Synthesis of potential dual binding site acetylcholinesterase inhibitors through an efficient solid phase approach based on the Mitsunobu reaction

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Abstract
A solid phase synthesis of a number of monoamino and diamino derivatives as potential dual binding site AChE inhibitors was developed. Our synthetic protocol was characterized by a series of three consecutive Mitsunobu reactions. The loading of the first building block, a dimethylaminophenol, onto the brominated Wang resin was efficiently carried out with DIAD and PPh₃ in THF, whereas the two subsequent Mitsunobu reactions, allowing the introduction of an aliphatic spacer linked to a second phenolic moiety, resulted in higher yields when performed with ADDP and PBu₃ in CH₂Cl₂. The scheme adopted in this synthesis could allow an easy and straightforward entry to libraries of amines of the general formulae A and B.

Keywords: Chemical library, solid phase synthesis, Mitsunobu reaction, acetycholinesterase inhibitors

Introduction
In the last decade combinatorial chemistry has represented the most promising approach to allow entry to a great number of biological targets arising from molecular biology and genomic studies.1 In fact, the possibility of synthesizing libraries of hundreds of compounds in a suitable scalable fashion has determined an epochal change in medicinal chemistry, especially in the fundamental processes of lead discovery and optimization.2 The positive impact that

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combinatorial chemistry has had in reducing the time necessary for the identification and optimization of leads, has favoured the development of suitable techniques and automated equipments to prepare libraries of compounds. In particular, two different approaches are currently followed for the preparation of libraries: the solid phase synthesis and the solid supported reagent-driven reactions.

Following the pioneering work of Merrifield, the solid phase synthesis exploded in the 90’s after the publication by Ellman and co-workers of the synthesis of a library of benzodiazepines that represented the first example of small organic molecules of pharmaceutical interest completely prepared on the solid phase. As witnessed by the huge number of papers published afterwards, combinatorial chemistry became an essential discipline in the field of medicinal chemistry both for academic institutions and pharmaceutical companies.

In this report we describe the design and the solid phase synthesis of a small library of compounds as potential inhibitors of acetylcholinesterase (AChE). AChE is a key enzyme involved in the termination of nervous signals through the hydrolysis of acetylcholine. Besides the more traditional therapeutic applications, such as the treatment of myasthenia gravis and glaucoma, AChE inhibitors have recently found a widespread clinical use in the symptomatic treatment of Alzheimer’s disease (AD), a progressive degenerative disorder characterized by reduced cholinergic transmission, formation of amyloid plaques and tangles and neuronal loss. However, most of the AChE inhibitors on the market suffer from severe side effects and therefore new compounds endowed with improved potency and reduced non-desirable effects are currently pursued.

Recently, our group has published the synthesis and the biological activity of a series of 7-substituted coumarin derivatives of general structure displaying a dual AChE/MAO inhibitory activity (Figure 1). The inhibitory potency towards the MAO, in particular the MAO-B isoform, was in the nanomolar range, whereas a lower potency, in the micromolar range, was found against AChE. The absence of protonated or quaternary nitrogen atoms in compounds and the prevalent hydrophobic character of the substituted coumarin moieties, suggested that the inhibitory activity of I could arise from an interaction at the peripheral binding site located at the entrance of the enzymic gorge ending with the primary (anionic) binding site. Molecular docking studies (data not shown) confirmed the correctness of this hypothesis.

![Figure 1](image-url) General structure of AChE/MAO dual inhibitors. X = one- or two-atom bridge; AH = aromatic or heteroaromatic moiety.
Aiming at the recovery of a second strong interaction at the primary binding site, able to significantly enhance the low AChE inhibitory potency observed in compounds I, we designed and prepared a small library of potential dual binding site inhibitors, represented by the general structures A and B in Figure 2.

Figure 2. General structures of targeted molecules.

Results and Discussion

Wang resin$^{14}$ was selected as the most appropriate solid support to efficiently load the starting building block, that is the 3,5-dihydroxy-dimethylaniline $^1$,$^5$, and to easily cleave the final compounds from the resin in mild conditions. Unfortunately, the direct loading of 1 onto the brominated Wang resin 10 (prepared according to path a) in Scheme 1) using the sterically hindered strong base KHMDS [potassium bis(trimethylsilyl)amide] to ensure the complete deprotonation of one phenolic group, gave, after cleavage, a mixture of different products (path b) Scheme 1).

Scheme 1
As verified in a similar reaction carried out in solution, the synthetic protocol b) depicted in Scheme 1 afforded both O- and C-alkylated (on the aromatic ring) products, the latter compound being formed in higher concentration. Thus, to overcome this problem, we decided to protect one of the two hydroxyl groups of 1 as a benzoate (3). Indeed, compound 3, obtained by reacting 1 with benzoyl chloride in 2N NaOH at 0 °C, was easily loaded in high yield and purity onto the resin 10 using KHMDS as a base and DMF as a solvent (Scheme 2). The resin loading level was evaluated by 1H-NMR and GC-MS, using 2,6-dimethoxytoluene and 3-dimethylaminophenol respectively as internal standards. Further attempts aimed at improving the efficiency of this step, allowed us to determine the best experimental conditions: 3 fold excess of both compound 3 and KHMDS, in DMSO, instead of DMF as solvent. No significant effect on the reaction yield was observed upon temperature changes.

Scheme 2

The second step in our synthetic scheme was the introduction of a suitable aliphatic spacer, as represented in Figure 2. Under the same experimental conditions of the first step, the alkylation reaction afforded two principal products, identified again as the O- and C-alkylated derivatives. Despite the wide range of reaction conditions explored, such as the use of different bases (DBU and NaH), solvents (DMF and DMSO), electrophiles (alkyl chlorides and bromides) and temperatures, the yields of the reactions were always unsatisfactorily low. Finally, we decided to apply the Mitsunobu reaction16 to overcome the previous drawbacks. As auspicated, by using PPh₃ (triphenylphosphine), DIAD (diisopropyl azodicarboxylate), and 1,3-propan- or 1,4-butan-diol in THF, the reaction allowed selective alkylation of the phenolic oxygen (Scheme 3). Starting from this encouraging result we tried to improve the efficiency of the reaction by testing different reagents and experimental conditions. The use of a 6 fold excess of ADDP [1,1’-(azodicarbonyl)dipiperidine] and PBu₃ (tributylphosphine) in CH₂Cl₂ gave the best results in terms of reaction yields and compound purity.

Scheme 3
The last step of our synthetic pathway was the introduction of an aromatic moiety, such as 1 or 3,4-dimethyl-7-hydroxycoumarin. The good results given by the Mitsunobu reaction in the previous step, prompted us to extend the same protocol also to this step (Scheme 4).

![Scheme 4](image)

Scheme 4

After exploring a wide range of reaction conditions, we realized that those found in the second step gave the best results. The subsequent deprotection and cleavage reactions furnished the desired compound in good yield and purity as assessed by ESI-MS and ^1H-NMR analyses. However, we targeted two chemical steps that could be improved to ameliorate the efficiency, yield and compound purity of our protocol, that are the protection of 1 and the coupling of the alkyl spacer. We decided therefore to use a different protecting group for 1, to monoprotect the alkyldiol in order to reduce possible side reactions and the amount of ADDP and PBu₃ needed to drive the reaction to completion. The TIPS (triisopropylsilyl) protecting group was selected because its introduction and subsequent removal are high yielding and chemoselective. To protect one hydroxyl group of the alkane diols we selected the silylated reagent TBDMSCl (t.butyldimethylsilyl chloride). Unfortunately, the subsequent loading of compound 5 onto the resin 10, carried out following the experimental conditions assessed previously, gave no improvement of the yield, since the reaction led to a number of undesired compounds as determined by GC-MS. Finally we successfully applied the Mitsunobu protocol also to load 5 onto the resin. The best results in terms of yield and purity were obtained using a 3 fold excess of 5, PPh₃ and DIAD in THF at room temperature.

![Scheme 5](image)

Scheme 5
The deprotection of the phenolic and alcoholic hydroxyls, made with TBAF (tetrabutylammonium fluoride), afforded the expected compounds in quantitative yields. The definitive overall synthetic pathway, outlined in Scheme 5, was validated with the preparation of compound 14 which was obtained in good yield and purity as assessed by TLC, ESI-MS and $^1$H-NMR analyses. The overall yield determined by $^1$H-NMR analysis, using 2,6-dimethoxytoluene as internal standard, was 50% as a result of 7 consecutive steps with a purity greater than 80%.

The synthetic pathway described above was applied to the preparation of a small library of potential AChE inhibitors (Scheme 6), that was carried out in parallel using the organic synthesizer Quest 210. The quaternization of compounds 20-23 was made in solution after cleavage of the tertiary amines from the resin, using a 20 fold excess of CH$_3$I in CH$_3$CN. The ESI-MS and the $^1$H-NMR data of compounds 14-27 highlight the efficacy of the reported method. The yields and the purity of all compounds were comparable with those determined above for derivative 14.

Although the solid phase synthesis enables a rapid preparation of libraries of compounds, the work needed to assess the best reaction conditions is often tedious and time consuming. Indeed, the optimization of the synthetic protocol for the preparation of the compounds depicted in Figure 2 required a full exploitation of different chemical strategies. Among the wide variety of
the chemical reactions and experimental conditions explored, the Mitsunobu protocol offered the best results in terms of purity and yields, as verified by ESI-MS (Figure 3) and $^1$H-NMR analyses.

\[ \text{Figure 3. Representative examples of ESI-MS: a) 18: 499.3 [M+H]^+; b) 23: 474.2 [M+H]^+, 496.2 [M+Na]^+.} \]

In this regard, it must be underlined that even if the synthesis of the library was carried out through a sequence of the same type of reactions, the experimental conditions adopted in each step, i.e. stoichiometry, reagents and solvents, were different depending on the reactivity of the species involved in the reaction. In fact, in the first step, the loading of compound 5 was performed in THF using DIAD and PPh$_3$ as the coupling reagents. As outlined by the $^1$H-NMR spectra and GC-MS analyses of the products obtained from the resin cleavage, those experimental conditions guaranteed the complete transformation of the starting material and the formation of the desired product in good yield and purity. The optimization of the next two steps was more time consuming, because of the low reactivity of the diol. In fact, differently from the first step, the SN type reactions were not suited for the coupling of 2 with a dibromoalkane, because the reaction gave, again the two O- and C-alkylated products. Moreover, using the cheaper and safer Mitsunobu protocol adopted in the first step, the yield of the reaction was low, because of the reduced reactivity of the diol. In fact, to ensure a complete transformation of the starting material we had to use different reagents such as ADDP and PBu$_3$, that favored the
formation of a more reactive intermediate. Furthermore, the CH₂Cl₂ solvent gave better results compared to THF and diverse THF/CH₂Cl₂ mixtures.

Conclusions

The synthetic method proposed in this report allowed the facile and straightforward preparation of a small library of bioactive compounds in a parallel fashion. The protocol may be applied to the preparation of larger libraries containing a variety of structural modifications in the spacer, in the N-alkyl groups and in the phenolic moieties. Properly designed structural variations should allow to explore and map, in full details, the key binding regions at both anionic and peripheral binding sites of both the AChE and BChE targeted enzymes. More particularly, a fully exploration of the possible interactions at the AChE peripheral binding sites should be pursued and preferred, since, as highlighted in a recently published paper, AChE inhibitors that interact at those binding sites could diminish the β-amyloid aggregation and this surely constitutes an additional pharmacological action, highly desirable for the treatment of Alzheimer’s disease. The compounds described in this work will be therefore tested as AChE and BChE inhibitors, both as free amines and as methylammonium quaternary salts. The results from the ongoing studies of enzyme inhibition, SAFIR and molecular docking will be reported in due course.

Experimental Section

General Procedures. Wang resin-100-200 mesh, loading level 0.83 mmol/g- was purchased from Novabiochem. Unless otherwise stated, reagents and solvents were obtained from commercial sources and used without further purification. Column chromatography was carried out using Merck 60 (0.063-0.200 mm) silica gel. Flash chromatography was performed on Merck 60 (0.015-0.040 mm) silica gel, according to the procedure of Still. Thin-layer chromatography was carried out on Merck 60 F254 250-µm silica gel plates. Reagent grade THF was distilled under N₂ from sodium/benzophenone ketyl, whereas CH₂Cl₂ was distilled on calcium hydride under N₂. Melting points were determined by the capillary method on a Stuart Scientific SMP-3 apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin Elmer FT-IR spectrophotometer. Unless otherwise indicated ¹H-NMR spectra were recorded in Acetone-d₆ at 300 MHz on a Brucker 300 instrument. Chemical shifts are reported in δ (ppm) downfield from an internal solvent peak and J values are in hertz. The ¹H-NMR for the test compound 14 was recorded in DMSO-d₆, using 2,6-dimethoxytoluene as internal standard [δ: 1.95 (s, 3H), 3.72 (s, 6H), 6.57 (d, 2H, J = 8.32), 7.08 (t, 1H, J = 8.35)]. ESI-MS data were acquired on a Agilent 1100 series LC/MSD Trap. Standard electrospray operating conditions were as follows: spray voltage 5.5kV, capillary temperature 275°C, capillary voltage 20V, tube lens offset voltage 40V. The
sample solutions were infused via a Harvard syringe pump at a constant flow rate. Data were acquired in the positive MS/MS product ion mode.

**3-(Dimethylamino)-5-hydroxyphenylbenzoate (3).** 2.7 g (17.6 mmol) of 5-dimethylamino-1,3-benzenediol (1) were solubilized in a 2N solution of NaOH (17.6 mL). To this solution was added, at 0 °C under magnetic stirring, benzoylchlo ride (0.68 mL, 5.9 mmol) diluted in 1 mL of DMF. After 20 min. the mixture was extracted with AcOEt. The organic layer was separated, dried over Na2SO4, concentrated under vacuum and purified by flash chromatography to give 0.5 g (50%) of 3 as a dark orange oil. 1H-NMR δ: 2.92 (s, 6H), 6.11 (t, 1H, J = 2.0), 6.13-6.16 (m, 2H), 7.59 (t, 2H, J= 7.3), 7.71 (tt, 1H, J = 7.3, 1.6), 8.13-8.17 (m, 2H), 8.27 (s, 1H).

**3-(Dimethylamino)-5-(triisopropylsilanyloxy)phenol (5).** To a solution of 1 (8.4 g, 55 mmol) in anhydrous CH2Cl2 (210 mL), were added imidazole (9.4 g, 137.5 mmol), 4-dimethylaminopyridine (DMAP) (3.4 g, 27.5 mmol) and triisopropilsilyl chloride (TIPSCl) (12.9 mL, 60.5 mmol). The mixture was stirred at room temperature for 2 hours, washed with H2O, dried over Na2SO4 then purified by column chromatography, using, as the eluents, CHCl3/hexane 8:2, then CHCl3 up to the elution of the first spot and finally CHCl3/AcOEt 8:2. The eluate corresponding to the desired product was concentrated under vacuum to give 4.9 g (65%), of a pure pink oil that solidifies on standing, mp 67-68°C. 1H-NMR (CDCl3) δ: 1.10 (d, 18H, J = 6.6), 1.16-1.33 (m, 3H), 2.88 (s, 6H), 4.75 (b, 1H), 5.80 (t, 1H, J = 2.1), 5.83 (t, 1H, J =2.1), 5.88 (t, 1H, J = 2.1). IR (cm⁻¹) 3293, 2944, 2867, 1616, 1587, 1150, 1029, 884.

**5-(N-Benzyl-N-methylamino)-benzene-1,3-diol (8).** To a solution of 1,3,5-trihydroxybenzene (7.8 g, 61.7 mmol) in DMF (108 mL) and H2O (46 mL) was added under argon N-benzylmethylamine (10.1 mL, 78.5 mmol), then heated to 50 °C. After 24 hours the mixture was concentrated under vacuum to obtain a dark oil, that was purified by column chromatography using CHCl3/AcOEt/petroleum ether 4:4:2, as eluent, to give 7.2 g (60%) of an oil of sufficient analytical purity. 1H-NMR (DMSO-d6) δ: 2.89 (s, 3H), 4.41 (s, 2H), 5.54-5.58 (m, 3H), 7.14-7.21 (m, 3H), 7.26-7.31 (m, 2H), 8.79 (s, 2H).

**3-(N-Benzyl-N-methylamino)-5-(triisopropylsilanyloxy)phenol (9).** To a solution of 8 (6.9 g, 30 mmol), in anhydrous CH2Cl2 (114 mL), were added imidazole (5.1 g, 75 mmol), DMAP (1.8 g, 15 mmol) and TIPSCl (7.1 mL, 33 mmol). The reaction mixture was stirred for 2 hours at room temperature, then washed with H2O. The organic layer was dried over Na2SO4, concentrated and purified by column chromatography, using, as the eluents, CHCl3/exane 8:2, then CHCl3 up to the elution of the first spot and finally CHCl3/AcOEt 8:2 to furnish 3.8 g (50%) of a red oil. 1H-NMR (CDCl3) δ: 1.04 (d, 18H, J = 6.0), 1.08-1.16 (m, 3H), 2.99 (s, 3H), 4.47 (s, 2H), 4.57 (s, 1H), 5.78 (t, 1H, J = 1.9), 5.84 (t, 2H, J = 1.9), 7.17-7.32 (m, 5H). IR (cm⁻¹) 3375, 2944, 2866, 1615, 1584, 1505, 1152, 1024, 883, 821.

**Brominated Wang resin (10).** The Wang resin (1.0 g, 0.8 mmol)) was swelled in anhydrous CH2Cl2 (15 mL) for 30 min. then filtered. To the resin was added anhydrous CH2Cl2 (5 mL) and CBr4 (0.6 g, 1.9 mmol), followed by cooling to 0 °C. A solution of PPh3 (0.45 g, 1.7 mmol) in CH2Cl2 (3 mL) was slowly added and the mixture was gently stirred for 5 min, before allowing
the temperature to rise up to room temperature. The reaction mixture was gently stirred at room temperature for 4 hours, the resin filtered off, washed with CH$_2$Cl$_2$ (3 x 15 mL), dried under vacuum and employed in the coupling step within 48 hours of preparation.

3-(tert-Butyl-dimethyl-silanyloxy)propan-1-ol (12), 4-(tert-butyl-dimethyl-silanyloxy)-butan-1-ol (13). To a suspension of NaH (0.52 g, 13.1 mmol) in THF (26 mL) was added 1,3-propandiol or 1,4-butandiol (13.1 mmol, 0.9 mL and 1.16 mL respectively), followed after 45 min by t-butyldimethylsilyl chloride (TBDMSCl) (2.0 g, 13.3 mmol). The mixture was stirred at room temperature for 45 min, thus diluted with Et$_2$O (200 mL). The organic layer was extracted with a 10% solution of K$_2$CO$_3$, washed with brine, dried over Na$_2$SO$_4$, then concentrated under vacuum to give a yellow oil, that was purified by flash chromatography using hexane/AcOEt 8:2 as eluent.

*Compound 12.* Yield: 77%. $^1$H-NMR (CDCl$_3$) δ 0.07 (s, 6H), 0.90 (s, 9H), 1.78 (qn, 2H, J = 5.5), 2.59 (b, 1H), 3.78-3.85 (m, 4H). IR (cm$^{-1}$) 3357, 2949, 2930, 2858, 1256, 1098, 837.

*Compound 13.* Yield: 82%. $^1$H-NMR (CDCl$_3$) δ 0.06 (s, 6H), 0.89 (s, 9H), 1.60-1.68 (m, 4H), 2.59 (b, 1H), 3.62-3.68 (m, 4H). IR (cm$^{-1}$) 3357, 2942, 2931, 2859, 1256, 1101, 837.

**Coupling of 3 to the brominated Wang resin (10).** To a round bottom flask, flame dried, were added under argon 3 (0.12 g, 0.47 mmol), anhydrous DMSO (2 mL) and a 0.5 M solution of KHMD5 in toluene (0.95 mL, 0.47 mmol). The mixture was stirred for 5 min then added to the brominated Wang resin 10 (0.2 g, 0.16 mmol), previously swelled in DMSO (4 mL). After 4 hours, the resin was filtered and washed with DMSO (3 x 4 mL), THF (3 x 4 mL) and MeOH (3 x 4 mL) and then dried under vacuum. The resin was recovered, swelled in anhydrous THF (4 mL), filtered, suspended in THF (2 mL) and treated with a saturated solution of MeONa in anh. MeOH (0.12 mL). The reaction mixture was stirred for 4 hours, filtered and washed with THF/H$_2$O 1:1 (3 x 4 mL), THF/2N HCl 1:1 (3 x 4 mL), THF/H$_2$O 1:1 (3 x 4 mL), THF (3 x 4 mL), MeOH (3 x 4 mL), ethyl ether (3 x 4 mL).

**Synthesis of the library reported in Scheme 6 (eps 14-23)**

2.0 g (1.7 mmol) of Wang resin were equally divided in ten reaction tubes of the Quest 210 apparatus and swelled with anhydrous THF (4 mL *per tube*) for 30 min. In two separated, flame dried, round bottom flasks were placed 2.5 mmol of 5 (0.64 g) or 9 (0.96 g). To each round bottom flask were added under argon THF (20 mL) and DIAD (0.49 mL, 2.5 mmol). These two solutions, divided in 5 aliquots, were added to the resin (1 aliquot *per tube*). To each tube was added PPh$_3$ (0.13 g, 0.5 mmol) and the mixture was stirred for 18 hours under nitrogen. The resins were filtered and washed with THF (3 x 4 mL), DMF (3 x 4 mL), THF (3 x 4 mL). To the resins were added THF (1 mL) and a 1 M solution of TBAF in THF (1 mL) and allowed to react for 1 hour. The solvent was filtered and the resin washed with THF (3 x 2 mL), THF/H$_2$O 1:1 (3 x 2 mL), THF (3 x 2 mL), CH$_2$Cl$_2$ (3 x 2 mL), MeOH (3 x 2 mL) and finally dried under vacuum over P$_2$O$_5$. The resins were swelled in CH$_2$Cl$_2$ for 30 min, filtered and treated with the TBDS-protected diols 12 and 13 (0.51 mmol, 97 mg and 104 mg respectively), using CH$_2$Cl$_2$ (2 mL). To
each tube were added ADDP (0.13 g, 0.51 mmol), PBu₃ (0.13 mL, 0.51 mmol) and the mixtures were stirred for 18 hours. After filtering and washing with CH₂Cl₂ (3 x 2 mL), THF (3 x 2 mL), DMF (3 x 2 mL), THF (3 x 2 mL), CH₂Cl₂ (3 x 2 mL), the coupling was repeated twice in the same conditions. At this point, the resin, filtered and washed as above, was suspended in THF (1 mL) and treated with TBAF (1 mL) for 1 hour, thus filtered and washed with THF (3 x 2 mL), THF/H₂O 1:1 (3 x 2 mL), THF (3 x 2 mL), CH₂Cl₂ (3 x 2 mL), MeOH (3 x 2 mL) and finally dried under vacuum over P₂O₅. The resin was swelled in CH₂Cl₂ for 30 min before the final coupling with 5, 9 or 7. Thus, to the selected tubes were added 5 (0.16 g, 0.51 mmol), 9 (0.2 g, 0.51 mmol) and 7 (0.97 g, 0.51 mmol), followed by CH₂Cl₂ (2 mL), ADDP (0.13 g, 0.51 mmol), PBu₃ (0.13 mL, 0.51 mmol). After filtering and washing as described before, the coupling was repeated twice. The resin carrying the TIPS protected compounds was suspended in THF (1 mL) and treated with a 1 M solution of TBAF in THF (1 mL). The solvent was filtered and the resin washed with THF (3 x 2 mL), THF/H₂O 1:1 (3 x 2 mL), THF (3 x 2 mL), CH₂Cl₂ (3 x 2 mL). To the resin swelled in CH₂Cl₂ were added a 50% solution of TFA in CH₂Cl₂ (2 mL). After 1 hour stirring under nitrogen, the resin was filtered and washed with the same mixture of cleavage (3 x 2 mL). Each solution was evaporated to dryness and to the residue was added toluene (2 mL) that was in turn removed under vacuum. The last step was repeated 3 times. The oil obtained were washed with CH₂Cl₂ and ether to obtain the desired compounds in a sufficiently pure form either in a solid or in an oily form.

3-(Dimethylamino)-5-{3-[3-(dimethylamino)-5-hydroxyphenoxy]propoxy}phenol (14). ¹H-NMR δ 2.15 (qn, 2H, J = 6.2), 2.86 (s, 12H), 4.08 (t, 4H, J = 6.2), 5.87 (s, 6H), 8.06 (s, 2H). MS (ESI), m/z calcd for C₁₉H₂₆N₂O₄: 346.4. Found: 347.2 [M+H]⁺.

3-(Dimethylamino)-5-{4-[3-(dimethylamino)-5-hydroxyphenoxy]butoxy}phenol (15). ¹H-NMR δ 1.87-1.91 (m, 4H), 2.86 (s, 12H), 3.96-4.00 (m, 4H), 5.82-5.84 (m, 6H), 7.95 (b, 2H). MS (ESI), m/z calcd for C₂₀H₂₈N₂O₄: 360.4. Found: 361.2 [M+H]⁺.

3-(N-Benzyl-N-methylamino)-5-{3-[3-(dimethylamino)-5-hydroxyphenoxy]propoxy}phenol (16). ¹H-NMR δ 1.84-1.87 (m, 2H), 2.86 (s, 6H), 2.98 (s, 3H), 3.93-3.97 (m, 4H), 4.51 (s, 2H), 5.81-5.88 (m, 6H), 7.21-7.33 (m, 5H), 7.97 (b, 2H). MS (ESI), m/z calcd for C₂₅H₃₀N₂O₄: 422.5. Found: 423.3 [M+H]⁺.

3-(N-Benzyl-N-methylamino)-5-{4-[3-(dimethylamino)-5-hydroxyphenoxy]butoxy}phenol (17). ¹H-NMR δ 1.83-1.84 (m, 4H), 2.98 (b, 4H), 4.51 (s, 4H), 5.80-5.84 (m, 2H), 7.21-7.33 (m, 5H), 7.96 (b, 2H). MS (ESI), m/z calcd for C₂₆H₃₂N₂O₄: 436.5. Found: 437.3 [M+H]⁺.

3-(N-Benzyl-N-methylamino)-5-{4-[3-(N-Benzyl-N-methylamino)-5-hydroxyphenoxy]butoxy}phenol (18). ¹H-NMR δ 2.07-2.10 (m, 2H), 2.97 (s, 6H), 4.02 (t, 4H, J = 6.2), 4.50 (s, 4H), 5.82 (t, 2H, J = 1.9), 5.86 (d, 4H, J = 1.9), 7.21-7.32 (m, 10H), 8.02 (s, 2H). MS (ESI), m/z calcd for C₃₁H₃₄N₂O₄: 498.6. Found: 499.3 [M+H]⁺.

3-(N-Benzyl-N-methylamino)-5-{4-[3-(N-Benzyl-N-methylamino)-5-hydroxyphenoxy]butoxy}phenol (19). ¹H-NMR δ 1.83-1.84 (m, 4H), 2.98 (s, 6H), 3.92 (b, 4H), 4.51 (s, 4H), 5.80-5.84 (m,
(2H), 5.85-5.90 (m, 4H), 7.21-7.33 (m, 10H), 7.95 (s, 2H). MS (ESI), m/z calcd for C_{32}H_{36}N_{2}O_{4}: 512.6. Found: 513.2 [M+H]^+.

7-[[3-(3-Dimethylamino-5-hydroxyphenoxy)propoxy]-3,4-dimethyl-2H-chromen-2-one (20). 1H-NMR δ 2.11 (s, 3H), 2.24 (qn, 2H, J = 6.0), 2.40 (s, 3H), 2.86 (s, 6H), 4.12 (t, 2H, J = 6.0), 4.29 (t, 2H, J = 6.0), 5.83-5.84 (m, 3H), 6.88 (d, 1H, J = 2.5), 6.94 (dd, 1H, J = 8.8, 2.5), 7.66 (d, 1H, J = 8.8), 7.97 (b, 1H). MS (ESI), m/z calcd for C_{22}H_{25}NO_{5}: 383.4. Found: 384.2 [M+H]^+.

7-{{[3-(3-Dimethylamino-5-hydroxyphenoxy)butoxy]-3,4-dimethyl-2H-chromen-2-one (21). 1H-NMR δ 1.92-2.01 (m, 4H), 2.18 (s, 3H), 2.37 (s, 3H), 2.90 (s, 6H), 4.00 (t, 2H, J = 5.6), 4.08 (t, 2H, J = 6.2), 5.86 (d, 2H, J = 1.9), 5.91 (d, 1H, J = 1.9), 6.82 (s, 1H), 6.85 (d, 1H, J = 2.5), 7.48 (d, 1H, J = 8.8), 7.97 (s, 1H). MS (ESI), m/z calcd for C_{23}H_{27}NO_{5}: 397.5. Found: 398.2 [M+H]^+.

7-(3-{3-[Benzyl(methyl)amino]-5-hydroxyphenoxy}propoxy)-3,4-dimethyl-2H-chromen-2-one (22). 1H-NMR δ 2.11 (s, 3H), 2.21 (qn, 2H, J = 6.2), 2.39 (s, 3H), 2.98 (s, 3H), 4.09 (t, 2H, J = 6.2), 4.25 (t, 2H, J = 6.2), 4.50 (s, 2H), 5.84-5.85 (m, 1H), 5.87-5.88 (m, 2H), 6.86 (d, 1H, J = 2.5), 6.92 (dd, 1H, J = 8.8, 2.5), 7.21-7.33 (m, 5H), 7.65 (d, 1H, J = 8.8), 7.98 (s, 1H). MS (ESI), m/z calcd for C_{28}H_{29}NO_{5}: 459.5. Found: 460.3 [M+H]^+.

7-(4-{3-[Benzyl(methyl)amino]-5-hydroxyphenoxy}butoxy)-3,4-dimethyl-2H-chromen-2-one (23). 1H-NMR δ 1.86-1.96 (m, 4H), 2.11 (s, 3H), 2.39 (s, 3H), 2.98 (s, 3H), 3.97 (t, 2H, J = 6.0), 4.16 (t, 2H, J = 6.0), 4.50 (s, 2H), 5.83 (d, 1H, J = 1.9), 5.86 (d, 2H, J = 1.9), 6.84 (d, 1H, J = 2.5), 6.91 (dd, 1H, J = 8.8, 2.5), 7.21-7.33 (m, 5H), 7.64 (d, 1H, J = 8.8), 7.96 (s, 1H). MS (ESI), m/z calcd for C_{29}H_{31}NO_{5}: 473.5. Found: 474.2 [M+H]^+.

Preparation of methylammonium quaternary salts (24-27)
The symmetrical dimethylaminoresorcinol derivatives 14 and 15, and the coumarin derivatives 20 and 21 were dissolved in CH_3CN and treated with 20 equivalent of CH_3I at reflux for 20 hours. The solvent was removed under vacuum to afford the methylammonium quaternary salts 24-27, that were treated with anh. ethyl ether and CH_2Cl_2 to remove unreacted starting materials. The compounds so obtained were sufficiently pure and, without further purifications, are currently under screening as AChE and BChE inhibitors.
3-{4-{(3,4-Dimethyl-2-oxo-2H-chromen-7-yl)oxy}butoxy}-5-hydroxy-N,N,N-trimethylbenzenaminium iodide (27). $^1$H-NMR (DMSO-d$_6$) $\delta$ 1.87 (b, 4H), 2.06 (s, 3H), 2.35 (s, 3H), 3.50 (s, 9H), 4.04 (b, 2H), 4.09-4.15 (m, 2H), 6.49 (s, 1H), 6.80 (s, 1H), 6.91 (d, 1H, $J = 2.5$), 6.94 (s, 2H), 7.68 (d, 1H, $J = 8.5$), 10.27 (s, 1H).

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References