Design, synthesis and biological evaluation of new substituted benzofuran-based derivatives *via* C−H bond activation

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Abstract: A series of biologically active disubstituted benzofuran derivatives (**3a**–**d**) have been designed and synthesized via C–H bond activation reaction. The chemical structures of all final compounds were confirmed by spectroscopic methods. *In vitro* anti acetylcholinesterase (AChE) activities of these novel compounds were evaluated and showed low to moderate results. Among them, compound **3d** moderately inhibited AChE activities with 68.12 % value.

Keywords: carbon–carbon coupling; benzofuran; acetylcholinesterase; Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative disorder that influences the life quality of the elderly population of the world.[1] Although the patomechanism of AD has not been clearly recognized, the typical pathological marks are amyloid-*β* (A*β*) deposits, oxidative stress, and decreased levels of acetylcholine (ACh) in the brain.[2,3] ACh is an important neurotransmitter responsible for memory. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are responsible enzymes for the metabolic hydrolysis of ACh at the cholinergic synapses.[4,5] Acetylcholinesterase (AChE) inhibitor drugs involving donepezil, rivastigmine and galantamine have been widely used for the treatment of AD.[6]

Numerous investigations have been carried out with an aim to find more efficient anti-acetylcholinesterase compounds with the new concept, mostly inspired by the dual-binding mode of action in donepezil. Among the large variety

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of anti-acetylcholinesterase compounds, benzofurans play an important role in the increasing of the levels of acetylcholine.[7,8] In addition, benzofuran derivatives are noted as the essential building block in bioactive substances[9] with broad range of biological and pharmaceutical activities such as anticancer,[10] anti-bacteria (1),[11] antifungal (2),[12] anti-inflammatory[13] and angiogenesis[14] (Fig. 1).

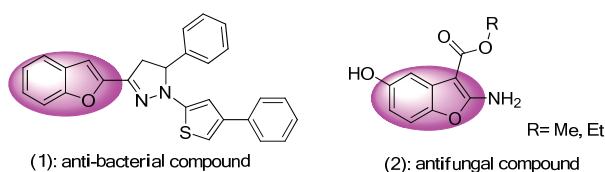(1): anti-bacterial compound   (2): antifungal compound

Fig. 1. Biologically active compounds containing benzofuran framework: 1 – anti-bacterial and 2 – antifungal compound.

On the other hand, the formation of C−C bonds is one of the most common processes in all areas of organic and drug synthesis.[15,16] Direct C–H bond activation reaction has become an efficient and clean strategy for C–C coupling, because of its conciseness and economic aspect, according to the concepts of "green chemistry".[17,18] Different transition-metals are widely used for this purpose.[19] Among them, palladium, or palladium complexes are commonly used in many coupling reactions such as Suzuki–Miyaura,[20] Sonogashira,[21] Heck,[22] Hiyama[23] and Negishi.[24,25]

The binding site on AChE is a deep and narrow gorge and it consists of several domains: catalytic, anioinic, acylic, oxyanionic, and peripheral anionic.[26] The most important of them are the catalytic active sites (CAS), where ACh hydrolysis happens and the peripheral anionic site (PAS) is placed near the entrance of the gorge and associated with the formation of amyloid plaques.[27] Thus, AChE is a target with dual functionality: ACh hydrolysis and amyloid beta (Aβ) peptide aggregation. Because of its importance, AChE is a focus of many intensive and extensive drug discovery studies during the last two decades.[28] These studies could be grouped into three directions: the leading optimisation of known AChEIs, hybrids between them and search for new scaffolds. One of the most useful structure-based computational methods in the discovery of novel hits, binding to a specific target, is the molecular docking.[29–31]

In the present study, we conducted a docking-based virtual screening Auto Dock database for the novel active inhibitors, in order to identify the novel hits binding to AChE.

Considering the importance of benzofuran derivatives in acetylcholineesterase inhibitor agents and following our research on the green synthesis of of biologically active heterocycles,[32–35] herein, we decided to prepare novel disubstituted benzofurans via direct C−H activation reaction, with hopes to possess better anti acetylcholinesterase potencies (Scheme 1).
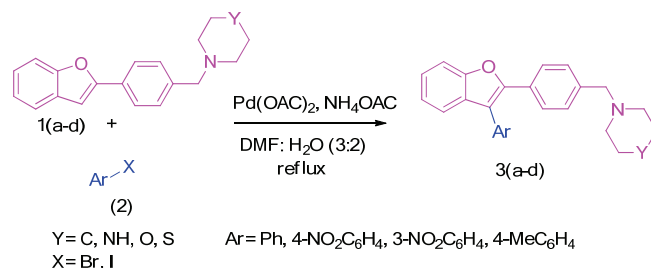
Scheme 1. Synthesis of novel disubstitud benzofurans via direct C−H bond activation reaction.

EXPERIMENTAL

Chemicals

All chemicals were purchased from Merck Company and were used without further purification.

Apparatus

Melting points were measured on an Electro thermal 9100 apparatus and they did not need corrections. The elemental analyses for C, H and N were performed using a Heraeus CHN-O-Rapid analyzer. IR spectra were recorded on a Shimadzu IR-460 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured (DMSO-d_6) with Bruker DRX-500 Avance (at 500.1 and 125.8 MHz) with TMS as an internal standard. Benzofuran derivatives **1a–d** were synthesized according to the reported procedure.[26]

Biological activity assays

AChE (E.C.3.1.1.7, Type VeS, from Electric eel), Ellman's reagent (DTNB), butyryl-thiocholine iodide (BTCh) and acetylthiocholine (ATC) were purchased from Sigma-Aldrich. 5,50-Dithiobis-(2-nitrobenzoic acid) (DTNB), potassium dihydrogen phosphate, dipotassium hydrogen phosphate potassium hydroxide and sodium hydrogen carbonate were purchased from Fluka.

Donepezil (Sigma) was used as reference drug for AChE inhibition. The AChE inhibitory activities of compounds **3a–d** were determined by using previously reported method.[30]

*General procedure for the synthesis of disubstituted benzofuran **3a–d***

A mixture of 1-(4-(benzofuran-2-yl)benzyl)derivatives **1a–d** (1.0 equiv.), NH$_4$OAc (3.0 equiv.), aryl halide (1.5 equiv.) and Pd(OAc)$_2$ (10 mol %) in DMF:H$_2$O (mole ratio 3:2, 5 mL) was refluxed for 8 h. The reaction progress was monitored by TLC using hexane/ethyl acetate (mole ratio 4:2) as an eluent. After completion of the reaction, the mixture was cooled to room temperature and then H$_2$O (4 mL) was added. The solid product was filtered, washed with cold water (3 mL), in order to obtain essentially pure products. The crude product was purified by flash chromatography on silica gel.

The structure of all the products was characterized by IR, ^{1}H- and ^{13}C-NMR spectra, along with the elemental analysis data and they have been identified by the comparison of spectral data and the melting point, for those obtained in authentic samples (see Supplementary material to this paper).

RESULTS AND DISCUSSION

Initially, we studied the coupling reaction of 1-(4-(benzofuran-2-yl)benzyl)-piperidine (1 mmol) and bromobenzen (1.5 mmol) in the absence and in the presence of Pd(OAc)$_2$. The catalyzed-reaction was optimized by the various ligand, base and solvents, presented in Table I. In the absence of catalyst, the coupling reaction did not proceed and no product was observed, even after the prolonged reaction time (Table I, entry 1). Since, the synthesis of disubstituted benzofuran failed in the absence of Pd(OAc)$_2$ catalyst, the effect of Pd(OAc)$_2$ was also investigated in various conditions (Table I, entries 2–12). With respect to the solvent system, the best result was obtained using DMF:H$_2$O (mole ratio 3:2) (Table I, entry 10). Additionally, we have attempted different ratios of Pd(OAc)$_2$ (3, 5, 10, 12, and 15 mol %) and observed that 10 mol % of the catalyst was suitable for the optimum conversion (Table I, entries 10–12). The increase in the mole ratio of Pd(OAc)$_2$ also did not improve the yield of reaction (Table I, entry 12).

TABLE I. Screening solvent, ligand and base conditions synthesis of the synthesis of 1-(4-(3--phenylbenzofuran-2-yl)benzyl)piperidine. Reaction conditions: 1 mmol of 1-(4-(benzofuran--2-yl)benzyl)piperidine (1a) and 1.5 mmol of bromobenzen (2)
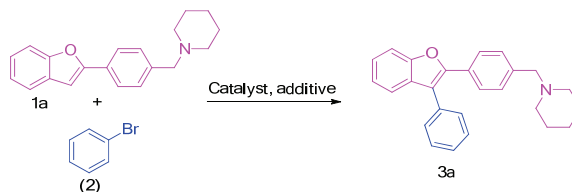
Entry	Content of Pd(OAc)$_2$, mol %	Ligand	Additive (content, mol)	Solvent (mole ratio)	Time h	Yield[a] %
1	–	–	K$_2$CO$_3$ (2)	DMF	24	0
2	10	PPh$_3$	K$_2$CO$_3$ (3)	Dioxane	12	50
3	10	PPh$_3$	K$_2$CO$_3$ (3)	Dioxane	14	55
4	5	PPh$_3$	Na$_2$CO$_3$ (3)	Dioxane:H$_2$O (2:1)	12	50
5	5	1,10-Phenanthroline	K$_2$CO$_3$ (3)	DMF	24	20
6	10	1,10-Phenanthroline	Na$_2$CO$_3$ (2)	DMF:H$_2$O (2:1)	24	30
7	5	PPh$_3$	Cs$_2$CO$_3$ (3)	DMA	24	40
8	10	10-Phenanthroline	Cs$_2$CO$_3$ (3)	DMF	24	30
9	15	PPh$_3$	NH$_4$OAc (2)	Dioxane	24	60
10	10	PPh$_3$	NH$_4$OAc (3)	DMF:H$_2$O (3:2)	12	80
11	5	PPh$_3$	NH$_4$OAc (3)	DMF:H$_2$O (3:2)	14	70
12	12	PPh$_3$	NH$_4$OAc (3)	DMF:H$_2$O (3:2)	12	80

[a]Yields refer to pure isolated yields

Screening the ratios of starting materials revealed that when 1.5 mmol of bromobenzen was used, the reaction proceeded successfully and gave the corresponding product in good yield.

A base screen revealed that NH_4OAc (3 mmol) gave the best reactivity in this coupling reaction (Table I, entries 9 and 10).

We were pleased to find that the use of the $Pd(OAc)_2$ in the presence of NH_4OAc and PPh_3, as base and ligand, respectively in $DMF:H_2O$ (mole ratio 3:2) provided the desired 1-(4-(3-phenylbenzofuran-2-yl)benzyl)piperidine (**3a**) in 80 % isolated yield (Table I, entry 10).

Furthermore, we have also examined the coupling reaction of 1-(4-(benzo-furan-2-yl)benzyl)piperidine, with iodobenzen in the same conditions. It is interesting that the yield of the compound **3a** increases to 88 % after 8 h reflux (Table II, entry 1).

Encouraged by this result, we synthesized several disubstituted benzofurans by the reaction of various substituted benzofurans with aryl halides, *via* the coupling reaction. The results are presented in Table II.

TABLE II. Synthesis of 2,3-diarylbenzofurans
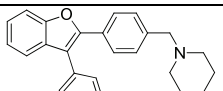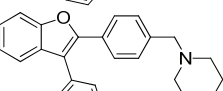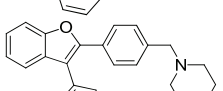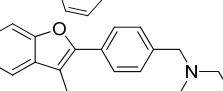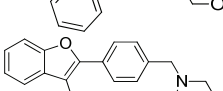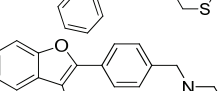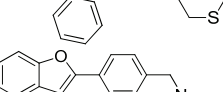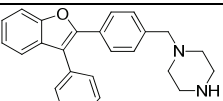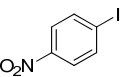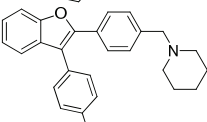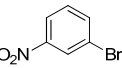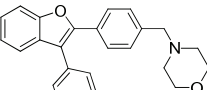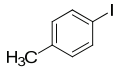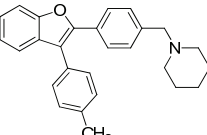
Entry	Ar–X	Product	Time, h	Yield[a], %
1			8	88
2			12	80
3			11	67
4			12	65
5			10	78
6			12	72
7			8	70

TABLE II. Continued

Entry	Ar–X	Product	Time, h	Yield[a], %
8			10	75
9			8	70
10			16	68
11			24	65

[a]Isolated yields

In the continuation of the research, the newly synthesis compounds **3a**–**d** were evaluated for their AChE inhibitory activities, using the modified colorimetric Ellman's method.[29] The stock solutions of the target compounds were prepared in a mixture of DMSO (1 mL) and ethanol (9 mL) and diluted with 0.1 M MKH_2PO_4/K_2HPO_4 buffer (pH 8.0) to obtain the final concentrations. Next, 20 mL of substrate (acetylthiocholine iodide 0.075 M) was added to the test solution to obtain the final concentration of 466 mM. The spectrophotometric measurements were performed on a UV Unico double beam spectrophotometer.

The inhibitory potency of the synthesized compounds **3a**–**g** toward AChE were determined, and compared with donepezil as the reference drug, reported in Table III.

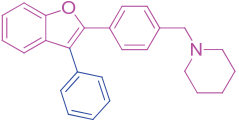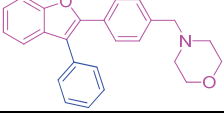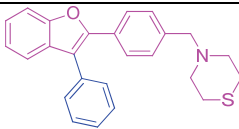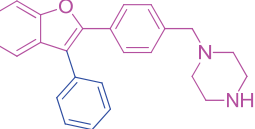TABLE III. *In vitro* inhibitory activity of compounds **3a**–**g** against AChE
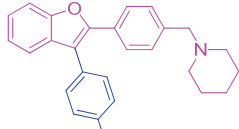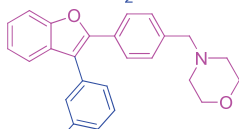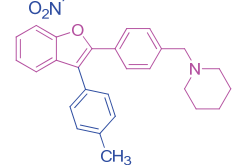
Entry	Compound	AChE IC_{50}[a], μM
1		24.46±1.8
2		34.25±2.1

TABLE III. Continued

Entry	Compound	AChE IC_{50}^{a}, µM
3		38.12±3.1
4		68.12±3.5
5		39.12±3.2
6		56.36±3.4
7		54.35±3.3

[a]Data are expressed as Mmean ± *SD* (three independent experiments)

These results show that the heterocycles containing nitrogen were more favourable than the heterocycles with more than one heteroatom. In this regard, the compounds **3d** with piperazine substituent, exhibited better activities against the AChE with 68.12 % inhibition. No improvements were observed in thiomorpholine, morpholine and piperidine derivatives (Table III).

Molecular docking analysis

The modeling study that was performed in this paper showed intensive interactions between **3a**, **3b**, **3c**, **3d** and human acetylcholinesterase, Fig. 2. The lowest-energy complexes were accepted as the calculated binding energy and its inhibition constant (K_i) value was used to define the binding affinity of the inhibitors and listed in Table I. The geometry of docking, obtained with the each of them with human acetylcholinesterase was shown in Fig. 1A–D, respectively. The four inhibitors were able to form hydrogen bonds (HBs) with the amino acid residues of the enzyme, hydrophobic and electrostatic interactions. In addition, the molecular docking results showed that other amino acids residues are involved in the interactions with the four inhibitors, Table IV.
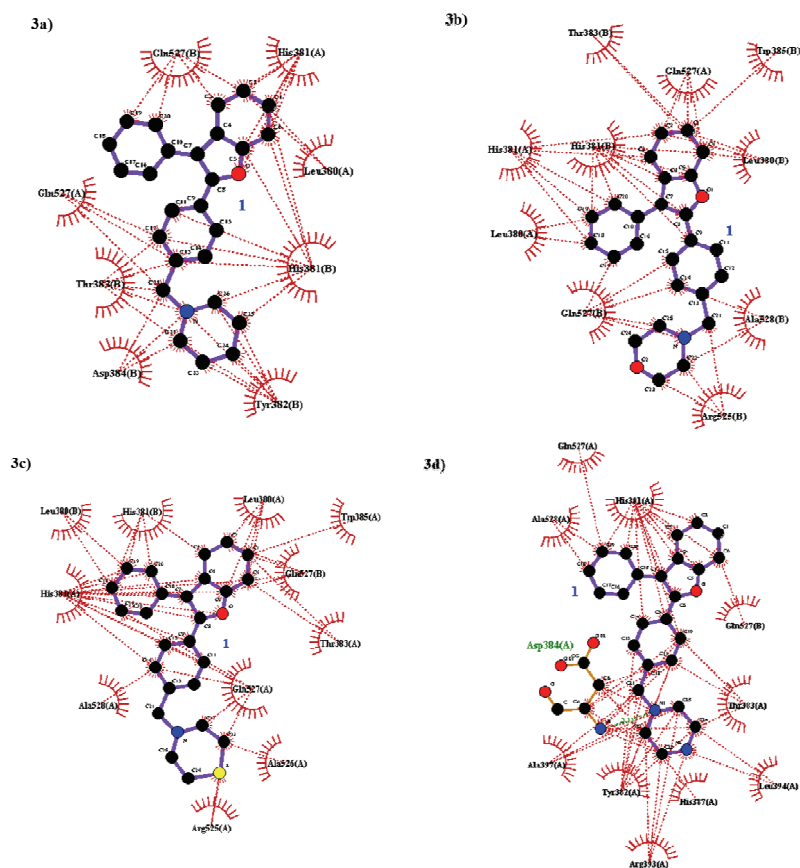
Fig. 2. LIGPLOT of hydrophobic and polar contacts between **3a**, **3b**, **3c** and **3d** and amino acid residues in acetylcholinesterase.

TABLE IV. AutoDock results (free binding energy, inhibition constant and intremolecular energy) of the novel inhibitors docked with acetylcholinesterase

Cmpd.	Free binding energy, kcal*/mol	K_i / nM	Intermolecular energy, kcal/mol
3a	−10.70	14.33	−11.30
3b	−10.18	34.45	−10.78
3c	−10.12	37.8	−10.72
3d	−11.02	8.39	−11.61

During all these interactions, the hydrogen bond between ligand and enzyme is the most important, because in most cases it can determine the binding strength and the location of ligand, whereas the hydrophobic interaction of some certain groups can affect the inhibition specialty to a large extent. The calculated inter-action energies of all complexes are in negative, which shows that **3a**, **3b**, **3c** and

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* 1 kcal = 4184 J

3d are potent inhibitor of acetylcholinesterase. In agreement with the experimental results, the inhibitory potency of the synthesized compound **3d** toward AChE was higher than the other compounds.

CONCLUSIONS

To summarize, we have designed and synthesized some novel disubstituted benzofuran derivatives *via* carbon–carbon coupling reaction. The preliminary biological activities screening tests indicated that these synthesized derivatives **3a**–**g** were identified to be moderately anti-acetylcolinestrase active. These researches showed the key role of benzofuran scaffold in AChE inhibition.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: .https://www.shd-pub.org.rs/index.php/JSCS/index, or from the corresponding author on request.

ИЗВОД
ДИЗАЈН, СИНТЕЗА И ИСПИТИВАЊЕ БИОЛОШКЕ АКТИВНОСТИ НОВИХ ДЕРИВАТА СУПСТИТУИСАНИХ БЕНЗОФУРАНА ДОБИЈЕНИХ АКТИВАЦИЈОМ С–Н ВЕЗЕ

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Серија биолошки активних дисупституисаних деривата бензофурана (**3a**–**d**) је синтетисана применом реакција које се одвијају активацијом С–Н везе. Структура свих финалних производа је потврђена спектроскопским методама. Резултати испитивања *in vitro* резултата инхибиције активности ацетилхолин естераза (AChE) показала су да нова једињења показују ниску до умерену инхибиторну активност. Од тестираних једињења дериват **3d** показује умерену инхибиторну активност од 68,12 %.

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