Rational design of central selective acetylcholinesterase inhibitors by means of a "bio-oxidisable prodrug" strategy†

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This work deals with the design of a "bio-oxidisable prodrug" strategy for the development of new central selective acetylcholinesterase inhibitors. This prodrug approach is expected to reduce peripheral anticholinesterase activity responsible for various side effects observed with presently marketed AChE inhibitors. The design of these new AChE inhibitors in quinoline series is roughly based on cyclic analogues of rivastigmine. The key activation step of the prodrug involves an oxidation of an *N*-alkyl-1,4-dihydroquinoline **1** to the corresponding quinolinium salt **2** unmasking the positive charge required for binding to the catalytic anionic site of the enzyme. The synthesis of a set of 1,4-dihydroquinolines **1** and their corresponding quinolinium salts **2** is presented. An *in vitro* biological evaluation revealed that while all reduced forms **1** were unable to exhibit any anticholinesterase activity ($IC_{50} > 10^6$ nM), most of the quinolinium salts **2** displayed high AChE inhibitory activity (IC_{50} ranging from 6 μM to 7 nM). These preliminary *in vitro* assays validate the use of these cyclic analogues of rivastigmine in quinoline series as appealing chemical tools for further *in vivo* development of this "bio-oxidisable prodrug" approach.

Introduction

Alzheimer's disease (AD), the most common form of dementia affecting elderly people, is a complex irreversible neurological affection clinically characterized by a progressive loss of memory and impairment of cognitive functions. While 1% to 3% of the population aged under 65 years are affected, more than 30% of people over 85 years of age are affected by this neurodegenerative disorder. Given the ageing of the population, AD is becoming one of the most important healthcare problems in developed countries.[1] Although the origin of AD is still under debate, amyloid plaques and neurofibrillary tangles observed in post-mortem brain studies in neurocortex and hippocampus regions are held responsible for the devastating clinical effects of the disease.[2]

Another important and significant aspect of neurodegeneration in the brain of AD patients is the loss of the basal forebrain cholinergic system, thought to play a crucial role in producing cognitive impairments and memory deficiency. Therefore, enhancement of the central cholinergic transmission has been considered as a promising approach for the symptomatic treatment of AD. Among the diverse strategies explored,[3] acetylcholinesterase (AChE) inhibitors have been extensively studied. This approach has provided the sole clinically effective method for a palliative treatment of mild to moderate AD.[4]

Over the past decade, several AChE inhibitors such as tacrine,[5] donepezil,[6] rivastigmine[7] and more recently galanthamine[8] have been launched on the market. Although all AChE inhibitors employed in AD therapy exhibit a preferential action in the central nervous system (CNS), the manifestation of peripheral activity in the course of the treatment causes serious adverse effects on peripheral organs and seriously limits the therapeutic potential of these cholinesterase inhibitors. Therefore, the design of central selective AChE inhibitors free from adverse peripheral effects remains a major challenging therapeutic goal.

The strategy disclosed in this work brings into play a "bio-oxidisable prodrug" which, after having crossed the blood-brain barrier (BBB), is expected to be converted to the parent drug in the CNS, *via* a redox-activation process.[9] Our aim is to report herein the design, synthesis and preliminary *in vitro* biological evaluation of these new potential central selective AChE inhibitors.

Results and discussion

"Bio-oxidisable prodrug" design of cyclic rivastigmine analogues

The design of this "bio-oxidisable prodrug" approach is closely connected with the action mechanism of AChE inhibitors. Whereas donepezil and galanthamine are competitive inhibitors, rivastigmine displays a pseudo-irreversible inhibition involving the carbamylation of the serine hydroxyl group located at the "esterasic site" of the enzyme. Both classes of these inhibitors share in common a tertiary amine which is known to play a central role in the mechanism of AChE inhibition. At physiological pH, the protonation of this amine results in the formation of a positive

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† Electronic supplementary information (ESI) available: Analytical data and purification conditions of **11a–g**, **1a–g**, **2a–g**, ^{1}H and ^{13}C NMR spectra of **3**, **4**, **6–9**, **10b–f**, **11a–f**, **1a–d,f**, **2a–f**, preparation of AChE and detailed procedure for AChE inhibition assay. See DOI: 10.1039/b903041g

charge which binds to the "catalytic anionic site" of the enzyme (Fig. 1). This binding element, although not sufficient, is essential in the design of these inhibitors. It is also conceptually important to note that while the charged bioactive form cannot cross the BBB by passive diffusion, both central and peripheral cholinergic activities commonly observed with these inhibitors stem from the existence of an acid–base equilibrium by permitting the neutral inactive form to cross this physiological barrier.

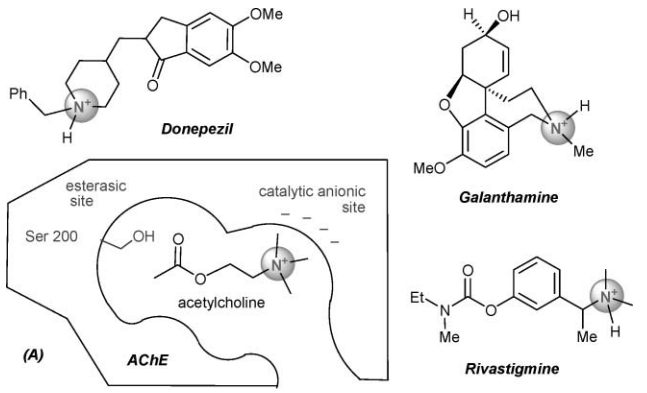


Fig. 1 AChE inhibitors clinically used for the symptomatic treatment of AD. (*A*) Schematic representation of the active site of AChE showing the "anionic" and "esterasic" sites of the enzyme.

On the basis of these mechanistic considerations, a prodrug approach was envisaged to design central selective AChE inhibitors by temporarily masking the positive charge at the periphery. To reach this goal, we focused our attention on cyclic rivastigmine analogues **1** as appealing prodrug candidates (Fig. 2). Although 1,4-dihydroquinoline carbamates **1** are structurally closely related to rivastigmine, these analogues mainly differ in that they possess a non-protonable enamine nitrogen atom at physiological pH and consequently cannot bind to the "anionic site" of the enzyme. After gaining access to the brain through the BBB by passive diffusion, oxidation of the 1,4-dihydroquinoline **1** would generate the corresponding quaternary quinolinium salt **2**,[10] allowing the latter to interact with the "anionic site" by unmasking the positive charge. The resulting permanently charged quinolinium salt **2** can no longer re-cross the BBB, in contrast to most other previously reported AChE inhibitors for which both neutral and charged forms are in equilibrium at physiological pH. This ionic species

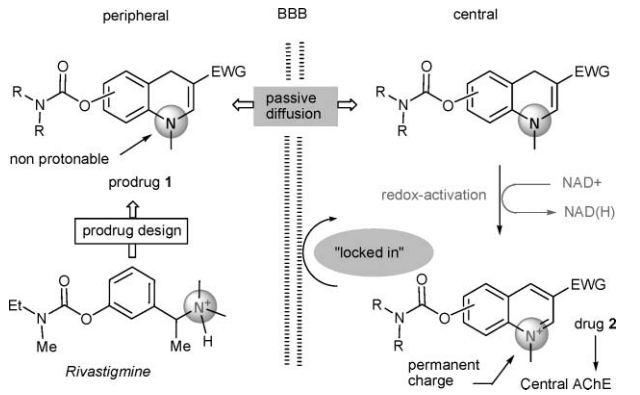
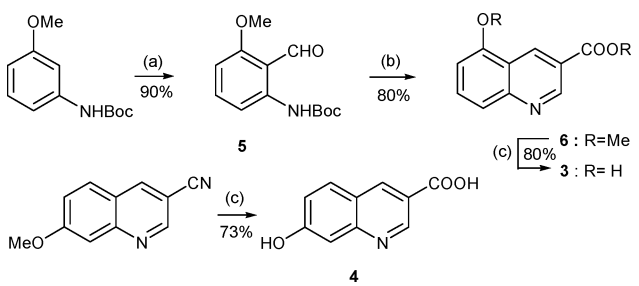


Fig. 2 Design of a "bio-oxidisable prodrug" **1** for central selective AChE inhibitory activity (EWG = electron withdrawing group).

"locked-in" into the brain is expected to display a central selective AChE inhibitory activity, thus exerting ameliorating effects on cholinergic deficits without showing excessive peripheral effects. This ideal scenario may be compromised if peripheral oxidation of the 1,4-dihydroquinoline **1** takes place. Indeed, redox activation of the prodrug **1** closely related to the conversion of the NAD(P)H ⇆ $NAD(P)^+$ coenzyme system may arise easily everywhere in the body. However, due to its high hydrophilicity, the resulting quinolinium salt **2** which might be formed prematurely in the periphery is expected to be easily eliminated from the peripheral circulation by the kidneys and bile. The presence of an electron withdrawing group (EWG) at C-3 is essential to the stabilization of the enamine function in prodrug **1**. This stabilizing element may be helpful to find the delicate balance between stabilization of the prodrug **1** at the periphery and its activation in the CNS. Although 1,4-dihydropyridine-type compounds have been widely developed by Bodor and Prokai for targeting neuroactive compounds to the brain,[11] the use of this redox chemical delivery system to design new prodrug strategies has remained almost unexplored (Fig. 2).[12]

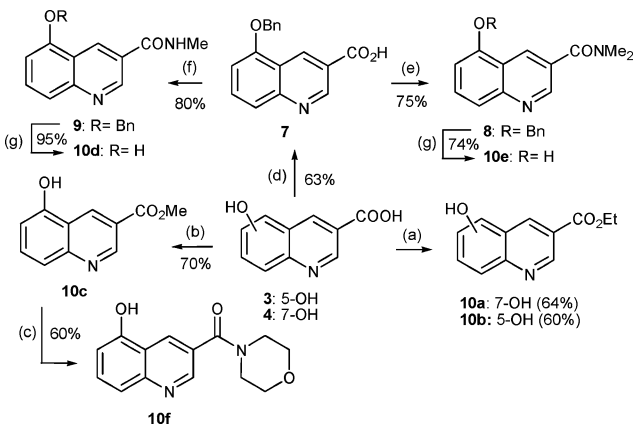
Preparation of 1,4-dihydroquinolines 1a–g and quinolinium salts 2a–g

These cyclic rivastigmine analogues can be described as consisting of a quinoline scaffold incorporating a carbamate moiety and an electron withdrawing group at C-3. To optimize both interactions at "esterasic" and "anionic" sites of the enzyme, we first investigated the relative position between the carbamate and positive charge of the quinolinium salt **2** by installing the carbamate successively at C-5 and C-7 positions of the quinoline ring. The preparation of both 5- and 7-hydroxyquinoline intermediates **3** and **4** is depicted in Scheme 1. The requisite 5-hydroxyquinoline **3** was obtained in a three-step sequence from *N*-Boc-3-methoxy aniline in a 65% overall yield. The reaction sequence started with the *ortho*-lithiation of *N*-Boc-3-methoxy aniline under the reaction conditions previously reported by Woggon.[13] Trapping the resulting lithiated species at –78 °C with DMF afforded the 2-aminobenzaldehyde **5** in 90% isolated yield. The latter compound was reacted with methyl *trans*-3-methoxyacrylate, according to a modified Friedländer reaction, to yield the desired quinoline **6** in 80% yield. Both *O*-demethylation and ester hydrolysis steps were achieved in a one-pot procedure in refluxing aqueous HBr, furnishing quinoline **3** in 80% yield. Using the above conditions, 7-hydroxyquinoline **4** was straightforwardly prepared in 73% yield in a single step from the known 3-cyano-7-methoxyquinoline.[14]



Scheme 1 Preparation of 5- and 7-hydroxyquinolines **3** and **4**. *Reagents and conditions*: (a) *t*-BuLi/Et_2O/–10 °C/3h then DMF/–78 °C to rt over 18h; (b) methyl *trans*-methoxyacrylate/HCl 3M/MeOH/reflux/3h; (c) aqueous HBr (48%)/reflux/24h for **3**, 12h for **4**.

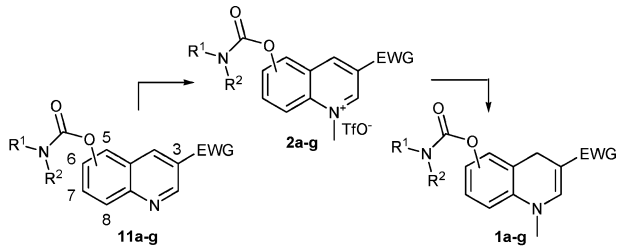
The functionalization of this quinoline scaffold was then investigated (Scheme 2). We first undertook to install various electron withdrawing groups at C-3 to find a good compromise between stability of the prodrugs **1** and AChE inhibitory activity of the related quinolinium salts **2**. To this end, quinoline esters **10a–c** were readily prepared in 60–70% yields by esterification of the corresponding quinoline carboxylic acids **3** and **4**. Quinoline amide **10f** was easily obtained in 60% yield, according to a literature procedure,[15] by reacting ester **10c** with morpholine hydrochloride in the presence of $AlMe_3$. Attempts to use this former one-step procedure during the preparation of quinoline amides **10d,e** failed. To thwart this failure, a protection/deprotection sequence of the phenol function of **3** was therefore investigated. The so-prepared *O*-benzylated quinoline **7** was subsequently converted into quinoline amides **8** and **9** in 75 and 80% yields respectively by conventional activation methods of the carboxylic acid with thionyl chloride or oxalyl chloride. The *O*-debenzylation step, conducted under hydrogen atmosphere, was accompanied by a partial reduction of the quinoline ring which could be easily re-oxidized by means of air bubbling to finally afford quinolines **10d** and **10e** in 95 and 74% yields respectively.

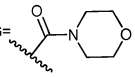


Scheme 2 Functionalization of the quinoline scaffold: screening of various electron withdrawing groups (EWGs) at C-3. *Reagents and conditions:* (a) EtOH/$SOCl_2$/reflux/12h; (b) **4**/MeOH/H_2SO_4/reflux/24h; (c) morpholine hydrochloride/$AlMe_3$/toluene/rt/3h; (d) **3**/BnBr/K_2CO_3/DMF/65 °C/36h; (e) $SOCl_2$/reflux/1h then CH_2Cl_2/Me_2NH/reflux/12h; (f) $(COCl)_2$/few drops of DMF/CH_2Cl_2/1h/rt then CH_2Cl_2/methylamine (2M in THF)/reflux 12h; (g) EtOH/5% Pd/C/1atm of H_2/rt/3h then bubbling of air through the solution.

The last steps include the carbamylation of quinolines **10a–f**, their quaternization and the regioselective reduction of the corresponding quinoliniun salts **2** into 1,4-dihydroquinolines **1** (Table 1). The carbamylation of quinolines **10a–f** occurred with fair to good yields, with only the exception of quinoline **10f** which afforded **11f** in only 18% isolated yield (Table 1, entry 6). In all cases, quinolinium salts **2a–g** were obtained in almost quantitative yields under mild conditions using methyl trifluorosulfonate as methylating agent. The desired 1,4-dihydroquinolines **1a–g** were obtained in moderate yields (46–62%) by regioselective reduction of **2a–g** in the presence of sodium dithionite under basic conditions.[16] Alternatively, quinolinium salt **2c** could be regioselectively reduced by means of *N*-benzyl-1,4-dihydronicotinamide (BNAH) in somewhat better yield (Table 1, entry 3).[17] Although

Table 1 Functionalization of the quinoline scaffold: carbamylation,[a] quaternization[b] and reduction[c] steps



11a: EWG=CO_2Et; R^1=R^2=Me (C-7)
11b: EWG=CO_2Et; R^1=R^2=Me (C-5)
11c: EWG=CO_2Me; R^1=R^2=Me (C-5)
11d: EWG= CONHMe; R^1=R^2=Me (C-5)
11e: EWG= $CONMe_2$; R^1=R^2=Me (C-5)
11f: EWG=  R^1=R^2=Me (C-5)
11g: EWG=CO_2Me; R^1= Et and R^2=H (C-5)

Entry	Carbamylation[a]	Quaternization[b]	Reduction[c]
1	**11a**: 90%	**2a**: >95%	**1a**: 47%
2	**11b**: 78%	**2b**: >95%	**1b**: 46%
3	**11c**: 85%	**2c**: >95%	**1c**: 47% (67%)[d]
4	**11d**: 65%	**2d**: >95%	**1d**: 53%
5	**11e**: 55%	**2e**: >95%	**1e**: 60%
6	**11f**: 18%	**2f**: >95%	**1f**: 46%
7	**11g**: 94%	**2g**: >95%	**1g**: 62%

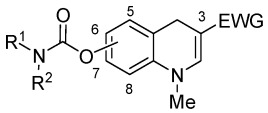
[a] Ethyl isocyanate or dimethylcarbamoyl chloride/THF/NaH/12h/reflux. [b] TfOMe/CH_2Cl_2/rt/2h. [c] $Na_2S_2O_4$/Na_2CO_3/CH_2Cl_2/H_2O/rt/2h. [d] *N*-Benzyl-1,4-dihydronicotinamide/CH_2Cl_2/rt/1h.

1,4-dihydroquinolines **1** have to be reactive enough to undergo an *in vivo* oxidation, these prodrug candidates should also display a satisfactory chemical stability for further *in vivo* development. From this point of view, we were pleased to notice that all 1,4-dihydroquinolines **1a–g** could be handled without any special caution and stored for several weeks without significant degradation.

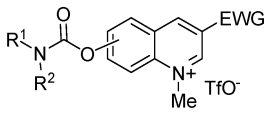
In vitro biological evaluation (AChE inhibitory activity)

AChE inhibitory activity of these newly designed compounds was determined by Ellman's colorimetric assay[18] on human AChE using donepezil as a reference compound. Whereas quinolinium salt **2a** bearing the carbamate moiety at C-7 revealed a moderate AChE inhibitory activity (IC_{50} = 0.5 μM, Table 2 entry 2), quinolinium salt **2b** proved to be much more potent (IC_{50} = 7 nM, Table 2 entry 3), designating C-5 as an appropriate attachment position to reach optimal inhibition. For comparison, donepezil, the most potent marketed AChE inhibitor, showed an IC_{50} value 7 times higher than that obtained for compound **2b** (Table 2 entry 1). Surprisingly, quinolinium salt **2c** is about 15-fold less potent than **2b** (Table 2 entries 3, 4), indicating that inhibitory activity is noticeably affected by slight modification of the ester group at C-3. We then focused our attention on the influence of the stabilizing group at C-3 on the AChE inhibitory activity, by replacing ethyl ester by different amides. With the exception of quinolinium salt **2f**, which exhibited a rather modest inhibition (IC_{50} = 0.86 μM, Table 2 entry 7), both quinolinium salts **2d,e** showed high AChE inhibitory activity (IC_{50} = 29 nM and 15 nM, Entries 5, 6). Lastly, the nature of the carbamate was briefly investigated by replacing *N,N*-dimethylcarbamate by *N*-ethylcarbamate. It turned out that

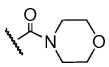
Table 2 *In vitro* inhibition of AChE by 1,4-dihydroquinoline **1a–g** and quinolinium salts **2a–g**



1a-g (IC_{50} > 10^6 nM)



2a-g (IC_{50} = 6μM to 7nM)

Entry	Carbamate position	R^1	R^2	EWG	IC_{50} (nM)
1					Donepezil: 50
2	7	Me	Me	COOEt	**1a**: $>10^6$ **2a**: 500
3	5	Me	Me	COOEt	**1b**: $>10^6$ **2b**: 7
4	5	Me	Me	COOMe	**1c**: $>10^6$ **2c**: 110
5	5	Me	Me	CONHMe	**1d**: $>10^6$ **2d**: 29
6	5	Me	Me	$CONMe_2$	**1e**: $>10^6$ **2e**: 15
7	5	Me	Me		**1f**: $>10^6$ **2f**: 860
8	5	Et	H	COOMe	**1g**: $>10^6$ **2g**: 6000

the resulting quinoliniun salt **2g** is 60-fold less potent than **2c** (Table 2 entry 8).

More importantly, whereas a large number of quinolium salts **2** displayed modest to excellent AChE inhibitory activities (IC_{50} = 6 μM–7 nM), all corresponding reduced forms **1a–g** proved to be completely inactive up to the limit of solubility (IC_{50} $>10^6$ nM, Table 2 entries 2–8). These preliminary *in vitro* assays strongly support our working hypothesis that while the positive charge in quinolinium salts **2** gives rise to a critical binding element at the "anionic site" of the enzyme, this positive charge is masked in the reduced forms **1** thanks to the low basicity of the enamine nitrogen atom which remains non-protonated at physiological pH. With such acetylcholinesterase inhibitors **2** and their corresponding inactive reduced forms **1** in hand, we have at our disposal promising chemical tools for further *in vivo* biological evaluation of this "bio-oxidisable prodrug" strategy.

Conclusion

A series of new carbamylated quinolinium salts **2**, structurally related to cyclic analogues of rivastigmine, exhibited high AChE inhibitory activity with IC_{50} values in the nanomolar range. Interestingly, the corresponding reduced forms **1** proved to be inactive in inhibiting AChE. These results pave the way for the development of a novel "bio-oxidisable prodrug" by means of such redox systems with the aim to reduce undesirable peripheral activity reported with marketed AChE inhibitors. At this stage, the key to success in this "bio-oxidisable prodrug" strategy lies in the preferential redox activation of prodrug **1** in the CNS, *versus* periphery. An *in vivo* study of this critical activation step will be reported in due course by investigating the central and peripheral cholinergic activity profiles in mice after administering the prodrug.

Experimental

General methods

Melting points (°C) were determined with a WME Köfler apparatus. IR spectra were recorded on a PERKIN ELMER IRFT 1650 spectrometer. Liquids were applied as a film between KBr windows and solids were dispersed in KBr tablets. Absorption bands are given in cm^{-1}. NMR spectra were recorded on a BRUKER AVANCE-300 (300 MHz) spectrometer: ^1H at 300 MHz, ^{13}C at 75 MHz and ^{19}F at 282.5 MHz using $CDCl_3$ or DMSO-d_6 as solvents and with the residual solvent signals as internal standards unless otherwise indicated. The following abbreviations are used to describe peak patterns: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Elemental analyses were carried out at the University of Rouen (Microanalytical Service Laboratory) on a CARLO ERBA 1160. Measurement accuracy is around ±0.4% on carbon. Mass spectra (EI, CI, FAB) were recorded on aJEOL JMS AX-500. Analytical thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel $60F_{254}$) from Merck laboratory or on alumina gel plates [DC-Alufolien Aluminiumoxid $150F_{254}$ neutral (Typ T)] from Merck laboratory. Flash chromatography on silica gel was carried out using silica (70–230 mesh ASTM) from Merck laboratory. Flash chromatography on alumina was carried out with aluminium oxide, activated, neutral, 50–200 micron from Acros Organics. Anhydrous dioxane, THF and Et_2O were distilled from sodium-benzophenone ketyl. Toluene, Et_3N, DMF, CH_2Cl_2 and CH_3CN were distilled over CaH_2 before use.

5-Hydroxyquinoline-3-carboxylic acid (3). A solution of **6** (7.3 g, 33.6 mmol) in an aqueous solution of HBr (150 mL, 48%) was heated under reflux for 24 hours. After cooling the reaction mixture to room temperature, the pH was adjusted to 2 with 2M aqueous NaOH. After filtration of the insoluble matter, the pH was adjusted between 5 and 6, and the resulting precipitate was filtered off and dried at 70 °C to afford **3** (5.25 g, 80%) as a brown solid. Mp >260 °C. ^1H NMR (DMSO-d_6) δ 10.91 (s, 1H), 9.25 (s, 1H), 9.04 (s, 1H), 7.70 (dd, J = 7.9 Hz and 8.3 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.02 (d, J = 7.5 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 166.7 (C), 154.8 (C), 150.3 (C), 150.1 (CH), 133.5 (CH), 133.1 (CH), 122.4 (C), 119.3 (CH), 118.5 (C), 109.8 (CH). Found: C, 63.2; H, 3.85; N, 7.4. Calc. for $C_{10}H_7NO_3$: C, 63.49; H, 3.73; N, 7.40%.

7-Hydroxy 3-quinolinecarboxylic acid (4). A solution of 3-cyano-7-methoxyquinoline[15] (20 g, 110 mmol) in an aqueous solution of HBr (300 mL, 48%) was stirred under reflux for 12 hours. The reaction mixture was then cooled to room temperature and neutralized by adding an aqueous solution of KOH (20%). The resulting precipitate was filtered and dried under vacuum to afford **4** (15.2 g, 73%) as a brown powder. Mp >260 °C. ^1H NMR (DMSO-d_6) δ 10.68 (s, 1 H), 9.20 (d, J = 2 Hz, 1 H), 8.84 (d, J = 2 Hz, 1 H), 8.06 (d, J = 9 Hz, 1 H), 7.34 (s, 1 H), 7.29 (d, J = 9 Hz, 1 H). HRMS calc. for (M^+) $C_{10}H_7NO_3$: 189.0426. Found: 189.0423.

***N*-(2-Formyl-3-methoxyphenyl)-2,2-dimethylpropanamide (5)[14].** To a solution of *N*-(3-methoxyphenyl)-2,2-dimethylpropanamide (1 g, 4.48 mmol) in Et_2O (20 mL) was added dropwise *t*-BuLi (7.04 mL, 9.86 mmol, 1.4 M in hexane) at −15 °C. This mixture was stirred for 3h at −10 °C. A solution of DMF in Et_2O (5 mL)

was added dropwise at −78 °C. The resulting mixture was stirred overnight at room temperature. The reaction was quenched by addition of a saturated aqueous solution of NH_4Cl (20 mL). After phase separation, the aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were washed with saturated aqueous NaCl and dried ($MgSO_4$). Flash chromatography (eluent: CH_2Cl_2) provided pure compound **5** (1.0 g, 90%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 10.88 (s, 1H), 10.35 (s, 1H), 8.00 (d, J = 7.9 Hz, 1H), 7.43 (t, J = 7.9 Hz, 1H), 6.44 (d, J = 7.9 Hz, 1H), 3.83 (s, 3H), 1.44 (s, 9H). IR ν_{max} (KBr): 3265, 1728, 1403, 1148 cm^{-1}. HRMS calc. for (M^+) $C_{13}H_{17}NO_4$: 251.1158. Found: 251.1162.

Methyl 5-methoxyquinoline-3-carboxylate (6). To a solution of **5** (7.8 g, 31 mmol) and methyl *trans*-3-methoxyacrylate (7.4 mL, 68.4 mmol) dissolved in 150 mL of methanol was slowly added 3M aqueous hydrochloric acid (100 mL). The resulting mixture was stirred under reflux for 3 hours. The reaction mixture was then cooled to room temperature and neutralized by adding Na_2CO_3. The aqueous solution was extracted with CH_2Cl_2 (4 × 150 mL). The combined organic layers were dried ($MgSO_4$), filtered and evaporated under vacuum. The crude product was then filtered on silica gel (EtOAc:cyclohexane, 50:50) to afford **6** (5.6 g, 80%) as a yellow powder. Mp 102 °C. ^{1}H NMR ($CDCl_3$) δ 9.38 (s, 1H), 9.18 (s, 1H), 7.68 (m, 2H), 6.85 (m, 1H), 3.99 (s, 3H), 3.98 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 166.1 (C), 156.1 (C), 150.6 (C), 150.5 (CH), 133.9 (CH), 132.3 (CH), 122.0 (C), 121.4 (CH), 119.5 (C), 105.1 (CH), 55.9 (CH_3), 52.5 (CH_3). IR ν_{max}/cm^{-1} (KBr) 1728, 1281, 1110, 815. Found: C, 66.2; H, 5.05; N, 6.4. Calc. for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45%.

5-(Benzyloxy)quinoline-3-carboxylic acid (7). To a solution of compound **3** (0.1 g, 0.53 mmol) in dry DMF (5 mL) was added benzyl bromide (132 µL, 1.11 mmol) and finely powdered K_2CO_3 (183 mg, 1.3 mmol). This mixture was stirred at 65 °C under N_2 for 36 hours. The reaction was worked up by pouring the solution into water (10 mL) and EtOAc (10 mL). After phase separation, the aqueous layer was extracted with EtOAc (3 × 10 mL). The organic layers were combined, washed with water and brine, and dried ($MgSO_4$). Filtration and evaporation under vacuum gave 0.14 g of a viscous brown oil which was treated with ethanolic KOH (150 mg, 2.66 mmol in 10 mL of ethanol) and heated under reflux for 3 hours. After evaporation of ethanol, the product was dissolved in 5 mL of water and washed with diethyl ether (2 × 10 mL). The aqueous layer was neutralized with 3 N aqueous HCl. The acid was filtered and dried under vacuum to afford compound **7** (93 mg, 63%) as a brown powder. ^{1}H NMR (DMSO-d_6) δ 9.30 (s, 1H), 9.04 (s, 1H), 7.83 (dd, J = 8.5 and 7.9 Hz, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.56 (d, J = 7.0 Hz, 2H), 7.35–7.48 (m, 3H), 7.27 (d, J = 7.7 Hz, 1H), 5.37 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 166.8 (C), 154.5 (C), 150.6 (CH), 149.7 (C), 136.6 (C), 132.0 (CH), 131.9 (CH), 128.7 (CH), 128.2 (CH), 127.9 (CH), 124.7 (C), 121.0 (CH), 119.0 (C), 107.0 (CH), 70.1 (CH_2). HRMS calc. for (M^+) $C_{17}H_{13}NO_3$: 279.0895. Found: 279.0889.

5-(Benzyloxy)-3-(*N*,*N*-dimethylcarboxamido)quinoline (8). A solution of compound **7** (0.5 g, 1.79 mmol) in thionyl chloride (15 mL) was heated under reflux for 1h. After evaporation of thionyl chloride, the residue was dissolved in dry CH_2Cl_2 (15 mL) and dimethylamine (4.5 mL, 8.96 mmol, 2M in THF) was added at 0 °C under N_2 atmosphere. The reaction mixture was then heated under reflux overnight and evaporated. Flash chromatography on silica gel of the residue (eluent: CH_2Cl_2 then CH_2Cl_2/*i*-PrOH: 9/1) provided pure compound **8** (412 mg, 75%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1H), 8.72 (s, 1H), 7.60–7.75 (m, 2H), 7.30–7.55 (m, 5H), 6.97 (d, J = 7.5 Hz, 1H), 5.23 (s, 2H), 3.16 (s, 3H), 3.04 (s, 3H). Found: C, 74.45; H, 5.9; N, 9.0. Calc. for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14%.

5-(Benzyloxy)-3-(*N*-methylcarboxamido)quinoline (9). To a suspension of compound **7** (1 g, 3.6 mmol) in dry CH_2Cl_2 (50 mL) were added 3 drops of dry DMF. The solution was stirred under N_2 at 0 °C for a few minutes before adding dropwise oxalyl chloride (2.2 mL, 25.2 mmol). The reaction mixture was stirred for 1 hour at room temperature and then evaporated under reduced pressure. The residue was dissolved in dry CH_2Cl_2 (30 mL) and this solution was added dropwise to a solution of methylamine (9 mL, 18 mmol, 2M in THF) in dry CH_2Cl_2 (50 mL) pre-cooled to 0 °C. The reaction mixture was then heated under reflux overnight. After cooling to room temperature, water (50 mL) was added. After phase separation, the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried ($MgSO_4$) and filtered. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel (eluent: EtOAc) to afford compound **9** (849 mg, 80%) as an orange solid. Mp 60 °C (degrad.) ^{1}H NMR ($CDCl_3$) δ 9.22 (d, J = 2.1 Hz, 1H), 8.90 (d, J = 2.1 Hz, 1H), 7.50–7.67 (m, 2H), 7.26–7.45 (m, 5H), 7.16 (br, 1H), 6.85 (d, J = 7.2 Hz, 1H), 5.13 (s, 2H), 2.97 (d, J = 4.7 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 166.7 (C), 154.7 (C), 149.8 (C), 149.0 (CH), 136.1 (C), 131.3 (CH), 130.1 (CH), 128.7 (CH), 128.3 (CH), 127.6 (CH), 126.3 (C), 121.3 (CH), 119.5 (C), 106.3 (CH), 70.6 (CH_2), 26.9 (CH_3). IR ν_{max}/cm^{-1} (KBr) 1640, 1265, 1091, 813. Found: C, 73.8; H, 5.5; N, 9.45. Calc for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58%.

Ethyl 7-hydroxyquinoline-3-carboxylate (10a). To a solution of compound **4** (15 g, 79 mmol) in EtOH (500 ml) was added dropwise $SOCl_2$ (40 mL). The resulting mixture was stirred under reflux for 12 hours. After adding water (200 mL), the pH was adjusted to 7 with 20% aqueous Na_2CO_3. The mixture was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were dried ($MgSO_4$), filtered and evaporated to afford compound **10a** (11 g, 64%) as a pale brown powder. Mp 182 °C. ^{1}H NMR ($CDCl_3$) δ 9.24 (d, J = 2 Hz, 1H), 8.70 (d, J = 2 Hz, 1H), 7.66 (d, J = 9 Hz, 1H), 7.36 (s, 1H), 7.02 (dd, J = 9 and 2 Hz, 1H), 4.48 (q, J = 7 Hz, 2 H), 1.48 (t, J = 7 Hz, 3H). HRMS calc. for (M^+) $C_{12}H_{11}NO_3^+$: 217.0739. Found: 217.0728.

Ethyl 5-hydroxyquinoline-3-carboxylate (10b). To a solution of compound **3** (15 g, 79 mmol) in EtOH (500 ml) was added dropwise $SOCl_2$ (40 mL). The resulting mixture was stirred under reflux for 12 hours. After adding water (200 mL), the pH was adjusted to 7 with 20% aqueous Na_2CO_3. The mixture was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were dried ($MgSO_4$), filtered and evaporated to afford compound **10b** (10.5 g, 60%) as a yellow solid. Mp 240 °C (degrad.) ^{1}H NMR (DMSO-d_6) 11.01 (s, 1H), 9.26 (d, J = 2.3 Hz, 1H), 9.06 (d, J = 1.9 Hz, 1H), 7.73 (dd, J = 8.1 and 8.1 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.04 (d, J = 7.7 Hz, 1H), 4.40 (q, J = 7.0 Hz, 2H), 1.38 (t, J = 7.2 Hz,

3H). IR ν_{max}/cm^{-1} (KBr) 1713, 1377, 1258, 1111. Found: C, 66.05; H, 5.5; N, 6.5. Calc. for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45%.

Methyl 5-hydroxyquinoline-3-carboxylate (10c). To a suspension of **3** (3.5 g, 17.3 mmol) in methanol (250 mL) was added concentrated H_2SO_4 (1.5 mL). The reaction mixture was heated under reflux for 24 hours and then evaporated to half volume before adding 100 mL of water. After neutralization with 2M aqueous NaOH, the mixture was extracted with EtOAc (4 × 200 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), filtered and evaporated under vacuum. Flash chromatography on silica gel (eluent: CH_2Cl_2:EtOAc 50:50) provided **10c** (2.54 g, 70%) as a yellow powder. Mp: 200 °C (degrad.) ^{1}H NMR (DMSO-d_6) δ 11.04 (s, 1H), 9.24 (s, 1H), 9.05 (s, 1H), 7.71 (dd, J = 8.1 and 8.1 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.02 (d, J = 7.7 Hz, 1H), 3.93 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 165.3 (C), 154.6 (C), 150.2 (C), 149.3 (CH), 133.1 (CH), 133.0 (CH), 121.0 (C), 119.1 (CH), 118.1 (C), 109.6 (CH), 52.4 (CH_3). IR ν_{max}/cm^{-1} (KBr) 1721, 1277, 1115. Found: C, 65.12; H, 4.55; N, 6.85. Calc. for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89%.

5-Hydroxy-3-(*N*-methylcarboxamido)quinoline (10d). A solution of compound **9** (391 mg, 1.34 mmol) in ethanol (50 mL) was stirred under H_2 atmosphere for 3 hours at room temperature in the presence of 5% Pd/C (276 mg, 0.13 mmol). The catalyst was then removed by filtration and ethanol was evaporated under reduced pressure. The ^{1}H NMR analysis of the crude product showed partial reduction of the quinoline ring. The mixture was dissolved in ethanol and air was bubbled through the solution until complete re-oxidation of the product. After evaporation of solvent, compound **10d** was obtained (263 mg, 95%) as a brownish powder. Mp 200 °C (degrad.) ^{1}H NMR ($CDCl_3$) δ 10.82 (s, 1H), 9.22 (d, J = 2.3 Hz, 1H), 8.97 (d, J = 2.1 Hz, 1H), 8.83 (br, 1H), 7.64 (dd, J = 8.3 and 7.9 Hz, 1H), 7.50 (d, J = 8.3, 1H), 6.99 (d, J = 7.5 Hz, 1H), 2.84 (d, J = 4.5 Hz, 3H). IR ν_{max}/cm^{-1} (KBr) 1622, 1320, 1279, 812. Found: C, 65.4; H, 4.9; N, 13.7. Calc. for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.98; N, 13.85%.

5-Hydroxy-3-(*N*,*N*-dimethylcarboxamido)quinoline (10e). A solution of compound **8** (275 mg, 0.90 mmol) in ethanol (40 mL) was stirred under H_2 atmosphere for 3 hours at room temperature in the presence of 5% Pd/C (191 mg, 0.09 mmol). The catalyst was then filtered and ethanol was evaporated under reduced pressure. The ^{1}H NMR analysis of the crude product showed partial reduction of the quinoline ring. The mixture was dissolved in ethanol and air was bubbled through the solution until complete re-oxidation of the product. After evaporation of solvent and purification by flash chromatography on silica gel, compound **10e** was obtained (144 mg, 74%) as a brown powder. ^{1}H NMR (DMSO-d_6) δ 10.88 (s, 1H), 8.98 (d, J = 2.0 Hz, 1H), 8.63 (d, J = 2.0 Hz, 1H), 7.72 (dd, J = 8.1 and 7.9 Hz, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.08 (d, J = 7.7 Hz, 1H), 3.10 (br, 6H). ^{13}C NMR (DMSO-d_6) δ 168.2 (C), 154.3 (C), 148.7 (CH), 148.0 (C), 132.0 (CH), 130.5 (CH), 128.2 (C), 118.7 (CH), 118.6 (C), 109.8 (CH), 36.0 (CH_3), 39.6 (CH_3). Found: C, 66.5; H, 5.5; N, 12.9. Calc. for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.96%.

4-(5-Hydroxyquinolin-3-yl-carbonyl)morpholine (10f). To a suspension of compound **10c** (300 mg, 1.48 mmol) in toluene (60 mL) pre-cooled to 0 °C were added $AlMe_3$ (1.6 mL, 3.2 mmol, 2M in heptane) and freshly distilled morpholine (270 μL, 3.2 mmol). The reaction mixture was stirred at room temperature for 3h. The reaction was quenched with a saturated aqueous solution of NH_4Cl, washed with water, and the aqueous solution was extracted with CH_2Cl_2 (2 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was washed with heptane to provide pure compound **10f** (240 mg, 60%) as a brown powder. ^{1}H NMR ($CDCl_3$) δ 8.91 (d, J = 2.1 Hz, 1H), 8.76 (d, J = 1.9 Hz, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.48 (dd, J = 7.9 and 8.3 Hz, 1H), 6.90 (d, J = 7.5 Hz, 1H), 3.40–4.00 (m, 8H). ^{13}C NMR ($CDCl_3$) δ 168.7 (C), 154.0 (C), 148.6 (C), 148.0 (CH), 132.1 (CH), 131.9 (CH), 126.2 (C), 119.3 (C), 119.2 (CH), 110.3 (CH), 66.9 (CH_2). Found: C, 65.2; H, 5.6; N, 10.75. Calc. for $C_{14}H_{14}N_2O_3$: C, 65.11; H, 5.46; N, 10.85%.

General procedure for the carbamylation of 10a–f (procedure A). To a solution of hydroxyquinoline **10** (1 mmol) in dry THF (50 mL) was added portionwise NaH (1.1 mmol) and the mixture was stirred at room temperature for one hour. Dimethylcarbamoyl chloride or ethyl isocyanate (1.1 mmol) was added dropwise and the resulting mixture was heated under reflux overnight. A saturated aqueous solution of NH_4Cl (30 mL) was added and after phase separation the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash chromatography to afford the desired carbamylated quinolines **11a–g**. All spectroscopic data, ^{1}H and ^{13}C NMR spectra are provided in the Electronic Supplementary Information (ESI†).

General procedure for the quaternisation of 11a–g (procedure B). To a stirred solution of carbamylated quinoline **11a–g** (1 mmol) in CH_2Cl_2 (10 mL) was added methyl trifluoromethanesulfonate (1.2 mmol) at room temperature. The resulting mixture was stirred for 2h at room temperature after which CH_2Cl_2 was evaporated *in vacuo* to afford the quinolinium salts **2a–g**. All spectroscopic data, ^{1}H and ^{13}C NMR spectra are provided in the Electronic Supplementary Information (ESI†).

General procedure for the reduction of 2a–g (procedure C). To a mixture of CH_2Cl_2 (6 mL) and water (6 mL) was added quinolinium salt **2** (0.22 mmol) and the resulting solution was vigorously stirred under nitrogen (water and CH_2Cl_2 were degassed by nitrogen bubbling before use). The following procedure was repeated twice: a solution of sodium dithionite (1mL; 190 mg/mL) and a solution of sodium carbonate (1 mL, 70 mg/mL) were added. The resultant biphasic solution was vigorously stirred at room temperature for 1 hour. After adding glacial acetic acid to reach pH 6 and phase separation, the aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated under vacuum to afford 1,4-dihydroquinolines **1a–g**. All spectroscopic data, ^{1}H and ^{13}C NMR spectra are provided in the Electronic Supplementary Information (ESI†).

***In vitro* AChE inhibition assay.** Inhibitory activity against AChE was evaluated according to Ellman's method.[18] Full experimental details are provided in the Electronic Supplementary Information (ESI†).

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