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Synthesis, Antioxidant, and Antiacetylcholinesterase Activities of Sulfonamide Derivatives of Dopamine-Related Compounds

Hülya Göçer[1,2], Akın Akıncıoğlu[1,2], Necla Öztaşkın[2], Süleyman Göksu[2]*, and İlhami Gülçin[2]

[1] Central Researching Laboratory, Agri Ibrahim Cecen University, Agri, Turkey
[2] Faculty of Science, Department of Chemistry, Atatürk University, Erzurum, Turkey

A series of sulfonamides were synthesized from dopamine derivatives. The reactions of amines with methanesulfonyl chloride followed by *O*-demethylation with BBr_3 afforded phenolic sulfonamides. The antioxidant activities of the synthesized phenolic sulfonamides were investigated by thiocyanate method, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\bullet}$), *N*,*N*-dimethyl-*p*-phenylenediamine ($DMPD^{\bullet+}$), and superoxide anion ($O_2^{\bullet-}$) radical scavenging, reducing power, and ferrous ion (Fe^{2+}) chelating assays. Sulfonamides **13**–**16** showed around 75–85% inhibition on linoleic acid peroxidation. On the other hand, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol, and trolox indicated an inhibition of 90.0%, 85.73%, 73.33%, and 85.73% on peroxidation, respectively, in the same system at the same concentration (10 μg/mL). Also, the inhibition effects of the synthesized compounds on acetylcholinesterase (AChE) activity were evaluated. AChE was effectively inhibited by sulfanomides **13**–**16**, with K_i values in the range of 33.04 ± 4.3 to 131.68 ± 8.8 nM.

Keywords: Antiacetylcholinesterase activity / Antioxidant activity / Dopamine / Phenolic compound / Radical scavenging / Sulfonamides

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Introduction

Sulfonamides are prominent biologically active compounds [1]. In the last decades, a variety of sulfonamide drugs have been developed and appeared in the markets. The carbonic anhydrase inhibitor brinzolamide (**1**), known as azopt, has been used to lower intraocular pressure in patients with open-angle glaucoma or ocular hypertension [2]. Dorzolamide (trusopt, **2**) is used as an anti-glaucoma agent by decreasing the production of aqueous humor [3]. A sulfonamide drug sultiame (sulthiame, **3**), an anticonvulsant, is reported for treatment of epilepsy and West syndrome [4]. In addition, the monoamine hormone dopamine (**4**) is a neurotransmitter and plays an important role in the central nervous system-related disorders such as schizophrenia and Parkinson's disease [5, 6]. Antioxidant properties of dopamine (**4**) and related monoamine hormones have previously been reported by Yen and Hsieh [7] (Fig. 1).

Free radicals, known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxide ($O_2^{\bullet-}$), hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), and nitric oxide ($NO^{\bullet}$) radicals, are highly reactive molecules with unpaired electrons that can quickly bind to nearby molecules. ROS are generated as by-products of cellular metabolism, primarily in the mitochondria. Free radicals are very reactive molecules; they can attack the molecules in adjacent cells, and probably the damage caused by them is unavoidable and is dealt with by repair processes [8–10]. The importance of ROS has attracted increasing attention over the past decades. In the presence of low concentrations of free radicals, ROS is important for normal cellular redox status, tissues function, and intracellular signaling processes [10]. When cellular

Correspondence: Prof. İlhami Gülçin, Faculty of Science, Department of Chemistry, Atatürk University, 25240 Erzurum, Turkey.
E-mail: igulcin@atauni.edu.tr
Fax: +90 442 2360948

Additional correspondence: Prof. Süleyman Göksu,
E-mail: sgoksu@atauni.edu.tr

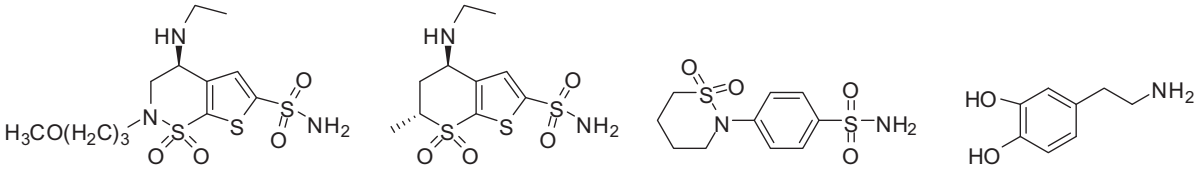


Figure 1. Some sulfonamide drugs (**1**–**3**) and dopamine (**4**).

production of ROS suppresses its antioxidant capacity, there is damage to cellular macromolecules such as lipids, proteins, etc. [11, 12]. They may cause DNA damage and, as a result, the mutation can occur. If ROS are not adequately scavenged by cellular antioxidant components, they can cause free-radical chain reactions, subsequently damaging the cellular biomolecules such as carbohydrates, proteins, lipids, and nucleic acids. Finally, they lead to disease conditions [13]. It was reported that ROS had been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer [14–17]. Chemically, oxidative stress is associated with increased production of oxidizing species such as $O_2^{\bullet -}$ and $HO^{\bullet}$, or a significant decrease in the effectiveness of antioxidant defenses. Biologically, it was defined as an imbalance between prooxidant production and the antioxidant capacity of the cell to prevent oxidative injury [13, 18]. In human metabolism, oxidative stress is thought to be involved in the development of many chorionic diseases or may exacerbate their symptoms [19, 20] including atherosclerosis, heart failure [21], autism [22], cancer [23], myocardial infarction [24], Parkinson's and Alzheimer's diseases [25], schizophrenia [26], bipolar disorder [27], and chronic fatigue syndrome [22].

An antioxidant molecule inhibits the oxidation of other molecules. On the other hand, oxidation process is known as a chemical reaction that transfers hydrogen atom or electrons from a substance to an oxidizing agent [28, 29]. The description of antioxidant is originally related to molecules that prevent the consumption of oxygen by human tissues. In other words, an antioxidant is a molecule or species that slows or prevents the oxidation of another molecule [30]. Physiologically there is an appropriate prooxidant–antioxidant balance in normal cells. This balance can be shifted toward the prooxidants when levels of antioxidants are diminished or when the production of ROS is increased greatly. This case is called as oxidative stress [28, 31].

Phenolic compounds are a class of chemical compounds containing a hydroxyl group (–OH) bonded directly to an aromatic hydrocarbon ring. They are esteemed as free radical scavengers, and their antioxidant properties are related to their chemical structure. Particularly, these properties of phenols depend on their hydrogen- or electron-donating ability and, most importantly, their ability to delocalize the unpaired electron within the aromatic structure [30–33]. They are known as an integral part of both human and animal diets. Also, phenolic compounds constitute the majority of natural antioxidants [34–36].

On the other hand, excess amount of ROS, which causes oxidative stress, is associated with pathology of many diseases including Alzheimer's disease. This disease is a degenerative disease of the central nervous system characterized especially by premature senile mental deterioration. It is the most common form of dementia and a progressive neurologic disorder characterized by cognitive deficit and behavioral abnormalities in the patient [37]. It was first described by German neuropathologist Alois Alzheimer in 1906 and was named after him [38]. So far, pathogenesis of Alzheimer's disease has not been completely clarified. The only known valid hypothesis being accepted is the lack of enough amount of acetylcholine (ACh), a neuromediator. Therefore, the compounds that have acetylcholinesterase (AChE) inhibitory effects were used for the treatment of Alzheimer's disease. However, most of these drugs have undesired side effects. Thus, the development and utilization of new effective antioxidants as well as AChE compounds are desired. In a previous report, it is suggested that the use of antioxidants may reduce the progression of Alzheimer's disease and minimize neuronal degeneration by inhibiting AChE, which are main enzymes in pathogenesis of Alzheimer's disease [39].

On the other hand, ACh acts as an excitatory neurotransmitter for voluntary muscles in the somatic nervous system and as a preganglionic and a postganglionic transmitter in the parasympathetic nervous system of vertebrates and invertebrates [40, 41]. Acetylcholinesterase (AChE. EC 3.1.1.7), a serine protease, is responsible for ACh hydrolysis and plays a fundamental role in impulse transmission by terminating the action of the neurotransmitter ACh at the cholinergic synapses and neuromuscular junction. It is associated with the normal neurotransmission by catalyzing the hydrolysis of ACh to acetate and choline, and acts to remove ACh from the

synaptic cleft [42]. It plays a vital role in the CNS, where it catalyzes the hydrolysis of the neurotransmitter ACh [43].

As sulfonamides and dopamine-related compounds show useful biological activities, in the present work we focused on the synthesis of phenolic sulfonamide derivatives of dopamine (**4**) related compounds **13–16**. There are various methods for evaluation of antioxidant capacity of pure compounds, which involve different mechanisms [44, 45]. Therefore, in the present study, we report the synthesis of phenolic compounds **13–16** and we determined the antioxidant activity of the synthesized phenolic compounds by thiocyanate method, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\bullet}$), *N*,*N*-dimethyl-*p*-phenylenediamine ($DMPD^{\bullet+}$), and $O_2^{\bullet-}$ scavenging activities, reducing power, and ferrous ion (Fe^{2+}) chelating activities. Another main goal of this research was to determine the AChE enzyme inhibitory effects of phenolic compounds **13–16** as alternative drugs for the treatment of Alzheimer's disease.

Results and discussion

Chemistry

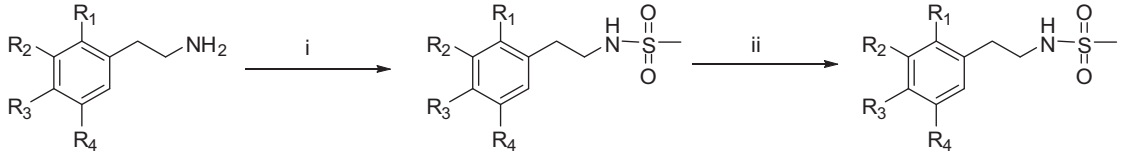
Our synthesis commences from commercially available methoxylated dopamine derivatives **5–8**. The reactions of free amines with methanesulfonyl chloride (MsCl) in the presence of Et_3N have previously been reported [46]. By following this procedure, the reactions of amines **5–8** with MsCl in the presence of Et_3N at 0–25°C for 15 h afforded novel sulfonamides **9**, **10**, and known compounds **11** [47] and **12** in moderate yields. Cleavage of arylmethyl ethers is an integral functional group transformation for the synthesis of biologically or synthetically important phenols. *O*-Demethylation of arylmethyl ethers can be achieved by HBr [48, 49] or BBr_3 [50, 51]. Because of the solubility problems of compounds **9–12**, we know very well from our earlier studies that the latter procedure is the most convenient one for *O*-demethylation of **9–12**. Therefore, the reactions of compounds **9–12** with BBr_3 in CH_2Cl_2 at 0–25°C for 20 h gave novel phenolic sulfonamide derivatives of dopamines **13–15** and a known compound **16** in high yields (Scheme 1). The structures of all synthesized compounds were characterized by ^1H and ^{13}C NMR spectroscopic techniques. The functional groups were characterized by IR.

Antioxidant properties

The antioxidant capacity of a phenolic compound is based on their free radical scavenging ability, donate hydrogen atom or electron, or chelate metal ions [52, 53]. The structure of a phenolic compound is a key determinant of their biological activities such as radical scavenging activity and metal chelating activity. For example, antioxidant activity of a phenolic acid depends on the number and position of the hydroxyl groups (–OH) [54]. Monohydroxy benzoic acids with the –OH moiety at the *ortho*- or *para*- position to the carboxyl group (–COOH) show no antioxidant activity, though the same is not true for the *meta*-hydroxybenzoic acid [18, 54, 55]. The antioxidant properties of phenolic acids or phenolic compounds increase with increasing degree of hydroxylation. For example, trihydroxylated gallic acid shows a marked antioxidant activity. Also, as can be seen in syringic acid, substitution of the hydroxyl groups at the 3- or 5-position with methoxyl groups reduces the antioxidant activity [10, 54].

The ferric thiocyanate method is the most effective antioxidant method and measures the amount of peroxide, which is the primary product of oxidation produced during the initial stages of oxidation [56, 57]. Total antioxidant activity of phenolic sulfonamides **13–16** and standard compounds was determined by using lipid peroxidation in the linoleic acid system. As seen in Table 1, phenolic sulfonamides **13–16** and standard compounds exhibited effective inhibition on linoleic acid peroxidation. At the 10 μg/mL concentration, percentage inhibition of linoleic acid peroxidation of phenolic sulfonamides **13–16** and standards decreased in the following order: butylated hydroxyanisole (BHA) (90.0%) > butylated hydroxytoluene (BHT) (85.7%) = trolox (85.7%) ≈ **15** (85.6%) ≈ **14** (85.5%) ≈ **13** (84.0%) > **16** (75.0%) > α-tocopherol (73.3%) and at the control value reached its maximum absorbance (144 h).

The reducing ability of a biologically active compound may serve as a significant indicator of its potential antioxidant



5 R_1=R_2=OMe, R_3=R_4=H
6 R_2=R_4=OMe, R_1=R_3=H
7 R_2=R_3=OMe, R_1=R_4=H
8 R_1=OMe, R_2=R_3=R_4=H

9 R_1=R_2=OMe, R_3=R_4=H, 77%
10 R_2=R_4=OMe, R_1=R_3=H, 75%
11 R_2=R_3=OMe, R_1=R_4=H, 73%
12 R_1=OMe, R_2=R_3=R_4=H, 78%

13 R_1=R_2=OH, R_3=R_4=H, 84%
14 R_2=R_4=OH, R_1=R_3=H, 81%
15 R_2=R_3=OH, R_1=R_4=H, 77%
16 R_1=OH, R_2=R_3=R_4=H, 82%

Scheme 1. Synthesis of sulfonamides. (i) $MeSO_2Cl/NEt_3$, CH_2Cl_2, 0–25°C, 15 h; (ii) BBr_3, CH_2Cl_2, 0–25°C, 20 h.

Table 1. The percentage inhibition of lipid peroxidation, Fe^{3+}–Fe^{2+} reductive potential, FRAP assay, and cupric ions (Cu^{2+}) reducing ability of phenolic sulfonamides (**13–16**) and reference antioxidants at the same concentrations (10 μg/mL)

Antioxidants	Fe^{3+} reducing ability[a)]	FRAP assay[a)]	Cu^{2+} reducing ability[a)]	Inhibition of lipid peroxidation[b)]
BHA	1.632 ± 0.279	2.143 ± 0.110	1.173 ± 0.066	90.0
BHT	1.407 ± 0.112	1.023 ± 0.217	1.140 ± 0.098	85.7
α-Tocopherol	0.727 ± 0.078	1.238 ± 0.267	0.330 ± 0.061	73.3
Trolox	0.675 ± 0.167	0.856 ± 0.040	0.268 ± 0.027	85.7
13	0.305 ± 0.106	0.444 ± 0.036	0.203 ± 0.106	84.0
14	2.411 ± 0.225	1.569 ± 0.095	1.901 ± 0.037	85.5
15	0.652 ± 0.212	0.413 ± 0.013	0.255 ± 0.039	85.6
16	2.454 ± 0.345	2.418 ± 0.059	2.617 ± 0.019	75.0

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.
[a)] The values were expressed as absorbance. High absorbance indicates high reducing ability.
[b)] The values were expressed as percentage of inhibition of linoleic acid emulsion peroxidation.

capacity. An antioxidant compound can donate electrons to reactive radicals or ROS. It can reduce them into more stable and unreactive species [58]. The reducing power of phenolic sulfonamides **13–16** was investigated by Fe^{3+}–Fe^{2+} transformation, FRAP, and CUPRAC assays.

As shown in Table 1, phenolic sulfonamides **13–16** had powerful ferric ions (Fe^{2+}) reducing ability. In this study, it was found that reducing powers of phenolic sulfonamide compounds **16** (2.454 ± 0.345) and **14** (2.411 ± 0.225) were higher than those of all used standard compounds such as BHA (1.632 ± 0.279), BHT (1.407 ± 0.112), α-tocopherol (0.727 ± 0.078), and trolox (0.675 ± 0.167). The reducing power of the phenolic sulfonamides **13–16** and standard antioxidants decreased in the following order: **16** ≈ **14** > BHA > BHT > α-tocopherol > trolox ≈ **15** > **13**. It was reported that Fe^{3+}–Fe^{2+} reducing ability is frequently used as a marker of electron-donating ability. This bioanalytical method is an important mechanism of phenolic antioxidants [10].

In the present study, we use the CUPRAC assay, which is based on reduction of Cu^{2+} to Cu^{+} by phenolic sulfonamides **13–16**. The CUPRAC method has been used to determine the reducing power of antioxidant compounds, especially phenolic compounds [59]. As seen in Table 1, cupric ion (Cu^{2+}) reducing power of phenolic sulfonamides **13–16** and standard compounds decreased in the following order: **16** (2.617 ± 0.019) > **14** (1.901 ± 0.037) > BHA (1.173 ± 0.066) ≈ BHT (1.140 ± 0.098) > α-tocopherol (0.330 ± 0.061) > trolox (0.268 ± 0.027) ≈ **15** (0.255 ± 0.039) > **13** (0.203 ± 0.106). In the CUPRAC assay, higher absorbance values indicate higher cupric ions (Cu^{2+}) reducing ability.

The FRAP assay measures the Fe^{3+}–Fe^{2+} reducing ability of the substance and was initially proposed for the measurement of total antioxidant capacity [60]. According to results obtained from FRAP assay (Table 1), reducing power of phenolic sulfonamides **13–16** and standard compounds decreased in the following order: **16** (2.418 ± 0.059) > BHA (2.143 ± 0.110) > **14** (1.569 ± 0.095) > α-tocopherol (1.238 ± 0.267) > BHT (1.023 ± 0.217) > trolox (0.856 ± 0.040) > **13** (0.444 ± 0.036) ≈ **15** (0.413 ± 0.013). In the FRAP assay, higher absorbance values indicate higher Fe^{3+}-TPTZ reducing ability. Also, of the used reduction methods, the highest reduction ability was observed for the phenolic sulfonamide **16**. Benzie and Strain reported that the FRAP assay offers a well-known index of antioxidant, or reducing, potential of samples or pure compounds. At low pH values, a Fe^{3+}–TPTZ complex is reduced to the ferrous (Fe^{3+}) form (Fe^{2+}–TPTZ), which has an intense blue color and maximum absorption at 593 nm [10].

Ferrous ion chelating activities of phenolic sulfonamides **13–16**, and standard antioxidants are presented in Table 2. As a priority, ferrozine constitutes a complex with unbounded Fe^{2+}. Thus, a decrease occurs in the amount of ferrozine–Fe^{2+} complex [61]. The data obtained from Table 2 clearly demonstrate that phenolic sulfonamides **13–16** possess a marked capacity for iron binding. In this assay, phenolic sulfonamides **13–16** interfered with the formation of the ferrous–ferrozine complex. According to the results, phenolic sulfonamides **13–16** have effective metal chelating abilities. IC_{50} value for metal chelating activity of phenolic sulfonamides **13–16** was in the range of 5.5–11.50 μg/mL (Table 2). On the other hand, these values were found similar to those of standard compounds such as BHA (9.90 μg/mL), BHT (6.30 μg/mL), α-tocopherol (12.50 μg/mL), and trolox (12.35 μg/mL). Accordingly, lower IC_{50} values indicate high metal binding ability. It suggests that phenolic sulfonamides **13–16** have chelating effects and are able to capture ferrous ion before ferrozine agent.

$DPPH^{\bullet}$, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$, and $O_2^{\bullet-}$ scavenging assays are frequently used for the determination of the radical scavenging ability of various plant samples or pure compounds [59]. In the present study, the radical scavenging

Table 2. Concentration required for 50% scavenging (IC_{50}) of ferrous ions (Fe^{2+}) chelating activity, $DPPH^{\bullet}$ scavenging activity, $ABTS^{\bullet+}$ scavenging activity, $DMPD^{\bullet+}$, and $O_2^{\bullet-}$ scavenging activity of phenolic sulfonamides (**13–16**) and standard radical scavengers such as BHA, BHT, α-tocopherol, and trolox

	Metal chelating		$DPPH^{\bullet}$ scavenging		$ABTS^{\bullet+}$ scavenging		$DMPD^{\bullet+}$ scavenging		$O_2^{\bullet-}$ scavenging	
Antioxidants	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
BHA	9.90	0.858	8.35	0.957	4.82	0.926	18.72	0.922	36.47	0.908
BHT	6.30	0.938	25.67	0.965	4.92	0.945	–	0.985	30.13	0.927
α-Tocopherol	12.50	0.904	7.62	0.950	4.91	0.954	–	0.917	20.38	0.913
Trolox	12.35	0.827	7.79	0.876	6.36	0.935	18.73	0.913	43.32	0.949
13	11.50	0.926	12.38	0.913	4.88	0.907	25.67	0.922	9.90	0.937
14	5.77	0.896	7.79	0.875	5.09	0.935	15.40	0.935	10.83	0.982
15	11.50	0.971	11.75	0.951	4.74	0.916	16.12	0.930	57.75	0.949
16	5.51	0.971	5.50	0.943	4.20	0.914	13.32	0.926	12.60	0.947

$DPPH^{\bullet}$, 1,1-diphenyl-2-picryl-hydrazyl free radicals; $ABTS^{\bullet+}$, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid radicals; $DMPD^{\bullet+}$, *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride radicals, $O_2^{\bullet-}$, superoxide anion radicals; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.

ability of phenolic sulfonamides **13–16** was evaluated by $DPPH^{\bullet}$, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$, and $O_2^{\bullet-}$ scavenging assays.

$DPPH^{\bullet}$ assay is esteemed as a distinguished method for the evaluation of radical scavenging activity of an antioxidant compound. A radical compound is stable and does not have to be generated as in other radical scavenging assays [62]. Concentration required for 50% DPPH free radical scavenging (IC_{50}) for phenolic sulfonamides **13–16** and standards increased in the following order: **16** (5.50 μg/mL, r^2: 0.943) < α-tocopherol (7.62 μg/mL, r^2: 0.950) < **14** (7.79 μg/mL, r^2: 0.875) = trolox (7.79 μg/mL, r^2: 0.876) < BHA (8.35 μg/mL, r^2: 0.957) < **15** (11.75 μg/mL, r^2: 0.951) < **13** (12.38 μg/mL, r^2: 0.913) < BHT (25.67 μg/mL, r^2: 0.965). These results clearly showed that all phenolic sulfonamides **13–16** had effective scavenging effect on DPPH radicals. Lower IC_{50} values indicate high DPPH free radical scavenging ability. Phenolic groups easily stabilize a radical formed on phenolic carbon with their resonance structure. In phenolic sulfonamides **13–15**, phenolic groups also have two hydroxyl units. On the other hand, phenolic sulfonamide **16** has one hydroxyl unit. Abstracting hydrogen atoms from phenolic hydroxyl groups may occur easily.

ABTS radical cation decolorization assay is another effective method for the measurement of radical scavenging activity of pure molecules [63]. IC_{50} values belonging to scavenging of ABTS radical by phenolic sulfonamides **13–16** and standard antioxidants increased in the following order: **16** (4.20 μg/mL, r^2: 0.914) ≤ **15** (4.74 μg/mL, r^2: 0.916) ≤ BHA (4.82 μg/mL, r^2: 0.926) ≤ **13** (4.88 μg/mL, r^2: 0.907) ≤ α-tocopherol (4.91 μg/mL, r^2: 0.954) ≤ BHT (4.92 μg/mL, r^2: 0.945) ≤ **14** (5.09 μg/mL, r^2: 0.935) ≤ trolox (6.36 μg/mL, r^2: 0.935). At the same manner, lower IC_{50} values indicate high ABTS radical scavenging ability (Table 2).

In the presence of a suitable oxidant solution, DMPD can form a stable and colored radical cation ($DMPD^{\bullet+}$) at acidic pH [64]. As seen in Table 2, $DMPD^{\bullet+}$ radical inhibition by phenolic sulfonamides **13–16** and standard antioxidants (BHA and trolox) increased in the following order: **16** (13.32 μg/mL, r^2: 0.926) < **14** (15.40 μg/mL, r^2: 0.935) ≤ **15** (16.12 μg/mL, r^2: 0.930) < BHA (18.72 μg/mL, r^2: 0.922) ≈ trolox (18.73 μg/mL, r^2: 0.913) < **13** (25.67 μg/mL, r^2: 0.922). Lower IC_{50} values indicate high $DMPD^{\bullet+}$ radical scavenging ability. $DMPD^{\bullet+}$ method is a sensitive method. Its reproducibility dramatically decreased when hydrophobic antioxidants such as α-tocopherol or BHT were used. For this reason, both standard antioxidants were not used in this assay [65].

Superoxide anion radicals are also highly reactive radicals formed in biological systems such as mitochondrial respiration and NADPH oxidase. They have been implicated as two highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [33]. As it can be seen in Table 2, the inhibition of superoxide radical formation by phenolic sulfonamides **13–16** and standard antioxidants increased in the following order: **13** (9.90 μg/mL, r^2: 0.937) < **14** (10.83 μg/mL, r^2: 0.982) < **16** (12.60 μg/mL, r^2: 0.947) < α-tocopherol (20.38 μg/mL, r^2: 0.913) < BHT (30.13 μg/mL, r^2: 0.927) < BHA (36.47 μg/mL, r^2: 0.908) < trolox (43.32 μg/mL, r^2: 0.949) < **15** (57.75 μg/mL, r^2: 0.949). Lower IC_{50} values indicate high superoxide radical scavenging ability.

Acetylcholinesterase inhibition

Inhibition of AChE enzyme was determined on commercially available purified AChE (E.C. 3.1.1.7, Product no: C0663-50 UN, Sigma–Aldrich) from human erythrocyte based on the method of Ellman et al. [65]. It was reported that the

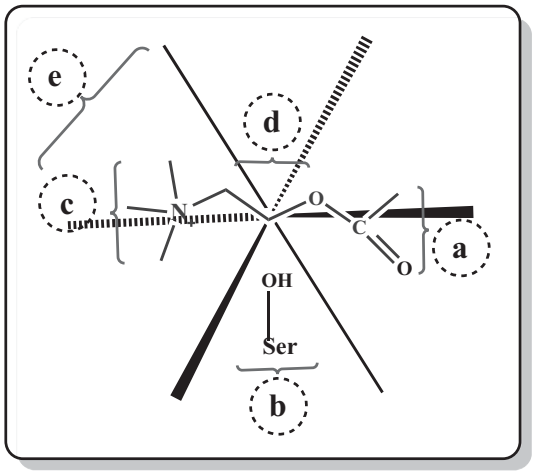


Figure 2. The interaction between acetylcholine and active sites of acetylcholinesterase enzyme.

inhibition of AChE has been used for the determination of neurotoxic properties of chemicals capable of interfering with normal neurotransmission of the parasympathetic and sympathetic nervous system. It is well known that some chemical compounds like organophosphate and carbamate pesticides [40, 66–68], enantiomeric inhibitors [43], metals, and non-pesticide contaminants have also been reported to inhibit AChE enzymatic activity [42, 69–72]. Also, it was demonstrated that the main AChE inhibitory effects was primarily associated with aromatic compounds and, to lesser degree, with aliphatic compounds [42].

In an important study, the three-dimensional structure of AChE from the Pacific electric ray (*Torpedo californica*) electric organ has been determined by X-ray analysis. The active site of AChE also consists of at least five major binding sites (Fig. 2):

a. Firstly an oxyanion hole stabilizes the tetrahedral intermediate.
b. Then an esteratic site, which is comprised of the active site serine residue, attacks the esteratic carbonyl group (C=O).
c. An anionic substrate binding site that contains a small number of negative charges but many aromatic residues, where the quaternary ammonium pole of ACh molecule and of various active site ligands binds through a preferential interaction of quaternary *N* atoms by *Z* electrons of aromatic groups.
d. An active site-selective aromatic binding site that is contiguous with or near the esteratic and anionic loci and that is important in binding aryl substrates and active site ligands.
e. A peripheral anionic binding site that may bind to the hydrophobic part of the leaving group from the active site.

Table 3. Acetylcholinesterase enzyme inhibition parameters of phenolic sulfonamides (**13–16**)

Sulfonamides	IC_{50} (nM)	K_i (nM)	r^2
13	442.8 ± 33.01	33.04 ± 4.3	0.9771
14	368.4 ± 50.04	59.05 ± 5.6	0.9542
15	452.9 ± 43.11	43.11 ± 2.8	0.9709
16	771.3 ± 131.68	131.68 ± 8.8	0.9705
Donepezil hydrochloride	55.0	–	–

All of these studies clearly showed that an investigation into the mechanism of action of AChE may lead to the design of the mechanism-based inhibitors, which could be of future therapeutic use. Different types of AChE inhibitors have been studied for the treatment of Alzheimer's disease. Some of the AChE inhibitors differ in their mechanism of action, metabolism, and brain selectivity.

AChE was very effectively inhibited by phenolic sulfonamides **13–16**, with K_is in the range of 33.04 ± 4.3 to 131.68 ± 8.8 nM (Table 3). The K_i values of phenolic sulfonamides **13–16** were similar to each other. The differences between the highest and the lowest K_i values of phenolic sulfonamides **13–16** were only fourfold. The most active one was phenolic sulfonamide **13** demonstrating a 33.04 ± 4.3 nM K_i value. On the other hand, donepezil hydrochloride, which is used for the treatment of mild-to-moderate Alzheimer's disease and various other memory impairments, had been shown to lower AChE inhibition activity (IC_{50}: 55 nM) [73]. Donepezil hydrochloride had *N*-benzylpiperidine and an indanone moiety that shows longer and more selective action.

Conclusions

In conclusion, starting from commercially available methoxylated dopamine-related compounds **5–8**, three new phenolic sulfonamides **13–15** and a known sulfonamide **16** were synthesized. In our study, phenolic sulfonamides **13–16** demonstrated effective antioxidant properties in the following assays: total antioxidant activity in linoleic acid emulsion, Fe^{3+} and Cu^{2+} reducing capabilities, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$, and $O_2{}^{\bullet-}$ scavenging and metal chelating activities when they are compared to standard antioxidant compounds. Based on results obtained from this study, phenolic sulfonamides **13–16** had powerful antioxidant activity and can be used for minimizing or preventing lipid oxidation in food and pharmaceutical products and for prolonging the shelf life of pharmaceuticals and nutritional quality of foods. Also, phenolic sulfonamides **13–16** had effective AChE inhibition properties and they can be good candidates for the treatment of mild-to-moderate Alzheimer's disease and various other memory impairments.

Experimental

All chemicals and solvents are commercially available and were used after distillation or treatment with drying agents. Melting points were determined on a capillary melting apparatus (BUCHI 530) and are uncorrected. IR spectra were obtained from solutions in 0.1 mm cells with a Perkin-Elmer spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a 400 (100)-MHz Varian and 400 (100)-MHz Bruker spectrometer; δ in ppm, Me_4Si as the internal standard. Elemental analyses were performed on a Leco CHNS-932 apparatus. All column chromatography was performed on silica gel (60-mesh, Merck). PLC is preparative thick-layer chromatography: 1 mm of silica gel 60 PF (Merck) on glass plates.

Synthesis

Standard procedure for the synthesis of sulfonamides

Amine **5** (1.30 g, 7.17 mmol) was dissolved in CH_2Cl_2 (30 mL) and this solution was cooled to 0°C. To this solution were added Et_3N (0.87 g, 8.61 mmol) and $MeSO_2Cl$ (0.99 g, 8.61 mmol). The reaction mixture was stirred at room temp. for 15 h. After the solvent was evaporated, the residue was chromatographed on silica gel (30 g) column with 20% EtOAc–hexane. Sulfonamide **9** was synthesized as yellowish oil (1.44 g, 77% yield).

Sulfonamides **10**–**12** were also synthesized by this procedure with yields of 75%, 73%, and 78%, respectively.

*N-(2,3-Dimethoxyphenethyl)methanesulfonamide (**9**)*

Yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (t, 1H, Ar–H, $J = 7.9$ Hz), 6.84 (dd, 1H, Ar–H, $J = 1.3$ and 8.2 Hz), 6.78 (dd, 1H, Ar–H, $J = 1.3$ and 7.6 Hz), 4.68 (t, 1H, NH, $J = 5.2$ Hz), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.38 (q, 2H, CH_2–N, $J = 6.7$ Hz), 2.89 (t, 2H, CH_2, $J = 6.7$ Hz), 2.78 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.8 (C), 147.2 (C), 131.8 (C), 124.4 (CH), 122.4 (CH), 111.4 (CH), 60.6 (OCH_3), 55.7 (OCH_3), 44.0 (CH_3), 39.9 (CH_2–N), 30.9 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3287, 2952, 2924, 2854, 1738, 1717, 1619, 1586, 1479, 1465, 1362, 1315, 1268, 1219, 1186, 1150, 1080. Anal. calcd. for ($C_{11}H_{17}NO_4S$): C, 50.95; H, 6.61; N, 5.40; S 12.36. Found C, 50.96; H, 6.65; N, 5.38; S, 12.32.

*N-(3,5-Dimethoxyphenethyl)methanesulfonamide (**10**)*

White solid. m.p. 83–85°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (s, 3H, Ar–H), 4.35 (t, 1H, NH, $J = 5.5$ Hz), 3.78 (s, 6H, $2OCH_3$), 3.39 (q, 2H, CH_2–N, $J = 6.7$ Hz), 2.87 (s, 3H, CH_3), 2.82 (t, 2H, CH_2, $J = 6.7$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2 (2C), 140.1 (C), 106.9 (2CH), 98.7 (CH), 55.3 ($2OCH_3$), 44.2 (CH_3), 40.4 (CH_2–N), 36.7 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3478, 3289, 3053, 2931, 2845, 1736, 1597, 1547, 1463, 1431, 1342, 1314, 1265, 1206, 1149, 1071. Anal. calcd. for ($C_{11}H_{17}NO_4S$): C, 50.95; H, 6.61; N, 5.40; S 12.36. Found C, 50.93; H, 6.58; N, 5.46; S, 12.32.

*N-(3,4-Dimethoxyphenethyl)methanesulfonamide (**11**)*

White solid. m.p. 78–80°C. (lit. [47] m.p. 72°C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.81 (d, 1H, Ar–H, $J = 8.1$ Hz), 6.75–6.71 (m, 2H, Ar–H), 4.37 (t, 1H, NH, $J = 6.5$ Hz), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.37 (q, 2H, CH_2–N, $J = 6.7$ Hz), 2.83 (s, 3H, CH_3), 2.79 (t, 2H, CH_2, $J = 6.7$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.4 (C), 148.2 (C), 130.4 (C), 121.1 (CH), 112.2 (CH), 111.7 (CH), 56.18 (OCH_3), 56.16 (OCH_3), 44.8 (CH_3), 40.6 (CH_2–N), 36.3 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3564, 3280, 3008, 2935, 2838, 2283, 2052, 1731, 1608, 1592, 1517, 1465, 1420, 1317, 1263, 1237, 1193, 1147, 1077, 1026. Anal. calcd. for ($C_{11}H_{17}NO_4S$): C, 50.95; H, 6.61; N, 5.40; S 12.36. Found C, 50.88; H, 6.60; N, 5.45; S, 12.40.

*N-(2-Methoxyphenethyl)methanesulfonamide (**12**)*

Yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (dt, 1H, Ar–H, $J = 1.6$ and 7.7 Hz), 7.15 (dd, 1H, Ar–H, $J = 1.3$ and 7.3 Hz), 6.93–6.86 (m, 2H, Ar–H), 4.52 (bs, 1H, NH), 3.84 (s, 3H, OCH_3), 3.38 (q, 2H, CH_2–N, $J = 6.6$ Hz), 2.88 (t, 2H, CH_2, $J = 6.6$ Hz), 2.76 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7 (C), 131.1 (CH), 128.6 (CH), 126.5 (C), 121.1 (CH), 110.8 (CH), 55.6 (OCH_3), 43.6 (CH_3), 40.2 (CH_2–N), 31.3 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3565, 3291, 3010, 2935, 2838, 2637, 2483, 2293, 2051, 1905, 1601, 1588, 1495, 1465, 1439, 1410, 1317, 1244, 1198, 1149, 1120, 1076, 1053, 1034. Anal. calcd. for ($C_{10}H_{15}NO_3S$): C, 52.38; H, 6.59; N, 6.11; S 13.98. Found C, 52.41; H, 6.61; N, 6.08; S, 13.93.

Standard procedure for the synthesis of phenolic sulfonamides

A solution of sulfonamide **5** (1 g, 3,86 mmol) in CH_2Cl_2 (25 mL) was cooled to 0°C and then a solution of BBr_3 (7.36 g, 29.36 mmol) in CH_2Cl_2 (5 mL) was added dropwise under N_2(g) over 5 min. After the cold bath was removed, the mixture was stirred at RT under N_2(g) for 1 day. The mixture was added to ice-water (40 mL) and then the organic phase was extracted. The water phase was extracted with EtOAc (2×30 mL). The combined organic phases were dried over Na_2SO_4 and solvent was evaporated. The phenolic sulfonamide **13** (0.75 g, 84%) was obtained.

*N-(2,3-Dihydroxyphenethyl)methanesulfonamide (**13**)*

Brown oil. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.37 (bs, 1H, OH), 7.19 (bs, 1H, OH), 6.61 (dd, 1H, Ar–H, $J = 1.6$ and 7.6 Hz), 6.54–6.46 (m, 2H, Ar–H), 5.86 (bs, 1H, NH), 3.21 (q, 2H, CH_2–N, $J = 7.2$ Hz), 2.74 (t, 2H, CH_2, $J = 7.2$ Hz), 2.71 (s, 3H, CH_3). ^{13}C NMR (100 MHz, acetone-d_6) δ 145.4 (C), 144.4 (C), 126.3 (C), 122.6 (CH), 120.3 (CH), 114.5 (CH), 44.1 (CH_3), 39.8 (CH_2–N), 31.9 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3428, 3305, 3305, 3019, 2929, 2851, 1620, 1594, 1502, 1477, 1437, 1406, 1308, 1278, 1193, 1144, 1078. Anal. calcd. for ($C_9H_{13}NO_4S$): C, 46.74; H, 5.67; N, 6.06; S 13.86. Found C, 46.76; H, 5.71; N, 5.97; S, 13.91.

*N-(3,5-Dihydroxyphenethyl)methanesulfonamide (**14**)*

Standard procedure described above for **13** was applied to sulfonamide **10** to give **14** (81%). Brown solid. m.p. 84–86°C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.13 (bs, 2H, 2OH), 6.15 (d, 2H, Ar–H, $J = 2.1$ Hz), 6.13 (t, 1H, Ar–H, $J = 2.1$ Hz), 5.90 (t, 1H, NH, $J = 5.4$ Hz), 3.20 (q, 2H, CH_2–N, $J = 7.7$ Hz), 2.77 (s, 3H, CH_3), 2.62 (t, 2H, CH_2, $J = 7.7$ Hz). ^{13}C NMR (100 MHz, acetone-d_6) δ 159.3 (2C), 142.2 (C), 108.4 (2CH), 101.8 (CH), 45.3 (CH_3), 40.0 (CH_2–N), 37.2 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3261, 3014, 2957, 2924, 2851, 1711, 1602, 1456, 1362, 1307, 1145, 1076. Anal. calcd. for ($C_9H_{13}NO_4S$): C, 46.74; H, 5.67; N, 6.06; S 13.86. Found C, 46.70; H, 5.61; N, 6.10; S, 13.90.

*N-(3,4-Dihydroxyphenethyl)methanesulfonamide (**15**)*

Compound **15** was synthesized from **11** (77%). Brown solid. m.p. 88–90°C. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.64 (bs, 1H, OH), 7.60 (bs, 1H, OH), 6.62–6.60 (m, 2H, Ar–H), 6.45 (dd, 1H, Ar–H, $J = 1.9$ and 8.1 Hz), 5.79 (bs, 1H, NH), 3.14 (q, 2H, CH_2–N, $J = 7.4$ Hz), 2.71

(s, 3H, CH_3), 2.59 (t, 2H, CH_2, $J = 7.4$ Hz). ^{13}C NMR (100 MHz, acetone-d_6) δ 145.9 (C), 144.5 (C), 131.4 (C), 121.0 (CH), 116.8 (CH), 116.1 (CH), 45.7 (CH_3), 39.9 (CH_2–N), 36.7 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3522, 3299, 3053, 2926, 2851, 1725, 1626, 1605, 1527, 1443, 1362, 1309, 1265, 1191, 1144, 1075. Anal. calcd. for ($C_9H_{13}NO_4S$): C, 46.74; H, 5.67; N, 6.06; S 13.86. Found C, 46.75; H, 5.62; N, 6.01; S, 13.88.

*N-(2-Hydroxyphenethyl)methanesulfonamide (**16**)*

Compound **16** was synthesized from **12** (82%). Brown solid. m.p. 72–74°C. ^1H NMR (400 MHz, $CDCl_3$) δ 7.09–7.06 (m, 2H, Ar–H), 6.85–6.80 (m, 2H, Ar–H), 6.56 (bs, 1H, OH), 5.68 (t, 1H, NH, $J = 5.7$ Hz), 3.36 (q, 2H, CH_2–N, $J = 6.7$ Hz), 2.85 (t, 2H, CH_2, $J = 6.7$ Hz), 2.75 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.2 (C), 131.1 (CH), 128.3 (CH), 124.7 (C), 120.9 (CH), 115.7 (CH), 43.8 (CH_3), 39.8 (CH_2–N), 30.9 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3428, 3305, 3064, 3025, 2918, 2849, 1608, 1595, 1505, 1490, 1456, 1408, 1311, 1264, 1234, 1174, 1145, 1107, 1075. Anal. calcd. for ($C_9H_{13}NO_3S$): C, 50.21; H, 6.09; N, 6.51; S 14.90. Found C, 50.19; H, 6.06; N, 6.55; S, 14.89.

Biochemistry

General experimental procedures

Materials, methods, and instrumentations were previously reported [10, 18]. In the present study, the following experimental procedures were applied to evaluation of antioxidant and radical scavenging activities of phenolic sulfonamides **13–16**.

Total antioxidant activity – Ferric thiocyanate method

The antioxidant activity of phenolic sulfonamides **13–16** and standard antioxidants was determined according to the ferric thiocyanate method [74]. This method was described previously [57]. Peroxides are formed during the linoleic acid oxidation. These peroxides lead to oxidation of Fe^{2+} to Fe^{3+}. Cupric ions (Fe^{3+}) form a complex with SCN^-. The resulting complex has maximum absorbance at 500 nm. High absorbance indicates high linoleic acid emulsion oxidation. The percentage inhibition of lipid peroxidation in linoleic acid was calculated by the following equation:

$$\text{ILP}\,(\%) = 100 - \left(\frac{\lambda_C}{\lambda_S}\right) \times 100$$

where ILP is the inhibition of lipid peroxidation, λ_C is the absorbance of control reaction, and λ_S is the absorbance in the presence of phenolic sulfonamides **13–16** or standard compounds [75].

Reducing power abilities

Fe^{3+}–Fe^{2+} reducing power of phenolic sulfonamides **13–16** was measured according to the method of Gülçin [75]. The Fe^{3+}–Fe^{2+} reducing ability is spectrophotometrically measured by absorbance of Perl's Prussian blue complex at 700 nm. Cu^{2+} reducing power of phenolic sulfonamides **13–16** was determined according to the method of Apak et al. [76]. The second used reducing power assay is ferric reducing antioxidant power, which is based upon reduction of the Fe^{3+}–TPTZ complex under acidic conditions [77]. Increased absorbance of blue-colored ferrous form (Fe^{2+}–TPTZ complex) is spectrophotometrically measured at 593 nm [78].

Ferrous ion (Fe^{2+}) chelating activity

Ferrozine forms complexes with Fe^{2+}, but in presence of ion chelating agents, the complex formation is disrupted, resulting in a decrease in the red color of the complexes [79]. Fe^{2+} chelating activity of phenolic sulfonamides **13–16** and standard compounds was measured by inhibiting the formation of Fe^{2+}–ferrozine complex after treatment of test material with Fe^{2+}. Fe^{2+} chelating ability of phenolic sulfonamides **13–16** was determined by the absorbance of the Fe^{2+}–ferrozine complex at 562 nm [58].

Radical scavenging activities

The DPPH free radical scavenging activity of phenolic sulfonamides **13–16** was evaluated by the method of Blois [80]. The $ABTS^{\bullet+}$ radical scavenging activity of phenolic sulfonamides **13–16** was performed according to the method of Re et al. [81]. $ABTS^{\bullet+}$ has a characteristic absorbance at 734 nm. The DMPD radical scavenging ability of phenolic sulfonamides **13–16** was determined by the method of Gülçin [59]. This assay is based on the capacity of the extract to inhibit $DMPD^{\bullet+}$ cation radical formation. $O_2^{\bullet-}$ scavenging activity of phenolic sulfonamides **13–16** was determined by the riboflavin/methionine/illuminate assay. This assay is based on the capability of antioxidant compounds to inhibit the photochemical reduction of NBT [79]. The percentage of above-mentioned metal chelating and radical scavenging capabilities of phenolic sulfonamides **13–16** and standards was calculated using the following equation:

$$\text{Metal chelating or radical scavenging}\,(\%) = \left(1 - \frac{\lambda_C}{\lambda_S}\right) \times 100$$

where λ_C is the absorbance of control and λ_S is the absorbance in the presence of phenolic sulfonamides **13–16** or standard compounds [82].

Determination of anticholinesterase activity

The inhibitory effects of phenolic sulfonamides **13–16** on AChE activities were measured by slightly modifying the spectrophotometric method of Ellman et al. [65]. Acetylthiocholine iodide was used as substrate of the reaction. DTNB (5,5′-dithio-bis(2-nitrobenzoic)acid (product no: D8130-1G, Sigma–Aldrich)) was used for the measurement of the AChE activity. Briefly, 100 μL of Tris/HCl buffer (1 M, pH 8.0), 10 μL of sample solution dissolved in deionized water at different concentrations and 50 μL AChE (5.32×10^{-3} U) solution were mixed and incubated for 10 min at 25°C. Then 50 μL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 50 μL of acetylthiocholine iodide (10 mM, product no: 01480-1G, Sigma–Aldrich). The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide, at a wavelength of 412 nm [83].

In order to determine the effect of phenolic sulfonamides **13–16** on AChE, different phenolic sulfonamides **13–16** concentrations were added into the reaction medium. The enzyme activity was measured, and an experiment in the absence of drug was used as control. The IC_{50} values were obtained from activity (%) versus phenolic sulfonamides **13–16** concentration plots.

To determine the K_i constants in the media with phenolic sulfonamides **13–16** as inhibitor, the different substrate (ACh)

concentrations were used. Inhibitor solution was added into the reaction medium, resulting in three different fixed concentrations of inhibitors. Lineweaver–Burk graphs [84] were drawn using 1/*V* versus 1/[S] values. K_i constants were calculated from these graphs. Donepezil hydrochloride was used as a reference compound.

The authors have declared no conflict of interest.

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