by ^1H NMR until the starting material has disappeared (3 h). The reaction mixture was filtered and the beads washed several times with dry and degassed CH_2Cl_2. The resulting solution was concentrated in vacuum to afford the desired dihydroquinoline **5b** (25 mg, 95%) as a brownish oil. ^1H ($CDCl_3$, 300 MHz). δ 7.19 (s, 1H), 6.63 (d, J = 6.5, 1H), 6.57 (m, 2H), 6.32 (m, 2H), 5.91 (dd, J = 10.3, J = 4.6, 1H), 5.83 (t, J = 4.8, 1H), 5.38 (t, J = 4.8, 1H), 4.56 (s_{broad}, 1H), 4.28–4.08 (m, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.76 (s, 2H), 3.66 (d, J = 15.3, 1H), 3.29 (t, J = 12.9, 1H), 3.19 (s, 3H), 3.08–3.02 (m, 1H), 2.76–3.2.69 (m, 1H), 2.37 (s, 3H), 2.23–2.02 (m, 2H), 1.59–1.53 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.6, 167.7, 148.0, 146.6, 144.6, 144.1, 132.8, 131.9, 131.5, 129.4, 122.2, 121.5, 113.3, 113.1, 111.4, 98.2, 97.5, 86.12, 64.8, 64.8, 60.5, 56.4, 56.3, 56.0, 53.8, 48.1, 42.0, 39.0, 34.3, 31.1, 29.8. 27.6, 26.3. IR (neat) ν 3404, 2924, 2853, 1718, 1655, 1623, 1582, 1519, 1459, 1439, 1385, 1284, 1260, 1205, 1170, 1089, 1023 cm^{-1}. HRMS (ESI^+) calcd for $[M+H]^+$ $C_{32}H_{38}N_3O_7$ *m/z* 576.2710, found 576.2690.

4.1.14. Resin-bound quinolin-3-carboxyethyl ester (**6a**)

Ellman's dihydropyran resin (0.12 g, 0.94 mmol/g, 0.11 mmol) was first swelled in a 5 mL QUEST reaction vessel containing 1,2-dichloroethane (4 mL). Alcohol **3a** (0.12 g, 0.37 mmol) and PPTS (0.10 g, 0.41 mmol) were added and the mixture was stirred at 80 °C for 16 h before solvent was removed by filtration. The resin was then washed with DCM (1 × 5 mL), THF (2 × 5 mL), THF/H_2O (4 × 5 mL), THF (4 × 5 mL) and dried under vacuum for 20 h to yield **6a** (150 mg) as a brown resin. IR (KBr) ν 3023, 2921, 1718, 1600, 1493, 1451, 1206, 1156, 1068 cm^{-1}. Anal. calcd. N, 1.06, found N, 1.09 corresponding to 0.78 mmol of quinoline-3-carboxylic acid ester/g of resin.

4.1.15. Resin-bound quinoline-3-carboxylic acid (**6b**)

Resin **6a** (0.16 g, 0.78 mmol/g, 0.12 mmol) was first swelled in a 5 mL QUEST reaction vessel containing a mixture of THF (2.5 mL) and water (2.5 mL). After addition of $LiOH \cdot H_2O$ (0.10 g, 2.43 mmol), the reaction mixture was heated at 60 °C for 50 h and then the resin was filtered and washed with 1 N acetic acid/THF (2 × 5 mL), THF/H_2O (3 × 5 mL), THF (4 × 5 mL) and dried under vacuum for 20 h to yield **6b** (148 mg) as a white resin. IR (KBr) ν 3024, 2921, 1718, 1600, 1493, 1451, 1207, 1156, 1068, 1029, 756, 897, 538 cm^{-1}. Anal. calcd. N, 1.08, found N, 1.05 corresponding to 0.75 mmol of quinoline-3-carboxylic acid/g of resin.

4.1.16. Resin-bound 3-(O)-(quinolin-3-carboxy)-galantamine ester (**6c**)

Resin **6b** (100 mg, 0.75 mmol/g, 0.075 mmol) was first swelled in a 5 mL QUEST reaction vessel together with CH_2Cl_2 (4 mL), DCC (34.7 mg, 0.17 mmol) and DMAP (20.1 mg, 0.17 mmol). Galantamine

(29 mg, 0.10 mmol) was added and the resin was stirred at room temperature for 48 h, then filtered, washed with DCM (1 × 5 mL), THF (2 × 5 mL), THF/H_2O (3 × 5 mL), THF (4 × 5 mL) and dried under vacuum for 20 h to yield **6c** (110 mg) as a beige resin. IR (KBr) ν 3024, 2920, 1716, 1600, 1494, 1452, 1360, 1156, 1029 cm^{-1}; Anal. calcd: N, 1.72, found N, 1.18 corresponding to 0.44 mmol of galantamine-quinoline/g of resin.

*4.1.17. 3-(O)-[N-Boc-glycinyl] galantamine **(7a)***

To a solution of *N*-Boc-glycine (47 mg, 0.385 mmol) and galantamine (100 mg, 0.35 mmol) in dry CH_2Cl_2 (5 mL) was added under nitrogen atmosphere HBTU (160 mg, 0.42 mmol) and DMAP (51 mg, 0.42 mmol). The reaction was stirred for 16 h at 20 °C. The crude mixture was washed with saturated $NaHCO_3$ and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated to afford a yellow oil which was purified on silica gel chromatography using a mixture of $CHCl_3$ and MeOH (50:3) as eluent to afford the desired ester **7a** as white foam (135 mg, mp 76 °C, 68%). ^1H NMR ($CDCl_3$, 300 MHz) δ 6.64 (d, J = 8.2 Hz, 1H), 6.56 (d, J = 8.2 Hz, 1H), 6.30 (d, J = 10.3 Hz, 1H), 5.88 (dd, J = 10.3, 4.9 Hz, 1H), 5.36 (t, J = 4.9 Hz, 1H), 5.04 (s, 1H), 4.54 (s, 1H), 4.11 (d, J = 15.1 Hz, 1H), 3.87 (d, J = 5.4 Hz, 2H), 3.83 (s, 3H), 3.66 (d, J = 15.1 Hz, 1H), 3.29 (t, J = 13.1 Hz, 1H), 3.04 (d, J = 14.7 Hz, 1H), 2.69 (d, J = 16.3 Hz, 1H), 2.37 (s, 3H), 2.09 (m, 2H), 1.56 (dd, J = 13.7, 2.1 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3, 155.9, 146.6, 144.1, 131.9, 131.5, 129.3, 122.3, 121.5, 111.5, 86.2, 79.9, 64.6, 60.5, 56.1, 53.8, 48.1, 42.8, 41.8, 34.3, 28.4, 27.7. IR (neat) ν 2928, 1743, 1713, 1507, 1366, 1280, 1231, 1196, 1164, 1049, 1026 cm^{-1}. HRMS (ESI^+) calcd for $[M+H]^+$ $C_{24}H_{33}N_2O_6^+$ *m/z* 445.2337, found 445.2339.

*4.1.18. 3-(O)-[(N-Fmoc)-glycinyl] galantamine **(7b)***

To a solution of *N*-Fmoc-glycine (110 mg, 0.385 mmol) and galantamine (100 mg, 0.35 mmol) in dry CH_2Cl_2 (10 mL) was added under nitrogen atmosphere HBTU (160 mg, 0.42 mmol) and DMAP (51 mg, 0.42 mmol). The reaction was stirred for 16 h at 20 °C. The crude mixture was washed with saturated $NaHCO_3$ and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated to afford a yellow oil which was purified on silica gel chromatography using a mixture of $CHCl_3$ and MeOH (50:3) (R_f = 0.67) as eluent to afford the desired ester **7b** as white foam (146 mg, mp 110 °C, 74%). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, J = 7.4, 2H), 7.58 (dd, J = 7.1, 2.2, 2H), 7.38 (t, J = 7.4, 2H), 7.29 (t, J = 7.4, 2H), 6.62 (d, J = 8.2, 1H), 6.55 (d, J = 8.2, 1H), 6.30 (d, J = 10.3, 1H), 5.89 (dd, J = 10.3, 4.9, 1H), 5.64 (t, J = 5.5, 1H), 5.37 (t, J = 4.9, 1H), 4.54 (s br, 1H), 4.37 (d, J = 7.4, 2H), 4.21 (t, J = 7.0, 1H), 4.08 (d, J = 15.1, 1H), 3.96 (d, J = 5.1, 2H), 3.80 (s, 3H), 3.64 (d, J = 15.1, 1H), 3.25 (t, J = 13.1, 1H), 3.01 (d, J = 14.6, 1H), 2.70 (dt, J = 16.3, 1.2, 1H), 2.35 (s, 3H), 2.16–2.01 (m, 2H), 1.52 (dd, J = 13.7, 2.0, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.9, 156.4, 146.5, 143.86, 143.93, 141.2, 131.8, 131.5, 129.3, 127.7, 127.1, 125.1, 122.1, 121.4, 119.9, 111.4, 86.0, 67.0, 64.7, 60.4, 55.9, 53.7, 48.0, 47.1, 43.0, 41.8, 34.2, 27.5. IR (neat) ν 734, 761, 800, 1025, 1047, 1113, 1168, 1195, 1231, 1266, 1283, 1439, 1729, 2926, 3381 cm^{-1}. HRMS (ESI^+) calcd for $[M+H]^+$ $C_{34}H_{35}N_2O_6$ *m/z* 567.2495, found 567.2509.

*4.1.19. 3-(O)-glycinyl galantamine **(7c)***

3-(*O*)-[(*N*-Fmoc)-glycinyl] galantamine **7b** (125 mg, 0.22 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and dry piperidine (65 μL, 0.66 mmol) was added. The solution was stirred during 1h30 at 25 °C, thereafter additional dry piperidine (100 μL, 121 mmol) was added and the resulting mixture was stirred for a further 0.5 h. The mixture was then concentrated under reduced pressure without heating and purified by column chromatography on silica gel ($CHCl_3$:MeOH gradient from 99:1 to 9:1) to give the desired amine derivative **7c** (67 mg, 88%) as a colourless oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 6.66 (d, J = 8.2, 1H), 6.58 (d, J = 8.1, 1H), 6.33 (d, J = 10.3, 1H), 5.91 (dd, J = 10.2, 4.1, 1H), 5.37 (t, J = 5.0, 1H), 4.57 (s, 1H), 4.13 (d, J = 15.0, 1H), 3.83 (d, J = 3.8, 3H), 3.67 (d, J = 15.2, 1H), 3.40 (d, J = 7.3, 2H), 3.37–3.24 (m, 1H), 3.09 (s, 1H), 2.77–2.64 (m, 1H), 2.40 (d, J = 4.8, 3H), 2.20–2.01 (m, 2H), 1.57 (d, J = 13.6, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.1, 146.5, 144.0, 131.9, 131.3, 129.3, 122.5, 121.5, 111.3, 86.2, 64.0, 60.4, 55.9, 53.7, 48.1, 44.5, 41.8, 34.3, 28.7. ^1H NMR (CD_3CN, 300 MHz) δ 6.69 (d, J = 8.1, 1H), 6.56 (d, J = 8.1, 1H), 6.30 (dt, J = 10.3, 1.1, 1H), 5.82 (ddd, J = 10.3, 5.0, 1.1, 1H), 5.30 (s, 1H), 4.50 (td, J = 3.0, 1.1, 1H), 4.06 (d, J = 15.2, 1H), 3.77 (s, 3H), 3.60 (d, J = 15.2, 1H), 3.14–3.32 (m, 3H), 2.96 (dt, J = 14.4, 3.0, 1H), 2.50 (ddt, J = 16.5, 3.0, 1.4, 1H), 2.29 (s, 3H), 2.21–2.01 (m, 2H), 1.53 (ddd, J = 13.8, 3.8, 1.8, 1H). ^{13}C NMR (CD_3CN, 75 MHz) δ 175.0, 147.6, 144.7, 133.4, 131.9, 131.2, 123.3, 122.1, 112.8, 86.9, 64.6, 60.7, 56.6, 54.26, 48.9, 44.9, 41.9, 34.8, 28.3. IR (KBr) ν 661, 729, 763, 776, 798, 833, 907, 1000, 1025, 1047, 1074, 1114, 1152, 1202, 1166, 1231, 1264, 1283, 1438, 1506, 1591, 1623, 1727, 2797, 2837, 2921, 3380 cm^{-1}. HRMS (ESI^+): $[M+H]^+$ $C_{19}H_{25}N_2O_4$ *m/z* 345.1814, found 345.1812.

4.2. In vitro AChE inhibition assay

The inhibitory activity of both compound **5b** and galantamine against AChE was measured according the spectrometric method of Ellman [21]. Acetylthiocholine iodide and 5,5-dithiobis-(2-nitrobenzoic) acid (DTNB) were purchased from Sigma Aldrich. AChE from human erythrocytes (buffered aqueous solution, ≥500 units/mg protein (BCA), Sigma Aldrich) was diluted in 20 mM HEPES buffer pH 8, 0.1% Triton X-100 such as to have enzyme solution with 0.25 units/mL enzyme activity. In the procedure, 100 μL of 0.3 mM DTNB dissolved in phosphate buffer pH 7.4 were added into the 96 wells plate followed by 50 μL of test compound solution and 50 μL of enzyme solution. After 5 min of preincubation, the reaction was then initiated by the injection of 50 μL of 10 mM acetylthiocholine iodide solution. The hydrolysis of acetylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine, at a wavelength of 412 nm every minute for 10 min using a 96-well microplate plate reader (TECAN Infinite M200, Lyon, France). Test compounds were dissolved in analytical grade DMSO. Donepezil was used as reference standard. First screening of AChE from human erythrocytes activity were carried out rat a 10^{-5} M, 10^{-5} M and at 10^{-6} M concentration of compounds respectively under study. For the compounds with significant inhibition (≥50%) after 4 min of reaction, IC_{50} values were determined graphically from 6 points inhibition curves using the Origin software.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.022.

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