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Research Article

# SOME NOVEL SCHIFF BASE DERIVATIVES AS PROMISING CHOLINESTERASE INHIBITORS WITH ANTIOXIDANT ACTIVITY AGAINST ALZHEIMER'S DISEASE: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION

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***Abstract:*** *In this study, five novel Schiff base derivatives (6-10) except for 9 were synthesized for the first time, characterized, and tested for their inhibition activities against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Also, the Antioxidant activities of these molecules were examined by DPPH and ABTS assays. Their molecular structures were characterized by three spectroscopic techniques. In AChE assay, compound 6 (95.87±1.59 % inhibition) inhibited this enzyme better than galanthamine (76.98±0.42 % inhibition). In BChE assay, compound 10 with an 87.92±1.08% inhibition value in the series indicated the highest activity compared to galanthamine (76.30±0.28 % inhibition). In ABTS radical scavenging assay, compounds 7, 8, and 9 except for 6 and 10 indicated higher antioxidant activities compared to butylated hydroxytoluene (BHT). It is believed that these results may contribute to the design and synthesis of novel antioxidant agents, AChE, and BChE inhibitors.*

***Keywords****: Schiff base, Antioxidant agent, Cholinesterase inhibitor, Alzheimer's disease.*

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## 1. Introduction

Alzheimer's disease (AD), which is a fatal neurodegenerative disease, is the most common cause of dementia in the aged population [1-3]. It is a nervous system disorder in which damage occurs in neurons in the brain, which manifests itself with progressive loss of cognitive functions such as attention, speech, and decision-making ability, especially memory loss [4, 5]. The pathology of progressive and cognitive dysfunction associated with aging in AD remains unclear, and therefore there is still no definitive radical treatment for AD [6]. Today, approximately 35 million people worldwide are affected by AD, and it is presumed that this number will reach approximately 65 billion in 2030 and 115 million in 2050. [7]. These data demonstrate the importance of developing an efficacious therapy.

The most significant pathological features of AD are β-amyloid extracellular plaques, intracellular neurofibrillary tangles formed by excessive phosphorylation of tau protein, loss of cholinergic neurons in the basal forebrain, and oxidative stress [8, 9]. Inadequate cholinergic transmission plays a significant role in the emergence of cognitive, functional, and behavioral symptoms in AD. Therefore, cholinesterase inhibitors (ChEIs) are used to increase the decreased amount of acetylcholine (ACh) in the brain in the cholinergic hypothesis [10, 11]. This hypothesis is the only currently accepted hypothesis

to explain the nature of this disease [12, 13]. The mechanisms of drugs currently employed in the therapy of this disease are based on this hypothesis [14, 15]. The treatments are usually planned to increase the function of the cholinergic system with either receptor agonists or ChEIs [16, 17]. Until now, ChEIs such as tacrine, donepezil, rivastigmine, and galanthamine has been approved by the US Food and Drug Administration (FDA) for use as AChE inhibitors [18].

In order to fully elucidate the etiology of this disease, other hypotheses such as the amyloid hypothesis, the tau hypothesis, and the oxidative stress hypothesis have been proposed, in addition to the cholinergic hypothesis [19]. In the oxidative stress hypothesis, it has been suggested that AD occurs as a result of degeneration and death in neurons due to increased oxidative stress [20]. In the treatment process of AD, the prevention of oxidative stress, which causes neuron degeneration and subsequent neuron death, is significant in terms of the therapy approach. Antioxidants are known to reduce oxidative stress [21, 22]. Therefore, the antioxidant activities of the designed compounds for this research were also investigated.

Nitrogen-containing heterocycles are found in the structure of many drug molecules and pharmaceutically active natural and synthetic molecules [23]. 4-Aminoantipyrine (4-AAP) and its derivatives constitute a significant class of nitrogen-containing heterocyclic compounds. They are known to display a wide range of various biological properties [24-30].

Encouraged by the aforementioned findings, herein we aimed to research the inhibition potency of novel Schiff bases against AChE and BChE. Also, the antioxidant activities of these compounds were evaluated by ABTS and DPPH assays. The inhibition activity results and antioxidant potencies of new Schiff bases were compared with standard molecules. Galanthamine as the standard compound for these enzymes was utilized. The inhibition activity results were given as % inhibition at 200 μM. Antioxidant activity values were given as $IC_{50}$ (μM) for these assays. BHT, butylated hydroyanisole (BHA), and α-Tocopherol (α-TOC) as the standard antioxidants in these assays were utilized. The molecular structures of new molecules were characterized by FT-IR, $^{1}$H NMR, and $^{13}$C NMR.

## 2. Materials and Methods

### 2.1. Chemistry and analysis

All chemicals employed in this manuscript were provided by commercial suppliers. A digital melting point instrument was used for the determination of the melting points of the target molecules. NMR (the Bruker AVANCE III 500 MHz spectrometer) and FT-IR spectra (the Perkin-Elmer spectrophotometer) for the characterization of target molecules were used, respectively.

### 2.2. The synthesis of aryl Sulfonates

Aryl sulfonate derivatives (**1-5**) were obtained and characterized in one of our previous studies. The synthesis procedure is given in detail in that study [31].

### 2.3. The preparation of Schiff bases

The synthesis procedure of novel compounds (**6-10**) was given in our previous study [25, 28].

#### 2.3.1 2-(((Antipyrine-4-yl)imino)methyl)-5-(diethylamino)phenyl 4-chlorobenzenesulfonate (6)

Light yellow solid, yield: 80%, m.p. 174-175°C. FT-IR ($cm^{-1}$) $\upsilon_{max}$: 3073, 2977 (C-H arom.), 2932, 2874 (C-H aliph.), 1646 (C=O), 1590 (C=N), 1348 ($SO_2$ asym.), 1190 ($SO_2$ sym.). $^{1}$H NMR ($CDCl_3$): $\delta$ 9.32 (s, 1H, –CH=N), 7.91 – 7.80 (m, 3H, Ar-H), 7.54 – 7.40 (m, 4H, Ar-H), 7.37 – 7.24 (m, 3H, Ar-H), 6.61 – 6.52 (m, 2H, Ar-H), 3.37 (q, $J$ = 7.1 Hz, 4H, –N($CH_2CH_3$)$_2$), 3.09 (s, 3H, –N-$CH_3$),

2.38 (s, 3H, =C-$CH_3$), 1.18 (t, *J* = 7.2 Hz, 6H, –N($CH_2CH_3$)$_2$) ppm. $^{13}$C NMR ($CDCl_3$): *δ* 160.78 (C=O), 151.56 (C=N), 150.82, 150.44, 150.06, 140.41, 135.19, 133.49, 130.56, 129.30, 129.16, 127.75, 126.62, 124.15, 119.23, 117.51, 110.51, 105.41 (Ar-C and Pyr-C), 44.70 (–N($CH_2CH_3$)$_2$), 36.15 (–N-$CH_3$), 12.53 (–N($CH_2CH_3$)$_2$), 10.05 (=C-$CH_3$) ppm.

### 2.3.2 3-(((Antipyrine-4-yl)imino)methyl)phenyl 4-chlorobenzenesulfonate (7)

Yellow solid, yield: 81%, m.p. 162-163°C. FT-IR ($cm^{-1}$) $\upsilon_{max}$: 3085, 3012 (C-H arom.), 2982, 2930 (C-H aliph.), 1653 (C=O), 1573 (C=N), 1374 ($SO_2$ asym.), 1184 ($SO_2$ sym.). $^1$H NMR ($CDCl_3$): *δ* 9.63 (s, 1H, –CH=N), 7.81 – 7.74 (m, 2H, Ar-H), 7.65 (dt, *J* = 7.8, 1.2 Hz, 1H, Ar-H), 7.54 – 7.43 (m, 5H, Ar-H), 7.41 – 7.26 (m, 4H, Ar-H), 7.03 – 7.01 (m, 1H, Ar-H), 3.17 (s, 3H, –N-$CH_3$), 2.44 (s, 3H, =C-$CH_3$) ppm. $^{13}$C NMR ($CDCl_3$): *δ* 160.52 (C=O), 154.48 (C=N), 152.22, 149.88, 140.99, 140.18, 134.55, 133.86, 129.96, 129.86, 129.54, 129.24, 127.16, 127.15, 124.59, 123.55, 120.08, 117.9 (Ar-C and Pyr-C), 35.61 (–N-$CH_3$), 9.98 (=C-$CH_3$) ppm.

### 2.3.3 2-(((Antipyrine-4-yl)imino)methyl)-6-methoxyphenyl 4-chlorobenzenesulfonate (8)

Yellow solid, yield: 88%, m.p. 235-236 °C. FT-IR ($cm^{-1}$) $\upsilon_{max}$: 3090, 3008 (C-H arom.), 2935, 2841 (C-H aliph.), 1649 (C=O), 1567 (C=N), 1375 ($SO_2$ asym.), 1191 ($SO_2$ sym.). $^1$H NMR ($CDCl_3$): *δ* 9.42 (s, 1H, –CH=N), 7.89 – 7.83 (m, 2H, Ar-H), 7.63 (dd, *J* = 7.9, 1.2 Hz, 1H, Ar-H), 7.48 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.43 – 7.36 (m, 4H, Ar-H), 7.32 (t, *J* = 7.3 Hz, 1H), 7.29 – 7.21 (m, 1H, Ar-H), 6.98 (dd, *J* = 8.2, 1.3 Hz, 1H, Ar-H), 3.79 (s, 3H, –$OCH_3$), 3.15 (s, 3H, –N-$CH_3$), 2.43 (s, 3H, =C-$CH_3$) ppm. $^{13}$C NMR ($CDCl_3$): *δ* 160.03 (C=O), 152.39 (C=N), 152.93, 150.77, 140.20, 138.37, 134.77, 134.74, 132.80, 130.24, 129.25, 129.20, 127.44, 126.89, 124.40, 118.48, 118.40, 113.92 (Ar-C and Pyr-C), 56.12 (–$OCH_3$), 35.77 (–N-$CH_3$), 10.03 (=C-$CH_3$) ppm.

### 2.3.4 5-(((Antipyrine-4-yl)imino)methyl)-2-methoxyphenyl 4-chlorobenzenesulfonate (9)

Light yellow solid, yield: 79%, m.p. 232-233 °C. FT-IR ($cm^{-1}$) $\upsilon_{max}$: 3055, 2973 (C-H arom.), 2930, 2838 (C-H aliph.), 1646 (C=O), 1582 (C=N), 1365 ($SO_2$ asym.), 1182 ($SO_2$ sym.). $^1$H NMR ($CDCl_3$): *δ* 9.62 (s, 1H, –CH=N), 7.86 – 7.78 (m, 2H, Ar-H), 7.72 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.56 (dd, *J* = 8.4, 1.9 Hz, 1H, Ar-H), 7.53 – 7.43 (m, 3H, Ar-H), 7.42 – 7.35 (m, 2H, Ar-H), 7.35 – 7.24 (m, 1H, Ar-H), 6.86 (d, *J* = 8.5 Hz, 1H, Ar-H), 3.61 (s, 3H, –$OCH_3$), 3.14 (s, 3H, –N-$CH_3$), 2.44 (s, 3H, =C-$CH_3$) ppm. $^{13}$C NMR ($CDCl_3$): *δ* 160.78 (C=O), 151.95 (C=N), 154.67, 153.07, 140.62, 138.65, 134.74, 134.72, 131.55, 130.09, 129.19, 129.08, 128.92, 126.94, 124.41, 121.61, 118.32, 112.26 (Ar-C and Pyr-C), 55.71 (–$OCH_3$), 35.79 (–N-$CH_3$), 10.03 (=C-$CH_3$) ppm.

### 2.3.5 1-(((Antipyrine-4-yl)imino)methyl)naphthalen-2-yl 4-chlorobenzenesulfonate (10)

Yellow solid, yield: 89%, m.p. 214 °C. FT-IR ($cm^{-1}$) $\upsilon_{max}$: 3105, 3061 (C-H arom.), 2971, 2926 (C-H aliph.), 1640 (C=O), 1581 (C=N), 1344 ($SO_2$ asym.), 1165 ($SO_2$ sym.). $^1$H NMR ($CDCl_3$): *δ* 9.90 (s, 1H, –CH=N), 9.12 (d, *J* = 9.6 Hz, 1H, Np-H), 7.91 – 7.77 (m, 4H, Ar-H and Np-H), 7.58 – 7.48 (m, 5H, Ar-H and Np-H), 7.46 (dd, *J* = 8.5, 1.2 Hz, 2H, Ar-H), 7.41 – 7.33 (m, 1H, Ar-H), 7.32 – 7.22 (m, 2H, Ar-H), 3.22 (s, 3H, –N-$CH_3$), 2.44 (s, 3H, =C-$CH_3$) ppm. $^{13}$C NMR ($CDCl_3$): *δ* 160.03 (C=O), 152.60 (C=N), 152.23, 147.38, 140.44, 134.74, 133.62, 132.67, 131.45, 131.31, 130.49, 129.37, 129.22, 128.27, 127.60, 127.14, 126.81, 126.39, 125.65, 124.48, 122.13, 118.41 (Ar-C, Np-C and Pyr-C), 35.73 (–N-$CH_3$), 10.11 (=C-$CH_3$) ppm.

### 2.4. General procedure for determining the anticholinesterase activities of the target molecules

In this research, the inhibitory performance of new compounds towards cholinesterases was determined, respectively [32]. The method used in the present study was explained in detail in our previous studies [27, 29, 31, 33].

### 2.5. General procedure for determining the antioxidant activities of the target molecules

The antioxidant potential of new molecules in DPPH and ABTS assays was determined according to the methods of Blois et al. [34] and Re et al. [35], respectively. These two antioxidant activity assays have been given in detail in the previous studies of our group [27, 29, 31].

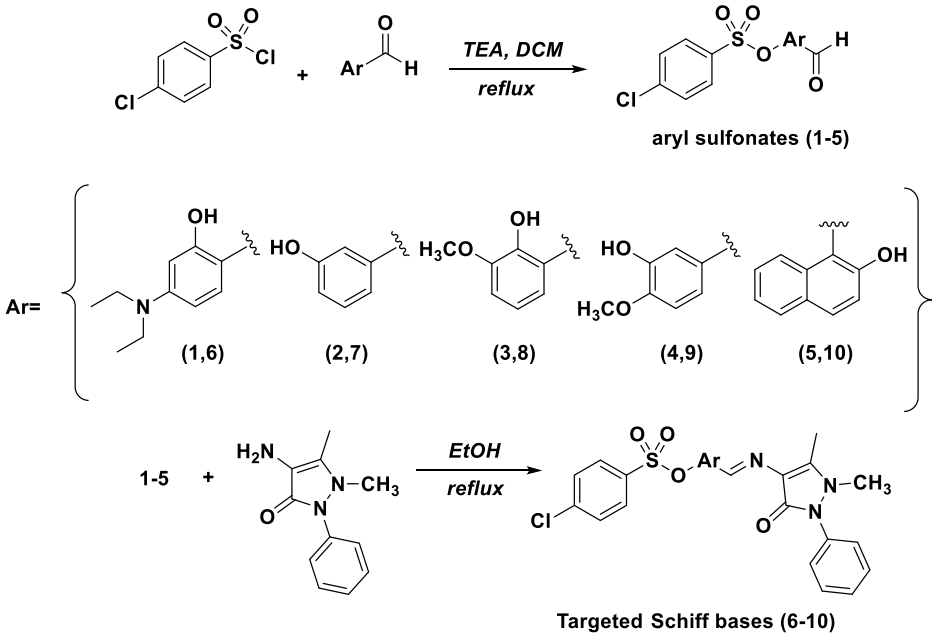
### 2.6. Statistical Analysis

Anticholinesterase and antioxidant and activity results of the target molecules in this research are stated as the mean ± SD of three parallel measurements. The statistical significance was forecasted employing a Student's t-test, where $p < .05$ was considered important.

## 3. Results

### 3.1. Chemistry

The designed Schiff base derivatives (**6**-**10**) for this study were obtained by two steps as outlined in Scheme 1. In the first synthesis step to acquire sulfonates (**1**-**5**), the *O*-sulfonylation of the phenolic aldehydes with 4-CBSC was carried out under reflux for 5. In the final step to obtain novel Schiff bases as the target molecules, compounds **1**-**5** were successfully reacted with 4-AAP in an ethanol medium for 2 h under reflux. In a literature search, we determined that the target molecules **6**, **7**, **8** ve **10** except for **9** were synthesized for the first time. On the other hand, the intermediates (**1**-**5**) used in this research to obtain the target compounds were obtained and characterized in one of our previous studies [31]. In that study, the anticholinestrase and antioxidant activities of the intermediates were investigated.




**Scheme 1**. The synthesis procedure of Schiff base derivatives (**6**-**10**)

In this research, all newly obtained compounds were absolutely characterized. FT-IR spectra of novel Schiff base derivatives observed that the two important bands, C=N group and C=O group stretching bands at 1567-1590 $cm^{-1}$ at 1640-1653 $cm^{-1}$ respectively. Respectively, the asymmetric and symmetric $SO_2$ group stretching bands were also determined at 1344-1375 and 1165-1191 $cm^{-1}$. On the other hand, $^1H$ NMR spectra of the newly obtained compounds, -CH=N proton signal were observed at 9.32-9.90 ppm. The protons of -N-$CH_3$ and =C-$CH_3$ were observed as a singlet at 3.09-3.22 ppm and 2.38-2.44 ppm, respectively. Respectively, $^{13}C$ NMR spectra of compounds, the carbons of -N-$CH_3$ and =C-$CH_3$ resonated at 35.61-36.15 and 9.98-10.11 ppm. The signal of the C=O carbon was determined to resonate at 160.03-160.78 ppm. The signal of CH=N was detected at 151.56-154.48 ppm [25, 28].

### 3.2. Biological activity results

#### 3.2.1. The inhibition activity results

Nowadays, due to the predicted increases in the number of Alzheimer's patients, the increase in treatment costs, and the long treatment process, great importance is given to the development of novel drugs in the therapy of AD worldwide [1-5, 16-18]. In this research, we determined the inhibition activities of some new heterocyclic molecules (**6-10**) as the target molecules against AChE and BChE (Table 1).

**Table 1.** The inhibitory activity results of Schiff bases

| The target molecules | AChE | BChE |
|---|---|---|
| 6 | 95.87±1.59 | 67.72±1.22 |
| 7 | 75.21±1.01 | 66.03±1.08 |
| 8 | 66.65±0.42 | 37.92±0.43 |
| 9 | 63.77±1.96 | 34.88±0.80 |
| 10 | 80.23±0.14 | 87.92±1.08 |
| Galanthamine | 76.98±0.42 | 76.30±0.28 |

(**i**) In AChE assay, the results of our study displayed that all tested compounds had varying % inhibition values. Among them, compounds **6** (95.87±1.59 % inhibition) and **10** (80.23±0.14% inhibition) were determined to inhibit AChE more than galanthamine (76.98±0.42% inhibition). Compound **6**, a Schiff base derivative based on 4-(diethylamino)salicylaldehyde**,** showed the highest activity towards this enzyme. Other than this, compound **9 (**63.77±1.96% inhibition), a Schiff base derivative based on 3-hydroxy-4-methoxybenzaldehyde, exhibited the weakest activity against this enzyme.

(**ii**) In BChE assay, we found that the compound **10** (87.92±1.08% inhibition)**,** a Schiff base derivative based on 2-hydroxy-1-naphthaldehyde in this series showed the highest activity against BChE. Apart from this compound, compounds **6** (67.72±1.22% inhibition) and **7** (66.03±1.08% inhibition) showed the closest activities to galanthamine (76.30±0.28% inhibition). On the other hand, compound **9 (**34.88±0.80% inhibition), a Schiff base derivative based on 3-hydroxy-4-methoxybenzaldehyde, demonstrated the weakest activity against BChE

#### 3.2.1 The activity results of antioxidant agents

In the current research, the antioxidant activity results of all Schiff base derivatives were given in Table 2.

**Table 2.** The results of DPPH and ABTS assays

| $IC_{50}$ values (μM) | | |
|---|---|---|
| The target molecules | DPPH | ABTS |
| 6 | 532.79±2.61 | 64.82±1.05 |
| 7 | 283.24±2.02 | 25.67±0.93 |
| 8 | 478.78±3.25 | 37.79±0.37 |
| 9 | 476.09±4.49 | 24.88±0.75 |
| 10 | 294.99±2.18 | >1000 |
| BHA | 47.44±0.60 | 16.20±0.20 |
| α-TOC | 48.37±0.58 | 16.19±0.17 |
| BHT | 203.50±0.66 | 41.56±0.57 |

(**i**) In DPPH radical scavenging assay, the tested molecules showed antioxidant activities in the range of 283.24 and 532.79 μM. Among these molecules, compound **7** ($IC_{50}$=283,24 μM), which is a Schiff base derivative based on 3-hydroxy benzaldehyde, exhibited the best antioxidant activity. However, these molecules indicated weaker antioxidant activities than BHT ($IC_{50}$=203.50 μM), BHA ($IC_{50}$=47.44 μM) and α-TOC ($IC_{50}$=48.37 μM).

(**ii**) In ABTS radical scavenging assay, all Schiff base derivatives except for compound **10** demonstrated activities in the range of 24.88 and 64.82 μM. Among these molecules in the series, compounds **7** ($IC_{50}$=25.67 μM), **8** ($IC_{50}$=37.79 μM), and **9** ($IC_{50}$=24.88 μM) showed higher antioxidant activity than BHT ($IC_{50}$=41.56 μM). When the results given in Table 2 were investigated, we found that compound **9** exhibited high antioxidant activity. However, all tested compounds displayed lower activities than BHA ($IC_{50}$=16.20 μM) and α-TOC ($IC_{50}$=16.19 μM).

## 4. Conclusion

AD is the most common age-related neurodegenerative disease and has become an important public health problem in most areas of the world. Substantial progress has been made in understanding the basic neurobiology of AD and, as a result, novel drugs for its therapy have become available. ChEIs, which increase the availability of ACh in central synapses, has become the main approach to symptomatic therapy. In this research, we synthesized and characterized five novel heterocyclic Schiff bases derived from 4-AAP as potential inhibitors of AChE and BChE with antioxidant activity. The inhibition potential of these molecules, which were characterized by three spectroscopic methods, against cholinesterases was investigated. We found that some of them inhibited these enzymes more than galanthamine. Amongst the screened molecules, compound **6** and **10** for AChE has been determined to be the most efficacious inhibitor. Compound **10** was also determined to be the best inhibitor for BChE. In ABTS radical scavenging assay, we determined that many compounds showed better antioxidant activities than BHT. In DPPH radical scavenging assay, we determined that the same molecules indicated lower antioxidant activities than standard antioxidants.

**Conflict of interest:**

The article's authors declare that there is no conflict of interest between them.

**The Declaration of Ethics Committee Approval**

The author declares that this document does not require ethics committee approval or any special permission. Our study does not cause any harm to the environment.

**Authors' Contributions:**

E. Ç: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing-original draft preparation, Writing - review&editing (%30)

M. B: Validation, Writing-original draft preparation (%20)

G. T: Conceptualization, Investigation, Writing-original draft preparation (%20)

R. Ç: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing-original draft preparation, Writing - review&editing (%30)

**Compliance with Research and Publication Ethics**

This study was carried out by obeying research and ethics rules.

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