DOI 10.1007/s11094-019-02000-4
Pharmaceutical Chemistry Journal, Vol. 53, No. 4, 19, 2019 (Russian Original Vol. 53, No. 4, April, 2019)

DESIGN AND SYNTHESIS OF 2-SUBSTITUTEDPHENYL BENZO[D]THIAZOLE DERIVATIVES AND THEIR β-AMYLOID AGGREGATION AND CHOLINESTERASE INHIBITORY ACTIVITIES

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Original article submitted November 5, 2018.

The occurrence of amyloid-β (Aβ) and reduced cholinergic tranmission are two major hallmarks of Alzheimer's disease (AD). Therefore, a series of new 2-phenylbenzo[*d*]thiazoles substituted with azole/piperazine moieties were designed, synthesized, and evaluated as potential dual inhibitors of Aβ aggregation and cholinesterase (ChE) activities. *In vitro* studies showed that compound **2m** containing an imidazole ring strongly inhibited $A\beta_{1-40}$ (49.2%) and $A\beta_{1-42}$ aggregation (60.6%). All derivatives exhibited weak inhibitory activities against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Therefore, compound **2m** may represent promising therapeutic option for inhibiting Aβ-mediated pathology in AD.

Keywords: benzothiazole; Alzheimer's disease; beta amyloid; cholinesterase.

1. INTRODUCTION

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disease and is the most common type of dementia known since ancient times [1 –3]. Distinctive plaques and neurofibrillary tangles in the brain tissue of dementia patients were described for the first time by Alois Alzheimer, a German psychiatrist, in 1906. Today, these findings are accepted as characteristic features of AD [4, 5]. Although many hypotheses regarding the pathophysiology of AD have been proposed, its pathogenesis cannot be still completely explained [6]. Reduced cholinergic transmission, beta amyloid (Aβ) protein aggregation, hyperphosphorylation of tau protein, increased oxidative stress, inflammation, and exposure to toxic metal ions have been implicated as the causes or contributors to the progression of AD [7 – 11]. Increasing cholinergic functions, inhibiting Aβ aggregate formation, and reducing tau protein hyperphosphorylation constitute the main treatment approaches for AD [12 – 14].

Research has been primarily focused on cholinesterase (ChE) inhibitors that can increase cholinergic transmission. Tacrin, donepezil, rivastigmine, and galantamine developed as a result of these studies were approved by the American Food and Drug Administration (FDA) in 1996, 1996, 2000, and 2001, respectively, for the treatment of AD [15]. However, studies also showed that acetylcholinesterase (AChE) exhibits non-cholinergic functions related to the formation and storage of amyloid aggregates, thus playing a role in the early stages of senile plaque development. Therefore, the cholinergic and amyloid hypotheses cannot be independently considered [16 – 18]. After AD was demonstrated to be a multifactorial disease, extensive research has been focused on adopting a new strategy that involved the design of multi-target-directed ligands (MTDLs). This approach features the development of new molecules that inhibit both amyloid aggregation and ChE activity [18 – 20].

The benzothiazole moiety is an interesting scaffold for the design of new compounds for non-invasive diagnostics and treatment of AD [2, 8, 21 – 32]. Hybrid compounds containing a benzothiazole ring and tacrine exhibit significant AChE and Aβ aggregation inhibitory activity [2, 20, 33]. Previous studies have shown that various compounds containing both benzothiazole and piperazine rings exhibit strong AChE inhibition [22, 23]. In addition, several benzothiazole analogs have been screened as potential amyloid-binding diagnostic agents for various neurodegenerative diseases [28 – 32]. Based on the above information, a series of new hybrid compounds bearing benzothiazole and azole/piperazine moieties were designed, synthesized, and evaluated

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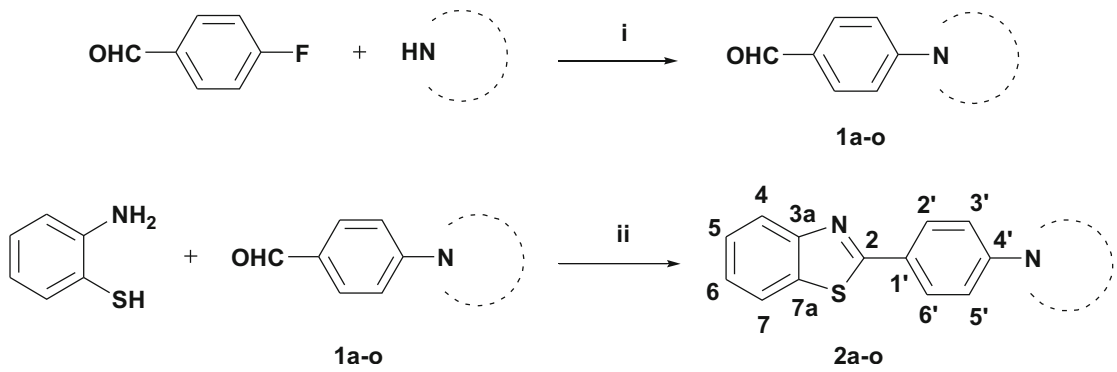


Scheme 1. Synthesis of the target compounds. Reagents and conditions: (i) K_2CO_3, DMSO, reflux (ii) DMSO, 140°C.

in this work as potential dual inhibitors for Aβ aggregation and ChE activity.

2. RESULTS AND DISCUSSION

2.1. Chemistry

In this study, a total of 15 2-(4-substitutedphenyl)benzo-[*d*]thiazole derivatives bearing azole or substituted piperazine moieties at the 4 position of the phenyl ring were synthesized, of which two have been previously reported (**2a** and **2l**) [29, 34, 35]. The target compounds, **2a**–**o**, were obtained using the synthetic route outlined in **Scheme 1**. The starting compounds, 4-substituted benzaldehyde derivatives **1a–o**, were obtained by aromatic nucleophilic substitution of 4-fluorobenzaldehyde with various azole and piperazine derivatives in the presence of potassium carbonate with yields of 70 – 81% [36]. The target compounds, 2-(4-substitutedphenyl) benzo[*d*]thiazole derivatives, **2a–o** were obtained by treating the substituted benzaldehydes **1a–o** with *o*-aminothiophenol at 140°C in moderate yields (55 – 79%). The structures of the target compounds were elucidated using infrared (IR), ^{1}H- and ^{13}C NMR, electrospray ionization-mass spectrometry (ESI-MS), and elemental analysis for the compounds not already described in the literature.

In the IR spectra of the target compounds, the absence of signals at approximately 2837 – 2812 and 1690 – 1657 cm^{-1}, which were seen in the spectra of the corresponding aldehydes, was evidence for the benzothiazole ring closure. In the ^{1}H-NMR spectra of the target compounds, signals observed as triplets or triplet of doublets at approximately 7.44 – 7.48 and 7.30 – 7.37 ppm were assigned to H_5 and H_6, whereas the signals observed as doublets at approximately 7.85 and 8.00 ppm were assigned to H_4 and H_7 of the benzothiazole ring, respectively. In addition, the ortho- (2,6-) and meta- (3,5-) phenyl protons at position 2 of benzothiazole were observed at approximately 7.90 and 8.00 ppm, respectively. Furthermore, in the ^{13}C-NMR spectra, C_4, C_7, C_6, C_5, C_{7a}, C_{3a}, and C_2 of the benzothiazole ring appeared at 122.32 – 122.34, 122.79 – 122.87, 125.49 – 125.59, 126.62 – 126.77, 134.44 – 134.51, 153.42 – 153.55, and 165.88 – 166.33 ppm, respectively. In the ESI-MS spectra, the protonated $[M+H]^+$ and sodiated $[M+Na]^+$ molecular ions of all compounds were observed. Elemental analysis agreed well with the theoretical chemical structures of the synthesized compounds.

2.2. Biological Activity

Inhibition of self-mediated $A\beta_{1–40}$ and $A\beta_{1-42}$ aggregation. Inhibitory effects of compounds **2a – 2o** on Aβ fibril formation were evaluated using the Thioflavin T method, and donepezil was used as a reference compound (Table 1). It was found that compounds **2a – 2o** bearing imidazole, triazole, and benzimidazole rings at the 4 position of the phenyl ring, respectively, showed good inhibitory activity on $A\beta_{1–40}$ (49.2 – 74.9%) and $A\beta_{1-42}$ (47.3 – 60.6%) aggregation. Furthermore, compound **2m** inhibited $A\beta_{1–40}$ aggregation better than donepezil ($p < 0.05$).

When the inhibitory activities of piperazine-substituted compounds (**2a – 2k**) on Aβ fibril formation were examined, the derivatives bearing small substituents, such as methyl, ethyl, and acetyl (**2a – 2c**) groups at the 4-position, showed good inhibitory effects on $A\beta_{1-42}$ fibril formation ($A\beta_{1-42}$: 63.7 – 82.7%). However, bulky substituents, such as cyclohexyl, phenyl, and benzyl groups, at the 4-position of piperazine reduced the inhibitory activity (**2d – 2k**). In particular, compound **2i** exhibited remarkable inhibitory activity on $A\beta_{1-40}$ (61.6%) and $A\beta_{1-42}$ (64.7%) fibril formation.

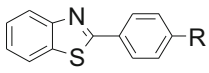
Cholinesterase inhibitory activity. Compounds **2a – 2o** were tested for their inhibitory activity towards AChE and BChE using the Ellman method and the results are summarized in Table 2. All compounds (except **2e** and **2o**) showed no inhibitory activity (7.9 – 48.7%) against either ChE at a concentration of 100 μM. The IC_{50} values of **2e** (IC_{50}: 78.09 μM for AChE, IC_{50}: 107.00 μM for BChE) and **2o** (IC_{50}: 46.84 μM for AChE and IC_{50}: 40.61 μM for BChE) were evaluated, clearly showing weak inhibitory activities towards both AChE and BChE.

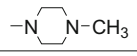
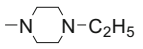
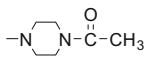
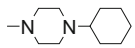
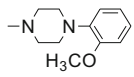
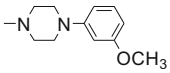
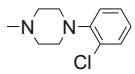
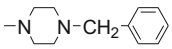
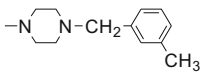
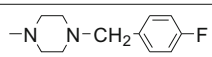
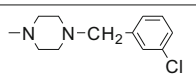
3. EXPERIMENTAL CHEMICAL PART

3.1. Materials and Methods

Melting points of the compounds were determined using a Stuart SMP20 melting point apparatus and the results are reported as uncorrected values. ATR-FTIR spectra were obtained using a MIRacle ATR accessory (Pike Technologies) in conjunction with a Spectrum BX FTIR spectrometer (Perkin Elmer) and positions of peaks are reported in cm^{-1}. The ^{1}H and ^{13}C NMR spectra (DMSO-d_6/$CDCl_3$) were recorded using Varian Mercury 400 FT NMR spectrophotometer with TMS as the internal standard (chemical shifts reported as δ, ppm). The ESI-MS spectra were measured using Micromass ZQ-4000 single-quadruple mass spectrometer. Elemental analyses (C, H, N, and S) were performed using Leco CHNS 932 analyzer.

TABLE 1. Effects of Compounds **2a – 2o** on the Inhibition of Self-Mediated $A\beta_{1-40}$ and $A\beta_{1-42}$ Aggregation



Comp	R	$A\beta_{1-40}$ aggregation ±SEM (%)	$A\beta_{1-42}$ aggregation ±SEM (%)
2a		81.3 ± 11.3	82.7 ± 8.1
2b		68.4 ± 6.5	70.5 ± 7.1
2c		55.6 ± 5.0*	63.7 ± 7.4*
2d		≥ 100	74.8 ± 8.2
2e		70.1 ± 4.4	86.8 ± 9.5
2f		≥ 100	69.7 ± 7.0
2g		≥ 100	≥ 100
2h		≥ 100	73.5 ± 7.2
2i		61.6 ± 5.2*	64.7 ± 7.2
2j		≥ 100	≥ 100
2k		≥ 100	≥ 100
2l		96.5 ± 10.5	≥ 100
2m		49.2 ± 6.8**	60.6 ± 6.8*
2n		60.9 ± 5.6*	47.3 ± 5.5**
2o		74.9 ± 13.3	60.6 ± 7.3*
Control[a]		100	100
Donepezil		83.1 ± 5.0	61.9 ± 8.7*

[a] The control of each assay was non-treated protein; data show mean ± SEM values for at least three independent experiments; * $p < 0.05$, ** $p < 0.001$.

3.2. Synthesis

General procedure for the preparation of 4-substitutedbenzaldehydes (1a – 1o). First, 4-fluorobenzaldehyde and various azole/piperazine derivatives were reacted using potassium carbonate as catalyst in DMSO as described in the literature [36].

General procedure for the preparation of 2-(4-substituted phenyl)benzo[*d*]thiazoles (2a – 2o). Initially, 4-substituted benzaldehydes **1a – 1o** (0.02 mol) and 2-aminothiophenol (0.02 mol) in 10 mL DMSO were heated under reflux at 140°C for 3 h. The mixture was then poured into 100 mL of ice-cold distilled water. The precipitated solid was collected by filtration and purified by crystallization from an appropriate solvent.

The synthesized compounds were characterized as follows:

2-[4-(4-methylpiperazin-1-yl)phenyl]benzo[*d*]thiazole (2a): m.p., 207°C (from methanol) (lit. [35], 211 – 212°C).

2-[4-(4-ethylpiperazin-1-yl)phenyl]benzo[*d*]thiazole (2b): yield, 0.49 g (75%); m.p. 215°C (from methanol); found: C, 70.29; H, 6.76; N, 12.86; S, 9,69; Anal. Calcd. for $C_{19}H_{21}N_3S$: C, 70.55; H, 6.54; N, 12.99; S, 9.91%; IR (ν_{max}, cm^{-1}): 3051 (C-H), 2979, 2943, 2805, 2767 (C-H), 1602, 1589, 1478, 1436, 1423 (C=N, C=C), 1223 (C-N); ^{1}H-NMR (400 MHz; DMSO-d_6; Me_4Si; δ_H, ppm): 1.02 (3H, t, -CH_3); 2.36 (2H, q, -CH_2-); 2.47 – 2.50 (4H, m, piperazine, overlapped with DMSO); 3.28 – 3.30 (4H, m, piperazine); 7.04 (2H, d, J = 9.2 Hz, $H_{3'}$, $H_{5'}$); 7.36 (1H, td, J_1 = 7.6 Hz, J_2 = 1.2 Hz, H_6); 7.46 (1H, t, J_1 = 8.0 Hz, H_5); 7.87 – 7.94 (3H, m, H_4, $H_{2'}$, $H_{6'}$); 8.03 (1H, d, J = 8.0 Hz, H_7). ^{13}C-NMR (100 MHz; DMSO-d_6; Me_4Si; δ_C, ppm): 11.94 (CH_2-$\underline{C}H_3$); 47.82 and 52.54 (piperazine); 52.33 ($\underline{C}H_2$-CH_3); 114.75; 121.41; 122.53; 124.03; 124.46; 126.06; 128.76; 134.68; 152.95; 154.33; 168.24. ESI-MS (*m/z*): 346.31 $[M+Na]^+$ (100%), 324.35 $[M+H]^+$.

2-[4-(4-acetylpiperazin-1-yl)phenyl]benzo[*d*]thiazole (2c): yield, 0.48 g (71%); m.p., 292°C (from DMF and H_2O); Found: C, 67.50; H, 5.79; N, 12.58; S, 9,29. Anal. Calcd. for $C_{19}H_{19}N_3OS$: C, 67.63; H, 5.68; N, 12.45; S, 9.50%; IR (ν_{max}, cm^{-1}): 3053 (C-H), 2994, 2840 (C-H), 1650 (C=O), 1600, 1479, 1434, 1424 (C=N, C=C), 1219 (C-N); ^{1}H-NMR (400 MHz; DMSO-d_6; Me_4Si; δ_H, ppm): 2.04 (3H, s, -CO-CH_3); 3.24 – 3.38 (4H, m, piperazine); 3.58 – 3.62 (4H, m, piperazine); 7.07 (2H, d, J = 9.2 Hz, $H_{3'}$, $H_{5'}$); 7.37 (1H, t, J = 7.2 Hz, H_6); 7.48 (1H, t, J = 7.2 Hz, H_5); 7.91–7.95 (3H, m, H_4, $H_{2'}$, $H_{6'}$); 8.04 (1H, d, J = 7.6 Hz, H_7). ESI-MS (*m/z*): 360.25 $[M+Na]^+$ (100%), 338.29 $[M+H]^+$.

2-[4-(4-cyclohexylpiperazin-1-yl)phenyl]benzo[*d*]thiazole (2d): yield, 0.58 g (77%); m.p., 234°C (from ethyl acetate); Found: C, 73.15; H, 7.10; N, 11.17; S, 8.46; Anal. Calcd. for $C_{23}H_{27}N_3S$: C, 73.17; H, 7.21; N, 11.13; S, 8.49%;

TABLE 2. Anti-Cholinesterase Activities of Compounds **2a –– 2o**

Comp	AChE inhibition ± SE (%)	BChE inhibition ± SE (%)	AchE IC_{50}[a] ± SE (μM)	BchE IC_{50}[a] ± SE (μM)
2a	8.2 ± 0.4	45.7 ± 2.1	–	–
2b	12.5 ± 7.2	48.7 ± 3.4	–	–
2c	nd	25.8 ± 4.9	–	–
2d	nd	33.0 ± 3.0	–	–
2e	56.3 ± 8.4	53.9 ± 4.3	78.09 ± 1.36	107.00 ± 2.78
2f	25.2 ± 3.8	nd	–	–
2g	37.6 ± 8.2	42.3 ± 1.3	–	–
2h	10.2 ± 4.4	24.4 ± 1.2	–	–
2i	48.1 ± 6.5	32.6 ± 2.1	–	–
2j	7.9 ± 2.1	33.9 ± 5.1	–	–
2k	22.8 ± 7.3	30.4 ± 4.5	–	–
2l	nd	47.7 ± 7.6	–	–
2m	20.8 ± 1.2	43.3 ± 4.4	–	–
2n	nd	34.9 ± 4.8	–	–
2o	72.8 ± 10.6	44.3 ± 6.5	46.84 ± 1.77	40.61 ± 1.59
Donepezil	–	–	6.16 ± 1.03[b]	4.94 ± 1.04

[a] IC_{50} values of compounds indicate the concentration that caused 50% enzyme activity loss; [b] IC_{50} (nM) ± SE;
nd = not determined; data show mean ± SE values for at least three independent experiments; * $p < 0.05$.

IR (ν_{max}, cm^{-1}): 2918, 2848 (C-H), 1605, 1478, 1438, 1427 (C=N, C=C), 1227 (C-N); 1H-NMR (400 MHz; $CDCl_3$; Me_4Si; δ_H, ppm): 1.11 – 1.93 (10H, m, cyclohexyl); 2.28 – 2.35 (1H, m, -N-CH-); 2.72 – 2.78 (4H, m, piperazine); 3.30 – 3.36 (4H, m, piperazine); 6.95 (2H, d, J = 8.0 Hz, $H_{3'}$, $H_{5'}$); 7.31 (1H, td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, H_6); 7.44 (1H, td, J_1 = 8.4 Hz, J_2 = 1.2 Hz, H_5); 7.85 (1H, d, J = 8.0 Hz, H_4); 7.95–8.01 (3H, m, H_7, $H_{2'}$, $H_{6'}$). ^{13}C-NMR (100 MHz; DMSO-d_6; Me_4Si; δ_C, ppm): 25.81; 26.26; 28.92; 63.55 (cyclohexyl); 48.27 and 48.77 (piperazine); 114.69; 121.40; 122.52; 123.93; 124.44; 126.05; 128.76; 134.67; 153.05; 154.34; 168.30. ESI-MS (*m/z*): 400.39 $[M+Na]^+$, 378.41 $[M+H]^+$ (100%).

2-[4-(4-(2-methoxyphenyl)piperazin-1-yl)phenyl]benzo-[*d*]thiazole (2e): yield, 0.64 g (79%); m.p., 218°C (from ethyl acetate); Found: C, 71.65; H, 5.84; N, 10.40; S, 8.09; Anal. Calcd. for $C_{24}H_{23}N_3OS$: C, 71.79; H, 5.77; N, 10.47; S, 7.99%; IR (ν_{max}, cm^{-1}): 3060 (C-H), 2951, 2829 (C-H), 1602, 1498, 1479, 1439, 1421 (C=N, C=C), 1223 (C-N), 1029 (C-O); ^{1}H-NMR (400 MHz; DMSO-d_6; Me_4Si; δ_H, ppm): 3.08–3.13 (4H, m, piperazine); 3.42–3.46 (4H, m, piperazine); 3.79 (3H, s, -OCH_3); 6.86 – 6.99 (4H, m, $H_{3''}$-$H_{6''}$); 7.11 (2H, d, J = 9.2 Hz, $H_{3'}$, $H_{5'}$); 7.36 (1H, td, J_1 = 7.6 Hz, J_2 = 0.8 Hz, H_6); 7.47 (1H, td, J_1 = 7.6 Hz, J_2 = 1.2 Hz, H_5); 7.91 – 7.95 (3H, m, H_4, $H_{2'}$, $H_{6'}$); 8.04 (1H, d, J = 8.0 Hz, H_7). ^{13}C-NMR (100 MHz; $CDCl_3$; Me_4Si; δ_C, ppm): 48.21 and 50.53 (piperazine); 55.45 (-O-CH_3); 111.38; 114.91; 118.32; 121.08; 121.43; 122.55; 123.38; 124.18; 124.49; 126.08; 128.81; 134.69; 151.34; 152.31; 153.07; 154.31; 168.25; 154.33; 168.24. ESI-MS (*m/z*): 424.29 $[M+Na]^+$ (100%), 402.33 $[M+H]^+$.

2-[4-(4-(3-methoxyphenyl)piperazin-1-yl)phenyl]benzo-[*d*]thiazole (2f): yield, 0.58 g (72%); m.p., 248°C (from chloroform and methanol); Found: C, 71.39; H, 5.95; N, 10.28; S, 7.78; Anal. Calcd. for $C_{24}H_{23}N_3OS$: C, 71.79; H, 5.77; N, 10.47; S, 7.99%; IR (ν_{max}, cm^{-1}): 2975, 2880, 2837 (C-H), 1602, 1479, 1434, 1425 (C=N, C=C), 1224 (C-N), 1036 (C-O); ^{1}H-NMR (400 MHz; $CDCl_3$; Me_4Si; δ_H, ppm): 3.36 (4H, bs, piperazine); 3.49 (4H, bs, piperazine); 3.81 (3H, s, -OCH_3); 6.46–6.64 (3H, m, $H_{2''}$, $H_{4''}$, $H_{6''}$); 7.01 (2H, d, J = 8.4 Hz, $H_{3'}$, $H_{5'}$); 7.21 (1H, t, J = 8.4 Hz, $H_{5''}$); 7.33 (1H, td, J_1 = 7.8 Hz, J_2 = 1.2 Hz, H_6); 7.45 (1H, td, J_1 = 7.8 Hz, J_2 = 1.2 Hz, H_5); 7.86 (1H, d, J = 8.0 Hz, H_4); 7.99 – 8.04 (3H, m, H_7, $H_{2'}$, $H_{6'}$). ^{13}C-NMR (100 MHz; $CDCl_3$; Me_4Si; δ_C, ppm): 47.95 and 49.12 (piperazine); 55.24 (-O-CH_3); 102.97; 109.16; 115.05; 121.43; 122.57; 124.55; 126.12; 128.84; 129.95; 134.66; 152.75; 160.66; 168.14. ESI-MS (*m/z*): 424.29 $[M+Na]^+$ (100%), 402.33 $[M+H]^+$.

2-[4-(4-(2-chlorophenyl)piperazin-1-yl)phenyl]benzo-[*d*]thiazole (2g): yield: 0.45 g (55%); m.p., 238°C (from chloroform); Found: C, 68.23; H, 4.83; N, 10.51; S, 7.91; Anal. Calcd. for $C_{23}H_{20}ClN_3S$: C, 68.05; H, 4.97; N, 10.35; S, 7.90%; IR (ν_{max}, cm^{-1}): 3069 (C-H), 2975, 2883, 2819 (C-H), 1603, 1588, 1477, 1438, 1420 (C=N, C=C), 1224 (C-N); ^{1}H-NMR (400 MHz; $CDCl_3$; Me_4Si; δ_H, ppm): 3.22–3.26 (4H, m, piperazine); 3.50–3.52 (4H, m, piperazine); 6.99 – 7.10 (4H, m, $H_{3'}$, $H_{5'}$, $H_{4''}$, $H_{6''}$); 7.26 (1H, m, $H_{5''}$, overlapped $CHCl_3$); 7.33 (1H, td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, H_6); 7.40 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz $H_{3''}$); 7.46 (1H, td, J_1 = 7.6 Hz, J_2 = 1.6 Hz, H_5); 7.86 (1H, d, J = 7.6 Hz, H_4); 8.01–8.04 (3H, m, H_7, $H_{2'}$, $H_{6'}$). ^{13}C-NMR (100 MHz; $CDCl_3$; Me_4Si; δ_C, ppm): 48.32 and 51.04 (piperazine); 115.04; 120.37; 121.43; 122.53; 124.07; 124.55; 126.13; 127.66; 128.84; 130.74; 134.60; 146.65; 146.89; 153.00; 168.22. ESI-MS *(m/z)*: 428.22 $[M+Na]^+$ (100%), 406.25 $[M+H]^+$.

2-[4-(4-benzylpiperazin-1-yl)phenyl]benzo[*d*]thiazole (2h): yield, 0.55 g (71%); m.p., 221°C (from acetone); Found: C, 75.03; H, 6.22; N, 10.95; S, 8.35; Anal. Calcd. for $C_{24}H_{23}N_3S$: C, 74.77; H, 6.01; N, 10.90; S, 8.32%; IR (ν_{max}, cm^{-1}): 3058, 3023 (C-H), 2886, 2830, 2786 (C-H), 1606, 1477, 1436, 1425 (C=N, C=C), 1251 (C-N); ^{1}H-NMR (400 MHz; $CDCl_3$; Me_4Si; δ_H, ppm): 2.63 (4H, bs, piperazine); 3.35 (4H, bs, piperazine); 3.59 (2H, s, -CH_2-); 6.94 (2H, d, J = 9.2 Hz, $H_{3'}$, $H_{5'}$); 7.26–7.36 (6H, m, H_6, $H_{2''}$, $H_{3''}$, $H_{4''}$, $H_{5''}$, $H_{6''}$); 7.44 (1H, t, J = 7.2 Hz, H_5); 7.85 (1H, d, J = 7.2 Hz, H_4); 7.95–8.01 (3H, m, H_7, $H_{2'}$, $H_{6'}$). ^{13}C-NMR (100 MHz; $CDCl_3$; Me_4Si; δ_C, ppm): 47.85 and 52.77 (piperazine); 62.99 (-CH_2-); 114.76; 121.41; 122.54; 123.99; 124.46; 126.06; 127.25; 128.33; 128.77; 129.19; 134.68; 153.01; 154.34; 168.27. ESI-MS (*m/z*): 408.25 $[M+Na]^+$, 386.29 $[M+H]^+$ (100%).

2-[4-(4-(3-methylbenzyl)piperazin-1-yl)phenyl]benzo-[*d*]thiazole (2i): yield, 0.58 g (72%); m.p., 186°C (from acetone); Found: C, 75.04; H, 6.24; N, 10.53; S, 8.13; Anal. Calcd. for $C_{25}H_{25}N_3S$: C, 75.15; H, 6.31; N, 10.52; S, 8.03%; IR (ν_{max}, cm^{-1}): 2918, 2845 (C-H), 1606, 1478, 1437, 1426 (C=N, C=C), 1249 (C-N); ^{1}H-NMR (400 MHz; DMSO-d_6; Me_4Si; δ_H, ppm): 2.29 (3H, s, -CH_3); 2.47 – 2.51 (4H, m, piperazine, overlapped DMSO); 3.28–3.32 (4H, m, piperazine); 3.47 (2H, s, -CH_2-); 7.01–7.13 (5H, m, $H_{3'}$, $H_{5'}$, $H_{2''}$, $H_{4''}$, $H_{6''}$); 7.20 (1H, t, J = 7.2 Hz, $H_{5''}$); 7.35 (1H, td, J_1 = 8.4 Hz, J_2 = 1.2 Hz, H_6); 7.46 (1H, td, J_1 = 8 Hz, J_2 = 1.2 Hz, H_5); 7.88 (2H, d, J = 8.8 Hz, $H_{2'}$, $H_{6'}$); 7.94 (1H, d, J = 7.6 Hz, H_4); 8.02 (1H, d, J = 7.6 Hz, H_7;). ^{13}C-NMR (100 MHz; $CDCl_3$; Me_4Si; δ_C, ppm): 21.40 (Ar-CH_3); 47.81 and 52.81 (piperazine); 63.02 (-CH_2-); 114.74; 121.40; 122.53; 123.96; 124.45; 126.05; 126.30; 127.99; 128.19; 128.76; 129.94; 134.68; 137.95; 153.01; 154.33; 168.27. ESI-MS (*m/z*): 422.26 $[M+Na]^+$, 400.30 $[M+H]^+$ (100%).

2-[4-(4-(4-fluorobenzyl)piperazin-1-yl)phenyl]benzo-[*d*]thiazole (2j): yield, 0.55 g (68%); m.p., 216°C (from acetone); Found: C, 71.73; H, 5.58; N, 10.56; S, 8.00; Anal. Calcd. for $C_{24}H_{22}FN_3S$: C, 71.44; H, 5.50; N, 10.41; S, 7.95%; IR (ν_{max}, cm^{-1}): 2841 (C-H), 1602, 1507, 1477, 1436, 1424 (C=N, C=C), 1249 (C-N); ^{1}H-NMR (400 MHz; $CDCl_3$; Me_4Si; δ_H, ppm): 2.60 (4H, bs, piperazine); 3.34 (4H, bs, piperazine); 3.54 (2H, s, -CH_2-); 6.93–7.04 (4H, m, $H_{3'}$, $H_{5'}$,

$H_{3''}$, $H_{5''}$); 7.30 – 7.36 (3H, m, H_6, $H_{2''}$, $H_{6''}$); 7.44 (1H, td, J_1 = 7.6 Hz, J_2 = 1.2 Hz, H_5); 7.85 (1H, d, J = 7.6 Hz, H_4); 7.94 – 8.20 (3H, m, H_7, $H_{2'}$, $H_{6'}$). ^{13}C-NMR (100 MHz; $CDCl_3$; Me_4Si; δ_C, ppm): 47.81 and 52.66 (piperazine); 62.10 (-CH_2-); 114.81; 115.15; 121.41; 122.54; 124.09; 124.48; 126.07; 128.77; 130.66; 134.67; 152.93; 154.32; 160.92; 163.35; 168.22. ESI-MS (*m/z*): 426.23 $[M+Na]^+$, 404.27 $[M+H]^+$ (100%).

2-[4-(4-(3-chlorobenzyl)piperazin-1-yl)phenyl]benzo-[*d*]thiazole (2k): yield**,** 0.59 g (70%); m.p., 199°C (from acetone); Found: C, 68.23; H, 5.25; N, 10.12; S, 7.63; Anal. Calcd. for $C_{24}H_{22}ClN_3S$: C, 68.64; H, 5.28; N, 10.01; S, 7.64%; IR (ν_{max}, cm^{-1}): 3055 (C-H), 2882, 2842 (C-H), 1606, 1477, 1435, 1425 (C=N, C=C), 1249 (C-N); ^{1}H-NMR (400 MHz; $CDCl_3$; Me_4Si; δ_H, ppm): 2.60–2.62 (4H, m, piperazine); 3.33 – 3.38 (4H, m, piperazine); 3.55 (2H, s, -CH_2-); 7.06 (2H, d, J = 8, $H_{3''}$, $H_{5''}$); 7.24–7.30 (3H, m, $H_{4''}$-$H_{6''}$); 7.32 (1H, td, J_1 = 8, J_2 = 1.2, H_6); 7.38 (1H; s; $H_{2''}$); 7.44 (1H, td, J_1 = 8, J_2 = 1.2, H_5); 7.85 (1H, d, J = 8, H_4); 7.96 – 8.02 (3H, m, H_7, $H_{2'}$, $H_{6'}$). ^{13}C-NMR (100 MHz; $CDCl_3$; Me_4Si; δ_C, ppm): 47.84 and 52.75 (piperazine); 62.30 (-CH_2-); 114.82; 121.41; 122.54; 124.48; 126.07; 127.18; 127.43; 128.77; 129.06; 129.59; 134.27; 134.67; 152.93; 154.32; 168.22. ESI-MS (*m/z*): 420 $[M+H]^+$ (100%), 422 $[M+H+2]^+$, 442 $[M+Na]^+$, 444 $[M+Na+2]^+$.

2-[4-(1H-pyrazol-1-yl)phenyl]benzo[*d*]thiazole (2l) [34]: m.p., 178°C.

2-[4-(1*H*-imidazole-1-yl)phenyl]benzo[*d*]thiazole (2m): yield, 0.59 g (70%); m.p., 199°C (from acetonitrile); Found: C, 69.73; H, 3.71; N, 15.25; S, 11.36; Anal. Calcd. for $C_{16}H_{11}N_3S$: C, 69.29; H, 4.00; N, 15.15; S, 11.56%; IR (ν_{max}, cm^{-1}): 3130, 3056 (C-H), 1606, 1529, 1486, 1440 (C=N, C=C), 1252 (C-N); ^{1}H-NMR (400 MHz; DMSO-d_6; Me_4Si; δ_H, ppm): 7.16 (1H, s, imidazole H_4); 7.46 (1H, td, J_1 = 7.6 Hz, J_2 = 0.8 Hz, H_6); 7.54 (1H, t, J_1 = 8 Hz, H_5); 7.84 – 7.89 (3H, m, H_4, $H_{3''}$, $H_{5''}$); 8.06 (1H, d, J = 8.4 Hz, H_7); 8.14 (1H, d, J = 7.2 Hz, imidazole H_5); 8.19 (2H, d, J = 8.8 Hz, $H_{2'}$, $H_{6'}$); 8.41 (1H, s, imidazole H_2). ^{13}C-NMR (100 MHz; DMSO-d_6; Me_4Si; δ_C, ppm): 117.75; 120.64; 122.34; 122.87; 125.59; 126.71; 128.68; 130.28; 131.00; 134.51; 135.60; 138.88; 153.52; 166.12. ESI-MS (*m/z*): 278.13 (100%) $[M+H]^+$, 300.12 $[M+Na]^+$.

2-[4-(1*H*-1, 2, 4-triazole-1-yl)phenyl]benzo[*d*]thiazole (2n): yield**,** 0.43 g (77%); m.p., 222°C (from acetone); Found: C, 67.87; H, 3.72; N, 20.08; S, 11.51; Anal. Calcd. for $C_{15}H_{10}N_4S$: C, 64.73; H, 3.62; N, 20.1; S, 11.52%; IR (ν_{max}, cm^{-1}): 3089 (C-H), 1606, 1529, 1503, 1487, 1439 (C=N, C=C), 1222 (C-N); ^{1}H-NMR (400 MHz; DMSO-d_6; Me_4Si; δ_H, ppm): 7.46 (1H, td, J_1 = 7.6 Hz, J_2 = 1.2 Hz, H_6); 7.54 (1H, td, J_1 = 7.6 Hz, J_2 = 1.2 Hz, H_5); 8.05–8.08 (3H, m, H_4, $H_{3'}$, $H_{5'}$); 8.15 (1H, d, J = 8 Hz, H_7); 8.25 (2H, d, J = 9.2 Hz, $H_{2'}$, $H_{6'}$); 8.29 (1H; s; triazole H_3); 9.42 (1H, s, triazole H_5). ^{13}C-NMR (100 MHz; DMSO-d_6; Me_4Si; δ_C, ppm): 119.80; 122.24; 122.82; 125.55; 126.62; 128.54; 131.83; 134.48; 138.46; 142.53; 152.59; 153.42; 165.88. ESI-MS (*m/z*): 279.24 $[M+H]^+$, 301.20 $[M+Na]^+$ (100%).

2-[4-(1*H*-benzimidazole-1-yl)phenyl]benzo[*d*]thiazole (2o): yield, 0.46 g (70%); m.p., 202°C (from acetonitrile); Found: C, 73.46; H, 4.17; N, 12.95; S, 9.71; Anal. Calcd. for $C_{20}H_{13}N_3S$: C, 73.37; H, 4.00; N, 12.83; S, 9.79%; IR (ν_{max}, cm^{-1}): 3058 (C-H), 1604, 1524, 1487, 1453 (C=N, C=C), 1225 (C-N); ^{1}H-NMR (400 MHz; DMSO-d_6; Me_4Si; δ_H, ppm): 7.31–7.39 (2H, m, benzimidazole H_5, H_6); 7.48 (1H, td, J_1 = 8 Hz, J_2 = 1.2 Hz, H_6); 7.56 (1H, td, J_1 = 7.2 Hz, J_2 = 1.2, H_5); 7.75 (1H, d, J = 8 Hz, benzimidazole H_4); 7.80 (1H, d, J = 6.8 Hz, benzimidazole H_7); 7.90 (2H, d, J = 9.2 Hz, $H_{3'}$, $H_{5'}$); 8.09 (1H, d, J = 7.6 Hz, H_4); 8.17 (1H, d, J = 7.6 Hz, H_7); 8.31 (2H, d, J = 8.4 Hz, $H_{2'}$, $H_{6'}$); 8.68 (1H, s, benzimidazole H_2). ^{13}C-NMR (100 MHz; DMSO-d_6; Me_4Si; δ_C, ppm): 110.87; 120.09; 122.42; 122.76; 122.97; 123.72; 124.02; 125.69; 126.77; 128.83; 131.70; 132.64; 134.60; 138.23; 143.14; 143.97; 153.56; 166.10. ESI-MS (*m/z*): 328.26 $[M+H]^+$, 350.22 $[M+Na]^+$ (100%).

EXPERIMENTAL BIOLOGICAL PART

Inhibition of self-mediated $A\beta_{1-40}$ and $A\beta_{1-42}$ aggregation. Inhibition of $A\beta_{1-40}$ and $A\beta_{1-42}$ aggregation was measured using the ThT method [37]. The inhibitor (100 μM) and $A\beta_{1-40/1-42}$ (5 μM) were incubated in the assay medium containing 0.01 M NaCl in 0.05 M potassium phosphate buffer (pH 7.4) at 37°C for 48 h. The 100 μM $A\beta_{1-40/1-42}$ ± inhibitor mixture was added to thioflavin T (ThT; 200 μM) in 50 mM glycine-NaOH buffer, pH 8.0 and the reduction in the fluorescence intensity at Exc: 448 nm Em: 490 nm was measured using an RF 5301 PC spectrofluorophotometer. Donepezil (100 μM) was used as the positive controls.

Cholinesterase inhibitory activity. AChE (electric eel), BChE (equine serum), acetylthiocholine iodide, and S-butyrylthiocholine iodide were obtained from Sigma-Aldrich and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) was obtained from Calbiochem (Los Angeles, CA, USA). The cholinesterase inhibitory activity of the target compounds were determined using Ellman's assay [38]. Reactions were initiated by addition of an enzyme into media containing the substrate (0.05 – 0.4 mM) and 0.125 mM DTNB in 100 mM 3(N-morpholino)propanesulfonic acid buffer, pH 8.0, at 25°C and monitored spectrophotometrically at 412 nm using UV-Vis Model 1700 Shimadzu PC spectrophotometer.

Statistical analysis. Statistical analysis was performed using GraphPad Prism software using one-way ANOVA analysis (Bonferroni's post-hoc test) in comparison to control, with *p*-values less than 0.05 considered statistically significant**.**

CONCLUSION

A series of new 2-phenylbenzo[*d*]thiazoles substituted with azole/piperazine moieties were designed and synthesized. All compounds were evaluated for their Aβ aggregation and ChE inhibitory activity, and three azole-substituted compounds **2m – 2o** (with imidazole, triazole, and benzimidazole rings, respectively) showed stronger inhibitory effects on $A\beta_{1-40}$ and $A\beta_{1-42}$ aggregation. Furthermore, piperazine-substituted compounds with small moieties such as methyl, ethyl, and acetyl (**2a – 2c**), were more promising than bulky moieties such as cyclohexyl, phenyl, and benzyl (**2d – 2k**), for Aβ aggregation inhibition (except for **2i**). However, no one compound exhibited good inhibitory activity against both AChE and BChE. Therefore, imidazole **2m** may be promising therapeutic option for inhibiting Aβ-mediated pathology in AD.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the Hacettepe University Scientific Research Fund (Project no: THD-2018 – 16569).

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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