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Research Article / Araştırma Makalesi

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW MULTI-TARGET 4-METHYLTHIAZOLE DERIVATIVES FOR TREATMENT OF ALZHEIMER'S DISEASE

ALZHEİMER HASTALIĞININ TEDAVİSİ İÇİN YENİ ÇOK HEDEFLİ 4-METİLTİYAZOL TÜREVLERİNİN TASARIMI, SENTEZİ VE BİYOLOJİK AKTİVİTELERİNİN DEĞERLENDİRMESİ

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ABSTRACT

Objective: *In this study, eight new 4-methylthiazole derivatives were designed and synthesized with the aim of exhibiting multi-target effects in the treatment of Alzheimer's disease. Their cholinesterase inhibitor activities, antioxidant properties, and metal chelator effects were tested in vitro.*
Material and Method: *The title compounds were obtained using 4-methylthiazole-2-amine as the starting material. According to method A, intermediate 1 was synthesized by reacting the starting material with 3-chloropropionyl chloride. Subsequently, the title compounds (2a-f) were obtained via substitution reactions with suitable amine derivatives. The title compounds that could not be obtained by method A were prepared from the reaction of starting material with appropriate propanoic acid derivatives using method B. Structural analysis of the synthesized and purified final compounds were elucidated using ^{1}H-NMR, ^{13}C-NMR, and HRMS methods. The cholinesterase inhibitor effects of all synthesized compounds were determined using the modified Ellman method. Additionally, the antioxidant properties of all the compounds were measured using the DPPH and ORAC methods, and their metal-chelator effects were analysed using UV spectroscopy.*
Result and Discussion: *The cholinesterase inhibitory effects of all title compounds were evaluated, and compounds 2c, 2f, and 3b were determined to be selective butyrylcholinesterase (BChE) inhibitors. Specifically, the 4-fluorobenzylpiperazine derivative compound 2c (IC_{50}=8.96 μM) is the most potent selective BChE inhibitor in the series. In addition, all final compounds exhibit antioxidant properties according to the ORAC test. Also, all compounds chelate with different amounts of Cu(II), Fe(II), and Zn(II) ions.*
Keywords: *Alzheimer's disease, antioxidant, cholinesterase inhibition, metal chelator, 4-methylthiazole*

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ÖZ

Amaç: *Bu çalışmada, sekiz yeni 4-metiltiyazol türevi bileşik Alzheimer hastalığının tedavisinde çoklu hedefe yönelik etki göstermesi hedeflenerek tasarlandı ve sentezlendi. Kolinesteraz inhibitör aktiviteleri, antioksidan özellikleri ve metal şelatör etkileri in vitro olarak test edildi.*

Gereç ve Yöntem: *Sonuç bileşikleri sentez edebilmek için 4-metiltiyazol-2-amin başlangıç maddesi olarak kullanıldı. Metot A'ya göre öncelikle başlangıç maddesi ve 3-kloropropiyonil klorürün tepkimesinden ara ürün 1 elde edilmiştir. Daha sonra ara ürün 1'in uygun amin türevleri ile yer değiştirme reaksiyonundan sonuç ürünlere (2a-f) ulaşılmıştır. Bu yöntem ile elde edilemeyen sonuç ürünler (3a-b) metot B'ye göre başlangıç maddesi ve uygun propanoik asit türevlerinin reaksiyonuyla hazırlanmıştır. Sentezlenen ve saflaştırılan sonuç bileşiklerin yapıları ^{1}H-NMR, ^{13}C-NMR ve HRMS yöntemleriyle aydınlatılmıştır. Yapıları aydınlatılan bileşiklerin kolinesteraz inhibitor etkileri modifiye Ellman yöntemiyle belirlenmiştir. Ayrıca tüm bileşiklerin antioksidan özellikleri DPPH ve ORAC yöntemiyle, metal şelatör etkiler ise UV-spektroskopisi ile ölçülmüştür.*

Sonuç ve Tartışma: *Sentezlenen bileşiklerin kolinesteraz inhibitör etkileri değerlendirildiğinde bileşik 2c, 2f ve 3b'nin selektif butirilkolinesteraz (BChE) inhibitörü olduğu belirlenmiştir. Özellikle 4-florobenzilpiperazin türevi bileşik 2c (IC_{50} = 8.96 μM) serinin en güçlü bir BChE inhibitörü olarak göze çarpmaktadır. Tüm sonuç bileşikler ORAC testine göre antioksidan özellik göstermektedir. Buna ek olarak tüm bileşikler test edilen metal iyonlarıyla değişen oranlarda şelasyon yapmaktadır.*

Anahtar Kelimeler: *Alzheimer hastalığı, antioksidan, kolinesteraz inhibisyonu, metal şelasyonu, 4-metiltiyazol*

INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is a severe and prevalent neurological disorder, characterised by cognitive deficits, memory loss and the deterioration in language and behaviour skills. As the global population ages, the number of cases of AD is increasing. Consequently, the burden on society, families and healthcare services is gradually mounting [1-3].

The development and progression of AD are related to multiple interconnected physiological and biochemical factors, which are not yet fully understood. Nevertheless, the literature reveals that low levels of neurotransmitters (particularly acetylcholine), amyloid-beta (Aβ) aggregates, oxidative stress, and metal concentrations play an interdependent key role in neurodegeneration [2,4]. According to the 'cholinergic hypothesis', the first hypothesis to explain the background of AD, high levels of acetylcholinesterase (AChE) in the brains of AD patients facilitate the hydrolysis of acetylcholine (ACh), leading to memory deficits in the early stages of the disease [5,6]. Indeed, the level of ACh is subject to regulation by two enzymes: AChE and BChE (butyrylcholinesterase). Physiological evidence demonstrates that AChE accounts for the majority (80%) of the regulatory process, with the remaining 20% being attributed to BChE. However, as the disease progresses, there is a marked shift in the balance of enzyme activity, with BChE increasing in activity and assuming a catalytic role. Based on these facts, BChE emerges as a promising candidate for the treatment of AD [7-9]. Up to now, the U.S. Food and Drug Administration (FDA) has only approved five drugs for the treatment of AD, four of which are acetylcholinesterase inhibitors, including Tacrine (delisted), Donepezil, Galantamine, Rivastigmine and the NMDA (N-methyl-D-aspartate) receptor antagonist Memantine (Figure 1). These drugs only provide limited symptomatic relief in memory and cognitive functions, but are incapable of slowing or halting progressive neurodegeneration [10]. As mentioned at the beginning of this article, the etiology of AD involves multiple targets or factors, and due to the complex pathogenesis of AD, the study of multi-target directed ligands (MTDLs) targeting the pathogenic network in AD may be a more beneficial strategy [11,12].

The antioxidant systems in the brain gradually weaken with age, especially in the case of AD. Oxidative imbalance can cause cell death by affecting the balance between antioxidants and free radicals and damaging crucial macromolecules. Furthermore, oxidative stress emerges as a central actor that can trigger many pathological cascades. Thus, antioxidants may represent great importance for the treatment of AD. Scientists have also found more metal ions, like Cu^{2+}, Zn^{2+} and Fe^{2+}, in the brains of people with AD than in the brains of healthy people. Therefore, reducing the levels of these biometals in the brain could be a potential treatment for AD. Considering this point, the present study focuses on MTDLs,

which have the ability to not only inhibit ChEs but also reduce the oxidative stress and metal concentrations.

Recently, thiazole-containing compounds have attracted attention as promising scaffolds for Alzheimer's disease due to their diverse biological activities such as cholinesterase inhibition, antioxidant activity, and metal-chelating properties. Several studies have reported thiazole derivatives as potential MTDLs for Alzheimer's therapy (Figure 2) [13-16].

In this study, a series of 4-methylthiazole derivatives were synthesized and their AChE/BChE inhibitory activity, antioxidant properties and metal chelating ability were evaluated using in vitro models. According to our previous anti-AD studies and the relevant literature, the thiazole ring with antioxidant properties and tertiary amines as cholinesterase-inhibitory pharmacophores were preferred [13-24]. As a result, eight new 4-methylthiazole-tertiary amine hybrids were designed and synthesized using the MTDL approach.

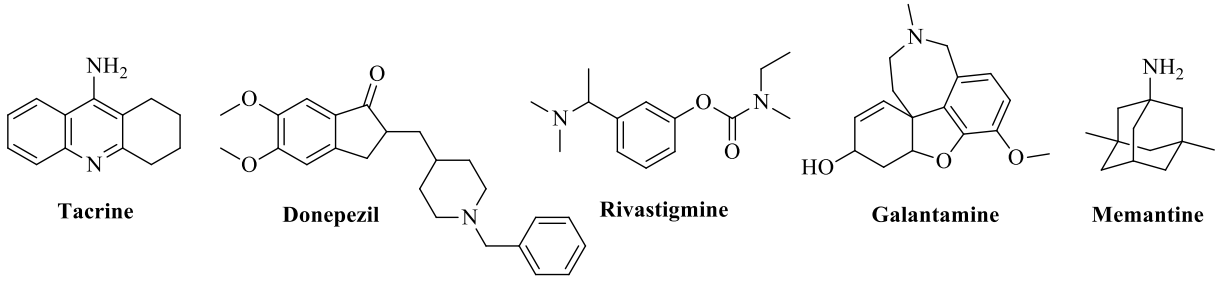

Figure 1. The structures of tacrine, donepezil, rivastigmine, galantamine, and memantine

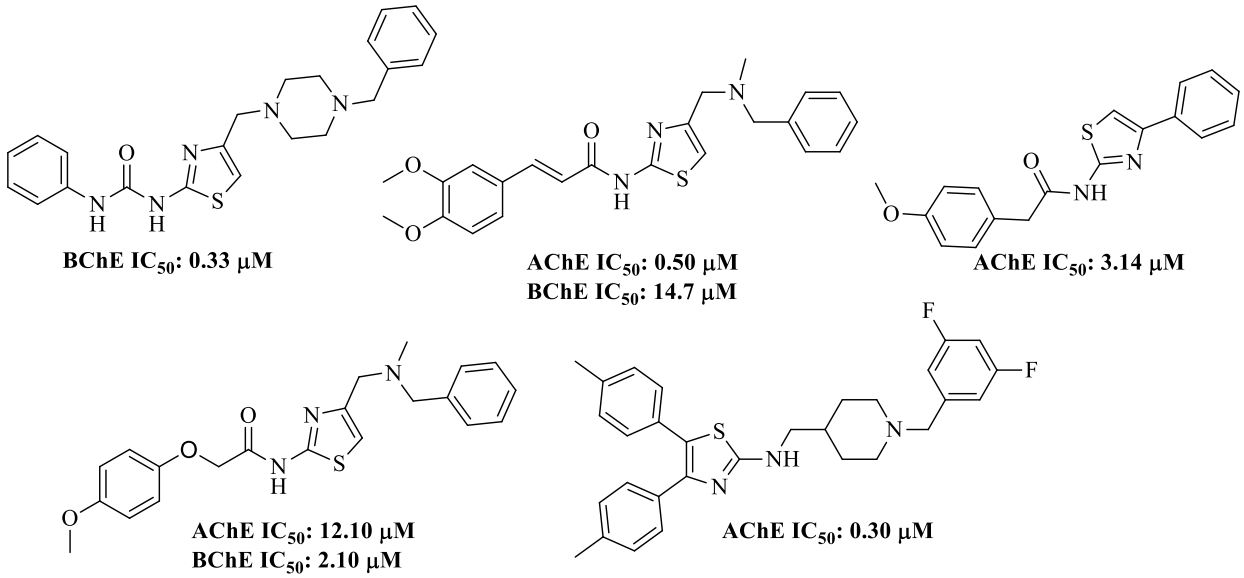

Figure 2. Representative thiazole-containing compounds reported for the treatment of AD

MATERIAL AND METHOD

Chemistry

All the chemicals used for the synthesis of the compounds were purchased from commercial suppliers and Merck 60 F254 plates were used for thin-layer chromatography. Melting points (mp) were recorded on a Schmelzpunkt SMP-II digital melting point apparatus. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR spectra were recorded using a Bruker Avance Neo 500 MHz FT-NMR spectrometer in DMSO-d_6 using tetramethylsilane as the internal standard (Faculty of Pharmacy, Ankara University). The high-

resolution mass spectra (HRMS) were obtained on a Waters LCT Premier XE Mass Spectrometer also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Faculty of Pharmacy, Gazi University).

3-Chloro-*N*-(4-methylthiazol-2-yl)propanamide (1)

To the solution of 500 mg (4.4 mmol) 4-methylthiazol-2-amine in 3 ml DMF, 53 mg (0.4 mmol) DMAP and 0.48 ml (3.3 mmol) TEA were added. After cooling the reaction mixture in an ice bath, 0.55 ml (5.7 mmol) of 3-chloropropionyl chloride was slowly added and mixed for 1 h at room temperature. The reaction mixture was poured into ice water, extracted with ethyl acetate (3×40 mL), the combined organic phases were dried over anhydrous Na_2SO_4, filtered and concentrated [18]. The precipitate was used in the next step without further purification. Yield 73%, light-yellow oil. HRMS (ESI) $[M+H]^+$ m/z for $C_7H_9ClN_2OS$ calculated: 204.0124, found: 204.0126.

General Procedure for the Preparation of Propanamide Derivatives (2a-f) (Method A)

A mixture of intermediate 1 (1 equivalent), the appropriate amine derivative (1 equivalent) and K_2CO_3 (2.5 equivalents) in ACN (20 ml) was refluxed for 1.5 h. After completion, the mixture was concentrated under reduced pressure then ice water was added to the flask, extracted with DCM (3×30 ml). The combined organic phases dried over anhydrous Na_2SO_4, filtered and concentrated. Subsequently, the crude product was solidified with suitable solvent, filtered and recrystallised from appropriate solvent [18].

3-(4-(4-Fluorophenyl)piperazin-1-yl)-*N*-(4-methylthiazol-2-yl)propanamide (2a)

According to the method A described above, compound 2a was synthesized and recrystallised with ethanol. Beige crystals. Yield: 18%. mp: 154 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.08 (s, 1H, NH), 7.05-7.01 (m, 2H, $H^{3'}$, $H^{5'}$), 6.95-6.92 (m, 2H, $H^{2'}$, $H^{6'}$), 6.72 (d, 1H, J=1 Hz, $H^{thiazole}$), 3.06 (t, 4H, J=4.9 Hz $H^{3piperazine}$, $H^{5piperazine}$), 2.70 (t, 2H, J=6.7 Hz, -N-C$\underline{H_2}$-CH_2-), 2.61 (t, 2H, J=6.7 Hz, -N-CH_2-C$\underline{H_2}$-), 2.54 (t, 4H, J=4.9 Hz, $H^{2piperazine}$, $H^{6piperazine}$), 2.25 (d, 3H, J=1 Hz, -CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 170.52, 157.65, 156.45 (d, J=234 Hz), 148.35 (d, J=1.5 Hz), 147.01, 117.54 (d, J=7.6 Hz), 115.68 (d, J=21.5 Hz), 107.94, 53.78, 52.81, 49.41, 33.18, 17.34. HRMS (ESI) $[M+H]^+$ m/z for $C_{17}H_{22}FN_4OS$ calculated: 349.1498, found: 349.1500.

3-(4-(4-Methoxyphenyl)piperazin-1-yl)-*N*-(4-methylthiazol-2-yl)propanamide (2b)

According to the method A described above, compound 2b was synthesized and recrystallised with ethanol. White crystals. Yield: 16%. mp: 139 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.09 (s, 1H, NH), 6.87 (d, 2H, J=9.1 Hz, $H^{3'}$, $H^{5'}$), 6.80 (d, 2H, J=9.1 Hz, $H^{2'}$, $H^{6'}$), 6.72 (d, 1H, J=0.8 Hz, $H^{thiazole}$), 3.67 (s, 3H, O-CH_3), 2.99 (t, 4H, J=4.8 Hz, $H^{3piperazine}$, $H^{5piperazine}$), 2.69 (t, 2H, J=6.8 Hz, -N-C$\underline{H_2}$-CH_2-), 2.61 (t, 2H, J=6.8 Hz, -N-CH_2-C$\underline{H_2}$-), 2.55 (t, 4H, J=4.8 Hz, $H^{2piperazine}$, $H^{6piperazine}$), 2.25 (d, 3H, J=0.8 Hz, -CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 170.54, 157.64, 153.34, 147.01, 145.84, 117.78, 114.70, 107.94, 55.64, 53.82, 52.94, 50.02, 33.17, 17.35. HRMS (ESI) $[M+H]^+$ m/z for $C_{18}H_{25}N_4O_2S$ calculated: 361.1698, found: 361.1711.

3-(4-(4-Fluorobenzyl)piperazin-1-yl)-*N*-(4-methylthiazol-2-yl)propanamide (2c)

According to the method A described above, compound 2c was synthesized and recrystallised with ethanol. White crystals. Yield: 30%. mp: 105 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.06 (s, 1H, NH), 7.32-7.29 (m, 2H, $H^{2'}$, $H^{6'}$), 7.14-7.11 (m, 2H, $H^{3'}$, $H^{5'}$), 6.71 (d, 1H, J=1 Hz, $H^{thiazole}$), 3.42 (s, 2H, piperazine-CH_2-phenyl), 2.62 (t, 2H, J=6.6 Hz, -N-C$\underline{H_2}$-CH_2-), 2.55 (t, 2H, J=6.6 Hz, -N-CH_2-C$\underline{H_2}$-), 2.40-2.34 (m, 8H, $H^{piperazine}$), 2.25 (d, 3H, J=1 Hz, -CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 170.53, 161.68 (d, J=240.6 Hz), 157.63, 146.99, 134.85 (d, J=2.7 Hz), 131.05 (d, J=8.1 Hz), 115.30 (d, J=20.9 Hz), 108.12, 61.53, 53.80, 52.93, 52.83, 33.11, 17.35. HRMS (ESI) $[M+H]^+$ m/z for $C_{18}H_{23}FN_4OS$ calculated: 363.1655, found: 363.1673.

3-(4-(4-Methoxybenzyl)piperazin-1-yl)-*N*-(4-methylthiazol-2-yl)propanamide (2d)

According to the method A described above, compound 2d was synthesized and recrystallised with ethanol. White crystals. Yield: 59 %. mp: 101 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.06 (s, 1H, NH), 7.18 (d, 2H, *J*=8.6 Hz, $H^{2'}$, $H^{6'}$), 6.86 (d, 2H, *J*=8.6 Hz, $H^{3'}$, $H^{5'}$), 6.72 (d, 1H, *J*=1 Hz, $H^{thiazole}$), 3.72 (s, 3H, O-CH_3), 3.35 (s, 2H, piperazine-CH_2-phenyl), 2.61 (t, 2H, *J*=6.6 Hz, -N-C$\underline{H_2}$-CH_2-), 2.54 (t, 2H, *J*=6.6 Hz, -N-CH_2-C$\underline{H_2}$-), 2.39 (bt, 4H, $H^{3piperazine}$, $H^{5piperazine}$), 2.37 (bt, 4H, $H^{2piperazine}$, $H^{6piperazine}$), 2.36 (s, 3H, -CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 170.55, 158.69, 157.62, 146.99, 130.49, 130.46, 113.97, 107.92, 61.89, 55.44, 53.82, 52.92, 52.85, 33.11, 17.35. HRMS (ESI) $[M+H]^+$ m/z for $C_{19}H_{27}N_4O_2S$ calculated: 375.1855, found: 375.1872.

3-(4-(Benzo[*d*][1,3]dioxol-5-ylmethyl)piperazin-1-yl)-*N*-(4-methylthiazol-2-yl)propanamide (2e)

According to the method A described above, compound 2e was synthesized and recrystallised with acetone-hexane. White crystals. Yield: 59 %. mp: 129 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.09 (s, 1H, NH), 6.83-6.71 (m, 4H, phenyl-H, $H^{thiazole}$), 5.97 (s, 2H, -O-CH_2-O-), 3.42 (s, 2H, piperazine-CH_2-phenyl), 2.61 (t, 2H, *J*=6.6 Hz, -N-C$\underline{H_2}$-CH_2-), 2.59 (t, 2H, *J*=6.6 Hz, -N-CH_2-C$\underline{H_2}$-), 2.39-2.32 (m, 8H, $H^{piperazine}$), 2.25 (d, 3H, *J*=0.8 Hz, -CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 170.54, 157.62, 147.65, 146.99, 146.56, 132.55, 122.34, 109.46, 108.26, 107.92, 101.20, 62.15, 53.81, 52.88, 52.86, 33.12, 17.35. HRMS (ESI) $[M+H]^+$ m/z for $C_{19}H_{25}N_4O_3S$ calculated: 389.1647, found: 389.1651.

***N*-(4-Methylthiazol-2-yl)-3-(4-phenylpiperidin-1-yl)propanamide (2f)**

According to the method A described above, compound 2f was synthesized and recrystallised with ethanol. White crystals. Yield: 59 %. mp: 106 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.19 (s, 1H, NH), 7.29-7.23 (m, 4H, $H^{2'}$, $H^{3'}$, $H^{5'}$, $H^{6'}$), 7.18 (t, 1H, *J*=7.1 Hz, $H^{4'}$), 6.72 (d, 1H, *J*=1 Hz, $H^{thiazole}$), 2.99 (d, 2H, *J*=11.2 Hz, $H^{2piperidine(e)}$, $H^{6piperidine(e)}$), 2.67 (t, 2H, *J*=6.7 Hz, -N-C$\underline{H_2}$-CH_2-), 2.59 (t, 2H, *J*=6.7 Hz, -N-CH_2-C$\underline{H_2}$-), 2.47-2.42 (m, 1H, $H^{4piperidine}$), 2.26 (d, 3H, *J*=1 Hz, -CH_3), 2.06 (t, 2H, *J*=11.2 Hz, $H^{2piperidine(a)}$, $H^{6piperidine(a)}$), 1.75-1.59 (m, 4H, $H^{3piperidine}$, $H^{5piperidine}$). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 170.65, 157.67, 147.02, 146.68, 128.79, 127.16, 126.46, 107.92, 54.09, 53.83, 42.23, 33.52, 33.23, 17.36. HRMS (ESI) $[M+H]^+$ m/z for $C_{18}H_{24}N_3OS$ calculated: 330.1640, found: 330.1654.

General Method for the Preparation of 3-Substitutedpropanoic Acid Intermediates

According to our previously reported method, corresponding amine derivatives (1.0 equivalent) and methyl acrylate (1.2 equivalent) in 10 ml of DCM were stirred overnight at room temperature. At the end of the reaction, the solvent was evaporated under reduced pressure, and the corresponding methyl ester was obtained. The methyl ester was hydrolysed by heating at 40 °C for 2 h with 5% NaOH solution to give the desired propanoic acid. After this period, the mixture was allowed to cool to room temperature and neutralised with HCl. The resulting precipitate was filtered and dried [18,19]. It was used without further purification.

General Procedure for the Preparation of Propanamide Derivatives (3a-b) (Method B)

The appropriate amount of 3-substituted propanoic acid (1.1 equivalent) was dissolved in 20 ml of DCM, then EDC (1.2 equivalent), DMAP (0.2 equivalent) and 4-methylthiazol-2-amine (1 equivalent) were added and the reaction was carried out overnight at room temperature. At the end of the reaction, the solvent was evaporated to dryness. The precipitate was poured into water, and the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure. To obtain pure product recrystallized from suitable solvent [18,19].

***N*-(4-Methylthiazol-2-yl)-3-(4-phenylpiperazin-1-yl)propanamide (3a)**

According to the method B described above, compound 3a was synthesized and recrystallised with ethanol. Beige crystals. Yield: 59 %. mp: 161 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.10

(s, 1H, NH), 7.22-7.19 (m, 2H, $H^{3'}$, $H^{5'}$), 6.92 (d, 2H, *J*=7.5 Hz, $H^{2'}$, $H^{6'}$), 6.77 (t, 1H, *J*=7.5 Hz, $H^{4'}$), 6.72 (d, 1H, *J*=1 Hz, $H^{thiazole}$), 3.11 (t, 4H, *J*=4.8 Hz, $H^{3piperazine}$, $H^{5piperazine}$), 2.70 (t, 2H, *J*=6.7 Hz, -N-C$\underline{H_2}$-CH_2-), 2.61 (t, 2H, *J*=6.7 Hz, -N-CH_2-C$\underline{H_2}$-), 2.56 (t, 4H, *J*=4.8 Hz, $H^{2piperazine}$, $H^{6piperazine}$), 2.25 (d, 3H, *J*=1 Hz, -CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 170.54, 157.65, 151.45, 147.01, 129.36, 119.25, 115,82, 107.94, 53.82, 52.84, 48.63, 33.17, 17.35. HRMS (ESI) $[M+H]^+$ m/z for $C_{17}H_{23}N_4OS$ calculated: 331.1593, found: 331.1599.

3-(4-Benzylpiperazin-1-yl)-*N*-(4-methylthiazol-2-yl)propanamide (3b)

According to the method B described above, compound 3b was synthesized and recrystallised with ethanol. White crystals. Yield: 59 %. mp: 149 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.04 (s, 1H, NH), 7.33-7.23 (m, 5H, phenyl H), 6.71 (d, 1H, *J*=1 Hz, $H^{thiazole}$), 3.44 (s, 2H, piperazine-CH_2-phenyl), 2.63 (t, 2H, *J*=6.6 Hz, -N-C$\underline{H_2}$-CH_2-), 2.54 (t, 2H, *J*=6.6 Hz, -N-CH_2-C$\underline{H_2}$-), 2.41 (bt, 4H, $H^{3piperazine}$, $H^{5piperazine}$), 2.36 (bt, 4H, $H^{2piperazine}$, $H^{6piperazine}$), 2.25 (s, 3H, -CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ (ppm): 170.54, 157.63, 147.00, 138.73, 129.25, 128.60, 127.33, 107.92, 62.50, 53.82, 53.06, 52.86, 33.13, 17.35. HRMS (ESI) $[M+H]^+$ m/z for $C_{18}H_{25}N_4OS$ calculated: 345.1749, found: 345.1759.

Biological Activity Assays

Cholinesterase Inhibition Assay

AChE (electric eel) and BChE (equine serum) enzymes used in cholinesterase inhibitory activity tests were purchased from Sigma Aldrich. As explained in detail in our previous studies, modified Ellman method was applied to determine the inhibition values of the synthesized title compounds. The IC_{50} values were calculated using GraphPad Prism software (Version 7.0) [13,18,25].

In Vitro Antioxidant Activity Assays (DPPH and ORAC-Fluorescein)

To determine the *in vitro* antioxidant activities of the compounds, DPPH and ORAC-FL assays were conducted according to our previous methods [13,14].

Metal Binding Studies

The experimental protocol for the ligand-metal binding evaluation was performed in accordance with the previously reported method. The UV-Vis absorption spectra of the metal-treated ligand and the control ligand solution were visualized and plotted as wavelength (nm) versus absorbance [13,14].

RESULT AND DISCUSSION

Chemistry

The synthetic routes to the final compounds (2a-f and 3a-b) were presented in Figure 3 and 4. Commercially available 2-amino-4-methylthiazole was used as the starting material. The key intermediate 1 was obtained by reaction of 2-amino-4-methylthiazole with 3-chloropropionyl chloride in DMF using TEA and DMAP. Subsequently, the title compounds 2a-f were synthesized by reacting intermediate 1 and appropriate amine derivatives in the presence of potassium carbonate (Figure 3). However, due to their very low yields, the title compounds containing phenylpiperazine and benzylpiperazine derivatives in their side chains (3a-b) could not be effectively synthesized by method A. Therefore, these compounds were prepared by a different synthetic method. According to method B, first the reaction of methyl acrylate with appropriate amines to yield the corresponding ester derivatives, then these derivatives were hydrolysed in a 5% NaOH solution to give the 3-substituted propanoic acid. Finally, compounds 3a-b were synthesized using 2-amino-4-methylthiazole with 3-substituted propanoic acid in the presence of EDC and DMAP, as shown in Figure 4.

In this study, eight new final compounds were synthesized. The structures of the compounds were confirmed by ^{1}H NMR, ^{13}C NMR and high-resolution mass spectroscopy (LC-HRMS). Spectral data were consistent with the assigned structures and all characterization data are provided in the

experimental section.

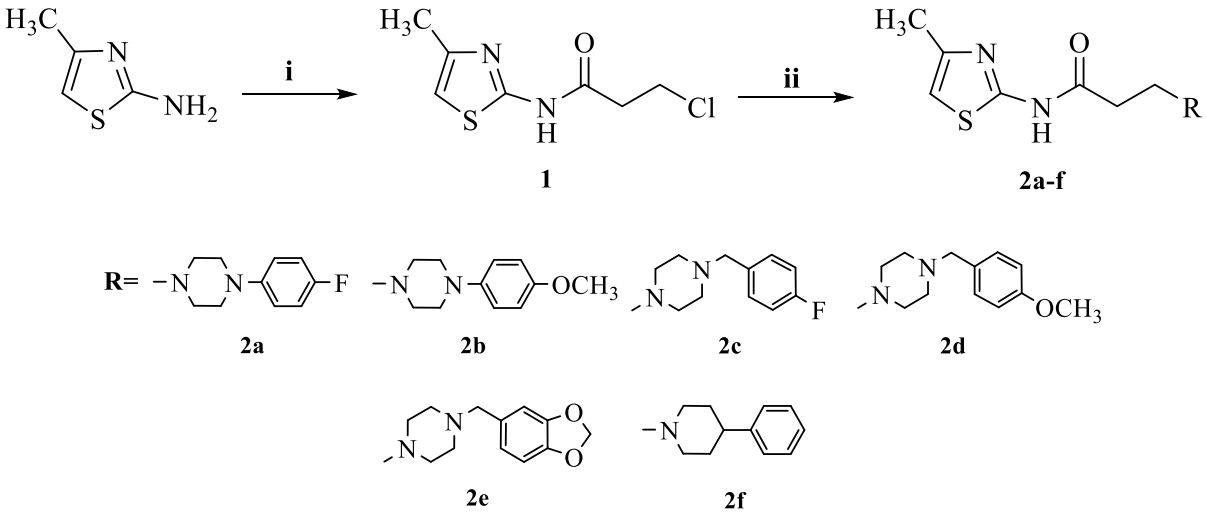

Figure 3. (Method A) Synthesis of the compounds 2a-f. Reagents and conditions: (i) 3-Chloropropionyl chloride, DMAP, TEA, DMF, 1 h, rt. (ii) Amine derivative, K_2CO_3, 1.5 h, reflux

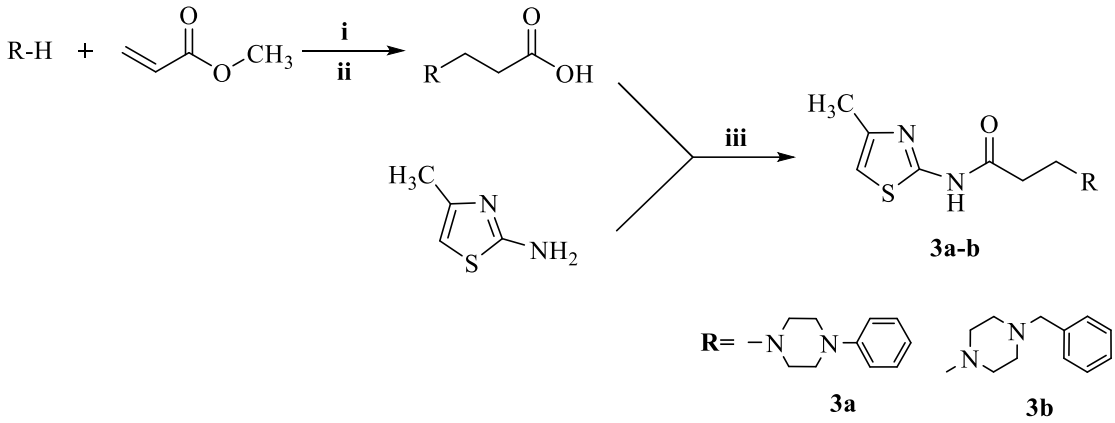

Figure 4. (Method B) Synthesis of compounds 3a-b. Reagents and conditions: (i) DCM, overnight, rt. (ii) %5 NaOH, 2 h, 40 °C. (iii) EDC, DMAP, DCM, overnight, rt

Biology

In Vitro Cholinesterase Inhibitor Activity

The inhibitory effects of the title compounds 2a-f and 3a-b against *ee*AChE (from electric eel) and *eq*BChE (from equine serum) were measured using modified Ellman spectrophotometric technique. Donepezil was used as a positive control and the results of the cholinesterase (ChE) inhibitory activity tests are presented in Table 1. According to the cholinesterase inhibitory activity results, the final compounds exhibited more potent inhibitory activity against *eq*BChE. Especially, compound 2c was the most active with an IC_{50} value of 8.96 μM, followed by 2f (IC_{50}= 19.60 μM) and 3b (IC_{50} = 36.62 μM), respectively. The structure–activity relationship (SAR) evaluation revealed that inhibitory activity of the final compounds was strongly influenced by tertiary amine fragment in the side chain. Therefore, a comparison of fluorobenzylpiperazine derivative compound 2c and benzylpiperazine derivative compound 3b showed that the BChE activity increased 4-fold when fluorine was introduced at the 4th position. The phenylpiperidine-derived compound 2f exhibited average BChE inhibitory activity.

Table 1. Cholinesterase inhibitory activities, ORAC and DPPH results of the synthesized compounds

Compd.	*ee*AChE		*eq*BChE		ORAC[a]	DPPH % inhibition (100 µM)
	% inhibition ± SD (100 µM)	IC_{50} (µM) ± SD	% inhibition ± SD (100 µM)	IC_{50} (µM) ± SD		
2a	20.5 ± 1.2	≥50	29.4 ± 0.7	≥20	4.77± 0.17	10.15 ± 2.08
2b	31.1 ± 0.4	≥50	42.9 ± 1.5	≥20	24.95 ± 0.54	9.21 ± 1.32
2c	27.4 ± 1.8	≥50	94.8 ± 0.5	8.96 ± 0.6	8.58 ± 1.34	22.17 ± 1.01
2d	28.1 ± 0.2	≥50	40.1 ± 4.4	≥20	1.03 ± 0.42	15.25 ± 1.11
2e	21.0 ± 0.6	≥50	48.1 ± 2.2	≥20	2.02 ± 0.20	13.12 ± 1.18
2f	16.9 ± 2.8	≥50	81.6 ± 2.5	19.60 ± 1.1	0.40 ± 0.01	14.06 ± 1.03
3a	13.3 ± 4.1	≥50	39.4 ± 0.5	≥20	4.45 ± 0.12	12.15 ± 1.34
3b	33.7 ± 2.3	≥50	87.4 ± 0.1	36.62 ± 1.6	1.50 ± 0.08	16.19 ± 1.65
Donepezil	98.0 ± 0.8	0.065 ± 0.002	88.0 ± 1.4	3.55 ± 0.07	nd[b]	nd
Trolox	-	-	-	-	1.00	-
Gallic acid	-	-	-	-	-	91.46 ± 0.52

[a]Data are expressed as µmol of Trolox equivalent/µmol of tested compounds
[b]Not determined
Data are the mean ± SD of three independent experiments

Antioxidant Activity

The radical scavenging (DPPH assay) and oxygen radical absorbance (ORAC-FL assay) capabilities of the compounds were examined. The antioxidant standards gallic acid and the vitamin E analogue, Trolox were utilised for the DPPH and ORAC-FL assays, respectively. As shown in Table 1, none of the final compounds exhibited a notable radical scavenging effect in comparison to gallic acid. However, all the molecules displayed extremely high ORAC-FL values, ranging from 0.40 to 24,95 Trolox equivalents. In addition, compound 2b (as 24.95 trolox equivalent), a 4-methoxypiperazine derivative, has a notably high ability to absorb oxygen radicals.

Metal-Chelating Properties of Title Compounds

The ability of the title compounds to chelate biologically relevant metal ions, such as Cu^{2+}, Fe^{2+} and Zn^{2+}, was investigated using UV–Vis spectroscopy with a wavelength range of 230–500 nm. The presence of complexes was indicated by the differences in the spectra of the metal-treated ligands and the ligand alone. As can be seen in Figure 5, the difference UV-Vis spectra were obtained by subtracting the absorbances of the ligand and metal from those of the metal-ligand mixture. All of the final compounds interacted with the tested metal ions at different rates. Furthermore, compounds 2a and 2e demonstrated particularly strong chelating properties against all tested ions.

Inspired by our previous studies as well as the reported literature, a series of 4-methylthiazole derivatives were designed and synthesized as potential MTDLs for the treatment of AD. The thiazole ring was selected as the core scaffold due to its reported biological activities and antioxidant properties. The linker part was designed considering the structural features of acetylcholine. Furthermore, tertiary amin moiety was incorporated as a basic center, since amine-containing structures are known to interact with the catalytic active site of cholinesterase enzymes [13-16].

These derivatives were evaluated by bioactivity tests in vitro, including AChE/BChE inhibition, antioxidant activity and metal-chelating abilities. The results of the in vitro cholinesterase inhibitor activity tests demonstrated that compound 2c exhibited the most potent and selective BChE inhibition (IC_{50} = 8.96 µM). Compounds 2f (IC_{50} = 19.60 µM) and 3b (IC_{50} = 36.62 µM) also showed moderate BChE inhibitor activity. Additionally, 2c (8.58) showed significant antioxidant activity compared to Trolox. Moreover, the results of the metal chelator activity tests indicated that compound 2c was a zinc

and iron chelator. Taken together, 2c was a promising compound addressing multiple factors associated with AD and deserved further structural optimization.

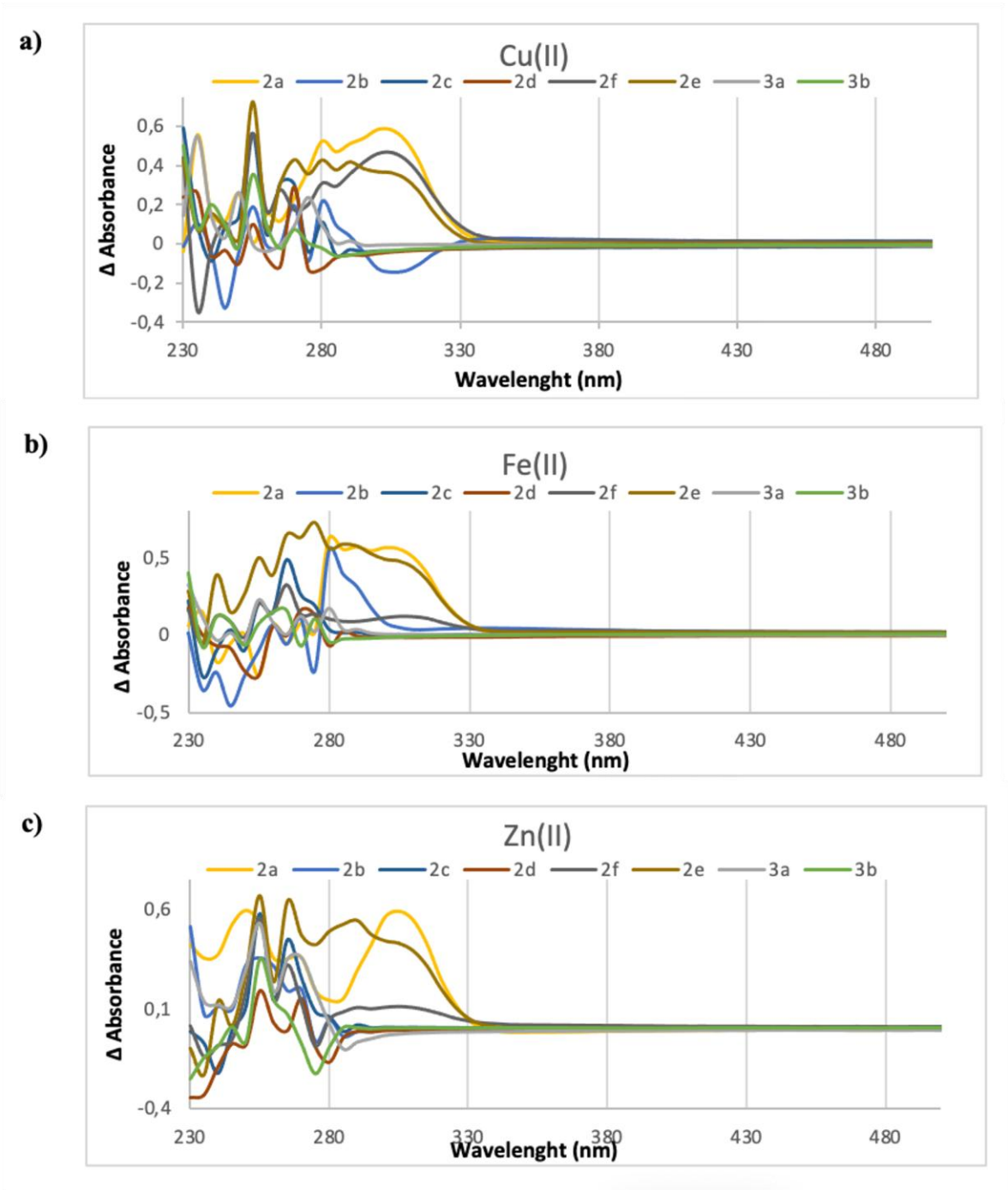

Figure 5. The difference UV-vis spectra between selected compounds and Cu(II) (a), Fe(II) (b), Zn(II) (c) ions

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AUTHOR CONTRIBUTIONS

Concept: M.B.; Design: M.B.; Control: M.B.; Sources: M.B.; Materials: M.B.; Data Collection and/or Processing: M.B.; Analysis and/or Interpretation: M.B.; Literature Review: M.B.; Manuscript Writing: M.B.; Critical Review: M.B.; Other: -

CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

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