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Synthesis and Molecular Docking Study of Novel Pyridine Derivatives as Cholinesterase Inhibitors

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Abstract

Alzheimer's disease (AD) which is the most commonly diagnosed cause of dementia in the elderly, is involved with a loss of presynaptic cholinergic function in the areas of the brain related to memory and learning. Preserving acetylcholine (ACh) levels by enhancement of cholinergic neurotransmission is a useful method for preventing AD occurrence and progression. In this study, a novel series of pyridine derivatives (2, 6-dimethyl-4-(2-aminophenyl)-3, 5-pyridine dicarboximide), were designed and synthesized as potent acetylcholinesterase (AChE) inhibitors. The structure of synthesized compounds was elucidated by 1HNMR, 13CNMR, FT-IR, and Mass spectroscopy techniques. Also, molecular docking studies were performed to evaluate the probable interaction between the synthesized compounds and the AChE enzyme. Based on the molecular docking study, all the proposed compounds could occupy the active sites of AChE and so may be considered as AChE inhibitors. According to the calculated binding energies, the representative compound 5c bearing para-fluorobenzyl substituent in amide moiety could be the best potent compound in this study. In-vitro and In-vivo studies are needed to confirm the In-silico results.

Keywords: Pyridine derivatives • Molecular docking • Anti-Cholinesterase • Alzheimer's disease

Introduction

Alzheimer's Disease (AD) is one of the neurodegenerative disorders and the most commonly diagnosed cause of dementia in the elderly. It is involved with a loss of presynaptic cholinergic function in the areas of the brain related to memory and learning. Preserving acetylcholine and butyrylcholine levels by enhancement of cholinergic neurotransmission is a useful method for preventing AD occurrence and progression. Hence, a promising strategy for drug development in AD treatment is to suppress acetylcholinesterase (AChE) from breaking down ACh. AD is also associated with the formation of the senile plaques mostly including amyloid-beta (Aβ) as well as Neuro Fibrillary Tangles (NFTs) formation in the patients' brain The other factors which are correlated with AD proposed to be oxidative stress, τ-protein aggregation and dyshomeostasis of bio-metals. It has been shown that the AChE enzyme owns two binding sites, including catalytic anionic site (CAS) and peripheral anionic sites (PAS). It is also assumed that blocking PAS of AChE slows down inhibition of Aβ aggregation besides; the anionic site accommodates the positive quaternary amine of acetylcholine and hydrolyzes it to acetate and choline. Accordingly, for the management of AD, the most effective agents are the multi-binding inhibitors which can prevent the self-assembly of Aβ and inhibit the catalytic activity of AChE. Therefore, current pharmacotherapy of AD is based on the amendment of cholinergic activity with cholinesterase inhibitors. A cholinomimetic drug can act as a cholinergic neurotransmitter that is impervious to acetylcholinesterase's lysing action [1].

It is proposed that the amine functional group is necessary for interaction with the catalytic site of AChE. Furthermore, the ligands with aromatic and heteroaromatic rings may establish favorable stacking interactions with PAS. A large number of multifunctional anti-AD molecules have been designed and synthesized from which, pyridine derivatives are the most attractive compounds due to their cholinesterase inhibiting ability. Pandolfi et al. synthesized a new series of pyridine derivatives with the amide groups and proposed them as anticholinesterase compounds. In the other study, 2-amino-4, 6-dimethylpyridine derivatives were considered for anticholinesterase property. Besides, the therapeutic role of the pyridine derivatives and pharmaceutical effects of 1, 4-dihydropyridine derivatives have been studied. Anticholinesterase activity of some imidazopyridine derivatives has also been

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investigated previously. El-Sayed et al. synthesized novel pyrrolizine-based compounds and evaluated their AChE inhibitor potency by in-vitro and in-vivo studies. Another study on pyrazoline-based compounds as potent AChE inhibitors has been reported by Tugrak et al. Their results indicated high AChE inhibitor potency for one of the synthesized compounds. Recently, a series of benzylpiperidine-linked 1, 3-dimethylbenzimidazolinone derivatives were reported as cholinesterase inhibitors and in vitro assays showed the effectiveness of most of the synthesized compounds on ChEs in the micro molar range. Based on the previous studies on cholinesterase inhibitors, herein, we describe the design, synthesis, and molecular docking simulation studies of new pyridine derivatives, as novel, and potential inhibitors of AChE [2].

Materials and Methods

General

All commercially available reagents and solvents in this research were of analytical grade purity and procured from Merck, Germany; Nifedipine was obtained from Zahravi Ltd. (Tabriz, Iran); 1HNMR and 13CNMR spectra were recorded on a Varian (Inova 500 MHz, USA) spectrometer and chemical shifts were expressed in (ppm) using tetramethylsilane (TMS) as the internal standard. The IR spectra were run on an Agilent (Magna-IR 559 spectrophotometer, USA using KBr disks). The mass spectra of the products were obtained with an Agilent (5977B GC/MSD Mass spectrometer, USA). Melting points were measured by (Gallenkamp electrothermal, UK) apparatus. The formation of the compounds was monitored by thin-layer chromatography Merck silica gel (60 F254).

General procedures

Oxidation of dihydropyridine ring: In a 250 mL flask, nifedipine (2mmol, 0.69 g) (compound 1) was solved in dichloromethane (30 mL). Then, MnO2 (28.75 mmol, 2.5g) was added and the reaction mixture was stirred for 4 h. Termination of the reaction and formation of the desired product was confirmed using TLC by the mixture of ethyl acetate/petrol ether (1:1) as the solvent. After completion of the reaction (24 h), the solid material was removed by filtration using a sintered glass funnel covered by diatomaceous earth. Next, the resulting residue was extracted by methanol (3 × 20 mL). Finally, the solvent was evaporated under vacuum to afford the pyridine derivative, compound 2 insufficient purity.

Hydrolysis of the ester groups: Compound 2 was dissolved in methanol (30 mL) and was added concentrated HCl. The solution was refluxed at 180 °C for 48 h. The formation of the desired product was monitored by TLC. The solvent was reduced to 30 mL and, the solid material was filtered and dried at room temperature. The crystallization of the product was carried out by chloroform to afford compound 3.

Amidation of the carboxylic acid groups: In a flat bottom flask, the compound 3 (1 mmol, 0.26 g) was solved in acetonitrile (15 mL) with the addition of a few drops of trimethylamine. Then, N-hydroxy benzotriazole (HOBT) (2 mmol, 0.27 g) and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDCI) (2 mmol, 0.31 g) were added and the mixture was stirred for 2 h. at room temperature. In this step, we used five different amines for an amidation reaction to synthesize five different derivatives. For this purpose, the appropriate amine (1-phenyl ethylamine, 4-methoxyaniline, 4-fluoro benzylamine, 4-methyl aniline, and benzylamine) was added to the final mixture and stirring continued for 24 h. After completion of the reaction (monitored by TLC; ethyl acetate/ petrol ether; 1:1), the mixtures were separately extracted by the solvents: 10% NaCl, 10% NaHCO3, and 10% Citric acid, (2 x 50 mL) respectively. In each step, the aqueous phase was removed and, the extraction was continued for the organic phase. At last, the organic phase was dried over NaHSO3 and removal of the solvent under reduced pressure, afforded the desired compounds: 4 (a-e). The formation of the products was confirmed by using TLC (ethyl acetate/petrol ether; 1:1).

Reduction of the nitro groups: The nitro groups of each compounds 4(a-e) were reduced to obtain the desired amine derivatives. For this reason, each of the compounds 4 (a-e) (1 mmol), was solved in methanol/water (50 mL, 7:1) and, then ammonium chloride (6 mmol, 0.32 g) was added and the mixture was stirred at room temperature. Then, Zn powder (2 mmol, 0.13 g) was added during 4-5 min until the reaction completed (monitored by TLC, ethyl acetate/ petrol ether, 1:1). Finally, the precipitated solid was removed by filtration and the solution was cooled on ice, until the desired products 5 (a-e) precipitated and were flittered off from the solutions. The synthesized compounds 4 (a-e) and 5 (a-e) are as follow.

Molecular docking study: To investigate the anticholinesterase activity of synthesized compounds, molecular docking study was performed using Autodock Vina 4.2 with 120 runs using the Lamarckian genetic algorithm (LGA). As the first step, the crystal structure of AChE enzyme complexed with donepezil (PDB Code: 1EVE) was retrieved from the protein data bank website as docking template for the compounds. Then, all the unbound molecules were eliminated from the pdb file. The prepared pdb file was used as the receptor. All the compounds structures were sketched and 3D-optimized by Marvine Sketch 15.8.1, 2015, ChemAxon. The grid box was set on the coordinates of the complexed ligand. After analysis of the results, the most desirable conformation with the minimum free energy of binding interaction with the receptor was represented using Discovery Studio visualizer 4.5 software.

Results and Discussion

The pyridine derivatives 4 (a-e) and 5 (a-e) were synthesized using Nifedipine as a precursor via the route outlined in Scheme 1.

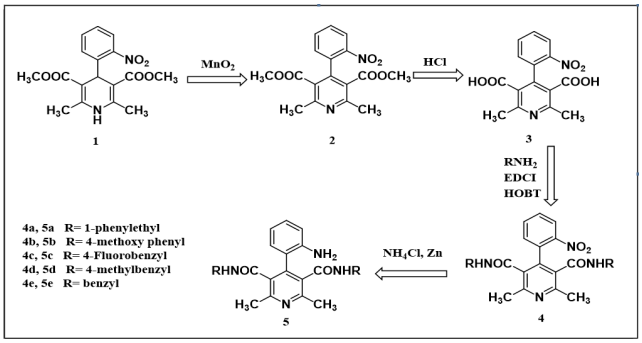

Figure 1: The synthesis procedure of designed compounds 4(a-e) and 5(a-e).

As is shown in, in the first step, active pharmaceutical ingredient nifedipine was oxidized by manganese dioxide to convert dihydropyridine to pyridine derivative. Then, the ester functional groups of compound 2, were hydrolyzed to the corresponding carboxylic acid groups by using 37% acid chloride. Next, we used five different amines to convert the carboxylic acid groups of the compound 3 to the amide groups in the presence of EDCI and HOBT (activating the carboxylic acid groups and converting them to the good leaving groups). Acetonitrile and trimethylamine (1:1) were used as the solvent in this reaction to afford the desired compounds (4a-e). The final reaction was performed by reducing the nitro groups of the compounds (4a-e), to the amino groups in the presence of zinc powder and ammonium chloride and methanol/water (1:1) as the solvent [3]. The target compounds (5a-e) were purified by thin-layer chromatography.

Molecular docking results

A computational method which is used for searching the mechanism and interactions between protein and molecules is molecular docking simulation. In this study, the interactions between the five pyridine derivatives and AChE enzyme was studied by molecular docking simulation using the Auto Dock Vina 4.2 program. Two important parameters for comparing the inhibition activity, binding energy (Kcal) and inhibition constant (μM) were evaluated in this study (Table 1). The binding energy indicates how strongly the molecule and the enzyme are bound together. The inhibition constant is calculated as Ki = exp ((ΔG × 1,000)/ (Rcal × T(K)), where ΔG (Kcal/mol) is the docking energy, R (cal) is 1.98719, and T (K) is 298.15. There is a reverse relation between the binding energy and the inhibition constant. With increasing the inhibition activity (more negative binding energy) of compounds, the inhibition constant will decrease. Based on the molecular docking study, all the compounds 5 (a-e) showed inhibition activity against the cholinesterase enzyme. The best results was found for the compound c with binding energy (ΔG = -11.6 Kcal/mol) and the inhibition constant (ki=0.336 μm). It looks that the presence of halogen atom has an important role in this regard.As represented in Fig. 1 the compound (5a) was located in the hydrophobic pocket surrounding three amino acids Phe329, 330, and Tyr333, and also bounded to the mid gorge recognition site via π-π interaction with Phe329. The amino group of the aniline ring was involved in a hydrogen bond with Tyr333 that is located at Peripheral Anionic Site (PAS), and another hydrogen bond was formed between the nitrogen of pyridine moiety of the compound and the hydroxyl group in Tyr69. The other parts of the compound participated in the hydrophobic interactions. The compound (5b) also fitted in the hydrophobic pocket of the enzyme as the compound (5a). In spite of the hydrogen bond interactions between the methoxy group of the compound and the two amino acids Ser199 and Gly117, the hydrophobicity of the methoxy group provided the mismatch hydrophobic interaction in the pocket, therefore, the binding energy of the compound (5b) (ΔG = -9.9 Kcal/mol) was less than the compound (ΔG = -10.2 Kcal/mol) and also less than all congeners in this study.

Compounds	ΔG (Kcal/mol)	Ki (μM)
5a	-10.2	0.351
5b	-9.9	0.357
5c	-11.6	0.336
5d	-10.2	0.35
5e	-10.8	0.344

Table 1: Molecular docking results of pyridine derivatives with AChE.

The compound 5c which was found as the most potent compound among the other synthesized compounds (ΔG = -11.6 Kcal/mol), also fitted in the hydrophobic pocket however in a different way [4]. The aniline ring of the compound made parallel stacking with the five-member ring of Trp83 and formed a π-π stacking interaction. On the other hand, a halogen bond was formed between the fluorine atom of the compound (5c) and the oxygen atom in Glu198. Moreover, two hydrogen bonds were established between the nitrogen in amide moiety of the compound and the hydroxyl groups in the amino acids Tyr120 and Ser121 [5]. In addition, there are a number of hydrophobic interactions between the compound and the amino acids Tyr120, 69; Trp278, 83, and Phe289, 329. The compound (5d) also adopted in the hydrophobic pocket (ΔG = -10.2 Kcal/mol) like the compound (5a). Furthermore, two π-π stacking interactions were formed between the phenyl rings of the compound and the amino acids Phe329 and Tyr333. In the case of compound (5e), a π-π stacking interaction was made between the aniline ring of the compound and the phenyl ring of Phe329. Likewise, two hydrogen bonds were formed between the nitrogen of amide moiety and the hydroxyl groups present in Ser121 and Tyr120. The binding energy for the compound (5e) was calculated (ΔG = -10.8 Kcal/mol).

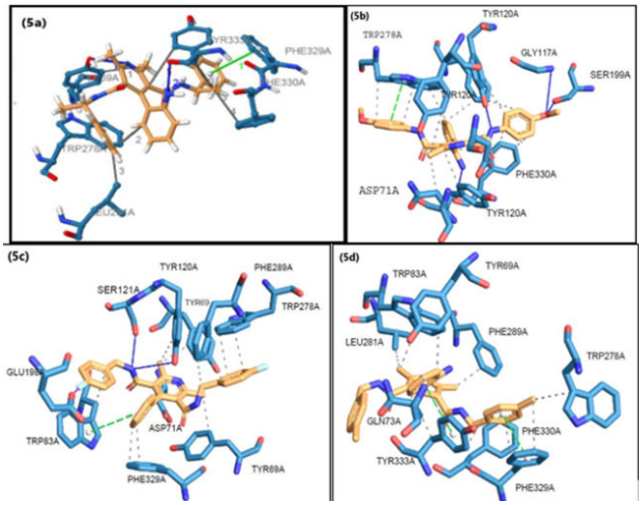

Figure 2a: Docking of compound 5a with the active site of AChE; hydrogen bonds (blue), hydrophobic interactions (gray), π-π stacking interaction (green); b: Docking of compound 5b with the active site of AChE; hydrogen bonds (blue), hydrophobic interactions (gray), π-πstacking interaction (green); c) Docking of compound 5c with the active site of AChE; hydrogen bonds (blue), hydrophobic interactions (gray), π-π stacking interaction (green) and halogen bond (pink); d) Docking of compound 5d with the active site of AChE; hydrophobic interactions (gray), π-π stacking interaction (green).

Conclusion

In conclusion, five new pyridine derivatives synthesized in this study were evaluated as AChE inhibitors by molecular simulations.

Based on molecular docking results, all the compounds were fitted in the hydrophobic pocket of the enzyme as well. Hydrogen bond interactions and π-π stacking interactions had an important role in the connection between AChE enzyme and the pyridine derivatives. In the case of the compound 5c, formation of a halogen bond between Florine atom of the compound and Oxygen atom of Glu198 in enzyme had an additional role in binding energy. Based on this results, the five synthesized compounds can serve as promising leads for inhibition of AChE enzyme. Certainly, these results will not be reliable until they are biologically tested. This is just a suggestion in introducing the compounds that are easily synthesized and have the potential to inhibit the enzyme cholinesterase. In-vitro and in-vivo studies are needed to confirm the in-silico results.

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