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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DESIGN, SYNTHESIS, AND BIOLOGICAL ASSESSMENT OF NOVEL *N'*-(BENZYLIDENE)PROPANEHYDRAZIDES AS MTDL FOR ALZHEIMER'S DISEASE

ALZHEİMER HASTALIĞI İÇİN MTDL OLARAK YENİ N'-PROPANHİDRAZİTLERİN TASARIMI, SENTEZİ VE BİYOLOJİK DEĞERLENDİRMESİ

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ABSTRACT

Objective: *In this study, following the multi-target directed ligands (MTDLs) strategy and drawing inspiration from the neuroprotective structure of ferulic acid, eight novel N'-(benzylidene)propanehydrazide derivatives were designed, synthesized, and tested to evaluate their cholinesterase inhibitory and antioxidant capacities.*

Material and Method: *To obtain the final compounds, first, corresponding key intermediates, 3-(substitutedamino)propanehydrazides, were prepared by the hydrolysis with hydrazine hydrate of methyl 3-(substitutedamino)propanoate intermediates. These intermediates had been prepared from the Michael addition of methyl acrylate and commercially available tertiary amine derivatives. Subsequently, the final compounds were synthesized from the reaction of the starting compounds 4-hydroxybenzaldehyde or 4-methoxybenzaldehyde and the corresponding key intermediates. Structural analysis of the synthesized and purified compounds was carried out using ^{1}H-NMR, ^{13}C-NMR, and HRMS. Then, all the final compounds were examined for their cholinesterase inhibitory effect using the modified Ellman method, their antioxidant effect using the DPPH and ORAC methods, and their metal chelator effect using UV-spectroscopy analysis. Moreover, physicochemical parameters were calculated using QikProp Schrödinger Suite 2023 to predict the druggability of all compounds.*

Result and Discussion: *Seven of the eight final compounds exhibited moderate cholinesterase inhibition at varying rates. Compounds* ***2a*** *(IC_{50} = 12.83 µM) and* ***2d*** *(IC_{50} = 16.02 µM) were identified as the most potent inhibitors for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), respectively. Moreover, all the final compounds exhibited antioxidant activity in the ORAC assay. Chelator effects of all compounds were also observed for Cu(II), Fe(II), and Zn(II) ions at varying rates. Additionally, the final compounds demonstrated acceptable lead-like properties according to in-silico predictions.*

Keywords: *Acylhydrazone, antioxidant, Alzheimer's disease, cholinesterase inhibition, metal-chelator*

ÖZ

Amaç: *Bu çalışmada, çoklu hedefe yönelik ligand (MTDL) stratejisi izlenerek ve nöroprotektif ferulik asit yapısından ilham alınarak, sekiz yeni N'-(benziliden)propanhidrazit türevleri tasarlandı, sentezlendi, kolinesteraz inhibitör ve antioksidan kapasitelerini değerlendirmek üzere test edildi.*

Gereç ve Yöntem: *Sonuç bileşiklerin eldesi için ilk olarak, kilit ara ürünler olan 3-*

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(amino)propanhidrazitler, metil 3-(sübstitüeamino)propanoat ara ürünlerinin hidrazin hidrat ile hidrolizinden hazırlandı. Bu ara ürünler ise metil akrilat ve ticari olarak mevcut tersiyer amin türevlerinin Michael katım tepkimesinden hazırlanmıştır. Hazırlanan kilit ara ürünlerin, seri hareket bileşikleri olan 4-hidroksibenzaldehit veya 4-metoksibenzaldehit ile katım tepkimesinden sonuç bileşikler sentezlenmiştir. Sentezlenen ve saflaştırılan bileşiklerin ^{1}H-NMR, ^{13}C-NMR ve HRMS analizleri ile yapı kontrolleri gerçekleştirilmiştir. Ardından, sonuç bileşiklerin modifiye Ellman yöntemiyle kolinesteraz inhibitör etki, DPPH ve ORAC yöntemleriyle antioksidan etki ve UV-spektroskopisi analiziyle de metal şelatör etki incelemeleri yapılmıştır. Ayrıca tüm bileşiklerin ilaç olabilirliklerini değerlendirebilmek amacıyla QikProp Schrodinger Suite 2023 kullanılarak fizikokimyasal parametreleri hesaplanmıştır.

Sonuç ve Tartışma: *Sekiz sonuç bileşikten yedisinin değişen oranlarda kolinesteraz inhibisyonu oluşturdukları bulunmuştur. Bileşik **2a** (IC_{50} = 12.83 µM) ve **2d** (IC_{50} = 16.02 µM) sırasıyla en iyi asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BChE) inhibitörü olarak belirlenmiştir. Bununla birlikte, ORAC testinde tüm bileşikler antioksidan etki göstermiştir. Tüm bileşiklerin Cu(II), Fe(II), ve Zn(II) iyonları için değişen oranlarda şelatör etkileri de gözlenmiştir. Ayrıca, final bileşikler, in-siliko tahminlere göre kabul edilebilir öncü benzeri özellikler göstermiştir.*

Anahtar Kelimeler: *Açilhidrazon, Alzheimer hastalığı, antioksidan, kolinesteraz inhibisyonu, metal-şelatör*

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, presenting a significant global public health challenge [1]. As the aging population increases, AD prevalence rises, imposing escalating burdens on individuals, families, and healthcare systems [2]. Marked by cognitive decline, memory loss, and various behavioral symptoms, AD detrimentally impacts independence and quality of life [3]. The societal consequences extend beyond emotional and economic strain on families to a global healthcare resource burden [4].

AD exhibits distinct pathophysiological features, including acetylcholine deficiency, neuroinflammation, oxidative stress, metal ion dyshomeostasis, and protein dysregulations [5-7]. While the precise etiology remains unclear, a multifactorial interplay of genetic, environmental, and lifestyle factors contributes to disease development [8]. Genetic studies identify key genes like amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) in familial AD, with sporadic AD associated with a complex genetic landscape [9]. Medicinal chemistry plays a vital role in the search for AD therapies, focusing on designing compounds targeting specific biological pathways. Recent advances highlight potential drug targets like Aβ aggregation, tau hyperphosphorylation, neuroinflammation, and oxidative stress [10]. Medicinal chemists strive to develop compounds selectively modulating these targets for disease-modifying interventions [11,12]. Existing AD therapies primarily manage symptoms and provide temporary cognitive relief using cholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists [13]. The therapeutic options within these drug classes are currently limited to donepezil, rivastigmine, galantamine, and the NMDA receptor antagonist memantine [7,14]. However, these treatments do not address fundamental disease processes, prompting the urgent need for innovative disease-modifying interventions [15,16].

Recent AD drug development emphasizes multitarget-directed ligands (MTDLs) over the traditional single-target approach [17]. Cholinesterase inhibition, particularly targeting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), is crucial in this strategy [18,19]. While AChE has historically received more attention, recent research underscores the significance of BChE inhibition as the disease progresses [20,21]. Cholinesterases remain principal targets for MTDL development, combining inhibitory activity with neuroprotective targets [12].

Oxidative stress (OS) is identified as an early and triggering pathology in AD, contributing significantly to neurodegeneration [22,23]. Compounds with antioxidant activity are considered valuable for MTDL development against AD. The metal hypothesis implicates Fe, Cu, and Zn ions in AD pathologies, suggesting metal chelators as valuable for drug development due to their effects on various AD mechanisms [24].

Our research team has devoted research efforts to reaching MTDLs against AD during the last

decade. With this purpose, we have studied various heterocyclic scaffolds such as phthalazinone, pyridazinone, pyridazine, benzoxazolone, benzothiazolone, and thiazole [25-31]. In this study, we preferred phenol and anisole instead of heterocyclic systems, drawing inspiration from the neuroprotective ferulic acid (FA) structure [32,33]. FA serves as a potential pharmacophore that exhibits multiple pharmacological properties, such as antioxidant, neuroprotection, modulation of Aß aggregation, and anti-inflammatory effects. However, FA lacks cholinesterase inhibitor activity. Despite this, numerous studies in MTDL design have incorporated FA, often hybridizing it with known cholinesterase inhibitors. According to molecular docking results of these hybrids, FA primarily engages in π-π stacking interactions via its phenyl core with key aromatic residues (Trp286 and Tyr341 for huAChE or Trp231 and Phe329 for huBChE) at the peripheral anionic site [34,35]. Phenol and anisole connected with tertiary nitrogen-bearing structures known for their ability to interact with the catalytic site of cholinesterase enzymes [36] by using propanehydrazide bridge, which could contribute to the antioxidant and metal chelator effects [37]. As a result, we designed and synthesized novel eight *N'*-(benzylidene)propanehydrazide derivatives (Figure 1).

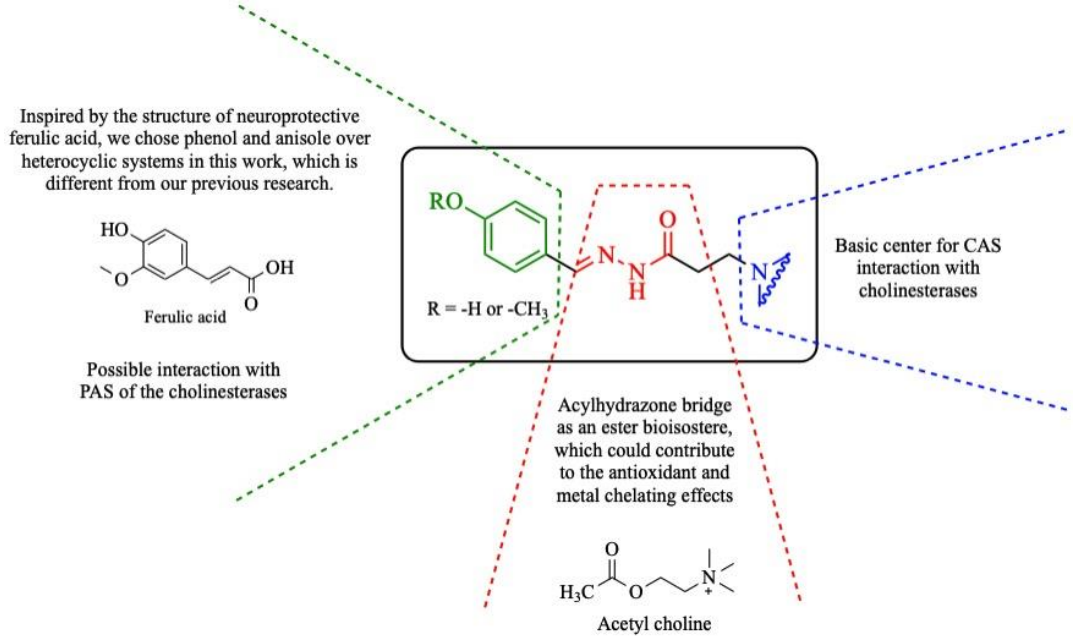

Figure 1. Design strategy and general structures of the final compounds

MATERIAL AND METHOD

Chemistry

Chemicals were acquired from commercial suppliers (Sigma Aldrich, Merck and Isolab) and Merck 60F254 plates were used for TLC. Schmelzpunkt SMP-II digital apparatus was used for melting point (mp) detection. NMR spectra were recorded using a Bruker Avance Neo 500 MHz FT-NMR spectrometer. Waters LCT Premier XE Mass Spectrometer operating in electrospray ionization (ESI) mode recruited to collect high resolution mass spectra data (HRMS). The mass spectrometer was also coupled to an AQUITY Ultra Performance Liquid Chromatography system with UV detector monitoring at 254 nm.

General method for the synthesis of 3-(substitutedamino)propanehydrazide intermediates (5a-h)

According to our previously reported method [27] corresponding amine derivative **(3a-d)** (1.0 g, 1 equivalent) in DCM (15 ml) and methyl acrylate (1.2 equivalent) was used to obtain methyl 3-(substitutedamino)propanoate intermediates **(4a-d)**. Without further purification prepared **4a-d** (1.0 equivalent) and $NH_2NH_2.H_2O$ (64%, 5.0 equivalent) were refluxed in EtOH (25 ml) for 4h. The reaction

mixture was concentrated under reduced pressure at the end of this period, then treated with diethyl ether, and the precipitated crude intermediate **(5a-d)** was filtered off and crystallized or washed with the appropriate solvent.

3-(4-Benzylpiperazin-1-yl)propanehydrazide (5a)

Recrystallized from diisopropyl ether-isopropyl alcohol. Light pinky solid. Yield: 70 %. mp: 73-75 °C. HRMS (ESI) [M + H]+ m/z for $C_{14}H_{23}N_4O$ calculated: 263.1872, found: 263.1875.

3-(4-(4-Fluorobenzyl)piperazin-1-yl)propanehydrazide (5b)

Washed with diethyl ether-petroleum ether. White solid. Yield: 46 %. mp: 58-60 °C. HRMS (ESI) [M + H]+ m/z for $C_{14}H_{22}FN_4O$ calculated: 281.1778, found: 281.1777.

3-(4-Phenylpiperidin-1-yl)propanehydrazide (5c)

Washed with diethyl ether-petroleum ether. Light pinky solid. Yield: 87 %. mp: 112-114 °C. HRMS (ESI) [M + H]+ m/z for $C_{14}H_{22}N_3O$ calculated: 248.1763, found: 248.1761.

3-(4-Benzylpiperidin-1-yl)propanehydrazide (5d)

Recrystallized from ethyl acetate. Light pinky solid. Yield: 43 %. mp: 110-112 °C. HRMS (ESI) [M +H]+ m/z for $C_{15}H_{24}N_3O$ calculated: 262.1919, found: 262.1913.

General procedure for the synthesis of *N′*-(4-hydroxy/methoxybenzylidene)propanehydrazide derivatives (1a-d and 2a-d)

4-Hydroxybenzaldehyde **(1)** or 4-methoxybenzaldehyde **(2)** (1.0 equivalent) and corresponding 3-(substitutedamino) propanehydrazide intermediate **(5a-d)** (1.0 equivalent) were dissolved in EtOH (20 ml). The mixture was either stirred at room temperature (rt) or refluxed until the starting compounds were no longer present. After completion, the mixture was concentrated under reduced pressure then diethyl ether-petroleum ether was added to the flask, precipitate was filtered. The filtered solid was recrystallized from the appropriate solvent.

***(E)*-3-(4-benzylpiperazin-1-yl)-*N′*-(4-hydroxybenzylidene)propanehydrazide (1a)**

As mentioned in the general method, compound **1** (300 mg, 2.46 mmol) and **5a** (644 mg, 2.46 mmol) were stirred overnight at rt. Tetrahydrofuran was used to recrystallize the product. White crystals. Yield: 79 %. mp: 210 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.24, 11.06 (two s, 1H, NH), 9.91, 9.88 (two s, 1H, OH), 8.04, 7.88 (two s, 1H, -N=CH-), 7.50, 7.47 (two d, J = 8.6 Hz, 2H, $H^{2,6}$), 7.39 – 7.17 (m, 5H, H^{phenyl}), 6.82, 6.81 (two d, J = 8.6 Hz, 2H, $H^{3,5}$), 3.46 (br s, 2H, piperazine-CH_2-phenyl), 2.77 (t, J = 6.6 Hz, 1H, -H_2CC=O), 2.72 – 2.58 (m, 2H, CH_2-C$\underline{H}_2$-piperazine), 2.48 – 2.21 (m, 9H, $H^{piperazine}$, -H_2CC=O). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 173.28, 167.58, 159.72, 159.51, 146.64, 143.44, 138.50, 138.48, 129.35, 129.16, 128.77, 128.62, 127.42, 125.76, 116.15, 116.10, 67.48, 62.38, 62.36, 54.12, 53.72, 52.88, 52.84, 52.71, 32.46, 30.04. HR-ESI-MS (m/z) calcd for $C_{21}H_{26}N_4O_2$ $[M+H]^+$ 367.2134, found 367.2138.

***(E)*-3-(4-(4-fluorobenzyl)piperazin-1-yl)-*N′*-(4-hydroxybenzylidene)propanehydrazide (1b)**

As mentioned in the general method, compound **1** (250 mg, 2.05 mmol) and **5b** (574 mg, 2.05 mmol) were stirred overnight at rt. Acetonitrile was used to recrystallize the product. Beige crystals. Yield: 59 %. mp: 197 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.20, 11.03 (two s, 1H, NH), 9.88 (br s, 1H, OH), 8.03, 7.87 (two s, 1H, -N=CH-), 7.50, 7.47 (two d, J = 8.7 Hz, 2H, $H^{2,6}$), 7.31 (dd, J = 14.0, 7.7 Hz, 2H, $H^{2',6'}$), 7.13 (td, J = 8.9, 3.7 Hz, 2H, $H^{3',5'}$), 6.81, 6.80 (two d, J = 8.7, 2H, $H^{3,5}$), 3.43, 3.41 (two s, 2H, piperazine-CH_2-phenyl), 2.74 (t, J = 7.3 Hz, 1H, -H_2CC=O), 2.62 – 2.55 (m, 2H, CH_2-C$\underline{H}_2$-piperazine), 2.49 – 2.30 (m, 9H, $H^{piperazine}$, -H_2CC=O). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 173.50, 167.68, 162.64, 160.71, 159.72, 159.71, 159.49, 146.59, 143.33, 134.86, 131.10, 131.04, 129.16, 128.74, 125.79, 125.74, 116.14, 116.10, 115.38, 115.22, 61.57, 54.22, 53.92, 53.05, 52.95, 32.61, 30.29. HR-ESI-MS (m/z) calcd for $C_{21}H_{25}FN_4O_2$ $[M+H]^+$ 385.2040, found 385.2041.

(*E*)-*N*'-(4-hydroxybenzylidene)-3-(4-phenylpiperidin-1-yl)propanehydrazide (1c)

As mentioned in the general method, compound **1** (250 mg, 2.05 mmol) and **5c** (506 mg, 2.05 mmol) were stirred overnight at rt. Tetrahydrofuran was used to recrystallize the product. White crystals. Yield: 44 %. mp: 241 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.23, 11.04 (two s, 1H, NH), 9.88 (br s, 1H, OH), 8.06, 7.89 (two s, 1H, -N=CH-), 7.52, 7.49 (two d, J = 8.6 Hz, 2H, $H^{2,6}$), 7.31 – 7.15 (m, 5H, H^{phenyl}), 6.82, 6.81 (two d, J = 8.6 Hz, 2H, $H^{3,5}$), 2.99 (t, J = 12.9 Hz, 2H, $H^{2piperidine(eq),}$ $H^{6piperidine(eq)}$), 2.79 (t, J = 7.2 Hz, 1H, -H_2CC=O), 2.69 – 2.60 (m, 2H, CH_2-C<u>H</u>$_2$-piperidine), 2.49 – 2.42 (m, 1H, $H^{4piperidine}$), 2.37 (t, J = 7.2 Hz, 1H, -H_2CC=O), 2.11 – 1.98 (m, 2H, $H^{2piperidine(ax)}$, $H^{6piperidine(ax)}$), 1.78 – 1.69 (m, 2H, $H^{3piperidine(eq),}$ $H^{5piperidine(eq)}$), 1.68 – 1.53 (m, 2H, $H^{3piperidine(ax),}$ $H^{5piperidine(ax)}$). HR-ESI-MS (m/z) calcd for $C_{21}H_{25}N_3O_2$ $[M+H]^+$ 352.2025, found 352.2024.

(*E*)-3-(4-benzylpiperidin-1-yl)-*N*'-(4-hydroxybenzylidene)propanehydrazide (1d)

As mentioned in the general method, compound **1** (250 mg, 2.05 mmol) and **5d** (535 mg, 2.05 mmol) were stirred overnight at rt. Tetrahydrofuran was used to recrystallize the product. Beige crystals. Yield: 53 %. mp: 224 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.21, 11.00 (two s, 1H, NH), 9.89 (br s, 1H, OH), 8.03, 7.87 (two s, 1H, -N=CH-), 7.50, 7.46 (two d, J = 8.6 Hz, 2H, $H^{2,6}$), 7.30 – 7.10 (m, 5H, H^{phenyl}), 6.81, 6.80 (two d, J = 8.6 Hz, 2H, $H^{3,5}$), 2.84 (d, J = 11.7 Hz, 2H, $H^{2piperidine(eq),}$ $H^{6piperidine(eq)}$), 2.73 (t, J = 7.2 Hz, 1H, -H_2CC=O), 2.60 – 2.52 (m, 2H, CH_2-C<u>H</u>$_2$-piperidine), 2.49, 2.47 (two d, J = 7.0 Hz, 2H, piperidine-CH_2-phenyl), 2.31 (t, J = 7.2 Hz, 1H, -H_2CC=O), 1.86 (t, J = 11.7 Hz, 2H, $H^{2piperidine(ax)}$, $H^{6piperidine(ax)}$), 1.55 – 1.38 (m, 3H, $H^{3piperidine(eq),}$ $H^{5piperidine(eq)}$, $H^{4piperidine}$), 1.22 – 1.08 (m, 2H, $H^{3piperidine(ax),}$ $H^{5piperidine(ax)}$). HR-ESI-MS (m/z) calcd for $C_{22}H_{27}N_3O_2$ $[M+H]^+$ 366.2182, found 366.2176.

(*E*)-3-(4-benzylpiperazin-1-yl)-*N*'-(4-methoxybenzylidene)propanehydrazide (2a)

As mentioned in the general method, compound **2** (223.4 µl, 1.84 mmol) and **5a** (481 mg, 1.84 mmol) were stirred overnight at rt. Tetrahydrofuran was used to recrystallize the product. White crystals. Yield: 57 %. mp: 148 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.28, 11.11 (two s, 1H, NH), 8.08, 7.92 (two s, 1H, -N=CH-), 7.62, 7.58 (two d, J = 8.8 Hz, 2H, $H^{2,6}$), 7.34 – 7.20 (m, 5H, H^{phenyl}), 6.99 (t, J = 8.8 Hz, 2H, $H^{3,5}$), 3.80, 3.79 (two s, 3H, -OCH_3), 3.45, 3.43 (two s, 2H, piperazine-CH_2-phenyl), 2.76 (t, J = 7.3 Hz, 1H, -H_2CC=O), 2.65 – 2.55 (m, 2H, CH_2-C<u>H</u>$_2$-piperazine), 2.49 – 2.07 (m, 9H, $H^{piperazine}$, -H_2CC=O). HR-ESI-MS (m/z) calcd for $C_{22}H_{28}N_4O_2$ $[M+H]^+$ 381.2291, found 381.2285.

(*E*)-3-(4-(4-fluorobenzyl)piperazin-1-yl)-*N*'-(4-methoxybenzylidene)propanehydrazide (2b)

As mentioned in the general method, compound **2** (223.4 µl, 1.84 mmol) and **5b** (514 mg, 1.84 mmol) were refluxed for 72 h. Ethylacetate-hexane was used to recrystallize the product. Light pinky crystals. Yield: 80 %. mp: 147 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.27, 11.11 (two s, 1H, NH), 8.08, 7.92 (two s, 1H, -N=CH-), 7.62, 7.58 (two d, J = 8.7 Hz, 2H, $H^{2,6}$), 7.35 – 7.25 (m, 2H, $H^{2',6'}$), 7.18 – 7.09 (m, 2H, $H^{3',5'}$), 6.99 (t, J = 8.7 Hz, 2H, $H^{3,5}$), 3.80, 3.79 (two s, 3H, -OCH_3), 3.43, 3.41 (two s, 2H, piperazine-CH_2-phenyl), 2.76 (t, J = 7.3 Hz, 1H, -H_2CC=O), 2.65 – 2.55 (m, 2H, CH_2-C<u>H</u>$_2$-piperazine), 2.49 – 2.12 (m, 9H, $H^{piperazine}$, -H_2CC=O). HR-ESI-MS (m/z) calcd for $C_{22}H_{27}FN_4O_2$ $[M+H]^+$ 399.2196, found 399.2194.

(*E*)-*N*'-(4-methoxybenzylidene)-3-(4-phenylpiperidin-1-yl)propanehydrazide (2c)

As mentioned in the general method, compound **2** (223.4 µl, 1.84 mmol) and **5c** (535 mg, 1.84 mmol) were stirred overnight at rt. Acetonitrile was used to recrystallize the product. White crystals. Yield: 75 %. mp: 165 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.30, 11.13 (two s, 1H, NH), 8.11, 7.94 (two s, 1H, -N=CH-), 7.63, 7.60 (two d, J = 8.8 Hz, 2H, $H^{2,6}$), 7.33 – 7.13 (m, 5H, H^{phenyl}), 7.00, 6.99 (two d, J = 8.8 Hz, 2H, $H^{3,5}$), 3.80, 3.79 (two s, 3H, -OCH_3), 3.00 (t, J = 11.7 Hz, 2H, $H^{2piperidine(eq),}$ $H^{6piperidine(eq)}$), 2.80 (t, J = 7.2 Hz, 1H, -H_2CC=O), 2.70 – 2.60 (m, 2H, CH_2-C<u>H</u>$_2$-piperidine), 2.49 – 2.42 (m, 1H, $H^{4piperidine}$), 2.38 (t, J = 7.2 Hz, 1H, -H_2CC=O), 2.11 – 2.00 (m, 2H, $H^{2piperidine(ax)}$, $H^{6piperidine(ax)}$), 1.78 – 1.69 (m, 2H, $H^{3piperidine(eq),}$ $H^{5piperidine(eq)}$), 1.68 – 1.55 (m, 2H, $H^{3piperidine(ax),}$ $H^{5piperidine(ax)}$). HR-ESI-MS (m/z) calcd for $C_{22}H_{27}N_3O_2$ $[M+H]^+$ 366.2182, found 366.2173.

(*E*)-3-(4-benzylpiperidin-1-yl)-*N*'-(4-methoxybenzylidene)propanehydrazide (2d)

As mentioned in the general method, compound **2** (223.4 µl, 2.05 mmol) and **5d** (479 mg, 1.84 mmol) were stirred overnight at rt. Acetonitrile was used to recrystallize the product. White crystals. Yield: 56 %. mp: 138 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 11.28, 11.09 (two s, 1H, NH), 8.08, 7.92 (two s, 1H, -N=CH-), 7.62, 7.58 (two d, J = 8.6 Hz, 2H, $H^{2,6}$), 7.30 – 7.10 (m, 5H, H^{phenyl}), 7.00, 6.99 (two d, J = 8.6 Hz, 2H, $H^{3,5}$), 3.80, 3.79 (two s, 3H, -OCH_3), 2.84 (t, J = 11.6 Hz, 2H, $H^{2piperidine(eq),}$ $H^{6piperidine(eq)}$), 2.74 (t, J = 7.2 Hz, 1H, -H_2CC=O), 2.61 – 2.53 (m, 2H, CH_2-C$\underline{H}_2$-piperidine), 2.49, 2.46 (two d, J = 7.0 Hz, 2H, piperidine-CH_2-phenyl), 2.32 (t, J = 7.2 Hz, 1H, -H_2CC=O), 1.86 (t, J = 11.6 Hz, 2H, $H^{2piperidine(ax)}$, $H^{6piperidine(ax)}$), 1.55 – 1.38 (m, 3H, $H^{3piperidine(eq),}$ $H^{5piperidine(eq)}$, $H^{4piperidine}$), 1.22 – 1.07 (m, 2H, $H^{3piperidine(ax),}$ $H^{5piperidine(ax)}$). HR-ESI-MS (m/z) calcd for $C_{23}H_{29}N_3O_2$ $[M+H]^+$ 380.2338, found 380.2344.

Biological Assays

Cholinesterase Inhibition Assay

AChE (electric eel) and BChE (equine serum) from Sigma Aldrich were employed in the assays, following the method previously reported by us [29,31]. To determine the IC_{50} values, GraphPad Prism software (Version 7.0) was used.

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

DPPH and ORAC-FL assays were conducted using our previously reported procedures [29,31]. In DPPH assay, test samples were assayed at 100 µM for 30 min incubation time. Assay was carried out in triplicate, and the mean±SD was computed.

Metal Binding Studies

Studies for the ligand-metal binding evaluation was carried out in accordance with our previously reported methodology [29,31]. The overlapping spectra of the metal-treated ligand and the control solution of the ligand were visualized, and the resulting wavelength (nm) vs. absorbance graphs were drawn.

Evaluation of In Silico Physicochemical Parameters

In order to obtain low energy conformations of the ligands as well as potential ionization states for pH 7.0 ± 2.0, LigPrep module was used. Table 3 shows the Qikprop predictions for the top-scoring states of each compound.

RESULT AND DISCUSSION

Chemistry

Route for the synthesis of intermediates and final compounds were presented in Figure 2. Corresponding key intermediates, 3-(substitutedamino)propane hydrazide intermediates **(5a-d)**, were prepared from the hydrolization with hydrazine hydrate of methyl 3-(substitutedamino)propanoate intermediates **(4a-d)** have been prepared from [27] the Michael addition of methyl acrylate and appropriate amine derivative **(3a-d)**. Finally, from the reaction of starting compound 4-hydroxybenzaldehyde **(1)** or 4-methoxybenzaldehyde **(2)** and corresponding key intermediate **(5a-d)**, *N'*-(4-hydroxybenzylidene) propanehydrazides **(1a-d)** and *N'*-(4-methoxybenzylidene) propanehydrazides **(2a-d)** were synthesized.

Eight final compounds were synthesized in this study. ^{1}H NMR and high-resolution mass spectra (LC-HRMS) were used for the chemical structure and purity verification of the compounds. Additionally, ^{13}C NMR data of the **1a** and **1b** were presented as representatives of the series. The data obtained from all analyses of the compounds matched the proposed structures. Acylhydrazones, in other words, (-C(O)-N-N=CH) structure can potentially have *E/Z* geometrical isomers (C=N), *cis/trans* amide conformers (C(O)-NH). During the characterization of title compounds with TLC and LC-HRMS techniques, only the presence of the *E* isomers, less hindered and favored ones was confirmed.

Additionally, energy calculations of the isomers confirm this observation (Figure 3). As a result of diastereomeric nature of title compounds (*E*, *cis* and *E*, *trans*), explicit sets of certain protons or duplication were observed in ^{1}H NMR spectra with a similar pattern for all compounds (Figure 4).

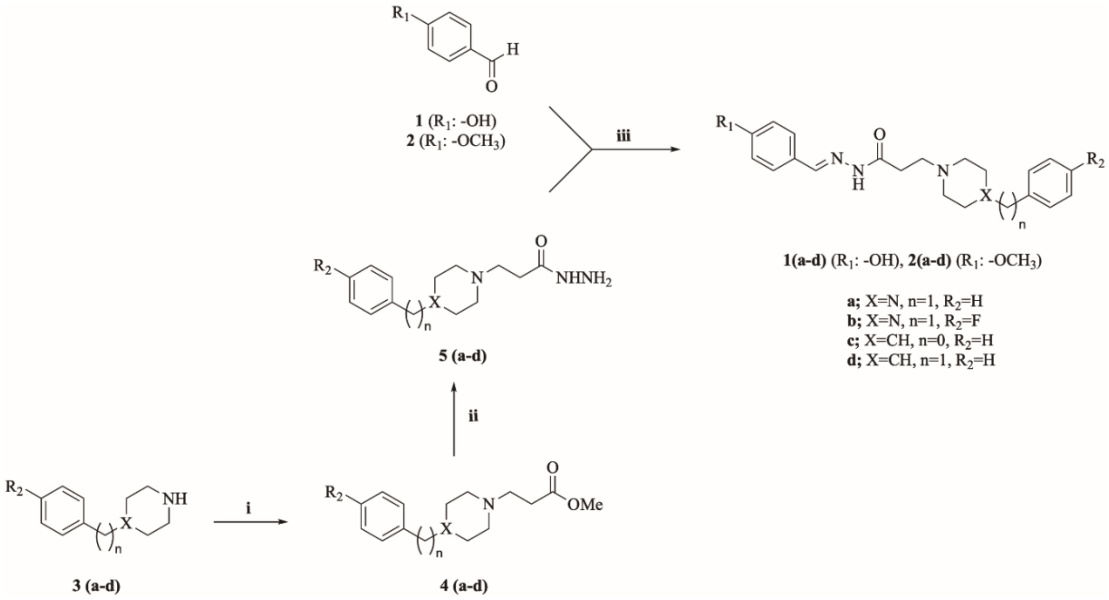

Figure 2. Synthesis of benzylidene propanehydrazide derivatives **1a-d** and **2a-d**. Reagents and conditions: **(i)** Methyl acrylate, DCM, rt, 24h; **(ii)** Hydrazine monohydrate, EtOH, reflux, 4h; **(iii)** EtOH, reflux, 4h

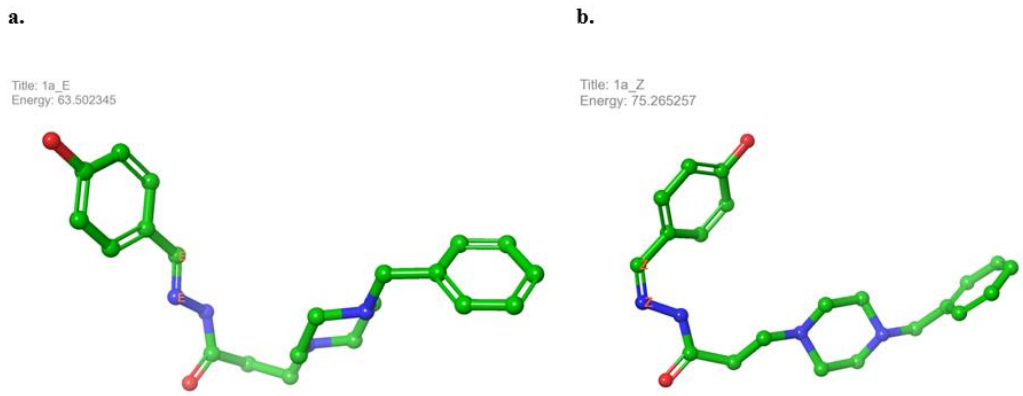

Figure 3. Calculated energies by LigPrep for *E* isomer **(a)** and *Z* isomer **(b)** of compound **1a**

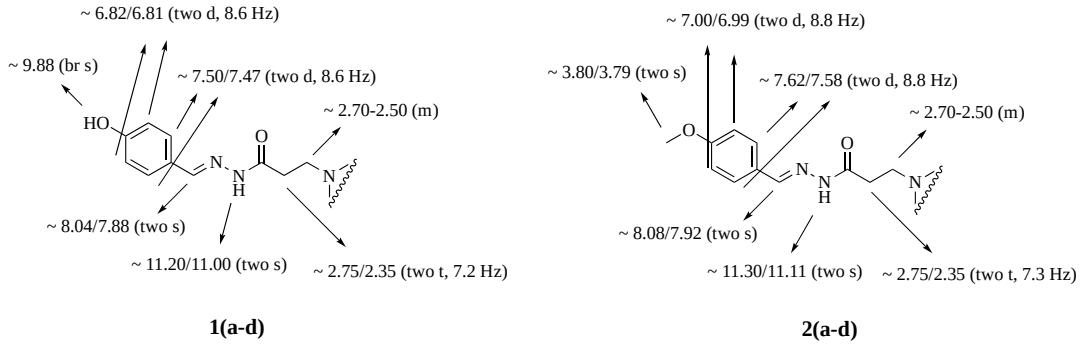

Figure 4. Common ^{1}H NMR peaks of benzylidene propanehydrazide series **1a-d** and **2a-d**

Biology

Cholinesterase Inhibition

By using modified Ellman's method, inhibition percentages of compounds on cholinesterases were evaluated at 10, 33.3 and 100 μM. Subsequently, the IC_{50} values of the final compounds were assayed and calculated. Donepezil and Galantamine were used as positive controls [38]. All the cholinesterase (ChE) inhibitory activity results were presented in Table 1. All the tested compounds, except **2c**, exhibited moderate AChE or BChE inhibitory activity. Among them, only compound **2a** (IC_{50} values for AChE = 12.83 μM and BChE = 72.78 μM) assigned as a dual ChE inhibitor. Interestingly, structure-activity relationship (SAR) results showed us that piperazine derivatives (**1a**, **1b**, **2a**, and **2b**) were preferable for AChE inhibition and piperidine derivatives (**1c**, **1d**, and **2d**) were preferable for BChE inhibition. Additionally, switching from phenol to anisole generally enhances ChE inhibition; just in **2c**, this effect plays its role contrarily.

Table 1. Cholinesterase inhibitory activity results of the synthesized compounds

Compound	***ee*AChE**				***eq*BChE**			
	%inh (10 μM)	%inh (33.3 μM)	%inh (100 μM)	IC_{50}	%inh (10 μM)	%inh (33.3 μM)	%inh (100 μM)	IC_{50}
1a	43.4 ± 0.8	72.8 ± 1.9	87.2 ± 1.1	16.72 ± 4.61	≤10	13.3 ± 3.5	33.1 ± 2.6	≥100
1b	21.4 ± 1.3	46.9 ± 0.4	74.2 ± 1.3	34.04 ± 4.61	≤10	12.5 ± 0.8	36.8 ± 1.3	≥100
1c	≤10	10.5 ± 0.7	36.8 ± 2.9	≥100	20.6 ± 2.1	41.2 ± 4.2	65.5 ± 1.4	59.56 ± 3.5
1d	≤10	29.8 ± 0.1	50.4 ± 3.2	≥100	21.0 ± 2.7	43.7 ± 0.4	66.5 ± 2.8	53.76 ± 4.2
2a	44.4 ± 1.2	73.0 ± 0.2	89.5 ± 1.4	12.83 ± 3.80	18.4 ± 1.5	45.1 ± 1.7	59.6 ± 2.6	72.78 ± 5.68
2b	23.1 ± 1.7	51.4 ± 1.4	78.4 ± 0.3	29.26 ± 4.45	≤10	25.5 ± 2.0	54.0 ± 0.2	≥100
2c	≤10	≤10	16.4 ± 0.4	≥100	26.6 ± 3.4	53.9 ± 1.3	42.2 ± 1.1	≥100
2d	≤10	24.0 ± 9.9	54.7 ± 1.1	≥100	42.9 ± 2.5	73.1 ± 1.0	89.4 ± 3.7	16.02 ± 1.2
Donepezil	98.7 ± 0.4	-	-	0.062 ± 0.002	89.9 ± 0.3	-	-	3.55 ± 0.07
Galantamine	91.9 ± 0.7	-	-	nd	29.9 ± 0.5	-	-	nd

Antioxidant Activity

Both the radical scavenging (DPPH assay) and oxygen radical absorbance (ORAC-FL assay) capabilities of the compounds were investigated. Antioxidant standarts, Gallic acid [39] and Trolox [40] were recruited for DPPH and ORAC-FL assays, respectively. Table 2 displays the results. At 100 μM, phenol derivatives **(1a-d)** showed low radical scavenging activity (between %12 and %33). However, at the same concentration, none of the anisole derivatives **(2a-d)** exhibited radical scavenging activity. Nonetheless, every molecule exhibited very high ORAC-FL values, which varied from 0.743 to 24.958 Trolox equivalents. Furthermore, phenol derivatives **(1a–d)**, in particular compound **1b** (as 24.958 trolox equivalent), have extremely significant capabilities to absorb oxygen radicals.

Metal Binding

Cu(II), Fe(II), and Zn(II) binding capabilities of the compounds were assessed by screening their absorptions in the 230-500 nm range. Any change in the metal-treated ligand's spectra relative to the spectra of the ligand alone, as determined by the UV-vis spectrophotometry method, is ascribed to complexation [41]. "Difference UV-vis spectra" were created for improved assessment. Figure 5 shows the "difference UV-vis spectra" that were obtained by subtracting the separate absorbances of the ligand and metal from the absorbance of the metal-ligand mixture. All compounds demonstrated chelation with every tested ion, though at different rates, as we saw. Interestingly, **1c** and **2d** showed significant Fe(II) and Cu(II) chelation. Additionally, **2d** showed significant Zn(II) chelation.

Table 2. Antioxidant activity results of the compounds

Compound	DPPH % inhibition (100 µM)	ORAC-FL
1a	12.15 ± 1.08	11.436 ± 0.381
1b	12.76 ± 0.88	24.958 ± 0.490
1c	22.67 ± 1.00	14.546 ± 0.535
1d	33.63 ± 1.47	2.556 ± 0.337
2a	≤10	1.153 ± 0.150
2b	≤10	1.159 ± 0.099
2c	≤10	0.692 ± 0.147
2d	≤10	0.743 ± 0.093
Gallic acid (100 µM)	92.71 ± 0.90	nd
Trolox	nd	1.000

The mean ± SD of three independent experiments. nd; not determined

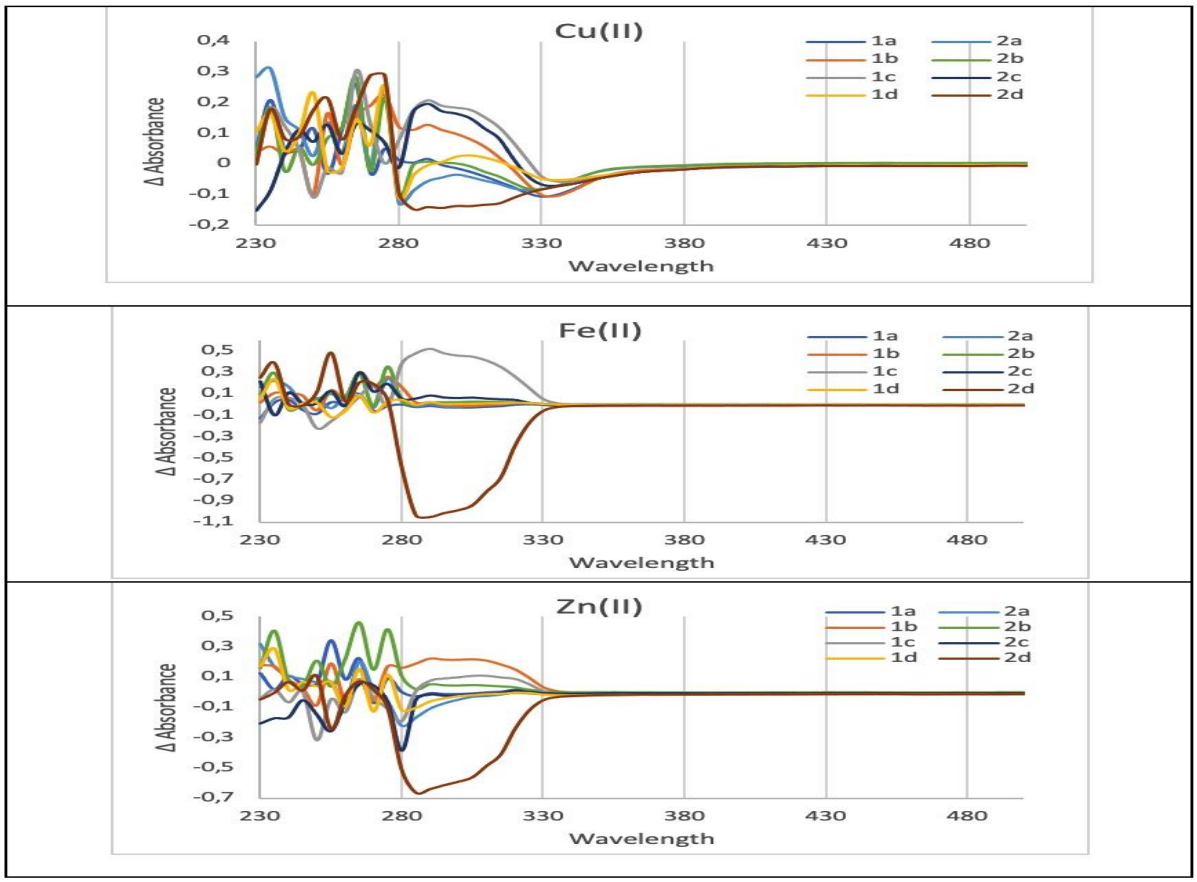

Figure 5. The UV–vis difference spectra between title compounds and metal ions

Evaluation of *in silico* physicochemical parameters

For the calculation of in-silico physicochemical properties and ADME predictions QikProp Schrodinger Suite 2023 was used [42]. The results are presented in Table 3. With QPlogBB values ranging from -1.029 to 0.149, all of the compounds were predicted to be BBB-permeable. Most of the compounds were forecasted to exhibit a CNS score of 1, falling within the range of -2 (inactive) to +2 (active). Exceptionally, **1c** and **1d**, but these compounds have not significant chlolinesterase inhibition.

All of the compounds follow Lipinski's rule of five [43]. Additionally, all the compunds, except **2d**, follow Jorgensen's rule of three [44]. QPlogS value of **2d** (-5.739) slightly over Jorgensen's but it is in the recommended range by the Qikprop manual. As a result, *in silico* ADME predictions revealed that the compounds generally have acceptable drug-likeness as well as significant BBB permeation capacity.

Table 3. Predicted physicochemical parameters of compounds

Descriptor	Compounds								Recommended value
	1a	1b	1c	1d	2a	2b	2c	2d	
MW	366.462	384.452	351.447	365.474	380.489	398.479	365.474	379.501	130 - 725
vol	1.259.398	1278.077	1237.221	1291.965	1313.241	1329.378	1280.682	1346.391	500 - 2000
n-rot	9	9	7	9	9	9	7	9	0 - 15
DHB	2.000	2.000	2.000	2.000	1.000	1.000	1.000	1.000	0 - 6
AHB	7.250	7.250	5.250	5.250	7.250	7.250	5.250	5.250	2 - 20
PSA	84.620	84.634	79.464	79.305	70.267	70.322	62.087	65.105	7 - 200
QPlogS	-2.752	-3.221	-5.006	-5.151	-3.197	-3.562	-5.203	-5.739	-6.5 - 0.5
QlogP$_{o/w}$	2.525	2.745	3.681	4.025	3.339	3.572	4.540	4.904	2.0 - 6.5
QPPCaco	39.905	37.195	151.930	151.811	132.013	131.449	687.218	500.498	<25 poor, >500 great
QlogBB	-0.537	-0.490	-0.886	-1.029	0.043	0.149	-0.102	-0.422	-3.0 - 1.2
CNS	1	1	0	-2	1	1	1	1	-2 (inactive) to +2 (active)
#metab	5	5	5	4	5	5	5	4	1 - 8
%HOA	70.385	71.129	87.548	89.554	84.453	85.783	100.000	100.000	>80% high <25% poor
VRF	0	0	0	0	0	0	0	0	-
VJR	0	0	0	0	0	0	0	1	-

MW: Molecular weight **vol:** Total solvent-accessible volume **n-rot:** Number of rotatable bonds **DHB:** Estimated number of hydrogen bond donors **AHB:** Estimated number of hydrogen bond acceptors (2.0-20.0) **PSA:** Van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl carbon atoms **QPlogS:** Predicted aqueous solubility **QlogPo/w:** Predicted octanol/water partition coefficient **QPCaco:** Predicted apparent Caco-2 cell permeability **QlogBB:** Predicted brain/blood partition coefficient **CNS:** Predicted central nervous system activity **#metab:** Number of likely metabolic reactions.**%HOA:**Predicted human oral absorption percent **VRF:** Number of violations of Lipinski's rule of five (The rules are: MW < 500, logP <5, DHB ≤ 5, AHB ≤ 10) **VJR:** Number of violations of Jorgensen's rule of three (QPlogS > -5.7, QP PCaco > 22 nm/s, # Primary Metabolites < 7)

In this study, we designed and synthesized novel *N'*-(benzylidene)propanehydrazide derivatives. Subsequently, their anti-cholinesterase, antioxidant, and metal-chelating abilities were evaluated with the aim of obtaining new hit compounds. Based on the ChE inhibitory activity results, all the tested compounds, except **2c**, exhibited moderate AChE or BChE inhibitory activity. Especially, **2a** (IC_{50} values for AChE = 12.83 μM and BChE = 72.78 μM) and **2d** (IC_{50} value for BChE = 16.02 μM) were determined as the most potent inhibitors for AChE and BChE, respectively. Additionally, **2a** (1.153) and **2d** (0.743) exhibited similar antioxidant activity to trolox. Unfortunately, the antioxidant capacities of the cholinesterase inhibitory active compounds were relatively lower than the inactive ones. Moreover, metal-binding studies indicated that all the compounds were chelators for copper, iron, and zinc ions. Especially, the active compound **2d** showed significant Zn(II) chelation. Besides, compounds demonstrated appreciable blood-brain barrier (BBB) permeation capacity along with acceptable lead-like properties according to in-silico predictions. Overall, **2a** and **2d** could be considered as a starting point for new structural modifications in the MTDL design against AD.

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AUTHOR CONTRIBUTIONS

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