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Synthesis of Novel Schiff Bases with Piperidine Rings and Investigation of Their Antioxidant Capacities and Anticholinesterase and Carbonic Anhydrase Enzyme Inhibition Properties

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The use of Schiff bases especially in chemistry, medicine, pharmacy, and various industries has increased the importance of these compounds. Schiff bases and their derivatives show important bioactive properties in a wide range. Compounds containing phenol and its derivatives in their molecular structures have the potential to capture free radicals associated with various diseases. In this study, five new Schiff bases (13–17) having piperidine rings containing phenol groups, which have the potential to be used as antioxidants, were synthesized for the first time using microwave energy. The antioxidant effects of the compounds used in the syntheses (7–12) and the obtained new Schiff bases (13–17) and their inhibitory abilities against some metabolic enzymes including acetylcholinesterase (AChE) and human carbonic anhydrases I and II (hCAs I and hCAs II) were determined. In addition to the experimental findings, molecular docking studies of the compounds against human acetylcholinesterase were performed in order to provide ideas on structure-based drug design (PDB ID: 4EY6). In light of the results obtained, it is thought that this study will be useful and guide in the food, medical, and pharmaceutical industries in the future.

1. Introduction

Free radicals are produced endogenously during the oxidation of carbohydrates, lipids, and proteins as part of the body's normal metabolic processes.[1] Exogenous factors such as sunlight, radiation, organic solvents, and environmental pollution also create free radicals.[2,3] Normally, antioxidants and free radicals exist in balance in metabolism, and since this balance is important for a healthy life, changing the current balance in favor of free radicals creates a predisposition to diseases.[4] Since free radicals are highly reactive species, they damage cells by interacting with lipids, nucleic acids, and proteins.[5] Free radicals cause changes in enzyme activities, conduction problems in the nervous system, and DNA damage and mutations.[6–8] Oxidative stress, which develops due to increases in reactive oxygen species (ROS) and free radicals, plays a role in the etiopathogenesis of many diseases such as atherosclerosis, rheumatologic diseases, diabetes, and cancer, as well as aging.[9,10] Hydroxyl radicals (OH•), superoxide anion radicals ($^1O_2$), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), hypochlorite ions (ClO^-), and singlet oxygen (O_2•) are the most common ROS.[11,12] ROS and free radicals are released during the conversion of nutrients into energy through oxygen during metabolic steps and cause various health problems, the effects of which can be lifelong.[13] As can be seen from the literature, preventing or delaying the development of ROS that causes various diseases is of critical importance.

Antioxidants reduce the formation of free radicals, protect living organisms from the dangerous effects of ROS, repair oxidative damage, eliminate damaged molecules, and prevent mutations.[14,15] Antioxidants are defined as substances that can delay or prevent the oxidation of substrate molecules. Antioxidant substances donate electrons to free radicals and thus minimize oxidative damage in biological systems.[16,17] Antioxidant defense mechanisms in living organisms reduce cellular damage by preventing ROS formation.[18,19] Nowadays, various synthetic and natural antioxidants are used in the food industry in particular.[20] Synthetic antioxidants are added to foods during processing to maintain their color, aroma, and freshness, as well as to prevent lipid oxidation in foods with high lipid content.[19] For example, propyl gallate (PG, E310), tert-butylhydroquinone (TBHQ, E319), butylated hydroxyanisole (BHA, E320), and butylated hydroxytoluene (BHT, E321) are examples of synthetic ones frequently used in the food industry.[21] In addition, phenolic compounds, vitamins (C and E), and carotenoids are natural antioxidants.[22] However, since synthetic antioxidants have been reported to cause health problems, researchers have turned to the synthesis of safer alternative antioxidant compounds.[5,23] It has been reported that diseases such as diabetes, glaucoma, cataracts, cancer, cardiovascular conditions, Parkinson's,

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and Alzheimer's are associated with ROS and free radicals, and antioxidant molecules inhibit some enzymes that have effects on the development of such diseases.[24–26]

Alzheimer's disease is a neurological disease in which memory loss and forgetfulness are common effects.[27] Considering the pathological feature of the disease, it has been observed that amyloid beta (Aβ) intercellular plaques are formed, there is cholinergic neuron loss in the forebrain, and acetylcholine, a neurotransmitter, is decreased.[28] Activating and increasing the number of cholinergic neurons that have decreased in the brains of these patients is considered a method of treating the disease.[29] Inhibition of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) has become popular for the management of various mental disorders, and cholinesterase inhibitors are used to alleviate the symptoms of neurological diseases such as dementia and Alzheimer's disease.[30] Rivastigmine, galantamine, and tacrine are drugs currently widely used as acetylcholine esterase enzyme inhibitors in the treatment of Alzheimer's disease.[31] However, these inhibitors generally have side effects such as increases in gastric acid and bronchial secretions, nausea, vomiting, diarrhea, and bradyarrhythmia.[32] Donepezil, which has a piperidine structure and is used as a drug in the treatment of Alzheimer's disease, has fewer side effects than other drugs and is highly specific for AChE in the central nervous system, which is an important advantage.[33] Many piperidine derivatives, such as donepezil, form the basic structure of pharmacologically active compounds and important drugs.[34]

Carbonic anhydrases (E.C.4.2.1.1, CAs), an important enzyme family, are metalloenzymes, and they catalyze the conversion between CO_2 and the bicarbonate ion (HCO_3^-), one of the most important reactions in life.[35] This reaction is involved in the removal of CO_2 from red blood cells, production of body fluids, pH regulation of the organism, water, and ion transport, and homeostasis in the central nervous system (CNS), eye, and inner ear. It is important in photosynthetic processes in some bacteria, plants, and algae and in many other physiological processes.[36,37] The clinical use of carbonic anhydrase (CA) inhibitors has attracted much attention in recent years due to their important physiological roles in biosynthetic reactions and tumorigenesis. Inhibitors of different isoforms of the CA enzyme family have begun to be applied clinically in the treatment of various diseases, including glaucoma, retinopathy, hemolytic anemia, epilepsy, obesity, and cancer.[38]

Heterocyclic chemistry constitutes an important subclass of organic chemistry. Heterocycles are defined as organic compounds containing at least one hetero atom such as nitrogen, oxygen, and sulfur.[30] Synthetic heterocyclic chemistry is used in various fields such as medicine, pharmacology, agriculture, dyes and pigments, polymer science, electronics, optics, and corrosion inhibitors.[39,40] Many biological compounds, including vitamins, hemoglobin, hormones, DNA, RNA, and others, contain heterocyclic rings.[41] Recent studies have shown that various heterocyclic structures have become critical structural components of numerous drugs.[42] Heterocyclic compounds, especially those carrying nitrogen atoms, are found

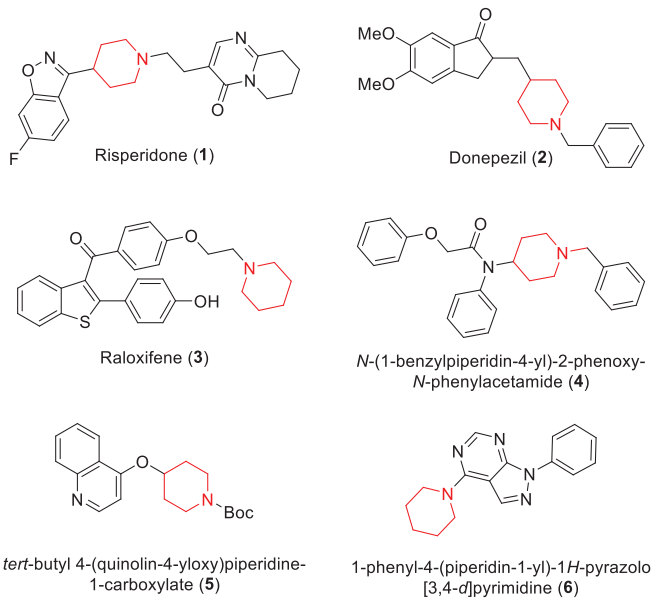


Figure 1. Some pharmacologically active molecules containing piperidine rings.

in nature and drugs and have important biological properties (anticancer, antimicrobial, analgesic, anti-inflammatory, etc.).[43,44]

Piperidine is a nonaromatic heterocyclic core with sp^3 hybridization, containing a six-membered ring that includes five methylene groups (—CH_2—) and one secondary amine group (—NH—).[45] Piperidine-containing compounds have long been synthesized and are considered one of the most essential synthetic medicinal blocks utilized in drug production.[46] Piperidine is utilized for a variety of purposes, including as a solvent, base, accelerator for rubber vulcanization, and a component in the production of pharmaceuticals.[47] Piperidine, a nitrogen-containing heterocyclic compound, and its derivatives are an important pharmacophore group widely used in many vital drugs. Many compounds with this ring system in their structure have antidepressive, anxiolytic, anticonvulsant, and antinociceptive effects.[48,49] The chemical structures of some pharmacologically active molecules containing piperidine rings are given in Figure 1. Of these, risperidone 1) is used as an antipsychotic,[50] donepezil 2) is used to treat Alzheimer's disease,[51] and raloxifene 3) is used for postmenopausal osteoporosis and breast cancer.[52] Additionally, piperidinyl pyrazolo pyrimidine 4) derivatives have been used as antihypertensives,[48] piperidine carboxylate (5) derivatives as antivirals against influenza,[53] and 1,4 disubstituted piperidine (6) derivatives as antimalarials against *Plasmodium falciparum*.[54]

Schiff bases and their derivatives have attracted remarkable attention due to their wide applications in molecular catalysis, nonlinear optical materials, functional materials, molecular recognition, corrosion, etc.[55,56] In addition, Schiff bases, like heterocyclic compounds, exhibit a wide range of biological activities, such as antiviral, antibacterial, anti-inflammatory, antitumor, antifungal, antimicrobial, anticancer, antiulcer, antioxidant, antimalarial, antiproliferative, and antipyretic.[57,58] These

compounds, in addition to their biological potentials, exhibit antioxidant properties by effectively scavenging ROS, and they are a subject of interest due to this ability.[59,60] In addition, heterocyclic compounds containing imine and phenol groups in their structures have the potential to be used as synthetic antioxidants due to their free radical scavenging abilities, and they are also drug candidates.[61–63]

Toxic organic solvents are used in many organic synthesis methods. In addition to increasing the cost of scientific research, such solvents are also known to have negative effects, especially on human health and the environment via pollution.[64] Reducing the amount of solvent used in chemical reactions or designing solvent-free reactions attracts the attention of researchers.[57,65] In recent years, the use of microwave energy in organic synthesis has become a popular topic as it provides great advantages.[66] This technique produces high yields in a shorter time, as well as reducing the formation of by-products and solvent consumption.[67,68] There are various synthetic methods for the synthesis of Schiff bases including microwave synthesis.[69] Microwave energy-assisted syntheses used in imine synthesis contribute to the development of cleaner and environmentally friendly synthesis methods, aligning with the targets of green chemistry.[70,71] Recently, the increase in studies on Schiff bases and the antioxidant activities of these compounds prompted us to conduct the present study.

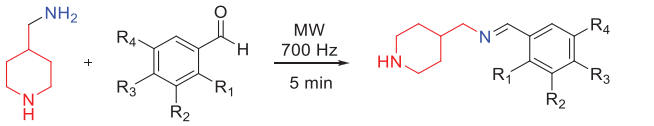
The aim here was to synthesize new Schiff bases (13–17) containing a piperidine ring using microwave irradiation synthesis, which is a fast, cheap, and very environmentally friendly technique. Another important aim was to examine the antioxidant abilities and anticholinesterase and CA enzyme inhibition properties of the starting compounds and the synthesized compounds. Furthermore, to provide insight into the experimental findings and the structure-based drug design, molecular docking studies of the compounds were performed on human AChE (PDB ID: 4EY6).

2. Results and Discussion

2.1. Chemistry

2.1.1. General

All reagents used were of high purity and were commercially available. The reactions were performed in a microwave oven (230 V/50 Hz, 700 W) and were monitored using TLC with the aid of UV light (254 nm). Crude products (13,14) were crystallized from petroleum ether, then directly filtered through filter paper, and purified. The viscous products (15–17) were not subjected to any purification process. The melting points of the solids products were measured with Gallenkamp melting point instruments. ^{1}H NMR and ^{13}C NMR spectra were obtained on 400 (100)-MHz Varian and Bruker spectrometers, and elemental analyses were performed on a Leco CHNS-932 instrument. UV–vis analyses were performed with a Shimadzu UV-1280 Multipurpose spectrophotometer.



8 R_1= R_2=R_4=H, R_3=OH
9 R_1= R_4=H, R_2=OMe, R_3=OH
10 R_1= R_4=H, R_2=OH, R_3=OMe
11 R_1=OH,R_2=OMe, R_3= R_4=H
12 R_1=OH, R_2= R_3=R_4=H

13 R_1= R_2=R_4=H, R_3=OH
14 R_1= R_4=H, R_2=OMe, R_3=OH
15 R_1= R_4=H, R_2=OH, R_3=OMe
16 R_1=OH,R_2=OMe, R_3= R_4=H
17 R_1=OH, R_2= R_3=R_4=H

Figure 2. Synthesis of new Schiff bases (13–17).

2.1.2. Synthesis of Schiff Bases

Schiff bases (13–17) were synthesized according to the procedures described in the literature.[57,72] The Schiff bases (13–17) were obtained from the reaction of carbonyl compounds with primary amines. For this, piperidin-4-ylmethanamine (7) was subjected to a condensation reaction with different aldehyde compounds (8–13). 4-Hydroxybenzaldehyde (8), 4-hydroxy-3-methoxybenzaldehyde (9), 3-hydroxy-4-methoxybenzaldehyde (10), 2-hydroxy-3-methoxybenzaldehyde (11), 3,4,5-trimethoxybenzaldehyde (12), and 2-hydroxybenzaldehyde (13) were used in the reactions. The piperidin-4-ylmethanamine (7) (1 mmol) and aldehyde compounds (8–13) (1 mmol) were placed in the reaction vessel and directly exposed to 700 W microwave radiation for 5 min. Thus, the desired molecules (13–17) were obtained in a short time and in a high yield (Figure 2). No solvent or catalyst was used in the reactions. The progress of the reactions was monitored on TLC plates under UV light. The new compounds were characterized using spectroscopic techniques. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz using $CDCl_3$ (Varian spectrometer).

2.1.3. Physical Properties and Spectral Data of the Synthesized Compounds

The protons belonging to the imine groups in the synthesized compounds generally gave singlet peaks in the range of 8.27–8.07 ppm in the ^{1}H NMR analysis. Moreover, the carbon atoms belonging to the imine groups generally gave peaks in the range of 165.17–160.87 ppm in the ^{13}C NMR analysis. Another result supporting the proposed structures was obtained with the aid of elemental analysis and these results are compatible with the structures of the proposed molecules.

4-(((Piperidin-4-ylmethyl)imino)methyl)phenol (13): It was obtained in 97% yield as a light brown solid. mp: 225–226 °C. R_f: 0.57 (EtOAc/petroleum ether: 30%). NMR (400 MHz, DMSO) δ 8.10 (s, 1H), 7.52 (t, J = 7.9 Hz, 2H), 6.75 (d, J = 8.6 Hz, 2H), 3.32 (d, J = 6.2 Hz, 2H), 2.89 (d, J = 12.0 Hz, 2H), 2.54–2.35 (m, 3H), 1.58 (dd, J = 21.6, 8.1 Hz, 2H), 1.11–0.98 (m, 2H). ^{13}C NMR (100 MHz, DMSO) δ 160.87, 160.82, 130.20, 127.79, 116.16, 67.83, 46.50, 38.16, 31.83. Elemental analysis for $C_{13}H_{18}N_2O$ Calcd. C, 71.53; H, 8.31; N, 12.83. Found C, 71.50; H, 8.35; N, 12.87.

2-Methoxy-4-(((piperidin-4-ylmethyl)imino)methyl)phenol (14): It was obtained in 97% yield as an orange solid, mp: 1117–118 °C. R_f: 0.50 (EtOAc/petroleum ether: 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 8.6 Hz, 3H), 7.37 (s, 3H), 7.04 (d, J = 8.1 Hz, 3H), 6.85 (d, J = 8.1 Hz, 3H), 5.18 (s, 5H), 3.88 (d, J = 7.5 Hz, 10H), 3.44 (d, J = 6.9 Hz, 7H), 3.14 (d, J = 11.9 Hz, 6H), 2.62 (dd, J = 28.0, 16.8 Hz, 6H), 1.92–1.63 (m, 11H), 1.35–1.14 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.62, 149.63, 147.89, 128.31, 124.15, 114.82, 108.60, 77.47, 67.89, 56.08, 46.09, 37.35, 31.06. Elemental analysis for $C_{14}H_{20}N_2O_2$ Calcd. C, 67.72; H, 8.12; N, 11.28. Found C, 67.76; H, 8.08; N, 11.25.

2-Methoxy-5-(((piperidin-4-ylmethyl)imino)methyl)phenol (15): It was obtained in 96% yield as a dark brown and viscous substance. R_f: 0.46 (EtOAc/petroleum ether: 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.31 (t, J = 2.9 Hz, 1H), 7.09 (dd, J = 8.3, 2.0 Hz, 1H), 6.83 (d, J = 8.3 Hz, 1H), 5.24 (brs, 2H, OH and NH), 3.87 (s, 3H, OMe), 3.48–3.32 (m, 2H), 3.12 (d, J = 12.4 Hz, 2H), 2.72–2.49 (m, 2H), 1.93–1.62 (m, 3H), 1.28–1.23 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.14, 150.04, 147.03, 129.91, 121.13, 113.82, 110.67, 67.78, 56.06, 46.18, 37.61, 31.43. Elemental analysis for $C_{14}H_{20}N_2O_2$ Calcd. C, 67.72; H, 8.12; N, 11.28. Found C, 67.69; H, 8.16; N, 11.24.

2-Methoxy-6-(((piperidin-4-ylmethyl)imino)methyl)phenol (16): It was obtained in 97% yield as a blood-red and viscous brown solid. mp: 147–148 °C. R_f: 0.17 (EtOAc/petroleum ether: 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 6.88–6.79 (m, 2H), 6.77–6.70 (m, 1H), 3.84 (s, 3H, OMe), 3.41 (dd, J = 15.2, 9.2 Hz, 2H), 3.03 (d, J = 12.0 Hz, 2H), 2.62–2.47 (m, 2H), 1.80–1.61 (m, 3H), 1.25–1.09 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.15, 152.77, 148.79, 122.94, 118.46, 117.80, 113.88, 65.38, 56.20, 46.46, 37.82, 31.54. Elemental analysis for $C_{14}H_{20}N_2O_2$ Calcd. C, 67.72; H, 8.12; N, 11.28. Found C, 67.76; H, 8.07; N, 11.31.

2-(((Piperidin-4-ylmethyl)imino)methyl)phenol (17): It was obtained in 96% yield as a yellow viscous substance. R_f: 0.22 (EtOAc/petroleum ether: 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.31–7.18 (m, 2H), 6.93 (dd, J = 12.1, 4.4 Hz, 1H), 6.87–6.80 (m, 1H), 3.42 (t, J = 9.1 Hz, 2H), 3.05 (d, J = 12.1 Hz, 2H), 2.57 (td, J = 12.2, 2.2 Hz, 2H), 1.81–1.62 (m, 3H), 1.25–1.09 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.17, 161.43, 132.35, 131.38, 118.92, 118.70, 117.18, 66.26, 46.56, 37.84, 31.68. Elemental analysis for $C_{13}H_{18}N_2O$ Calcd. C, 71.53; H, 8.31; N, 12.83. Found C, 71.57; H, 8.26; N, 12.86.

2.2. Biology

2.2.1. Antioxidant Activities

In addition to many natural antioxidants, including vitamins, carotenoids, polyphenols, and flavonoids, various synthetic antioxidants have also been discovered and their antioxidant capacities have been evaluated by different methods.[21,73] To date, various chemical tests, combined with the use of high-sensitivity automated detection devices, have been used to evaluate antioxidant activity through specific methods such as scavenging activity against different types of free radicals or ROS, reducing power, and metal chelation.[74] The antioxidant capacities of the amine compound (7), aldehyde compounds (8–12), and Schiff bases (13–17) were studied using the bioanalytical methods of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) cation radical (ABTS•+) and 1,1-diphenyl-2-picrylhydrazyl (DPPH•) scavenging activities and Fe^{3+}, Cu^{2+}, and Fe^{3+}-TPTZ complex reducing capacities.

2.2.2. Reducing Ability Assays

The Cu^{2+}, Fe^{3+}, and Fe^{3+}-TPTZ-complex reducing abilities of the amine (7), aldehyde derivatives (8–12), and the newly synthesized Schiff bases (13–17) were established as described previously.[5] All experiments to determine reducing abilities were conducted three times and the results were given as the arithmetic mean of these repetitions.

Various concentrations of the compounds (7–17) were placed into separate test tubes and 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL (1%) of potassium ferricyanide [$K_3Fe(CN)_6$] solutions were added to them. Then the obtained mixture was vortexed and incubated at 50 °C for 20 min. Following this, trichloroacetic acid (2.5 mL, 10%) was added to the tubes. Next, 2.5 mL of solution was taken from the tubes with the help of a pipette and transferred to another bottle and 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) were added followed by mixing. The absorbance values of the reducing effects of the compounds (7–17) and standards were recorded spectrophotometrically at 700 nm.

The Cu^{2+} reducing abilities of the amine (7), aldehyde derivatives (8–12), and new Schiff bases (13–17) were determined. For this purpose, 0.25 mL of $CuCl_2$ solution (10 mM), 0.25 mL of ethanolic neocuproine solution (7.5×10^{-3} M) and 250 µL of NH_4Ac buffer solution (1.0 M) at different concentrations (10–30 µg mL^{-1}) were transferred to test tubes containing samples of the amine (7), aldehyde compounds (8–12), and Schiff bases (13–17) separately. The total volume was made up of 2 mL of distilled water. Following this, 30 min of incubation was applied, and the absorbance values 200B of the samples were measured at 450 nm.

The Fe^{3+}-TPTZ complex reducing ability of the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) was determined. For this aim, 2.25 mL of TPTZ solution (10 mM in 40 mM HCl) was freshly prepared. It was then transferred to 2.5 mL of acetate buffer (0.3 M, pH 3.6) and 2.25 mL of $FeCl_3$ solution (20 mM). Next, different concentrations of the amine (7), aryl aldehyde derivatives (8–12), and Schiff bases (13–17) were transferred to the test tubes, which were incubated at 37 °C for 30 min. Finally, the absorbance values of the reducing power of the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) and standards were spectrophotometrically measured at 593 nm.

2.2.3. Radical Scavenging Capacities

The most commonly used spectrophotometric methods to determine the antioxidant capacity of compounds are DPPH$^•$ and $ABTS^{•+}$ scavenging methods.[75] The DPPH$^•$ scavenging effect of the amine (7), aldehyde derivatives (8–12), and Schiff

bases (13–17) were determined as described by Blois.[76] Briefly, 1 mL of DPPH$^{\bullet}$ solution (0.1 mM), which was prepared in ethanol and possessed violet/purple color depending on the concentration of the antioxidant, was added to the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) at different concentrations (10–30 µg mL^{-1}). Then they were incubated at room temperature for 30 min and their absorbance values were recorded at 517 nm.

The ABTS$^{\bullet+}$ radical cation scavenging assay is a way to calculate antioxidant capacity.[77] First, an aqueous solution of ABTS$^{\bullet+}$ (7.0 mM) was oxidized by oxidants like $K_2S_2O_8$ (2.5 mM) for the production of its radical cation (ABTS$^{\bullet+}$).[78,79] The ABTS$^{\bullet+}$ solution was diluted with a phosphate buffer (0.1 M, pH 7.4) prior to use, adjusting the absorbance value of the control to 0.750 ± 0.025 at 734 nm. Then 1 mL of ABTS$^{\bullet+}$ solution was added to 3 mL of the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) at different concentrations (10–30 µg mL^{-1}). After 30 min, the remaining absorbance of ABTS$^{\bullet+}$ was measured at 734 nm.

2.2.4. Acetylcholinesterase Inhibition Assay

Acetylcholinesterase inhibition of the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) was carried out according to Ellman's putative experiment as given previously.[80–82] Acetylthiocholine iodide (AChI) and 5,5′-dithiobis(2-nitro-benzoic acid) (DTNB) were used as substrate patterns for these cholinergic reactions. Briefly, 1 mL of Tris/HCl buffer (1.0 M, pH 8.0), 10 µL of different concentrations of the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) and 50 µL of AChE were mixed in a test tube. Then the sample was incubated at 25 °C for 15 min and 50 µL of DTNB solution (0.5 mM) was transferred to it. Then the reaction was started by adding 50 µL of AChI solution (10 mM) and absorbance was recorded at 412 nm. All experiments related to the AChE inhibition assay were performed three times and the results were given as the arithmetic mean of these repetitions.

2.2.5. Carbonic Anhydrase Purification and Inhibition Studies

Both hCA I and hCA II isoforms were purified using affinity chromatography including Sepharose-4B-L-tyrosine-sulfanilamide affinity material.[83] Both isoforms' activity was spectrophotometrically determined according to our Verpoorte's assay.[84] One CA unit is defined as the CA quantity that catalyzes *p*-nitrophenylacetate substrate to *p*-nitrophenolate product over 3 min at 348 nm (25 °C). The amount of protein was measured spectrophotometrically at 595 nm according to the Bradford method as bovine serum albumin equivalent.[85] SDS-PAGE was performed according to the Laemmli procedure, including 3% and 10% acrylamide concentrations to check enzyme purity.[86] All experiments related to the carbonic anhydrase inhibition assay were performed three times and the results were given as the arithmetic means of these repetitions.

2.2.6. Molecular Docking Studies

Molecular docking studies are of great importance in drug design and pharmacology. This method of analysis is widely used and reliable to detect protein-ligand interactions and binding sites in a short time.[87,88] The crystal structure of recombinant human AChE in complex with (-)-galantamine (PDB ID: 4EY6) was retrieved from a protein database (https://www.rcsb.org/) (Figure 3).

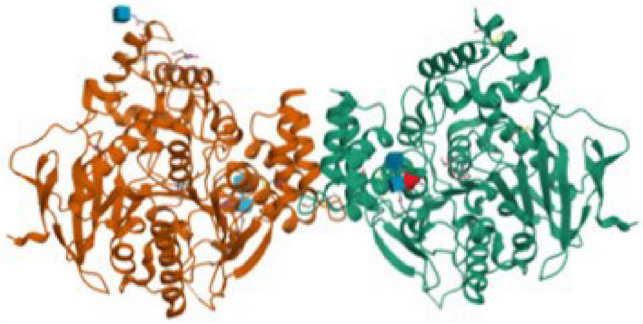


Figure 3. Crystal structure of recombinant human acetylcholinesterase enzyme in complex with (-)-galantamine (PDB ID: 4EY6).

In silico molecular coupling interactions were investigated by AutoDock Tools to compare the activity of Schiff bases against the properties of AChE (PDB code: 4EY6).[89] The 3D structures of the molecules were obtained by drawing their 2D structures. The energy of the molecules was minimized with the software Avogadro and converted to PDB format. In addition, the ligands and protein molecules were converted to pdbqt file formats using AutoDock tools 1.5.7. The crystal structure of AChE (pdb code: 4EY6) was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/). MGLTools was used to prepare the proteins and ligands for molecular docking studies. Water molecules were removed and polar hydrogen charges were added. Torsion angles were verified and Kollman charges were added and re-recorded in pdb format. To further complete the docking process, the active sites of the proteins were determined. Therefore, the grid parameters were selected as 60 × 60 × 60, Å x, y, z dimensions, 0.553 Å space, and −9.94, −43.48, 30.29 x, y, z centers for acetylcholinesterase (PDB ID: 4EY6). The Lamarckian genetic algorithm protocol was used for the molecular docking study. The automatic docking calculation approach was used to predict polar contacts, Van der Waals forces, and interactions between other noncovalent proteins and bound ligands. Files converted to protein-ligand complex were analyzed using the software BioVia Discovery Studio. The 2D and 3D binding interactions obtained based on the molecular docking results of compounds 13–17 in the active site of AChE (PDB: 4EY6) are given in Figure 4.

3. Results

3.1. Antioxidant Results

Free radicals have unpaired electrons and are extremely reactive and unstable.[90] Excessive formation of free radicals and ROS disrupts the structure of many cellular biomolecules, creating a risk of diseases such as cancer and cardiovascular diseases, as well as immunodeficiency and diabetes. Antioxidants, even at low con-

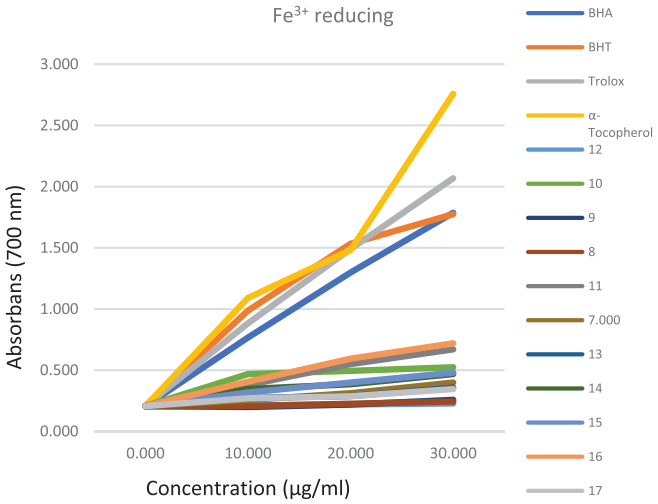


Figure 4. Fe^{3+} reducing potential of the componds (7–17) and standarts.

Table 1. The reducing ability of 30 µg mL^{-1} of compounds (Fe^{3+}-reducing methods).

Antioxidants	Fe^{3+} Reducing λ_{700}	r^2
BHA	1.79 ± 0.08	0.9991
BHT	1.78 ± 0.06	0.9498
Trolox	2.07 ± 0.10	0.9987
α-Tocopherol	2.76 ± 0.09	0.9678
7	0.40 ± 0.01	0.9979
8	0.24 ± 0.00	0.9806
9	0.26 ± 0.06	0.9986
10	0.52 ± 0.01	0.9565
11	0.67 ± 0.02	0.9934
12	0.23 ± 0.00	0.9973
13	0.36 ± 0.00	0.9534
14	0.47 ± 0.02	0.9767
15	0.48 ± 0.01	0.9930
16	0.72 ± 0.01	0.9902
17	0.35 ± 0.01	0.9581

centrations, can eliminate these undesirable harmful effects of ROS and free radicals.[91,92] There are many methods to determine the antioxidant activity of molecules.[93] In the present study, methods including $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activity and Fe^{3+}, Cu^{2+}, and Fe^{3+}-TPTZ complex and reduction abilities were used.

The reduction potentials of the amine (7), aldehyde derivatives (8–12), and newly synthesized Schiff bases (13–17) were determined by three different reduction tests. The reduction of Fe^{3+} by the amine (7), aldehyde derivatives (8–12), and new compounds (13–17) resulted in the formation of $Fe_4[Fe(CN)_6]$ complex showing absorbance at 700 nm.[94,95] As shown in Table 1 and Supplementary Figure S1A, the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) exhibited strong Fe^{3+}-reducing ability. These results showed that all chemicals used in the synthesis (7–12) and also the new compounds obtained (13–17) have the ability to reduce and neutralize free radicals and ROS.

Table 2. The reducing ability of 30 µg mL^{-1} of compounds (7–17) by Cu^{2+}.

Antioxidants	Cu^{2+} Reducing λ_{450}	r^2
BHA	2.29 ± 0.04	0.9938
BHT	1.66 ± 0.11	0.9981
Trolox	1.62 ± 0.02	0.9969
α-Tocopherol	0.73 ± 0.02	0.9631
7	0.11 ± 0.00	0.9794
8	0.07 ± 0.01	0.9899
9	0.31 ± 0.02	0.9937
10	0.36 ± 0.04	0.9988
11	0.77 ± 0.04	0.9689
12	0.10 ± 0.01	0.9727
13	0.12 ± 0.02	0.9746
14	0.48 ± 0.03	0.9997
15	0.63 ± 0.01	0.9852
16	0.81 ± 0.01	0.9843
17	0.23 ± 0.01	0.9522

The Fe^{3+}-reducing effects of 30 µg mL^{-1} amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) and standards were in the following order: α-Tocopherol (2.76 ± 0.09, r^2: 0.9678) > Trolox (2.07 ± 0.10, r^2: 0.9987) > BHA (1.79 ± 0.08, r^2: 0.9991) ≈ BHT (1.78 ± 0.06, r^2: 0.9498) > 16 (0.72 ± 0.01, r^2: 0.9902) > 11 (0.67 ± 0.02, r^2: 0.9934) > 10 (0.52 ± 0.01, r^2: 0.9565) > 15 (0.48 ± 0.01, r^2: 0.9930) ≈ 14 (0.47 ± 0.02, r^2: 0.9767) > 7 (0.40 ± 0.01, r^2: 0.9979) > 13 (0.36 ± 0.00, r^2: 0.9534) ≈ 17 (0.35 ± 0.01, r^2: 0.9581) > 9 (0.26 ± 0.06, r^2: 0.9986) > 8 (0.24 ± 0.00, r^2: 0.9806) ≈ 12 (0.23 ± 0.00, r^2: 0.9973). The results indicated that all compounds possessed marked reductive potentials. 11 and 16 demonstrated higher activity than the other compounds. In particular, 2-methoxy-6-(((piperidin-4-ylmethyl)imino)methyl)phenol (16) exhibited high reducing power (0.72 ± 0.01, r^2: 0.9902). This activity was higher than that of the other compounds (Table 1 and Figure 4).

The Cu^{2+}-reducing abilities of all compounds (7–17) are summarized in Table 2 and Figure 5. A good correlation was observed between the Cu^{2+}-reducing ability of amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) concentrations. The absorbance values of reducing ability exhibited by the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) at 30 µg mL^{-1} were in the following order: BHA (2.29 ± 0.04, r^2: 0.9938) > BHT (1.66 ± 0.11, r^2: 0.9981) > Trolox (1.62 ± 0.02, r^2: 0.9969) > 16 (0.81 ± 0.01, r^2: 0.9843) > 11 (0.77 ± 0.04, r^2: 0.9689) > α-Tocopherol (0.73 ± 0.02, r^2: 0.9631) > 15 (0.63 ± 0.01, r^2: 0.9852) > 14 (0.48 ± 0.03, r^2: 0.9997) > 10 (0.36 ± 0.04, r^2: 0.9988) > 9 (0.31 ± 0.02, r^2: 0.9937) > 17 (0.23 ± 0.01, r^2: 0.9522) > 13 (0.12 ± 0.02, r^2: 0.9746) ≈ 7 (0.11 ± 0.00, r^2: 0.9794) ≈ 12 (0.10 ± 0.01, r^2: 0.9727) > 8 (0.07 ± 0.01, r^2: 0.9899). In this reduction method, the highest reducing activity was exhibited by 16

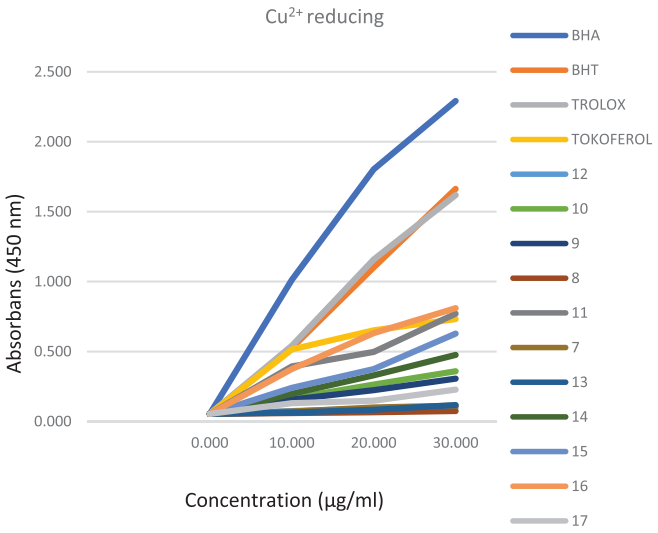


Figure 5. Cu^{2+} reducing potential of the componds (7–17) and standarts.

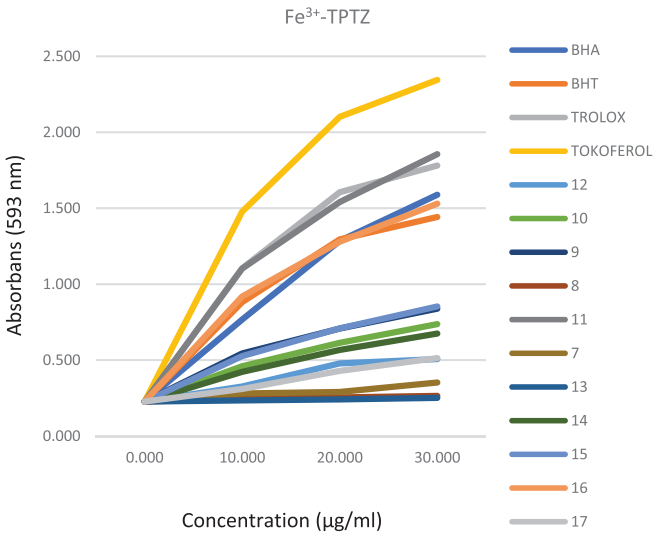


Figure 6. Fe^{3+}-TPTZ complex reducing ability of the compounds (7–17) and standarts.

Table 3. The reducing ability of 30 µg mL^{-1} of compounds (7–17) by Fe^{3+}-TPTZ reducing methods.

Antioxidants	Fe^{3+}-TPTZ Reducing λ_{593}	r^2
BHA	1.59 ± 0.02	0,9983
BHT	1.44 ± 0.04	0.9924
Trolox	1.78 ± 0.06	0.9896
α-Tocopherol	2.34 ± 0.04	0.9866
7	0.35 ± 0.10	0.9439
8	0.26 ± 0.01	0.9734
9	0.84 ± 0.02	0.9966
10	0.74 ± 0.03	0.9786
11	1.86 ± 0.04	0.9967
12	0.51 ± 0.04	0.9697
13	0.25 ± 0.03	0.9955
14	0.68 ± 0.03	0.9827
15	0.85 ± 0.01	0.9711
16	1.53 ± 0.08	0.9975
17	0.51 ± 0.02	0.9949

and the lowest by 8. Hydroxyl groups in phenolic rings increase the reducing activity properties of the compounds[19] (Table 2).

In addition to Fe^{3+} and Cu^{2+} reduction properties, the Fe^{3+}-TPTZ reducing ability of all compounds (7–17) were examined and the results are summarized in Table 3 and Figure 6. A good positive correlation was displayed between the reducing abilities in a concentration-dependent manner (10–30 µg mL^{-1}). At the 30 µg mL^{-1} concentration, the Fe^{3+}-TPTZ reducing ability of the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) and standards declined in the following order: α-Tocopherol (2.34 ± 0.04, r^2: 0.9866) > 11 (1.86 ± 0.04, r^2: 0.9967) > Trolox (1.78 ± 0.06, r^2: 0.9896) > BHA (1.59 ± 0.02, r^2: 0.9983) > 16 (1.53 ± 0.08, r^2: 0.9975) > BHT (1.44 ± 0.04, r^2: 0.9924) > 15 (0.85 ± 0.01, r^2: 0.9711) ≈ 9 (0.84 ± 0.02, r^2: 0.9966) > 10 (0.74 ± 0.03, r^2: 0.9786) > 14 (0.68 ± 0.03, r^2: 0.9827) > 17 (0.51 ± 0.02, r^2: 0.9949) > 12 (0.51 ± 0.04, r^2: 0.9700) > 7 (0.35 ± 0.10, r^2: 0.9439) > 8 (0.26 ± 0.01, r^2: 0.9734) ≈ 13 (0.25 ± 0.03, r^2: 0.9955). In this reduction method, the highest reducing activity was exhibited by 11 and 16 and the lowest by 8 and 13 (Table 3).

Like other reduction tests, this is a low-cost, rapid, stable, and selective method for pure compounds regardless of hydrophobicity and chemical content. The efficiency of a molecule's antioxidants can be determined using a variety of techniques. In the present study, common methods were selected, namely $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging activity and Fe^{3+}, Fe^{3+}-TPTZ complex, and Cu^{2+} reducing abilities. Moreover, as given in Table 1–3 and Supplementary Figure S2A, the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) demonstrated effective $DPPH^{\bullet}$ radical ability that was statistically significant ($p < 0.01$). The $DPPH^{\bullet}$ radical activity of the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) and positive controls increased depending on increased amine (7), aldehyde derivative (8–12), and Schiff base (13–17) concentrations.

The half maximal scavenging concentration (IC_{50}) of the amine (7), aldehyde derivatives (8–12), and Schiff bases (13–17) and standards toward $DPPH^{\bullet}$ radicals increased in the following order: 12 (1.41 ± 0.02, r^2: 0.9664 < 8 (1.39 ± 0.02, r^2: 0.9930) < 9 (1.39 ± 0.01, r^2: 0.9772) < 10 (1.38 ± 0.03, r^2: 0.9987) ≈ 15 (1.38 ± 0.01, r^2: 0.9921) < 7 (1.37 ± 0.02, r^2: 0.9999) < 14 (1.35 ± 0.04, r^2: 0.9567) ≈ 13 (1.35 ± 0.03, r^2: 0.9555) < 16 (1.34 ± 0.03, r^2: 0.9897) ≈ 17 (1.34 ± 0.03, r^2: 0.9802) < 11 (1.26 ± 0.03, r^2: 0.9695) < BHT (0.94 ± 0.10, r^2: 0.9961) < BHA (0.40 ± 0.01, r^2: 0.9912) < α-Tocopherol (0.09 ± 0.00, r^2: 0.9982) < Trolox (0.07 ± 0.00, r^2: 0.9997). The results showed that the Schiff bases (13–17) generally had better $DPPH^{\bullet}$ radical activity than the starting compounds. However, the most potent $DPPH^{\bullet}$ radical scavenging values were calculated for 11 (IC_{50}: 1.26 µg/mL; r^2: 0.9695), lower than those for BHT (0.94 ± 0.10, r^2: 0.9961) (Table 4 and Figure 7).

Table 4. IC_{50} (μg/mL) values for DPPH• scavenging abilities of all compounds (7–17) and standards.

Antioxidants	DPPH• Scavenging 517 nm	r^2
BHA	0.40 ± 0.01	0.9912
BHT	0.94 ± 0.10	0.9961
Trolox	0.06 ± 0.00	0.9997
α-Tocopherol	0.09 ± 0.00	0.9982
7	1.37 ± 0.02	0.9999
8	1.39 ± 0.02	0.9930
9	1.39 ± 0.01	0.9772
10	1.38 ± 0.03	0.9987
11	1.26 ± 0.03	0.9695
12	1.41 ± 0.02	0.9664
13	1.35 ± 0.03	0.9555
14	1.35 ± 0.04	0.9567
15	1.38 ± 0.01	0.9921
16	1.34 ± 0.03	0.9897
17	1.34 ± 0.03	0.9802

Table 5. IC_{50} (μg/mL) values for $ABTS^{•+}$ scavenging abilities of all compounds (7–17) and standards.

Antioxidants	$ABTS^{•+}$ Scavenging 734 nm	734 nm
BHA	0 ± 0	0 ± 0
BHT	0 ± 0	0 ± 0
Trolox5	0 ± 0	0 ± 0
α-Tocopherol	0.095 ± 0.00	0.095 ± 0.00
7	0.678 ± 0.06	0.678 ± 0.06
8	0.838 ± 0.03	0.838 ± 0.03
9	0.418 ± 0.00	0.418 ± 0.00
10	0.272 ± 0.03	0.272 ± 0.03
11	0.099 ± 0.03	0.099 ± 0.03
12	0.689 ± 0.01	0.689 ± 0.01
13	0 ± 0	0 ± 0
14	0 ± 0	0 ± 0
15	0 ± 0	0 ± 0
16	0 ± 0	0 ± 0
17	0.024 ± 0.01	0.024 ± 0.01

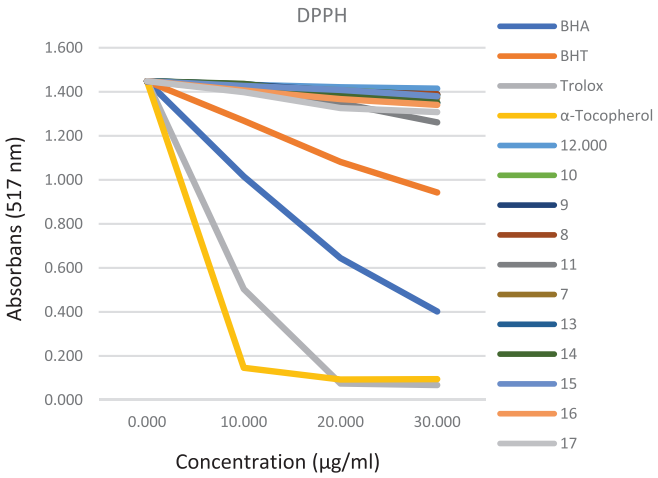


Figure 7. DPPH• scavenging abilities of all compounds (7–17) and standards.

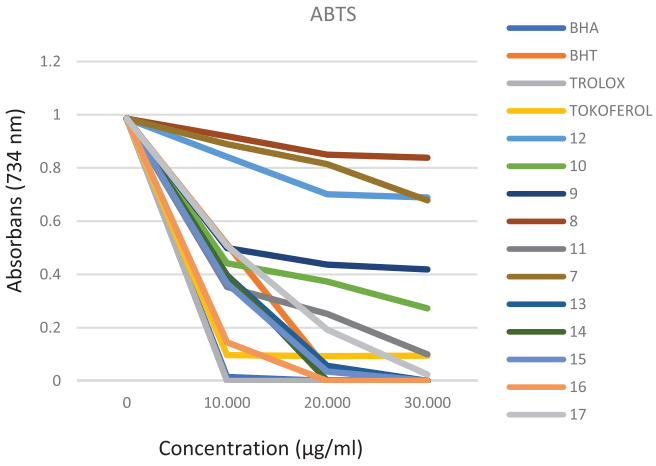


Figure 8. ABTS scavenging abilities of all compounds (7–17) and standards.

As shown in Table 5 and Figure 8, the order of all compounds (7–17) in terms of $ABTS^{•+}$ scavenging ability was as follows: 8 (0.84 ± 0.03, r^2: 0.9860) < 12 (0.69 ± 0.01, r^2: 0.9864) < 7 (0.68 ± 0.06, r^2: 0.9932) < 9 (0.42 ± 0.00, r^2: 0.9668) < 10 (0.27 ± 0.03, r^2: 0.9581) < 11 (0.10 ± 0.03, r^2: 0.9627) < α-Tocopherol (0.10 ± 0.00, r^2: 0.9351) < 17 (0.02 ± 0.01, r^2: 0.9999) < 13 (0 ± 0.0, r^2: 0.9999) ≈ 15 (0.00 ± 0.0, r^2: 0.9999) ≈ 14 (0.0 ± 0.0, r^2: 0.970) ≈ 16 (0.0 ± 0.0, r^2: 0.9775) ≈ BHT (0.0 ± 0.0, r^2: 0.9862) ≈ BHA (0.0 ± 0.0, r^2: 0.9386) ≈ Trolox (0.0 ± 0.0, r^2: 0.9333). The $ABTS^{•+}$ scavenging values calculated for imine compounds 13–17 were similar to those for BHT, BHA, and Trolox and lower than that for α-Tocopherol. The low IC_{50} values of the compounds reflect their effective $ABTS^{•+}$ scavenging ability.[96]

3.2. Results of Carbonic Anhydrase Inhibition Studies

hCA I and hCA II control acid-base balance through physiological pathways used for the treatment of cerebral edema, glaucoma, and epilepsy.[97] The amine (7), aldehyde derivatives (8–12), and novel Schiff bases (13–17) showed in vitro inhibitory effects against cytosolic hCA I, which is associated with cerebral and retinal edema and gastric and duodenal ulcers;[98,99] against hCA II, which is associated with edema, glaucoma, epilepsy, and mountain sickness;[98,100] and against AChE, which is linked to Alzheimer's disease due to its inhibitory activities.[101]

The CA inhibitory effects of all starting compounds (7–12) and the obtained Schiff bases (13–17) were determined by the esterase test and compared with those of acetazolamide (AZA).[102,103] Ellman's procedure[80] was used to determine the activity of the synthesized Schiff bases (13–17) against AChE and

Table 6a. Summarized inhibition parameters of all compounds (7–17) against hCA I and hCA II.

Compounds	IC_{50} [nM]			
	CA I	r^2	CA II	r^2
7	138.63	0.9910	115.53	0.9938
8	138.63	0.9910	138.63	0.9979
9	115.53	0.9936	77.02	0.9901
10	99.02	0.9910	69.31	0.9978
11	173.28	0.9979	115.53	0.9896
12	115.53	0.9924	69.32	0.9770
13	86.64	0.9937	99.021	0.9920
14	53.32	0.9906	115.53	0.9929
15	40.77	0.9945	99.02	0.9947
16	86.64	0.9947	77.02	0.9902
17	77.02	0.9919	99.02	0.9703
AZA	69.31	0.9936	38.51	0.9970

Table 6b. Summarized inhibition parameters of all compounds (7–17) against hCA I and hCA II.

Compounds	K_i [nM]	
	CA I	CA II
7	118.69 ± 7.48	182.60 ± 31.08
8	187.16 ± 41.34	133.72 ± 15.94
9	169.51 ± 27.65	94.77 ± 19.01
10	94.66 ± 15.44	74.04 ± 21.12
11	95.97 ± 9.63	109.58 ± 21.33
12	134.48 ± 9.86	64.40 ± 4.73
13	58.51 ± 12.62	86.03 ± 13.36
14	26.34 ± 2.21	122.29 ± 9.44
15	52.14 ± 10.73	118.90 ± 26.29
16	66.57 ± 11.57	109.97 ± 18.54
17	96.06 ± 24.70	92.91 ± 19.91
AZA	48.18 ± 14.97	59.92 ± 14.99

compared with the standard inhibitor tacrine. Further, the following insights can be given from the studied enzymes' inhibition results given in Tables 6a and 6b, and 7.

All compounds (7–17) exhibited effective inhibition profiles against the widespread cytosolic hCA I isozyme, with K_i values ranging from 26.34 ± 2.21 nM to 187.16 ± 41.34 nM. Compound 8 showed a lower inhibition profile (K_i: 187.16 ± 41.34 nM) when compared to the amine (7), aldehyde derivatives (8–12), and synthesized Schiff bases (13–17). However, within this series, compound 14 was the best inhibitor (K_i: 26.34 ± 2.21 nM) toward cytosolic hCA I isozyme in comparison with AZA (K_i: 48.17 ± 14.96 nM). Compounds 13 and 15 showed stronger inhibition ability than AZA as a standard and clinical CA inhibitor. All hCA isomers have a highly conserved active site, where a Zn^{2+} is coordinated by residues His94, His96, and His119 and an H_2O that is important for catalytic activity. Most hCA inhibitors have been identified as Zn-binding molecules. Overexpression of the hCA I isozyme has been associated with cerebral and retinal edema, while the hCA II isoform has been associated with altitude sickness, glaucoma, and epilepsy[104] (Table 6a and 6b).

All compounds (7–17) inhibited cytosolic and dominant hCA II isozyme with K_i of 64.40 ± 4.73–182.60 ± 31.08 nM. Among them, compound 12 showed an important inhibitory effect toward hCA II with K_i of 64.40 ± 4.73 nM. However, the inhibition profiles of all aldehyde derivatives (8–12) and Schiff bases (13–17) were lower than that of AZA (K_i: 59.91 ± 14.99 nM). Additionally, the selectivity index (hCA I / hCA II) for both hCA isoenzymes shows that the synthesized substances have a higher affinity for the hCA I isozyme rather than hCA II isoforms. However, the rest of the Schiff bases (13–17) demonstrated moderate inhibition on the ubiquitous and dominant cytosolic hCA II isoenzyme.

Table 7. The inhibition parameters of the aldehydes (8–13) and Schiff bases (13–17) toward AChE.

Compounds	IC_{50} [nM]		
	AChE	r^2	K_i
7	43.32	0.9927	5.21 ± 0.76
8	43.32	0.987	4.16 ± 0.25
9	33.00	0.9889	3.20 ± 0.13
10	30.13	0.9904	2.70 ± 0.30
11	33.00	0.9926	3.86 ± 0.56
12	26.66	0.9876	4.96 ± 0.54
13	17.32	0.9916	2.20 ± 0.07
14	13.59	0.9912	1.69 ± 0.13
15	12.37	0.992	1.63 ± 0.13
16	20.39	0.9912	1.63 ± 0.13
17	27.72	0.9905	4.07 ± 0.34
Tacrine	18.24	0.9938	4.14 ± 0.76

The AChE inhibition abilities of the amine (7), aldehyde derivatives (8–12), and synthesized Schiff bases (13–17) were given for the first time in the present study. The results are summarized in Table 4. Tacrine was used as a positive control for AChE inhibition with K_i of 4.14 ± 0.76 nM toward AChE. As presented in Table 7, the IC_{50} values of all Schiff bases (13–17) were in the range of 12.37 to 27.72 nM toward AChE. Most of the synthesized Schiff bases (13–15) had higher inhibitory effects than tacrine (IC_{50}: 18.24 nM). Some of the Schiff bases (16 and 17) and the starting compounds showed lower inhibitory effects than tacrine (IC_{50}: 46.20 nM).

3.3. Results of Molecular Docking Studies

The inhibitory effects of Schiff bases on AChE were investigated in vitro. A molecular docking study was performed to determine the possible binding positions, interactions, affinities, and complex structures of these compounds with AChE. The software Autodock 4.2 was used to determine the active site of AChE.

Molecular docking studies of compounds against AChE were performed. Recombinant human AChE complexed with (-)-galantamine (PDB ID: 4EY6) as the target protein was docked to

the active sites of different types of interactions. The 2D and 3D binding interactions of the synthesized molecules in the active site of AChE (PDB: 4EY6) are shown in Figure 9. The binding energy values range from –8.12 to –7.30 kcal mol^{-1}.

According to the molecular docking study results, compound 13 showed the lowest binding affinity, with –7.74 kcal mol^{-1}. Amino acids GLU202 and TYR72 have bond lengths of 2.04 and 1.88 Å, respectively, and are bound to compound 13 by conventional hydrogen bonding (Figure 9). The amino acid PRO88 had a carbon-hydrogen bond between the methoxy group in the phenyl ring, with a 3.54 Å bond length. A π-donor hydrogen bond existed between amino acid SER125 (2.69 Å). π–π T-shaped interactions were observed between TYR124 residue with 5.93 Å bond length. Moreover, compound 13 interacts with TRP86 (3.94 Å) via a π–alkyl interaction. All interactions are shown in detail in Figure 9.

According to the docking results, compound 14 showed the strongest inhibitory property with –8.12 kcal/mol the lowest binding affinity (Figure 9). Compound 14 had conventional hydrogen bonds with residues ASP74 (1.84 Å), PHE295 (1.94 Å), and ARG296 (1.87 Å) in the active site of the enzyme. Compound 14 formed a π–π -stacking interaction with amino acid residue TRP286 with an interplanar distance of 5.24 Å. In addition, compound 14 formed an alkyl interaction with LEU76 (4.42 Å) and VAL294 (4.62 Å). Compound 14 interacted with TYR341 (4.67 Å) and PHE338 (4.85 Å) via a π–alkyl interaction. Compound 14 formed the most stable complex, with a binding affinity of –8.12 kcal mol^{-1}, as a result of docking at the active site of AChE.

Figure 4 shows that compound 15 had good binding interactions with human AChE (PDB ID: 4EY6). Compound 15 showed the lowest binding affinity of –7.83 kcal mol^{-1}. ASP74 (3.20 Å), HIS447 (4.79 and 5.77 Å), and SER203 (5.00 and 4.42 Å) had conventional hydrogen bonds with compound 15. An amide–π stacked interaction was observed with GLY121 (4.54 Å) residue and π–alkyl interactions with HIS447 (6.71 Å), PHE338 (6.80 Å), PHE297 (5.93 Å), TRP236 (5.17 Å), and PHE295 (4.95 Å). All interactions are shown in detail in Figure 9.

According to the docking results, compound 16 showed the lowest binding affinity of –7.56 kcal/mol. The amino acid ASP74 had a conventional hydrogen bond with compound 16 with a binding length of 3.28 Å (Figure 4). A π–π T-shaped interaction was observed with TRP86 residue with 5.57 Å bond length and a π–sigma interaction was observed with TYR337 (4.29 Å) residue. Further, compound 16 interacted with TYR449 (5.27 Å) and TRP86 (5.08 Å) via a π–alkyl interaction. All interactions are shown in detail in Figure 9.

4. Discussion

Schiff bases are an important class of organic compounds extensively studied for their unique structural features, biological activities, and applications across disciplines such as chemistry, biochemistry, and medicine.[105] In medicinal chemistry, Schiff bases have attracted significant attention due to their many biological and pharmaceutical activities such as antifungal, antiviral, antitumor, antibacterial, antimalarial, anticancer, anti-inflammatory, reactive oxygen species (ROS) scavenging activities, and enzyme inhibition.[106–108] The biological activity of Schiff bases is due to the imine or azomethine (—C═N—) functional group as well as hydrophobic aromatic groups that can easily coordinate with metals to form versatile functional complexes.[109,110]

Recently, symmetric Schiff bases and their amine derivatives were tested against AChE, hCA I, and hCA II isoenzymes and the synthesized molecules were found to exhibit inhibitory effects.[111] In another study, novel Schiff bases derived from 2-aminopyridine were synthesized using a microwave technique. It was stated that all the synthesized Schiff bases showed DPPH• and nitric oxide radical scavenging power and could be useful in the development of a potential drug class.[112] It has been reported that the class of compounds known as Schiff bases containing the azomethine functional group exhibits scavenging activity, especially against superoxide anion radicals. As a result, it was observed that copper-containing Schiff base-metal complexes can almost completely eliminate superoxide anion radicals even at low concentrations.[113]

In a study conducted in 2020, eight sulfonamide derivative Schiff bases were synthesized and their inhibitory effects on AChE and CA I and II activities were determined using different bioanalytical assays such as antioxidant activities, radical scavenging tests with ABTS•+ and DPPH•, and metal reduction abilities with CUPRAC and FRAP tests. All compounds were reported to exhibit satisfactory enzyme inhibitory potential at nanomolar concentrations against AChE and CAI-II isoforms with Ki values ranging from 10.14 ± 0.03 to 100.58 ± 1.90 nM.[114]

Imine compounds are well-documented for their diverse biological activities. In this study, the antioxidant capacity and some metabolic enzyme inhibition abilities of Schiff bases synthesized by microwave method were reported for the first time. Here, five new Schiff base analogs were synthesized using an environmentally friendly methodology. The synthesis was achieved using a simple, efficient, and rapid method without the need for solvents or catalysts. Compared to other methods, microwave irradiation is the simplest way to synthesize new Schiff bases.

The antioxidant activities, acetylcholinesterase (AChE) inhibition, and carbonic anhydrase (CA) inhibition properties of the synthesized Schiff bases and their starting compounds were comprehensively evaluated. Results revealed that these compounds effectively inhibited metabolic enzymes, including AChE, hCA I, and hCA II, which are critical in drug design, toxicology, and medicine. Molecular docking studies were also performed to complement the experimental findings, focusing on AChE inhibition, the most pronounced activity among the synthesized compounds. AChE inhibitors are widely used in the treatment of neurological and psychiatric disorders.

Docking studies evaluated the binding energies of Schiff bases on the AChE enzyme, revealing the following activity order for the compounds based on their binding affinities: Compound 14 (–8.12 kcal mol^{-1}) > Compound 15 (–7.83 kcal mol^{-1}) > Compound 13 (–7.74 kcal mol^{-1}) ≥ Compound 16 (–7.56 kcal mol^{-1}) > Compound 17 (–7.30 kcal mol^{-1}). Compound 14, with the high-

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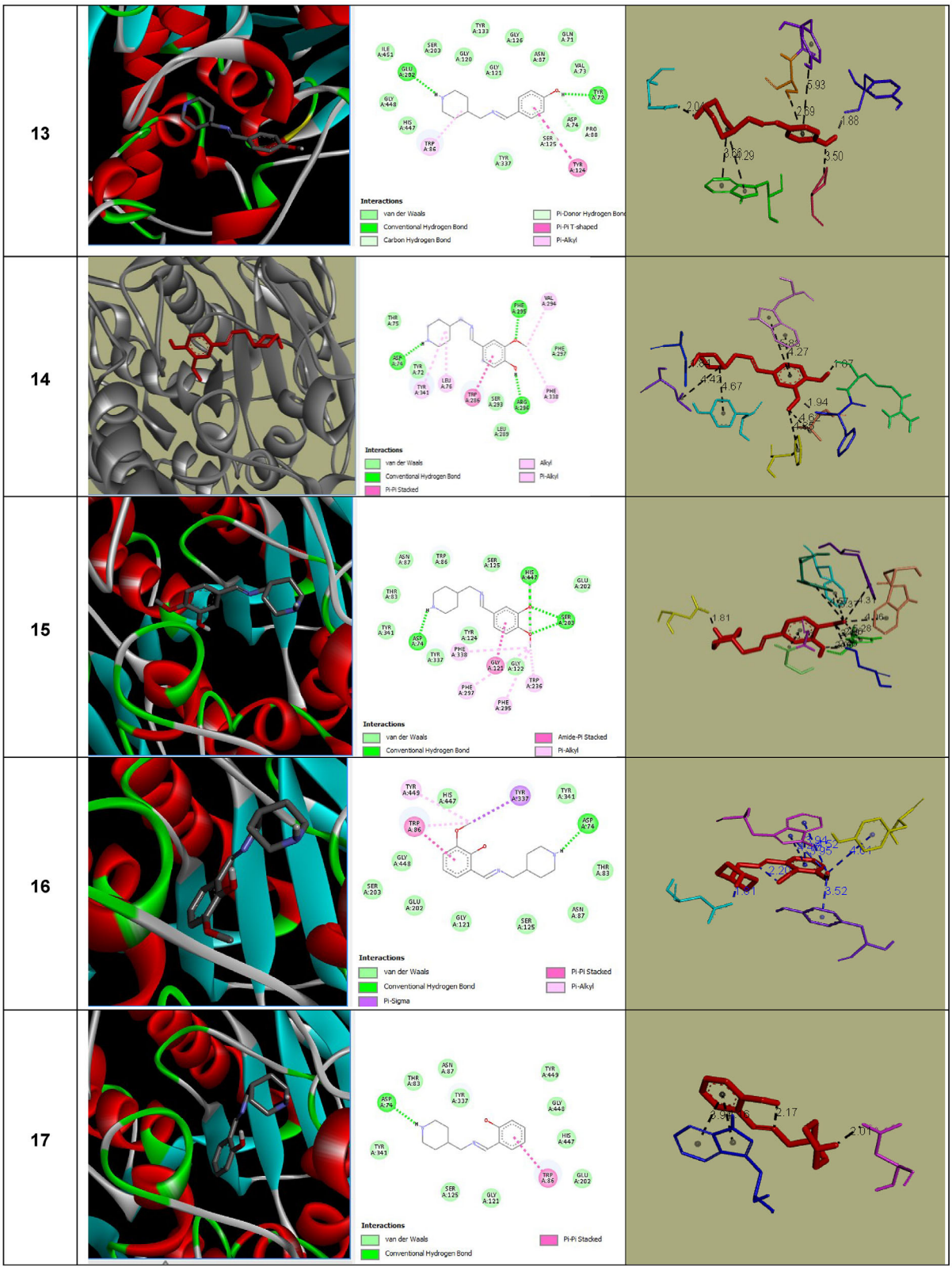

Figure 9. 2D and 3D binding interactions of compounds 13–17 within the active site of AChE (PDB: 4EY6).

est binding energy (–8.12 kcal mol^{-1}), exhibited the strongest activity. This order was consistent with biological test results, demonstrating a correlation between experimental and theoretical studies.

In conclusion, the newly synthesized Schiff bases offer a wide range of biological activities with both antioxidant and enzyme inhibitor properties. Their notable effects on AChE and hCA inhibition suggest that these compounds could serve as promising therapeutic agents for the treatment of various diseases, particularly those involving neurological and psychiatric disorders.

5. Conclusion

In this study, the synthesis of Schiff bases containing piperidine rings and the biological activities of these compounds such as antioxidant, AChE inhibition, and CA inhibition were investigated. The obtained results suggest that compounds that are particularly effective in terms of AChE inhibition can be used in the treatment of neurological diseases. In addition, the antioxidant properties of Schiff bases have an important potential in terms of reducing cellular damage due to oxidative stress. Docking studies revealed that compound 14 with the highest binding energy showed the strongest activity in AChE inhibition, indicating that this compound can be evaluated as a therapeutic agent. In conclusion, the newly synthesized Schiff bases can be suggested as potential drug candidates that can be used in the treatment of various diseases by exhibiting both antioxidant and enzyme inhibitor properties with their effects such as AChE and hCA inhibition. It is thought that these compounds may be effective in the treatment of neurological and psychotic diseases such as edema, epilepsy, glaucoma, and Alzheimer's disease.

Supporting Information

Additional supporting information includes spectral data and copies of ^{1}H NMR, ^{13}C NMR, and elemental analysis of all products.

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Conflict of Interests

The author declares no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords: Antioxidant activity · Aromatic aldehydes · Enzyme inhibition · Microwave · Piperidine · Schiff bases

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