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Biological Evaluation and Synthesis of Benzothiazole- Piperazine Derivatives as Probable Cholinesterase Inhibitors to Treat Alzheimer's Disease

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ABSTRACT

Background: Alzheimer's disease is the most prevalent cause of progressive dementia among older adults. One of the main causes of the disease is deficiency of the cholinergic system. Nevertheless, some acetyl cholinesterase inhibitors have played a significant role in controlling disease progression.

Aim and objectives: In continuation of the development of new acetylcholinesterase inhibitors, benzothiazole-piperazine derivatives as probable inhibitors were synthesized and evaluated.

Materials and Methods: Acylation of benzothiazole-2- amine with chloroacetyl chloride yielded 2-chloro-N-(benzothiazole-2-yl) acetamide which undergoes a substitution reaction with piperazine to produce intermediate 3. Reaction of intermediate 3 with the appropriate acyl chlorides or alkyl chloride produced the final compounds. Enzyme inhibitory potency was assessed by Ellman's test. The inhibitory activity of compounds on acetyl cholinesterase and butyryl cholinesterase enzymes was expressed as IC_{50}. Docking of the final derivatives in the active site of acetylcholinesterase (PDB ID: 1EVE) was performed.

Results: Unfortunately, the compounds failed to show inhibitory effects on cholinesterase enzymes with IC_{50}>100 µM values when compared with donepezil with IC_{50} values 0.014 ± 0.0012 and 14.4 ± 15 µM on acetyl cholinesterase and butyryl cholinesterase enzymes respectively. Compound **4c** with ΔG = -10.53 Kcal/mol was the best compound in docking studies.

Keywords: 2-Aminobenzothiazole; Acetylcholinesterase; butyrylcholinesterase; Ellman's test; Piperazine.

1. INTRODUCTION

Alzheimer's disease (AD) causes a progressive dementia in the geriatric people. The disease is associated with memory deficiency, anxiety and depression. Several factors including oxidative stress, deposit of abnormal amyloid beta-peptide (Aß) and degenerated cholinergic neurons in the brain are known as the etiology of the disease [1-4]. Acetyl cholinesterase (AchE) inhibitors, an enzyme responsible for the degradation of the acetylcholine in the synaptic gap, have been partially effective in treating Alzheimer's symptoms [1]. The most important scaffolds that have been shown inhibitory activity on this enzyme are thiazole [5], piperazine [3, 4], phthalimide [6-8], benzoxazole [9], benzothiazole [10-12], indanone [13], chalcone and coumarin based compounds and isatin [1, 14, 15] derivatives. Five ligand-binding sites including the catalytic triad, the oxygen anion hole, the catalytic anionic site (CAS), an acyl pocket, and the peripheral anionic site (PAS) have been identified on the AChE enzyme [2, 16]. According to the literature,

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structural requirements to design AChE inhibitors must contain a core ring, and a basic center to interact with PAS and CAS, respectively (Figure 1). The existence of a linker between the core and the basic center is also essential. In this study, benzothiazole and piperazine were considered as core ring systems, and basic centers respectively through a linker amide. Due to cholinesterase inhibitory effects reported for benzothiazol [10-12], piperazine [3, 4, 17] and benzothiazole-piperazine [10, 18-20] derivatives, these scaffolds are suitable candidates to design cholinesterase inhibitor agents. Some benzothiazole derivatives demonstrated anti-AD activity [16, 21-24] where sabeluzole with benzothiazole scaffold has been shown to delay the clinical progression of AD (Figure2). Consequently, benzothiazole–piperazine derivatives were prepared and evaluated on the AChE enzyme by Ozkay et al [16]. In this research, benzothiazole-piperazine derivatives were synthesized and assayed against (AChE), butyrylcholinesterase (BChE) enzymes through the Ellman method. Docking of the final derivatives in the active site of acetylcholinesterase (PDB ID: 1EVE) was performed in order to study interactions with the enzyme.

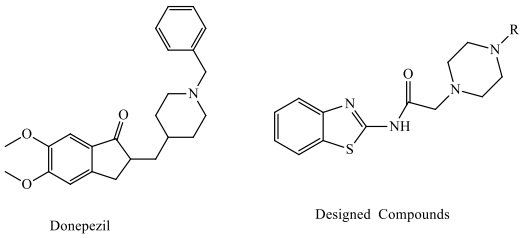


Figure-1 Structural exigencies in design of ACE inhibitors

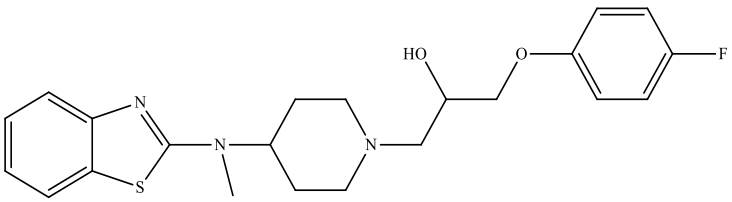


Figure-2 Structure of Sabeluzole

2. MATERIALS AND METHODS

Materials were prepared from the companies Merck (Germany) and Samchun (Korea) companies. Bruker400 MHz was used for recording proton nuclear magnetic resonance spectrometer (^{1}HNMR) spectra. Infrared (IR) (ν_{max} cm^{-1}) spectra were recorded by a WQF-510 IR spectrophotometer [China]. Electrothermal 9200 instrument [England] determined melting points of final products. Spectra of mass were registered using Agilent Technologies 5975C mass spectrometer (USA). Silicagel 60 plates (Germany) F_{254} were used for confirmation the end of the reaction.

2-1. N-(Benzo[d] thiazol-2-yl)-2-chloroacetamid (2)

After dissolving benzothiazole -2-amine **1** (0.05mol) in dry acetone (80 mL) an equimolar amount of chloroacetyl chloride was spilled and stirred in room temperature for 4h [25]. As soon as the reaction is complete, the, solvent was removed leaving a residue that was neutralized with sodium bicarbonate solution (5%). The mixture was filtered, and recrystallized from ethanol (10mL) after being washed with water (2 ×5mL) (Scheme 1).

2.2. N-(Benzo[d] thiazol-2-yl)-2-(piperazine-1-yl) aceamid (3)

To a solution of piperazine (0.04 mol) in anhydrous dichloromethane (60 mL), N-(benzo[d] thiazol-2-yl)-2-chloroacetamid 2 was added drop wise at 0 °C, (0.008 mol). The reaction was stirred for 8 h at 0 °C [26]. The pale yellow solution was washed with a saturated NaHCO3 solution (2 ×25 mL), dried with magnesium sulphate, filtered and concentrated under a vacuum. Absolute EtOH (15mL) was added and mixture filtered to remove a white residue. The solution was concentrated to afford 3 (Scheme 1).

2.3. N-(Benzo[d] thiazol-2-yl)-2-(4-benzoyle derivatives) piperazine-1-yl) aceamid (4a and 4b)

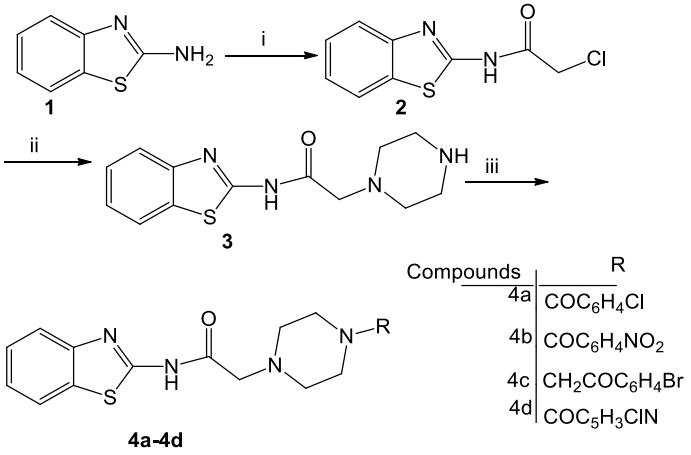
To compound 3 (0.02 mol) and triethylamine (Et_3N) in dry tetrahydrofuran (THF, 35 mL), [27] equimolar of benzoyl chloride derivatives (4-chlorobenzoyl chloride or 4-nitro benzoyl chloride) were added drop wise and stirred at room temperature until formation of product which was collected by filtration , and recrystallized from methanol (10mL)(Scheme 1).

2.4. N-(Benzo[d]thiazol-2-yl)-2-(4-(2-(4-bromophenyl)-2-oxoethyl) piperazine-1-yl) acetamide (4c)

Equimolar of compound **3** (0.02 mol), triethylamine and 2, 4´-dibromoacetophenone were mixed in acetonitrile solvent (CH_3CN) (35 mL) and refluxed for 24 h. Then acetonitrile was removed. The residue was recrystallized from ethyl acetate to obtain **4c** (Scheme 1).

2.5. Preparation of N-(benzo[d]thiazol-2-yl)-2-(4-(6-chloronicotinoyl) piperazine-1-yl) acetamide (4d)

6-Chloronicotinoyl chloride solution (0.02 mol) in THF (10 mL) was slowly spilled to solution of intermediate 3 (0.02 mol) in THF (15 mL) and pyridine. The solution was stirred at room condition for 2 h until formation of product which was collected, washed with water and recrystallized from methanol (Scheme 1).



Scheme -1 (i) Acetone, Chloroacetylchloride, Room temperature, (ii) piperazine, dichlorometan,0˚C, (iii) for 4a and 4b: Et_3N,THF,Benzoyl chloride derivatives, Room temperature; (iii) for 4c: Et_3N, CH_3CN, 2, 4´-dibromoacetophenone; (iii) for 4d: 6-Chloronicotinoyl chloride, THF, pyridine.

2.6. Inhibitory activity studies of ChEs

Activity of compounds **4a-d** against both AChE and butyry lcholinesterase (BChE) enzymes were assayed using Ellman's spectrophotometric method. Derivatives were dissolved in dimethylsulfoxide (DMSO) and diluted with phosphate-buffered saline (pH 8.0) PBS to yield a final concentration range. Donepezil and galantamin were used as references. The results are detailed in Table 1 [28, 29]. IC_{50} values were obtained by plotting inhibition percentage versus inhibitor concentrations.

2.7. Molecular docking

Hyperchem7.0 software was used for drawing three-dimensional (3D) structures of the ligands and subsequently optimized using the molecular mechanical force field (MM^+) and 3D geometry optimization calculations. The final conformations were calculated using the semi-empirical AM1 method and the Polak Ribiere conjugate gradient algorithm was used to optimize the molecular structures. These optimized structures were used by AutoDock Tools for the preparation of PDBQT files. Acetyl cholinesterase crystal structure in complex with donepezil (PDB ID: 1EVE; 2.5 Å) was obtained from the protein data bank (www.rcsb.org) [1, 8]. PDBQT file of protein was prepared by AutoDock Tools [30-34]. Gridbox dimensions were 56 × 62 × 58 with a 0.375 Å grid points spacing. The grid box was centered with the coordinates x =2.79 Å, y = 62.8 Å, z = 65.02Å. Standard procedure and default parameters of AutoDock 4.2 software were performed for docking. After 100 runs for each ligand, Root mean square deviation tolerance of 2.0 Å was used for conformation clustering. Conformations were ranked based on the binding free energy.

(3)

Yield: 30%; Pale yellow solid, 3423, 3295 (NH), 2945 (C-H, Aliphatic), 1694 (C=O) (cm^{-1}), ^{1}HNMR: ($CDCl_3$): δ 8.1 (NH, s), 7.86 (1H, d, *J*=8Hz), 7.82 (1H, d, *J*=8Hz), 7.49 (1H, t, *J*=8Hz), 7.37 (1H, t, *J*=8Hz), 3.38 (2H, s, CH_2), 3.74-3.71 and 3.57-3.54 (4H, m, H-piperazine), 2.72-2.65 (4H, m, H-piperazine), 2.25 (NH), m.p.110 -113 °C.

(4a)

Yield: 33%; White solid, 3399 (NH), 3050, 834 (C-H, Ar), 2936 (C-H, Aliphatic), 1697, 1637 (C=O), 1557, 1604, (C=C), 1261 (Ar-Cl) (cm^{-1}), ^{1}HNMR: (DMSO;d_6): δ 12.19 (1H, NH, s), 7.99 (1H, d, *J*=8Hz, H-benzothiazole), 7.75 (1H, d, *J*=8Hz, H-benzothiazole), 7.52 (2H, d, *J*=8Hz, H-chlorobenzoyl), 7.46-7.43 (3H, m, H-benzothiazole and H-chlorobenzoyl), 7.32 (1H, t, *J*=8Hz, H-benzothiazole), 3.43 (2H, s, CH_2), 3.39-3.35 (4H, m, piperazine), 2.62-2.56 (4H, m, piperazine) ; MS (m/z, %): 414 (M^+,5), 416 (M+2, 1.6) for $C_{20}H_{19}ClN_4O_2S$, M.W. 414.9g/mol, m.p.203-206 °C.

(4b)

Yield:45%; Yellow solid , 3416 (NH), 2839 (C-H, Aliphatic), 1638 (C=O), 1601,1439 (C=C), 1539, 1349 (NO_2) (cm^{-1}), ^{1}HNMR: (DMSO;d_6): δ 12.2 (1H, NH, s), 8.30 (2H, d, *J*=8Hz, H- nitrorobenzoyl), 7.99 (1H, d, *J*=8Hz, H-benzothiazole), 7.75 (1H, d, *J*=8Hz, H-benzothiazole) 7.68 (2H, d , *J*=8Hz, H- nitrorobenzoyl), 7.45 (1H, t, *J*=8Hz, H-benzothiazole), 7.32 (1H, t, *J*=8Hz, H-benzothiazole), 3.43 (2H, s, CH_2), 3.75- 3.69 (2H, m, piperazine), 2.67-2.55 (6H, m, piperazine) ; MS (m/z, %): 425 (M^+,10), for $C_{20}H_{19}N_5O_4S$, M.W. 425.4g/mol, m.p.190-193 °C.

(4c)

Yield: 50%; Yellow solid, 3409 (NH), 2864 (C-H, Aliphatic), 1667 (C=O), 1064 (Ar-Br) (cm^{-1}), ^{1}HNMR: (DMSO;d_6): δ 12.2 (1H, NH, broad), 8 (1H, d, *J*=8Hz, H-benzothiazole), 7.93 (2H, d, *J*=8Hz, H-benzoyl), 7.88-7.84 (2H, m, H-benzoyl), 7.77 (1H, d, *J*=8Hz, H-benzothiazole), 7.46 (1H, t, *J*=8Hz, H-benzothiazole), 7.33 (1H, t, *J*=8Hz, H-benzothiazole), 3.39 (2H, s, CH_2), 3.17(2H, s, CH_2), 3.1-2.99 (4H, m, piperazine), 2.55-2.51 (4H, m, piperazine) ; MS (m/z, %): 473 (M^+, 5), 475 (M+2, 2) for $C_{21}H_{21}BrN_4O_2S$, M.W. 473.3g/mol, m.p.136-139 °C.

(4d)

Yield: 46%; White solid, 3451 (NH), 3067, 896 (C-H, Ar), 2950 (C-H, Aliphatic), 1681 (C=O),1600 (C=C, Ar) (cm^{-1}), ^{1}HNMR: ($CDCl_3$): δ 8.49 (1H, s, H-pyridine), 7.85 (1H, d, *J*=8Hz, H-pyridine),7.82-7.76 (2H, m, H-

benzothiazole and pyridine), 7.48 (1H, t, *J*=8Hz, H-benzothiazole),7.44 (1H, d, *J*=8Hz, H-benzothiazole), 7.36 (1H, t, *J*=8Hz, H-benzothiazole), 3.45 (2H, s, CH_2), 3.9-4 and 3.73-3.63 (4H, m, piperazine), 2.9-2.65 (4H, m, piperazine); MS (m/z, %): 415 (M^+, 6), 417 (M+2, 3) for $C_{19}H_{18}ClN_5O_2S$, M.W. 415.9g/mol, m.p.169-171 °C.

Unfortunately, the compounds failed to show inhibitory effects on cholinesterase enzymes with IC_{50}>100 µM values when compared with donepezil with IC_{50} values 0.014 ± 0.0012 and 14.4 ± 15 µM on acetylcholinesterase and butyrylcholinesterase enzymes respectively.

Table 1- *In vitro* AChE and BChE inhibitory activity of the compounds.

Compounds	IC_{50} (µM) AChE	IC_{50} (µM) BChE
4a	>100	>100
4b	>100	>100
4c	>100	>100
4d	>100	>100
Donepezil	0.014 ± 0.0012	14.4 ± 15
Galantine	3 ± 0.18	31.6 ± 3

Estimated free binding energy values (kcal/mol), and the interactions with the active site of enzymes are shown in Table 2 and Figure 3.

Table2. Energy-based interactions and hydrogen bonds for the final compounds

Compounds	ΔG_{bind}(Kcal/mol)	Hydrogen bond (Distance, Å)
4a	-10.34	-
4b	-10.37	Phe 288 (1.806 Å), Arg 289 (2.093 Å)
4c	-10.57	His 440 (1.801 Å), Phe 330 (1.812 Å)
4d	-10.52	Phe 288 (1.888 Å)
Donepezil	-9.75	Phe 288

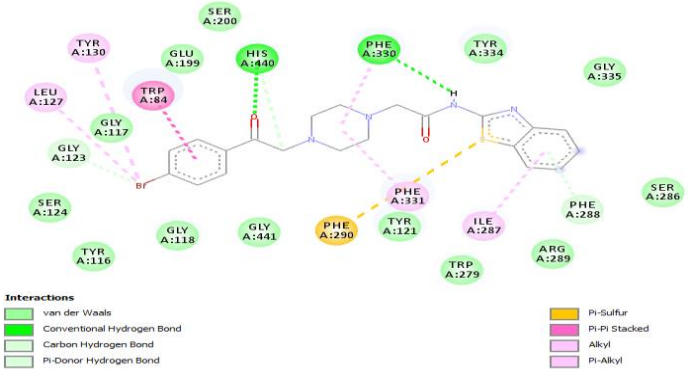


Figure 3. Docked conformation of compound 4c in the binding site of acetylcholinesterase

4. DISCUSSION

The studies of benzothiazoles demonstrated that the substituent modification at the C-2 position can result in change of activity [35]. As shown in Scheme 1, amino benzthiazole was acetylated with chloro acetyl chloride to produce 2-chloro-N-(benzo [d] thiazol) acetamides. Piperazine was nucleophilically substituted with N (benzo[d]thiazol) acetamides to produce N-(benzo[d] thiazol)-2-(piperazine-1-yl) aceamid. Final products were prepared through acylation or alkylation of piperazine nitrogen. Final compound structures were verified by ^{1}HNMR, (IR) and Mass spectra. The characteristic N-H bonds and carbonyl were observed in 3452-3399 and 1697-1637 cm $^{-1}$ regions, respectively. The ^{1}HNMR spectra exhibited aromatic peaks belonging to the benzothiazole ring in the range of 8-7.32 ppm. The peaks belonging to piperazine protons were seen at the range of 4-2.99 ppm and 2.9-2.51ppm. Downfield-shifted signals correspond to the amide region and the remaining correspond to the amine region of the piperazine ring. Compound **4d** which contains an electron withdrawing group in the amide site of the piperazine ring, exhibited a greater downfield shift. The singlet signals of CH_2 were observed in 3.45-3.17ppm range.

Ellman's test was used to assess the enzyme-inhibiting potency of final compounds. Unfortunately, the compounds (IC_{50}>100 µM values) exhibited no inhibitory effects on ChE enzymes in comparison with donepezil (IC_{50} values 0.014 ± 0.0012) and (14.4 ± 15µM) on acetylcholinesterase and butyrylcholinesterase enzymes respectively (Table1).

According to the findings of Ozkay et al the presence of methoxy substitution in the phenyl ring of benzothiazole likely has a positive effect on enzyme inhibition [16]. The benzothiazole-piperazine derivatives synthesized by Karaca et al revealed that benzothiazole-ring-unsubstituted compounds also have good inhibitory activity on the enzyme [36], thus, this conclusion cannot be considered definitive.

Comparing the results of benzothiazole-based derivatives in similar previous researches [16] and our study, it can be concluded that the presence of alkyl substitution on piperazine nitrogen is probably effective than acyl substitution in the inhibitory activity of acetylcholinesterase enzyme as seen in docking assay in present study (Table 2, compound 4c).

The free rotation around the carbon sp3 attached to piperazine may enhance the inhibitory effect, whereas the absence of rotation in this region renders the compounds ineffective. On the other hand, dimethylamino ethyl aliphatic located on the piperazine nitrogen enhanced the biological activity compared with phenyl or cyclohexy substituted piperazine. This suggests that linear aliphatic substitutions on piperazine-nitrogen probably exhibit better activity than aromatic or cyclic aliphatic substitutions [10].

In docking studies, compound **4c** with ΔG = -10.57 Kcal/mol was superior compound. Moreover, amino acids Phe 330 and His 440 were involved in the hydrogen binding with compound **4c.**

5. CONCLUSION

In this research, the methoxy substituent on the phenyl ring has been removed, acyl substitution has been used on the nitrogen of piperazine instead of alkyl substitution. These structural modifications likely account for inactivity of the compounds. Compound **4c** with ΔG = -10.53 Kcal/mol was the best compound in docking studies.

Conflicts of interest The authors declare no conflict of interest.

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التقييم البيولوجي وتخليق مشتقات البنزوثيازول–بيبيرازين كمثبطات محتملة لإنزيم الكولينستراز

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ملخص

مرض الزهايمر هو السبب الأكثر شيوعا للخرف التدريجي بين كبار السن. أحد الأسباب الرئيسية للمرض هو نقص الجهاز الكوليني. ومع ذلك، فقد لعبت بعض مثبطات الأسيتيل كولينستراز دورًا مهمًا في السيطرة على تطور المرض.

الهدف والغايات: استمرارًا لتطوير مثبطات الأسيتيل كولينستراز الجديدة، تم تصنيع وتقييم مشتقات البنزوثيازول–بيبيرازين كمثبطات محتملة.

المواد والطرق: أسفرت أسيلة بنزوثيازول–2– أمين مع كلوريد كلورو أسيتيل عن أسيتاميد 2–كلورو–ن–(بنزوثيازول–2–ييل) الذي يخضع لتفاعل استبدال مع البيبرازين لإنتاج وسيط 3. تفاعل الوسيط 3 مع كلوريدات الأسيل المناسبة أو كلوريد الألكيل ينتج المركبات النهائية. تم تقييم الفعالية المثبطة للإنزيم بواسطة اختبار إيلمان. تم التعبير عن النشاط المثبط للمركبات على إنزيمات الأسيتيل كولينستراز والبوتيريل كولينستراز بالرمز IC50. تم إجراء إرساء المشتقات النهائية في الموقع النشط للأسيتيل كولينستريز (معرف PDB: 1EVE).

النتائج: لسوء الحظ، فشلت المركبات في إظهار تأثيرات مثبطة على إنزيمات الكولينستريز بقيم IC50 > 100 ميكرومتر عند مقارنتها مع دونيبيزيل بقيم IC50 0.0012 ± 0.014 و14.4 ± 15 ميكرومتر على إنزيمات الأسيتيل كولينستراز والبوتيريل كولينستراز على التوالي. كان المركب c4 مع ΔG = -10.53 كيلو كالوري/مول هو المركب الأفضل في دراسات الالتحام.

الكلمات الدالة: 2–أمينوبنزوثيازول؛ أستيل كولينستريز ؛ بوتيريل كولينستراز ؛ اختبار إيلمان؛ بيبرازين.

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