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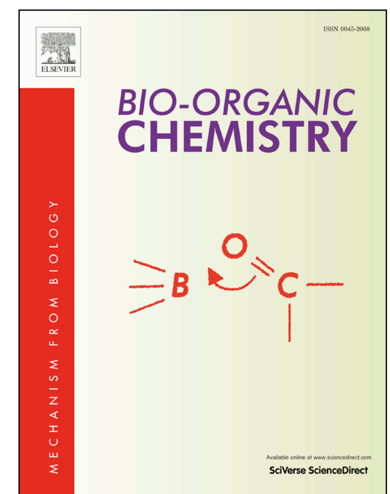

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Synthesis, molecular docking and cholinesterase inhibitory activity of hydroxylated 2-phenylbenzofuran derivatives

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ABSTRACT

We have designed, synthesized and evaluated a series of hydroxylated 2-phenylbenzofuran derivatives as potential cholinesterase inhibitors. Starting from a series of 2-phenylbenzofurans previously published, in this paper we present a complete synthesis and the influence on the activity of one or two hydroxyl groups located in *meta* or in *meta* and *para* positions respectively of the 2-phenyl ring and highlight the importance of position of hydroxyl groups. Moreover, simultaneous introduction of halogen at position 7 of the benzofuran scaffold resulted in an improved inhibitory activity against the enzyme. To further provide molecular insight and to identify the most probable ligand-binding site of the protein, docking studies were performed for the top-ranked compounds. Docking results revealed conserved ligand-binding residues and supported the role of catalytic site residues in enzyme inhibition.

Keywords: 2-Phenylbenzofurans, Cholinesterase Inhibitors, Docking studies

1. Introduction

Benzofuran derivatives are an important class of organic compounds that occur in natural products because of their pharmacological activities, including antitumoral properties. They can be used as anti-inflammatory, antimicrobial, antagonists of the angiotensin II receptor, analgesic, ligands of adenosine A1 receptor and so forth [1].

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Phenylbenzofurans are a very important molecule skeleton due to their synthetic versatility and their proved pharmacological properties [2]. They are synthetic compounds in which an additional phenyl ring is present in any position of the benzofuran nucleus. This could be easily obtained possible by two principal general methods: by a *C*-phenylation of a benzofuran [3] or by the construction of the benzofuran nucleus with the new ring already included on it [4]. In the present work we describe the second method, by a classical Wittig reaction [5,6], perhaps the most direct and general method known for preparing the desired 2-phenylbenzofurans.

Recently, benzofuran derivatives have showed enzymatic inhibition properties for example on monoamine oxidase [7], acetylcholinesterase (AChE) [8-10] and butyrylcholinesterase (BChE) [11, 12].

Acetylcholine (ACh) is a neurotransmitter that plays a role in the modulation of memory function in normal and neurodegenerative conditions [13]. In the cholinergic system, disruption in the levels of ACh is caused by hydrolytic action of cholinesterases (ChEs), a family of enzymes that play a role in ACh regulation and in the cholinergic signaling [14].

AChE and BChE appear to be simultaneously active in the synaptic hydrolysis of ACh, terminating its neurotransmitter action, and co-regulating levels of ACh. A well-documented strategy to restore the neurotransmitter level involves the use of cholinesterase inhibitors that suppress the ChEs enzymes and therefore increasing both the level and duration of the neurotransmitter action [15-17].

In our efforts to contribute to the development of novel compounds that may be useful in the treatment of neurodegenerative disorders such as Parkinson’s disease (PD) or Alzheimer’s disease (AD), we are focusing on 2-phenylbenzofuran derivatives [7,11,12,18,19].

In particular, with the aim of finding out structural features in the ChEs inhibitory activity, in the present work, we describe the synthesis of hydroxylated 2-phenylbenzofuran derivatives 7-bromine and 7-chlorine substituted and the importance of hydroxyl groups substitution in the 2-phenyl ring. We recently developed a series of 2-phenylbenzofurans which exhibited selective inhibitory property for BChE enzyme. Considering that the 7-chlorine-2-(3,5-dihydroxyethoxyphenyl)benzofuran and 7-bromine-2-(3,5-dihydroxyethoxyphenyl)benzofuran displayed highest inhibitory activity towards BChE with IC_{50} values of 6.23 μM and 3.57 μM respectively [11,12], in this paper we studied the influence on the activity of one or two hydroxyl groups located in *meta* or in *meta* and *para* positions respectively of the 2-phenyl ring in addition to the presence of a halogen at

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positions 7 of the benzofuran scaffold, which has been revealed as adequate position for substitution.

2. Experimental

2.1 Chemistry

2.1.1 General information

Starting materials and reagents were obtained from commercial suppliers (Sigma-Aldrich) and were used without further purification. 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 $CDCl_3$ 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). Elemental analyses were performed by using a Perkin Elmer 240B microanalyzer and are within 0.4% of calculated values in all cases. The analytical results indicate 98% purity for all compounds. Flash chromatography (FC) was performed on silica gel (Merck 60, 230-400 mesh); analytical thin-layer chromatography (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.

2.1.2. *General procedure for the preparation of 2-phenylbenzofuran (**1-4**).*

A mixture of 2-hydroxybenzyltriphenylphosphonium bromide (0.50 g, 1.11 mmol) and benzoyl chloride (0.12 mL, 1.11 mmol) in a mixed solvent (toluene 20 mL and Et_3N 0.5 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 **1-4**.

2.1.2.1. *7-chloro-2-(3-methoxyphenyl) benzofuran (**1**).* It was obtained with a yield of 56%. m.p. 43-45 °C. ^{1}H NMR (500 MHz, $CDCl_3$), *δ* (ppm), *J* (Hz) = 3.91 (s, 3H, OCH_3), 6.98-6.93 (m, 1H, H-4'), 7.01 (d, *J* = 1.2, 1H, H-3), 7.17 (td, *J* = 7.5, 3.5, 1H, H-5), 7.34-7.30 (m,

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1H, H-6'), 7.41-7.36 (m, 1H, H-6), 7.53-7.44 (m, 3H, H-4, H-2' and H-5'); ^{13}C NMR (125 MHz, $CDCl_3$), *δ* (ppm), *J* (Hz) = 55.46, 102.11, 110.65, 114.78, 116.73, 117.81, 119.50, 123.91, 124.53, 130.00, 130.88, 150.68, 131.20, 156.70, 160.04. Anal. calcd. for $C_{15}H_{11}ClO_2$: C, 69.64%; H, 4.29%. Found: C, 69.69%; H, 4.31%.

2.1.2.2. *7-bromine-2-(3-methoxyphenyl) benzofuran (**2**).* It was obtained with a yield of 81%. m.p. 45-47 °C. ^{1}H NMR (500 MHz, $CDCl_3$), *δ* (ppm), *J* (Hz) = 3.91 (s, 3H, OCH_3), 6.97 (d, *J* = 8.1, 1H, H-4’), 7.23 (s, 1H, H-3), 7.30 (d, *J* = 7.8, 1H, H-6'), 7.52 (t, *J* = 7.9, 1H, H-5’), 7.64 (s, 1H, H-2’), 7.45 (t, *J* = 7.7, 1H, H-5), 7.72 (d, *J* = 7.8, 1H, H-6), 7.83 (m, 1H, H-4); ^{13}C NMR (125 MHz, $CDCl_3$), *δ* (ppm) = 56.10, 100.92, 103.94, 108.46, 111.73, 118.76, 119.94, 123.12, 124.32, 127.20, 130.93, 149.83, 151.22, 152.96, 157.83;. Anal. calcd. for $C_{15}H_{11}BrO_2$: C,59.43%; H, 3.66%. Found: C, 59.50%; H, 3.72%.

2.1.2.3. *7-chlorine-2-(3,4-dimetoxyphenyl) benzofuran (**3**).* It was obtained with a yield of 51%. m.p. 117-120 °C; ^{1}H NMR (500 MHz, $CDCl_3$), *δ* (ppm), *J* (Hz) = 4.30 (s, 3H, OCH_3), 4.37 (s, 3H, OCH_3), 7.26 (s, 1H, H-3), 7.28 (t, *J* = 6.2, 1H, H-5), 7.52 (d, *J* = 7.8, 1H, H-5'), 7.63 (d, *J* = 7.8, 1H, H-6'), 7.74 (d, *J* = 1.1, 1H, H-6), 7.85-7.78 (m, 2H, H-4 e H-2'); ^{13}C NMR (125 MHz, $CDCl_3$), *δ* (ppm) = 55.97, 56.05, 100.41, 108.22, 111.37, 116.42, 118.32, 119.09, 122.85, 123.78, 123.97, 131.12, 149.25, 149.98, 150.42, 156.88; Anal. calcd. for $C_{16}H_{13}ClO_3$: C, 66.56%; H, 4.54%. Found: C, 66.61%; H, 4.57%

2.1.2.4. *7-bromine-2-(3,4-dimethoxyphenyl) benzofuran (**4**).* It was obtained with a yield of 79%; m.p. 139-141 °C; ^{1}H NMR (500 MHz, $CDCl_3$), *δ* (ppm), *J* (Hz) = 4.27 (s, 3H, OCH_3), 4.32 (s, 3H, OCH_3), 7.27 (d, *J* = 7.2, 2H, H-5' e H-6'), 7.41 (t, *J* = 7.7, 1H, H-5), 7.70 (s, 1H, H-2'), 7.73 (d, *J* = 7.8, 1H, H-6), 7.83-7.78 (m, 2H, H-3 e H-4); ^{13}C NMR (125 MHz, $CDCl_3$), *δ* (ppm) = 56.13, 56.19, 100.65, 103.88, 108.41, 111.52, 118.48, 119.81, 123.00, 124.28, 126.94, 130.87, 149.38, 150.12, 151.91, 156.88; Anal. calcd. for $C_{16}H_{13}BrO_3$: C, 57.68%; H, 3.93%. Found: C, 57.73%; H, 3.95%

2.1.3 *General procedure for the preparation of hydroxylated 2-phenylbenzofurans (**5-8**)*

A solution 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

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3 h. The solvent was evaporated under vacuum and the dry residue was purified by FC (dichloromethane/methanol 9.8:0.2) to give the desired compound **5-8** [20].

2.1.3.1. *7-chlorine-2-(3-hydroxyphenyl) benzofuran (**5**).* It was obtained with a yield of 94%. m.p. 110-112 °C. ^{1}H NMR (500 MHz, $CDCl_3$), *δ* (ppm), *J* (Hz) = 6.88-6.81 (m, 1H, H-4'), 7.27 (t, *J* = 7.8, 1H, H-5), 7.43-7.30 (m, 4H, H-6, H-2', H-5' e H-6'), 7.45 (s, 1H, H-3), 7.62 (d, *J* = 7.7, 1H, H-4), 9.75 (s, 1H, OH); ^{13}C NMR (125 MHz $CDCl_3$), *δ* (ppm): 102.52, 109.50, 111.32, 115.16, 115.82, 116.48, 120.16, 124.33, 124.45, 130.24, 130.68, 149.39, 156.22, 157.86; Anal. calcd. for $C_{14}H_9ClO_2$: C, 68.72%; H, 3.71%. Found: C, 68.75%; H, 3.75%.

2.1.3.2. *7-bromine-2-(3-hydroxyphenyl) benzofuran (**6**).* It was obtained with a yield of 93%. m.p. 92-95 °C. ^{1}H NMR (500 MHz, $CDCl_3$), *δ* (ppm), *J* (Hz) = 5.40 (s, 1H, OH), 7.13 (d, *J* = 8.1, 1H, H-4'), 7.26 (s, 1H, H-3), 7.35 (t, *J* = 7.8, 1H, H-6'), 7.57 (t, *J* = 7.9, 1H, H-5'), 7.65 (s, 1H, H-2'), 7.76-7.67 (m, 3H, H-4, H-5 and H-6); ^{13}C NMR (125 MHz $CDCl_3$), *δ* (ppm): 102.39, 104.06, 112.06, 116.22, 117.98, 120.20, 124.37, 127.50, 130.32, 130.50, 131.46, 152.05, 156.01, 156.29; Anal. calcd. for $C_{14}H_9BrO_2$: C, 61.78%; H, 3.58%. Found: C, 61.80%; H, 3.62%.

2.1.3.3. *7-chlorine-2-(3,4-dihydroxyphenyl) benzofuran (**7**).* It was obtained with a yield of 83%; m.p. 74-76 °C; ^{1}H NMR (500 MHz, $CDCl_3$), *δ* (ppm), *J* (Hz) = 4.57-5.58 (m, 2H, 2 x OH). 7.26 (s, 1H, H-3), 7.38 (d, 1H, *J* = 8.3 Hz, H-5'), 7.53-7.58 (m, 1H, H-2'), 7.66 (d, 1H, *J* = 8.3, H-6'), 7.77 (t, 1H, *J* = 11.4, 5.7 Hz, H-5), 7.83 (m, 2H, H-4 and H-6), ^{13}C NMR (125 MHz, DMSO), δ (ppm) = 100.54, 112.52, 115.97, 116.52, 118.81, 119.23, 123.40, 123.86, 124.08, 131.15, 143.82, 144.82, 150.47, 156.68; Anal. calcd. for $C_{14}H_9ClO_3$: C, 64.51%; H, 3.48%. Found: C, 64.54%; H, 3.52%.

2.1.3.4. *7-bromine-2-(3,4-dihydroxyethoxyphenyl) benzofuran (**8**).* It was obtained with a yield of 87%; m.p. 74-76 °C; ^{1}H NMR (500 MHz, DMSO), δ (ppm) = 3.12-4.06 (m, 2H, 2 x OH), 7.33 (d, *J* = 8.3, 1H, H-5'), 7.45 (d, *J* = 7.8, 1H, H-6'), 7.64 (s, 1H, H-3), 7.71-7.82 (m, 3H, H-4, H-5 and H-2'), 7.83 (d, *J* = 7.7, 1H, H-6); ^{13}C NMR (125 MHz, DMSO), δ (ppm) = 103.1, 106.9(2C), 108.8, 117.4, 119.8, 124.5, 124.8, 125.3, 139.6, 155.1, 156.1, 159.3(2C); Anal. calcd. for $C_{14}H_9BrO_3$: C, 64.51%; H, 3.48%. Found: C, 64.54; H, 3.52%.

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2.2. *Determination of cholinesterase inhibition*

The cholinesterase inhibition activity was performed using Ellman's method and analyzed as previously described [21] with slight modifications [11].

Acetylthiocholine iodide (AChI)/S-butyrylthiocholine iodide (BChI), and 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) (Sigma-Aldrich) were used for the determination of the AChE/BChE activities. Acetylcholinesterase was from *Electrophorus electricus* (AChE), while butyrylcholinesterase was from equine serum (BChE) (Sigma-Aldrich).

Total volume of the reaction mixture was 200 µL. It contained phosphate buffer (0.1 M, pH 8.0), enzyme solution, AChE (0.3 U/ml) or BChE (0.15 U/ml, DTNB (1.5 mM final concentration), and inhibitor, at different concentration, dissolved in 1% DMSO or DMSO alone (control). Finally, ATCI or BTCI (1.5 mM final concentration) as the substrate was added to the reaction mixture and the absorbance immediately monitored at 405 nm, with a plate reader FLUOstar OPTIMA (BMG Labtech, Offenburg, Germany). The activity of the enzymes was performed at 25 °C. Galantamine and tacrine were used as standard inhibitors. IC_{50} values were obtained from activity (slope) versus compound concentration plots. IC_{50} values displayed represent the mean ± standard deviation for three independent assays.

2.3. *Molecular modeling studies*

Three-dimensional (3D) protein structure of human BChE (PDB id: 4TPK) was accessed from protein data bank, and for the compounds the 3D-coordinates were generated using Open Babel software. Molecular docking of the compounds into hBChE protein was performed employing EADock DSS docking software incorporated in the SwissDock web server [22]. The docking procedure considers the entire protein surface as a potential target. Utilizing this procedure, a large number of ligand binding modes (~15000) were generated, with the simultaneous rough interaction energy estimation. The binding modes with favourable energies were then ranked and classified into different clusters, based on the full fitness scoring function. The most probable and consistent conformation was selected from 10 independent docking runs for each compound. The accuracy and prediction of correct binding mode depends on the number of free dihedral angles of the compound (only one in our case), with a success rate of 93% while considering the top five binding modes.

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3. Result and discussion

3.1. Chemistry

Benzofuran derivatives **1-8** were efficiently synthesized by a Wittig reaction, according to the protocol outlined in Figure 1. The desired Wittig reagents were readily prepared from the conveniently substituted *ortho*-hydroxybenzyl alcohols **a-b** [12,23,24] and PPh_3·HBr. (Figure 1, Scheme 1a). The key step for the formation of the benzofuran moiety was achieved by an intramolecular reaction between the *ortho*-hydroxybenzyltriphosphonium salt **c-d** [12] and the appropriate benzoyl chloride [5,6]. Hydrolysis of the methoxy groups of compounds **1-4** was performed by treatment with hydrogen iodide in acetic acid/acetic anhydride, to give the corresponding hydroxy derivatives **5-8** (Fig. 1, Scheme 2b) [20].

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

a

CHO, OH, R — $NaBH_4$/EtOH, 0 °C to rt, 2 h → OH, OH, R **a-b** — PPh_3 HBr/CH_3CN, 82 °C, 2 h. → $P^+Ph_3Br^-$, OH, R **c-d**

a, c: R = Cl
b, d: R = Br

b

$P^+Ph_3Br^-$, OH, R **c-d** — (O, Cl, OMe) toluene/NEt_3, 110 °C, 2 h; → OMe, O, R **1-2** — HI/AcOH/Ac_2O, 0°C to reflux, 4h → OH, O, R **5-6**

c-d — toluene/NEt_3, 110 °C, 2 h; (O, Cl, OMe, OMe) → OMe, OMe, O, R **3-4** — HI/AcOH/Ac_2O, 0°C to reflux, 4h → OH, OH, O, R **7-8**

1,3: R = Cl
2,4: R = Br

5,7: R = Cl
6,8: R = Br

Fig. 1. Protocol for synthesis of compounds **1-8**. (**a**) scheme 1 (**b**) scheme 2.

3.2. *Biological activity*

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The potential effects of the synthesized compounds **5-8** on AChE and BChE was performed on animal enzymes AChE and BChE, due to their lower cost and high degree of similarity with their respective human enzymes. Table 1 shows the inhibitory effect of the newly synthesized compounds **5-8** compared with those previously published by us **9-14** [11,12].

	AChE	BChE	Reference
Compounds	IC$_{50}$ (μM)		
5	28.21±1.26	13.42±2.57	
6	23.96±2.77	7.96±0.28	
7	56.27±2.07	15.54±2.62	
8	>100	10.86±0.35	
9	>100	30.3±1.90	[12]
10	>100	82.5±7.1	[12]
11	>100	6.23±0.43	[11]
12	100±6.10	3.57±0.25	[11]
13	50±3.30	25.7±1.60	[11]
14	37±2.6	18.41±0.93	[11]

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Galantamine	3.12±0.15	26.06±6.19
Tacrine	0.42±0.032	0.024±0.024

Table 1. Inhibition of AChE and BChE enzymes by compounds **5-14**. ChE inhibition is expressed as the mean ± SD (n = 3 experiments).

The experimental results showed that most of the compounds tested are selective BChE inhibitors with a varying efficiency. The IC_{50} values of BChE inhibition are similar to those showed by the most potent compounds previously described by us. As a rule, 2-phenylbenzofurans substituted at the 7-position were found to be more active molecules as BChE inhibitors than the corresponding 5-substituted derivatives or those derivatives unsubstituted in the benzene ring [12].

The compounds **6** and **8** bromine-substituted resulted somewhat better active respect to compounds chlorine-substituted **5** and **7** (Table 1).

Considering that 2-phenylbenzofurans with a bromine atom at position 5 in the benzofuran ring were inactive, the obtained results point out that the location of this substituent is at least as important as its nature for the activity [11,12].

In addition, in comparison to the previously obtained data, while the hydroxyl substituent in the *para* position on the 2-aryl (compounds **9** and **10**, IC_{50} value 30.3 and 82.5 and μM respectively) leads to much worse results, when that substituent moved in *meta* position (compounds **5** and **6**, IC_{50} value 13.42 and 7.96 μM respectively), the activity improve considerably [12].

Among all the benzofuran derivatives analyzed, maximum inhibitory activity against eqBChE enzyme were displayed by compounds **11** (IC_{50} = 6.23 μM) and **12** (IC_{50} = 3.57 μM), with two hydroxyl substituents in *meta* position on the phenyl-ring and with presence of chlorine and bromine atoms respectively at position 7 of benzofuran scaffold. EqBChE inhibitory activity displayed by these compounds was about 4- and 8- times higher than the reference compound, galantamine (IC_{50} = 26.06 μM).

The 2-phenylbenzofuran derivatives **7** and **8** with two hydroxyl substituents in *meta* and *para* positions of the 2-phenyl ring displayed lower inhibitory activity toward eqBChE (IC_{50} = 15.54 μM and IC_{50} = 10.86 μM), compared to isomers **11** and **12** with two hydroxyl groups in *meta* positions.

In the compounds with three hydroxyl substituents in the 2-phenyl ring (compounds **13** and **14**), we found lower inhibitory activity against eqBChE (IC_{50} = 25.70 and 18.41 μM)

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[11]. This fact suggests that little differences in the positions and number of hydroxyl groups in the 2-phenyl ring of the synthesized compounds could decrease or increase the inhibitory activity of the compounds against eqBChE. It has been shown previously that the position and number of hydroxyl group in the ligand can influence the magnitude of hydrogen bond interactions with the protein [25].

3.3. *Molecular and physicochemical properties*

Based on the experimental results, we performed docking calculations for the molecules **5-8** against protein hBChE.

In Table 2, we summarize the molecular and physicochemical properties of compounds that were calculated using SwissADME web tool [26] and compare them with reference drug molecules such as galantamine and tacrine.

Properties such as molecular weight (MW), volume, number of rotatable bonds [27], number of hydrogen bond (H-bond) acceptor and donor atoms, and polar surface [28] area (PSA), lipophilicity [29], water solubility [30] and drug likeliness [31,32] of the compounds are essential parameters that should be considered in drug development.

	5/9	**6/10**	**7/11**	**8/12**	**13**	**14**	**Galantamine**	**Tacrine**
MW (g/mol)	244.67	289.12	260.67	305.12	276.67	321.12	287.35	198.26
Volume (Å^3)	202.56	206.91	210.58	214.93	218.60	222.95	268.19	191.53
Rotatable bonds	1	1	1	1	1	1	1	0
H-bond acceptor atoms	2	2	3	3	4	4	4	1
H-bond donor atoms	1	1	2	2	3	3	1	1
Polar surface area (Å^2)	33.37	33.37	53.60	53.60	73.83	73.83	41.93	38.91
Lipophilicity (logP)	3.65	3.73	3.24	3.34	2.93	2.91	1.91	2.59
Water Solubility	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Soluble	Soluble
Drug likeliness (Lipsinky)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 2. Molecular properties of compounds under investigation

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The molecules investigated differ not only in the number of hydroxyl groups but also in their position in the phenyl ring and include a contemporary presence of either chlorine (**5**, **7**, **9**, **11**, **13**) or bromine (**6**, **8**, **10**, **12**, **14**) at position 7 of the benzofuran scaffold.

Molecules with identical halogen substitution of benzofuran moiety, number of hydroxyl group in the phenyl ring, but different in their position displayed same physicochemical properties. For example, molecules **5** and **9** differ only in the substitution position of hydroxyl group. All the molecules have only one rotatable bond each, thus displaying similar flexibility. With increasing the number of hydroxyl groups in the phenyl ring, the molecular weight, volume, number of hydrogen bond (H-bond) acceptor/donor atoms and polar surface area values also increased. While, an opposite trend was found for lipophilicity that provides measure the hydrophobic nature of the compound. Furthermore, all 2-phenylbenzofuran derivatives investigated here displayed moderate water solubility characteristics, and better lipophilicity with respect to the reference drug molecules.

Overall, based on the physiochemical and structure features investigated, all the molecules displayed druglikeness characteristics.

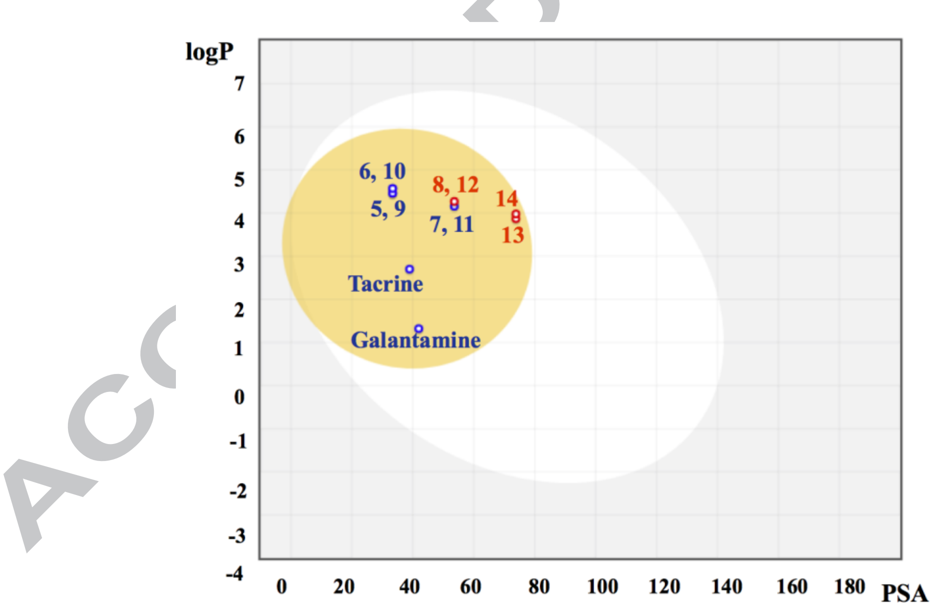

Fig. 2. A BOILED-Egg graphical output using polar surface area (PSA) vs lipophilicity (LogP) for the molecules investigated in this study. The region in yellow (yolk) represents high probability of brain penetration, while white represents the physicochemical space of gastrointestinal absorption. Blue dots represent molecule as substrate and red dots as non-substrates of the permeability glycoprotein.

Novel Brain Or IntestinaLEstimateD permeation (BOILED-Egg) method was employed to predict two crucial pharmacokinetic parameters namely human gastrointestinal absorption (HIA) and blood brain barrier (BBB) simultaneously, by utilizing two

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physicochemical descriptors (lipophilicity, polarity) of the molecule. It is encouraging to note that all the molecules investigated are classified in the same physiochemical space of tacrine and galantamine drugs, which indicate high probability of the molecules being absorbed in the brain (Fig. 2).

The molecules considered here displayed better lipophilicity and molecular flexibility with respect to galantamine and tacrine. Moreover, molecules with two or more hydroxyl groups displayed higher polar surface area and number of H-bond donor and acceptor atoms. Indeed, the relevance of molecular flexibility and polar surface area values of the molecules with oral bioavailability in rats has been tested for 434 pharmacia compounds [33]. All the molecules displayed druglikeness characteristic, a parameter that qualitatively assesses the possibility for a compound to become an oral drug. Interestingly, we found that all the molecules possess likelihood to become an oral drug.

Based on the experimental results and to further identify the most probable ligand-binding site of the protein hBChE, we performed docking calculations for the molecules **5-8**. Docking results marked all the molecules to bind at the same protein site (Fig. 3), and displaying similar binding energy value ranging between 7.4 to 7.8 kcal/mol.

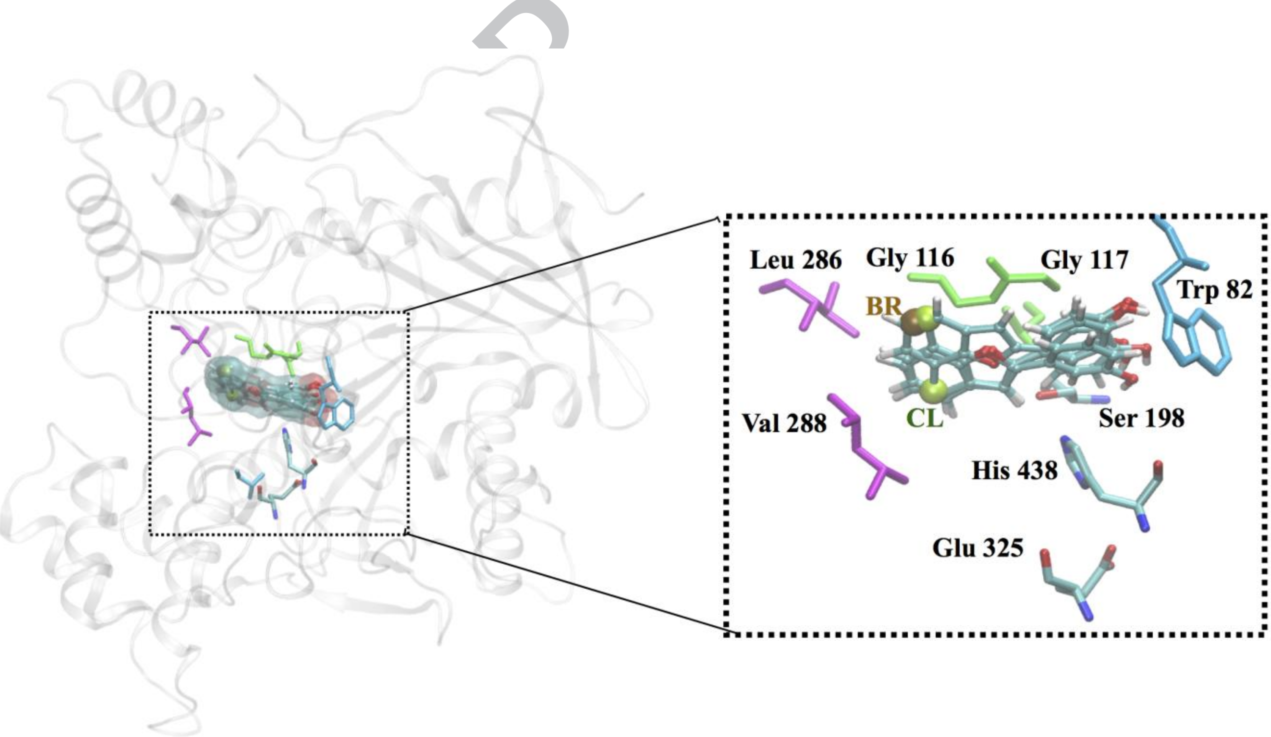

Fig. 3. Docking of molecules to hBChE protein. The most favourable docked position of molecules **5-8** in binding site of hBChE protein. The active site residues and molecules are shown in licorice and the protein in cartoon representation. On the right panel, zoomed view of the interaction region is shown.

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To explain the origin of the similarity in the binding energy values, we carefully probed the interaction site of the molecules in complex with hBChE protein, using Ligplot [34] software.

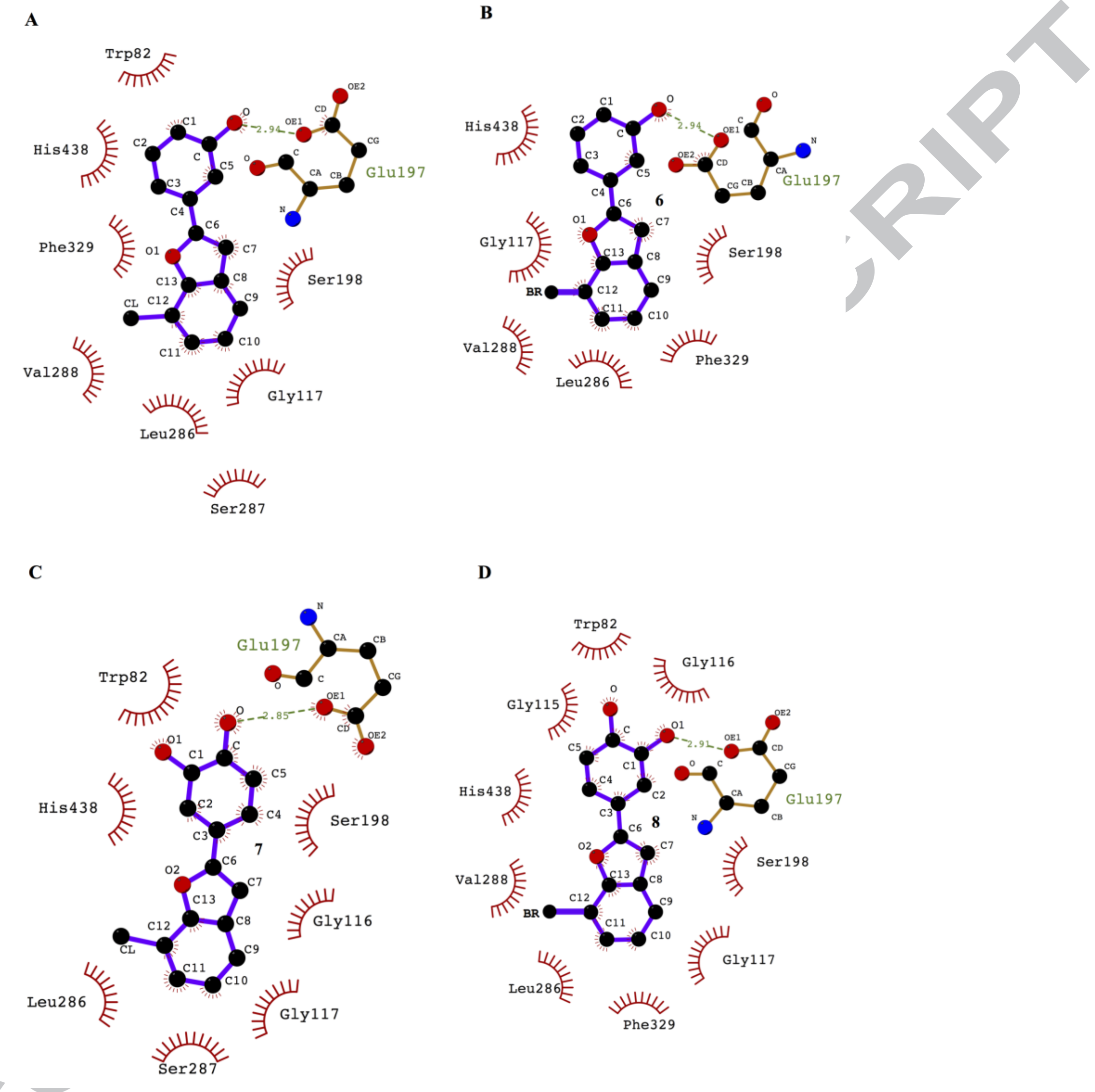

Fig. 4. Molecular interaction picture of hBChE protein complexes with molecule: (A) **5**, (B) **6**, (C) **7** and (D) **8**.

The binding site of hBChE protein is characterized by an acyl pocket, located at the bottom of a deep catalytic gorge; the oxyanion hole; the peripheral site, located at the lip of the gorge; and the choline-binding site also located within the gorge. In all the four ligand-protein complexes, we found involvement of catalytic triad residues (Ser198, His438), H-bond interactions with residue Glu197, acyl pocket residues (Leu286, Val288) and oxyanion hole residue (Gly117). Choline binding-site residue Trp82 was involved in interactions with molecules **5**, **7** and **8** (Fig. 4).

The docking binding energy values for these four complexes are quite similar, consistent with the not so different IC_{50} values calculated in our experiments. In accordance with our previously published results [11,12], the 2-pheynlbenzofuran derivatives with hydroxyl group/s in the phenyl ring exhibited rich interaction network involving the catalytic triad residues, which we propose to be an essential component for a molecule to display inhibitory activity against BChE enzyme. Different substitution position of hydroxyl group in the 2-phenyl group resulted in different interaction patterns, but remained consistent with participation of key residues to binding.

4. Conclusion

In this study, we have used the Wittig reaction as a key step for the efficient and general synthesis of a series of hydroxylated 2-phenylbenzofuran derivatives. All the compounds have been evaluated for their cholinesterase inhibitory activity. The experimental results showed that most of the benzofurans tested are selective BChE inhibitors with a varying efficiency. The IC_{50} values of BChE inhibition indicated compounds **6** and **8** with the presence of bromine at position 7 of the benzofuran scaffold as the most potent inhibitors with an IC_{50} value of 7.96 and 10.86 μM respectively. However, a thorough analysis of the results obtained, revealed that the maximum inhibitory activity was displayed by benzofurans derivatives with two hydroxyl substituents in *meta* position on the phenyl-ring and chlorine and bromine atoms respectively at position 7 of benzofuran scaffold (compounds **11** and **12**). Thus, our study provides a complete picture of the importance of number and position of the hydroxyl groups in the 2-phenyl ring, and among the compounds tested, two hydroxyl substituents in *meta* position to be the most functional for the cholinesterase inhibition. Moreover, comparative physicochemical and pharmacokinetic properties of the compounds with the commonly prescribed drugs revealed high probability of all the compounds to be absorbed in the brain and exhibiting promising druglikeness characteristics. Finally, molecular docking simulations assisted in explaining the structure-activity relationships of this type of compounds.

Conflict of interest

The authors declare no conflict of interest.

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Acknowledgements

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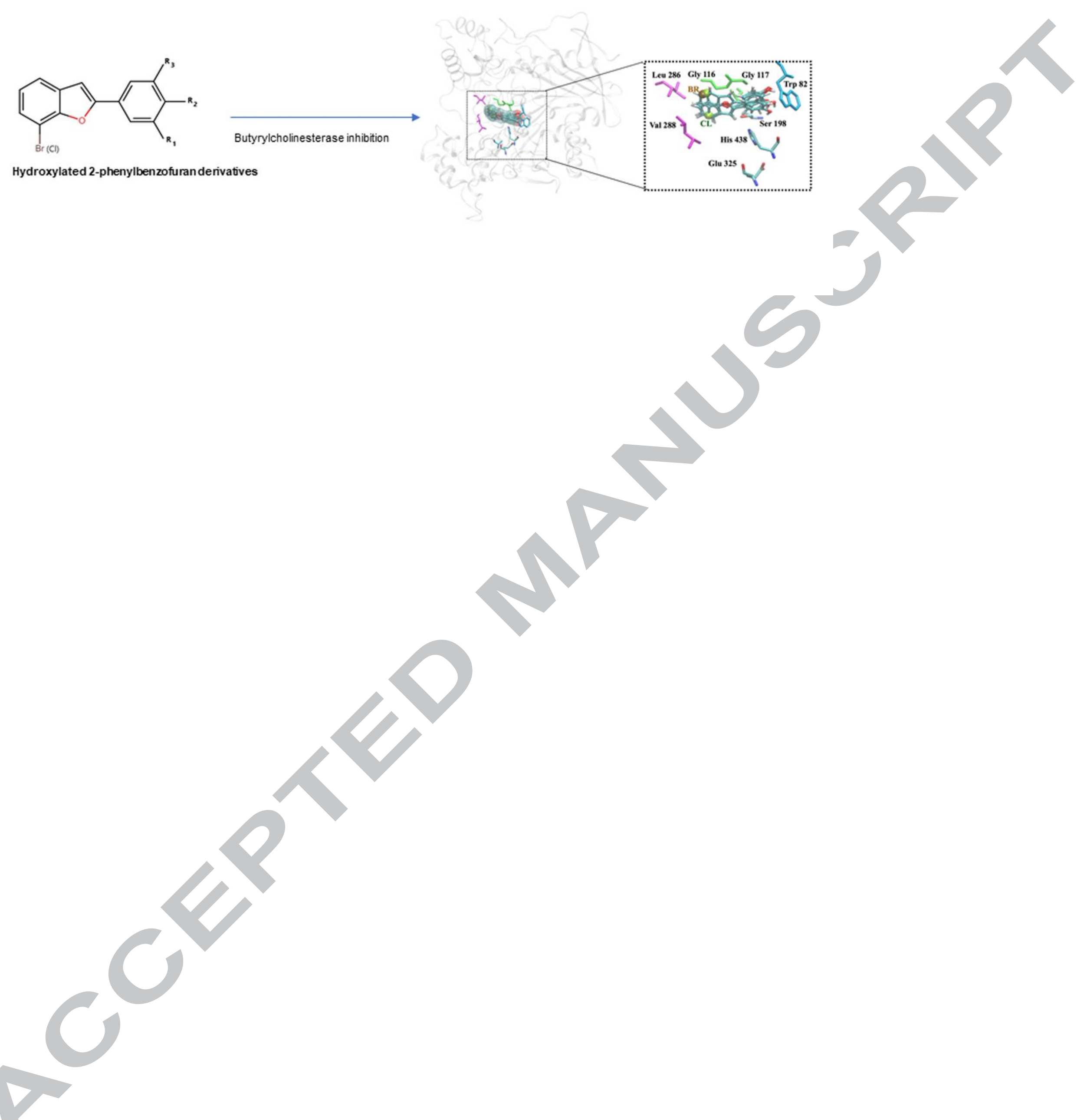
Graphical abstract



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Highlights

- Series of hydroxylated 2-phenylbenzofuran were synthesized.
- Compounds displayed activity against butyrylcholinesterase enzyme.
- Importance of number and position of hydroxyl subsitution in enzyme activity.
- Physicochemical properties of derivatives were characterised.
- Relationship between physicochemical properties and activity was found.
- Docking studies assisted in explaining the structure-activity.

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