Synthesis and biological activity of 2-phenylbenzofuran derivatives as butyrylcholinerase inhibitors

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Abstract:

Alzheimer disease (AD) is an irreversible and progressive brain disorder characterized by progressive memory loss and a wide range of cognitive impairments. An accepted strategy for its treatment is to restore the levels of acetylcholine by inhibiting cholinesterase enzymes, such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In the current paper we studied the interest of a series of 2- phenylbenzofuran derivatives as BChE inhibitors. The IC_{50} values of all the compounds were determined and compared with the standard inhibitor. This work was based in our previous results and is a preliminary screening for further design and synthesis of new potential compounds that could inhibit the activity of this enzyme involved in neurodegenerative diseases.

Keywords: Wittig reaction, 2-phenylbenzofuran, cholinesterase inhibitors

Introduction:

Acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) are cholinesterases enzymes involved in the cholinergic neurotransmission. Cholinesterases are a family of enzymes that catalyse the hydrolysis of ACh into choline and acetic acid and differ significantly in substrate specificity and activity in different brain regions. Inhibition of these enzymes is one of the major strategies for treatment Alzheimer's disease (AD). AD is an irreversible and progressive brain disorder characterized by progressive memory loss and a wide range of cognitive impairments. Although the precise cause of AD is not completely known, the deficit of acetylcholine seems to play a key role in pathogenesis of this disease. In fact, low levels of ACh appear to be a critical element in the development of cognitive and

neurodegenerative disorders in AD patients.[1] In this context, the inhibition of AChE and BChE and so the enhancement of cholinergic neurotransmission by preserving acetylcholine levels represents an effective way to overcome symptoms and progression of AD. BChE activity progressively increases in patients with AD, while AChE activity remains unchanged or declines. Therefore, compounds that selectively interact with BChE might have a relevant role in treatment of patients with advanced AD.

A lot of extracts from plant origin and synthetic compounds with anti-ChE activity have been characterized up to now.[2,3] However, a treatment for AD is not still present and therefore, a need for new drugs targeting this disease emerges.

In the search for finding new ChE inhibitors, we have recently reported 2-benzofurans derivatives and their inhibition activity towards both the enzymes were investigated.[4]

Benzofuran scaffold has drawn considerable attention over the last few years due to its profound physiological and chemotherapeutic properties.[5]

Our previous study has been the inspiration to design compounds with better inhibitory properties toward butyrylcholinesterase. In order to increase the strength of enzyme inhibition new 2-benzofurans were synthesized. We have used the Wittig reaction as a key step of a good methodology for the efficient and general synthesis of a selected series of 2-phenylbenzofurans. For all the compounds of the series, the IC_{50} values were determined. In this scenario, our findings could be extended to design and develop new potentially therapeutic molecules, especially useful in neurodegenerative diseases.

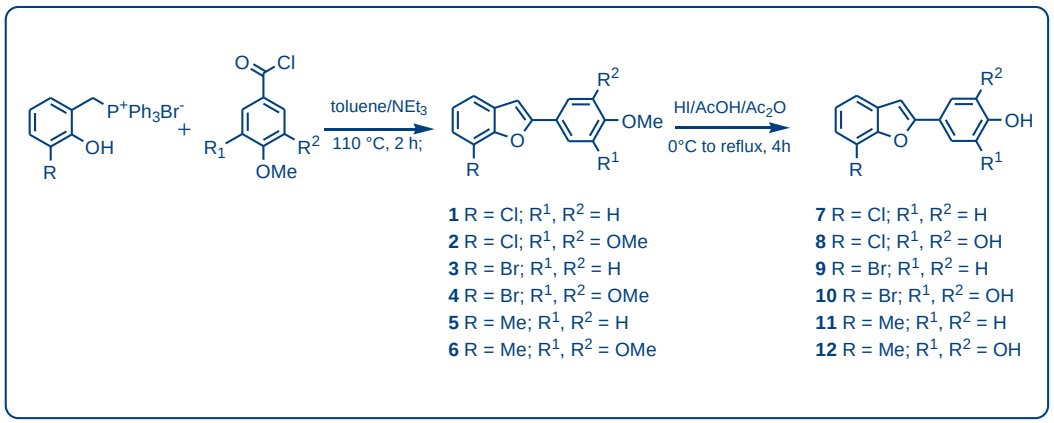
Based on the previous experimental results[4] and with the aim of finding novel and more selective AChE and BChE inhibitors, here we continue our studies, describing the synthesis, biological evaluation and docking studies of a new series of 2-phenylbenzofuran derivatives. Specifically the influence on the activity of one or three hydroxy groups located in different positions of the 2-phenyl and the presence of bromine and chlorine atoms or methyl group at position 7 of the benzofuran ring is studied.

Results and discussion

Compounds **1-12** were efficiently synthesized according to the synthetic strategy outlined in Scheme 1. The key step for the formation of the benzofuran moiety was achieved by an intramolecular Wittig reaction between *ortho*-hydroxybenzyltriphosphonium salt and the appropriate aroylchloride.[6]

The desired Wittig reagent was readily prepared from the conveniently substituted *ortho*-hydroxybenzyl alcohol and $PPh_3 \cdot HBr$.[4, 7-10]

Hydrolysis of the methoxy groups of compound **1–6**[4, 11-12] was performed by treatment with hydrogen iodide in acetic acid/acetic anhydride, to gave the corresponding hydroxy derivatives **7-12**.[13,14]



Scheme 1

The benzofuran structures were confirmed by ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis.

In table 1 we reported overall yields and melting points obtained for compounds **1-12**.

compounds	Yeld%	Mp	**compounds**	Yeld %	Mp
1	30	176-179°C	**7**	25	210-213°C
2	41	132-134°C	**8**	60	198-200°C
3	27	60-63°C	**9**	59	203-205°C
4	56	114-115°C	**10**	59	203-205°C
5	25	97-99°C	**11**	35	143-145°C
6	70	122-124°C	**12**	78	190-192°C

Table 1

All the tested compounds inhibited BChE activity and their IC_{50} values were calculated (Table 2). In our previous research,[4] compound **7** resulted to be the best inhibitor of the series with an IC_{50} (30.3 μM) comparable to galantamine value, used as reference, followed by compound **9** and **11** (IC_{50} values of 82.5 and 77 μM respectively).

compounds	% inhibition at 100 μM	IC_{50} (μM)
7*	77	30.3
8	95.3	25.7
9*	54	82.5
10	91	18.41
11*	58	77
12	99.5	19.8
Galantamine		28.3

*Data previously reported[4]

Table 2

Among the new series, compound **8** differs from compound **7** for the two hydroxyl groups in position 3 and 5 of the 2-phenyl ring, and the IC_{50} values of these compounds were quite similar.

Compounds **10** and **12** showed a much better inhibition if compared with the corresponding mono-hydroxy derivatives compounds **9** and **11**. In fact, the IC_{50} values of compounds **10** and **12** were 18.4 and 19.8 μM and therefore resulted to be 4.5 and 3.8 more active than compounds **9** and **10** (Table 2).

Thus, the introduction of the two additional hydroxylic groups in the position 3 and 5 of the phenyl ring resulted to increase the inhibitory activity of these series of benzofurans. Moreover, the presence of methyl, chlorine or bromine at position 7 of the benzofuran

scaffold results in a very little difference of enzyme inhibition, being compound **10** the more active inhibitor.

Conclusion

A series of 2-phenylbenzofurans compounds was designed, synthesized and evaluated as cholinesterase inhibitors.

Compounds **10** and **12** resulted to be the best BChE inhibitors of the studied series. These data strongly support the conclusion that the introduction of two hydroxy substituents in the position 3 and 5 of phenyl ring of benzofuran derivatives is essential to improve the enzymatic inhibition of these compounds. Thus, they could be the inspiration for the design of further more potent BChE inhibitors.

Materials and Methods

Starting materials and reagents were obtained from commercial suppliers (Sigma-Aldrich) and were used without further purification. All reactions were performed under N_2 atmosphere. Analytical thin layer chromatography (TLC) was carried out on silica gel 60 F254 plates (0.25 mm), visualized by exposure to UV light. Melting points (mp) are uncorrected and were determined with a Reichert Kofler thermopan or in capillary tubes in a Büchi 510 apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian INOVA 500 spectrometer using [D_6]DMSO or $CDCl_3$ as solvent. Chemical shifts (δ) are expressed in parts per million (ppm) using TMS as an internal standard. Coupling constants *J* are expressed in hertz (Hz). Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), and m (multiplet). Mass spectrometry was carried out with a Kratos MS-50 or a Varian MAT-711 spectrometer. Elemental analyses were performed by using a Perkin Elmer 240B microanalyzer and are within 0.4% of calculated values in all cases. Flash chromatography (FC) was performed on silica gel (Merck 60, 230-400 mesh); analytical TLC was performed on pre-coated silica gel plates (Merck 60 F254). Organic solutions were dried over anhydrous Na_2SO_4. Concentration and evaporation of the solvent after reaction or extraction was carried out on a rotary evaporator (Büchi Rotavapor) operating under reduced pressure.

Synthesis

General procedure for the preparation of 2-hydroxybenzyltri-phenylphosphonium bromide: A mixture of 2-hydroxybenzylalcohol (24.6 mmol) and $PPh_3 \cdot HBr$ (24.6 mmol) in CH_3CN (50 mL) was stirred under reflux for 2 h. The solid was filtered and washed with CH_3CN to give the desired compounds.

General procedure for the preparation of 2-phenylbenzofurans ***1-6***: a mixture of 2-hydroxybenzyltriphenylphosphonium bromide (1.11 mmol) and benzoyl chloride (1.11 mmol) in a mixed solvent (toluene 30 mL and Et_3N 0.6 mL) was stirred under reflux for 2 h. The precipitate was removed by filtration. The filtrate was concentrated, and the residue was purified by silica gel chromatography (hexane/EtOAc 9:1) to give the desired compounds.

General procedure for the preparation of hydroxylated 2-phenylbenzofurans ***7-12***: a mixture of the corresponding methoxy-2-phenylbenzofuran (0.11 g, 0.50 mmol) in acetic acid (5.0 mL) and acetic anhydride (5.0 mL), at 0 °C, was prepared. Hydriodic acid 57% (10.0 mL) was added drop-wise. The mixture was stirred under reflux temperature for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by FC (dichloromethanexane/methanol 9.8:0.2) to give the desired compounds.

Butyrylcholinesterase Activity

BChE activity was measured spectrophotometrically using Ellman's reagent according to the method previously reported.[4] The test is based to the hydrolysis of S-butyryltiocholine by BChE which produces thiocholine. The latter reacts with 5,5'-dithiobis-2-nitrobenzoate yielding a yellow coloured anion having an $\varepsilon_{405} = 1.36\ M^{-1}cm^{-1}$.

The reaction mixture contained 0.1 M phosphate buffer (pH 8.0), enzyme solution, 1.5 mM 5,5'-dithiobis-2-nitrobenzoate (DTNB) and inhibitor dissolved in DMSO at the desired concentrations or DMSO alone (control) in a final volume of 1 mL. Finally, S-butyrylthiocholine iodide (1.5 mM) was added to the reaction mixture and the absorbance immediately monitored at 405 nm. The velocity of the reactions was measured and BChE activity was calculated as a percentage of the velocity in the presence (100 μM) and in the absence of inhibitor and the IC_{50} values were calculated as concentration of compounds that produces 50 % of enzyme activity. Galantamine was used as standard inhibitor.

Acknowledgements

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