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Full Paper

Antioxidant Activity, Acetylcholinesterase, and Carbonic Anhydrase Inhibitory Properties of Novel Ureas Derived from Phenethylamines

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A series of ureas derived from phenethylamines were synthesized and evaluated for human carbonic anhydrase (hCA) I and II, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzyme inhibitory activities and antioxidant properties. The ureas were synthesized from the reactions of substituted phenethylamines with *N*,*N*-dimethylcarbamoyl chloride; then, the synthesized compounds were converted to their corresponding phenolic derivatives via *O*-demethylation. hCA I and II were effectively inhibited by the newly synthesized compounds, with K_i values in the range of 0.307–0.432 nM for hCA I and 0.149–0.278 nM for hCA II. On the other hand, the K_i parameters of these compounds for AChE and BChE were determined in the range of 0.129–0.434 and 0.095–0.207 nM, respectively. Phenolic ureas also showed good antioxidant activities.

Keywords: Acetylcholinesterase / Butyrylcholinesterase / Carbonic anhydrase / Enzyme inhibition / Urea

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Introduction

Ureas and their analogs are very important compounds in organic chemistry because of their applications in agriculture, pharmaceutical chemistry, and medicinal chemistry. They have considerable biological activities such as agro-defensive, anticonvulsant, and anti-irritant properties [1, 2]. Many aromatic and heterocyclic urea derivatives have shown acceptable anticancer activity and it has been demonstrated that these compounds are tubulin ligands [3]. There are many drugs in the market including urea moieties. For instance, ritonavir (**1**) is an antiretroviral drug used in the medication of HIV infection and AIDS [4]. Urea drug cabergoline (**2**) [5] has beneficial uses in the administration of prolactinomas. Another urea drug pheneturide (**3**) is an anticonvulsant drug [6]. Phenethylurea (**4**) and substituted phenethylureas had been reported to be used in temporary alleviation of sickle cell anemia and other human sickling diseases [7]. In addition, it has also been reported that sulfamide analogs of compound **4** show carbonic anhydrase inhibitory properties [8] (Fig. 1). In our earlier studies, we also reported the synthesis and biological properties of ureas [9] and sulfamide derivatives [10, 11].

Carbonic anhydrases (CA; EC 4.2.1.1), a ubiquitous group of zinc-bound proteins (metalloenzymes), have long been

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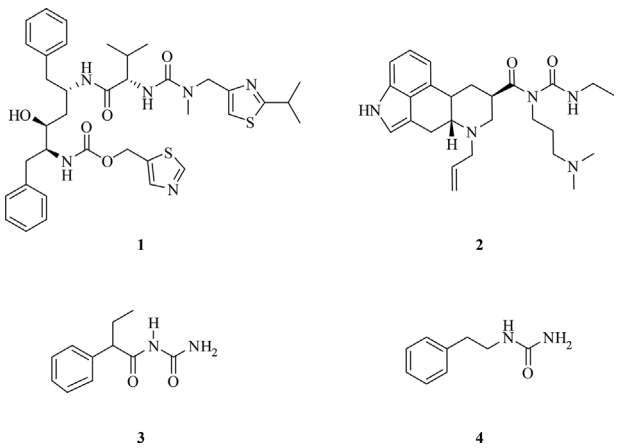


Figure 1. Some biologically active urea derivatives **1–4**.

known to catalyze the reversible hydration of carbon dioxide (CO_2) into bicarbonate (HCO_3^-) and a proton (H^+) at an extremely high rate and are involved in ion transport, as well as water and electrolyte balance in various tissues [12–14]. They are involved in many physiologic processes, among which pH buffering of extracellular and intracellular spaces [15, 16]. These enzymes were classified into several classes, which include six distinct genetic families, α-, β-, γ-, δ-, ζ-, and η-CAs [17, 18]. The family of human CAs (hCAs) comprises 16 different α-isoforms, of which several are cytosolic (CA I, II, III, VII, and XIII), five are membrane-bound isoforms (CA IV, IX, XII, XIV, and XV), two are mitochondrial (CA VA and VB), and one is secreted into saliva (CA VI). These enzymes are involved in various biochemical and metabolic processes, such as gluconeogenesis, lipogenesis, and ureagenesis [19–21]. Inhibition of CAs has pharmacologic applications in the field of antiglaucoma, anticonvulsant, anticancer, and anti-infective agents [22–24]. Because of this reason, extensive experimental studies of the physiologically important CA isoenzymes have been done to elucidate the fundamentals of its enzymatic actions. CA I and CA II are expressed mainly in erythrocytes and are widely distributed in the liver, pancreas, kidney, stomach, duodenum, and colon [25, 26].

Free radicals such as reactive oxygen species (ROS) are continually produced in our body as a by-product of many metabolic processes [27, 28]. In living organisms, various ROS can form in different ways. Normal aerobic respiration stimulates polymorphonuclear leucocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells [29, 30]. Under normal conditions, the body has its own antioxidant defense system comprising several antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase to detoxify these free radicals and ROS [31, 32]. They are also capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins, polyunsaturated fatty acids, and carbohydrates. Also, they may cause DNA damage that can lead to mutations [33]. Dietary antioxidants also play a crucial role in fighting these free radicals and ROS. However, there may be over-production of these free radicals leading to an imbalance between the generation and elimination of free radicals in the body [34, 35]. This situation is known as oxidative stress. This in turn results in oxidative damage to cellular components and biomolecules, thus marks the onset of many degenerative diseases related to aging such as cardiovascular disease, cancer, diabetes, and neurodegenerative diseases [36]. Since antioxidants are vital for their role to delay or inhibit oxidation of cellular components, adequate intake of these compounds in the diet will be beneficial to protect against oxidative damages to the cell. However, the use of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are still under evaluation in many countries due to their potential health hazard [37, 38]. Moreover, both synthetic antioxidant have been restricted by legislative rules due to doubts over their toxic and carcinogenic effects [39–41]. Therefore, there is a growing interest in natural and safer antioxidants for food applications, and a growing trend in consumer preferences [42, 43].

Increased oxidative stress and cholinergic dysfunction play an important role in the pathogenesis and progression of Alzheimer's disease (AD) [44]. Cholinesterases (ChE) are an enzyme family that catalyzes the hydrolysis of acetylcholine (ACh) or butrylcholine (BCh) into choline (Ch) and acetic acid or butyric acid, an essential process for the restoration of the cholinergic neurotransmission [45]. There are two cholinesterase types: acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8). AChE is mainly found at the neuromuscular junction and in animal synapses [46]. AChE is known to be abundant in the muscle, brain, and erythrocyte membrane, whereas BChE has a higher activity in liver, intestine, heart, kidney, and lung [47]. Both enzymes are responsible for the termination of cholinergic signaling by hydrolyzing ACh. Therefore, inhibition of both AChE and BChE and elimination of oxidative stress could be effective in the treatment and management of AD [48]. So, discovery of new inhibitors with more activity and less price is needed. So the search for novel compounds remains as an emerging demand for the treatment of AD [29].

It is well-known that there is a positive correlation between increased oxidative stress and cholinergic dysfunction. These both parameters play an important role in the pathogenesis and progression of Alzheimer's disease. Also, acetylcholinesterase [49] and carbonic anhydrase inhibitors [50] were used for treatment of glaucoma. Besides that phenolic compounds are well established CA isoenzymes, AChE, and BChE inhibition properties [51, 52].

As discussed above, urea and related compounds show beneficial biological activities, in the current study, we focused on the synthesis and biological screening of some novel ureas derived from phenethylamines. Therefore, urea derivatives **10–17** were synthesized and investigated for their

acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase inhibitory, and antioxidant properties.

Results and discussion

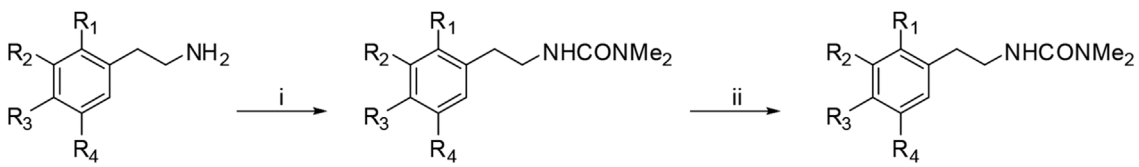
Chemistry

Urea derivatives of substituted phenethylamines can easily be prepared from the reactions of phenethylamines with *N*,*N*-dimethylcarbamoyl chloride (DMCC) [9]. In this context, phenethylamines **5–9** were reacted with DMCC in the presence of Et_3N at 0–25°C for 24 h to give ureas **10–14** in good yields. In addition, the synthesized ureas were converted to their phenolic derivatives. In the literature, conc. HBr and BBr_3 have been widely used in the synthesis of biologically active phenolic compounds via *O*-demethylation of arylmethyl ethers. From these studies, we very well know that HBr is the most effective reagent for *O*-demethylation of water soluble arylmethyl ethers [53, 54], while BBr_3 [55, 56] is the efficient one for *O*-demethylation of organic soluble arylmethyl ethers. Therefore, BBr_3 was chosen as a demethylation agent for the synthesis of the title phenolic compounds. Hence, novel phenolic ureas **15–17** were synthesized in moderate yields via *O*-demethylation of compounds **10–12** with BBr_3 at 0–25°C for 24 h (Scheme 1). Spectroscopic techniques such as ^1H, ^{13}C NMR, IR, and elemental analysis were used for the structural characterization of the novel compounds.

Biological assays

Antioxidant activity is reflected by the ability of pure compounds to inhibit the oxidation process [57]. As shown in Table 1, novel ureas **10–14** and phenolic derivatives **15–17** showed effective antioxidative potencies using different methods including ferric ions (Fe^{3+}), cupric ions (Cu^{2+}), and Fe^{3+}-TPTZ reducing powers, ferrous ions (Fe^{2+}) chelating ability, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities.

The reducing capabilities of bioactive compounds can be determined by means of the direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$ [58]. In this technique, the presence of reductants like novel ureas **10–14** and phenolic derivatives **15–17** would result in the reduction of Fe^{3+} to Fe^{2} [59]. As seen in Table 1, especially phenolic derivatives **15–17** demonstrated potent Fe^{3+} reducing capability and these diversities were statistically seen to be considerably important ($p < 0.01$). The reducing capacity of phenolic derivatives **15–17**, Trolox, and α-tocopherol increased constantly when the concentration of sample was increased. Reducing capacity of phenolic derivatives **15–17** and both standard compounds exposed the following order: Trolox (1.121 ± 0.001, r^2: 0.9016) > α-tocopherol (1.021 ± 0.001, r^2: 0.9748) > **16** (0.885 ± 0.002, r^2: 0.9684) ≥ **17** (0.868 ± 0.002, r^2: 0.9718) > **15** (0.524 ± 0.001, r^2: 0.9368) at the above-mentioned concentration. The results proved that phenolic derivatives **16,** which possess two hydroxyl groups (–OH) at the *ortho*-position had the best Fe^{3+} reducing ability in novel synthesized compounds (**10–17**). It was well-known that *ortho*-substitution of –OH group with electron-donating groups like methoxy groups ($–OCH_3$) can also increase the antioxidant activity [60, 61]. The CUPRAC method is a rapid, simple, selective, cost-effective, steady, and versatile antioxidant assay useful for a wide variety of phenolic compounds [62]. Cupric ions (Cu^{2+}) reducing power of 20 μg/mL concentration of novel ureas **10–14** and phenolic derivatives **15–17,** Trolox and α-tocopherol is shown in Table 1. It was detected that Cu^{2+} reducing capacity of novel ureas **10–14** and phenolic derivatives **15–17** was dependent on the concentration (10–30 μg/mL). Cu^{2+} reducing capability of phenolic derivatives **15–17** and both standard reducing agents at the same concentration (20 μg/mL) were given as the following order: α-tocopherol (1.335 ± 0.004, r^2: 0.9185) > Trolox (1.323 ± 0.003, r^2: 0.9935) > **16** (1.215 ± 0.003, r^2: 0.9459) > **17** (0.707 ± 0.003, r^2: 0.9362) > **15** (0.376 ± 0.003, r^2: 0.9448). Additionally, the FRAP assay is another frequently used reducing ability measurements method. According to results obtained from FRAP assay (Table 1), FRAP reducing power of phenolic derivatives **15–17** and standard compounds decreased in the following order: Trolox (1.825 ± 0.003, r^2: 0.9801) > α-tocopherol (1.644 ± 0.003, r^2: 0.9837) > **16** (1.533 ± 0.002, r^2: 0.9147) ≥ **17** (0.915 ± 0.001, r^2: 0.9862) > **15** (0.724 ± 0.001, r^2: 0.9895). In this method, higher



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

10 R_1=R_2=R_4=H, R_3=OMe, 77%
11 R_1=R_4=H, R_2=R_3=OMe, 74%
12 R_2=R_4=H, R_1=R_3=OMe, 80%
13 R_3=R_4=H, R_1=R_2=OMe, 60%
14 R_1=H, R_2=R_3=R_4=OMe, 67%

15 R_1=R_2=R_4=H, R_3=OH, 73%
16 R_1=R_4=H, R_2=R_3=OH, 69%
17 R_2=R_4=H, R_1=R_3=OH, 59%

Scheme 1. The synthesis of ureas and their phenolic derivatives. Reaction conditions: (i) NEt_3, CH_2Cl_2, 0°C, 30 min; then DMCC, 25°C, 24 h. (ii) BBr_3, CH_2Cl_2, 0°C, 1 h; then 25°C, 23 h.

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Table 1. Determination of reducing power of same concentration (20 μg/mL) of ureas 10–14 and phenolic derivatives 15–17 by FRAP methods, ferric ions (Fe^{3+}) reducing, and cupric ions (Cu^{2+}) reducing capacity by Cuprac method.

Antioxidants	Fe^{3+}-Fe^{2+}reducing		Cu^{2+}-Cu^{+} reducing		Fe^{3+}-TPTZ reducing	
	λ_{700}*	r^2	λ_{450}*	r^2	λ_{593}*	r^2
α-Tocopherol	1.021 ± 0.001	0.9748	1.335 ± 0.004	0.9185	1.644 ± 0.003	0.9837
Trolox	1.121 ± 0.001	0.9016	1.323 ± 0.003	0.9935	1.825 ± 0.003	0.9801
10	0.122 ± 0.002	0.9338	0.149 ± 0.003	0.9073	0.433 ± 0.002	0.9816
11	0.116 ± 0.001	0.9528	0.103 ± 0.002	0.9256	0.426 ± 0.001	0.9982
12	0.135 ± 0.002	0.9491	0.144 ± 0.002	0.9753	0.435 ± 0.002	0.9851
13	0.134 ± 0.001	0.9472	0.203 ± 0.006	0.9058	0.451 ± 0.001	0.9078
14	0.122 ± 0.003	0.9948	0.117 ± 0.004	0.9378	0.354 ± 0.004	0.9753
15	0.524 ± 0.001	0.9368	0.376 ± 0.003	0.9448	0.724 ± 0.001	0.9895
16	0.885 ± 0.002	0.9684	1.215 ± 0.003	0.9459	1.533 ± 0.002	0.9147
17	0.868 ± 0.002	0.9718	0.707 ± 0.003	0.9362	0.915 ± 0.001	0.9862

*Expressed as absorbance values.

absorbance values indicate higher reducing ability of the Fe^{3+}-TPTZ complex. In all reducing ability methods, phenolic derivatives **15–17** and both standards demonstrated same order.

In the presence of metal chelating compounds like Fe^{2+}, ferrozine–metal complex formation is broken down, resulting in a decrease in the red color of the Ferrozine-Fe^{2+} complex [63]. The data summarized in Table 2 display that taxifolin has a strong capability to bind Fe^{2+}. It is assumed that its main action as a peroxidation inhibitor may be involved in its Fe^{2+} linking capacity. It was reported that the molecules with structures including functional groups like –C–OH and –C=O can bind Fe^{2+} ions. Also, the compounds containing two or more of the following functional groups: –S–, –OH, –O–, –SH, $–NR_2$, C=O, –COOH, and $–H_2PO_3$ in a favor of structure-function configuration [64–66]. In this way, phenolic derivatives **15–17** can easily chelate Fe^{2+} ions. In this regard, phenolic derivatives **15–17** had also effective metal ions chelating effect. The distinction between phenolic derivatives **15–17** at different concentrations (10–30 μg/mL) and control value was fixed to be statistically important ($p < 0.01$, Table 2). IC_{50} values of phenolic derivatives **15–17** and standard compounds decreased in the following order: EDTA (11.18 μg/mL, r^2: 0.9506) > Trolox (18.73 μg/mL, r^2: 0.9036) > α-tocopherol (23.10 μg/mL, r^2: 0.9998) > **16** (24.75 μg/mL, r^2: 0.9326) ≥ **17** (68.28 μg/mL, r^2: 0.9646) > **15** (86.63 μg/mL, r^2: 0.9752). The most metal chelating effect was observed in phenolic derivative **16**, which contains two phenolic hydroxyl groups in the *ortho*-position, with K_i value of 24.75 μg/mL. Diphenolic structure in the *ortho*-position is generally favored of metal chelating configuration [67, 68].

$DPPH^{\bullet}$ become a steadier diamagnetic molecule after acceptation of an electron or hydrogen radical from an antioxidant compound [69]. DPPH radicals scavenging of phenolic derivatives **15–17** are given in Table 2. It is well-known that a radical can be stabilizing by the agency of resonance structure of the phenolic compounds. Half maximal concentrations (IC_{50}) values of phenolic derivatives **15–17** and standard compounds decreased in the following order: Trolox (12.83 μg/mL, r^2: 0.9609) > α-tocopherol (17.77 μg/mL, r^2: 0.9972) > **16** (24.75 μg/mL, r^2: 0.9177) ≥ **17** (57.01 μg/mL, r^2:

Table 2. Determination of half maximal concentrations (IC_{50}) of novel ureas 10–14, phenolic derivatives 15–17 and standards for $DPPH^{\bullet}$, $ABTS^{\bullet+}$ radical scavenging, and Fe^{2+} chelating activities.

Antioxidant compounds	$DPPH^{\bullet}$ scavenging	r^2	$ABTS^{\bullet+}$ scavenging	r^2	Metal chelating	r^2
α-Tocopherol	17.77	0.9972	5.58	0.9823	23.10	0.9998
Trolox	12.83	0.9609	3.36	0.9035	18.73	0.9036
10	225.1	0.9963	169.6	0.9903	69.31	0.9047
11	229.7	0.9354	170.3	0.9821	76.02	0.9588
12	231.5	0.9770	138.6	0.9785	36.47	0.9655
13	230.7	0.9836	172.4	0.9433	30.13	0.9101
14	221.1	0.9494	207.5	0.9241	15.75	0.9881
15	97.30	0.9822	91.50	0.9638	86.63	0.9752
16	24.75	0.9177	9.36	0.9576	24.75	0.9326
17	57.01	0.9148	21.87	0.9596	68.28	0.9646
EDTA	–	–	–	–	11.18	0.9506

0.9148) > **15** (97.30 μg/mL, r^2: 0.9822). Phenolic derivatives **16**, which possess two phenolic hydroxyl groups at the *ortho*-position demonstrated the most powerful DPPH• scavenging effect with IC_{50} value of 24.75 μg/mL in the used phenolic derivatives **15–17.** Previously, the role of –OH groups at the *ortho*-position in the antioxidant activity of phenolic acids has also been described [70]. The extra –OH group in phenolic compound provides greater stability along with higher antioxidant activity [71].

Another improved technique for determination of radical scavenging activity of pure compounds is $ABTS^{\bullet+}$ scavenging activity [72]. The generation of $ABTS^{\bullet+}$ defined here includes the direct production of the blue/green $ABTS^{\bullet+}$ chromophore due to the reaction between ABTS and oxidant molecule like potassium persulphate ($K_2S_2O_8$). ABTS radicals were generated in ABTS/$K_2S_2O_8$ system [73]. As shown in Table 2, phenolic derivatives **15–17** were efficient $ABTS^{\bullet+}$ scavengers in a concentration-dependent manner (10–30 μg/mL). The IC_{50} value for phenolic derivatives **15–17** in this analysis was 91.50 μg/mL (r^2: 0.9638) for **15**, 9.36 μg/mL (r^2: 0.9576) for **16**, and 21.87 μg/mL (r^2: 0.9596) for **17**. Moreover, IC_{50} values for Trolox and α-tocopherol were found to be 3.36 μg/mL (r^2: 0.9035), and 5.58 μg/mL (r^2: 0.9823), respectively. $ABTS^{\bullet+}$ scavenging efficacy of phenolic derivatives **15–17** and both standards increased in the following order: Trolox ≥ α-tocopherol > **16** > **17** > **15** (Table 2). As well as in DPPH• scavenging activity, a lower IC_{50} value indicates a higher $ABTS^{\bullet+}$ scavenging activity.

As shown in Table 3, K_i values were calculated for novel ureas **10–14** and phenolic derivatives **15–17** against both CA isoenzymes. Cytosolic hCA I is expressed in the body and can be found in high concentrations in the blood and gastrointestinal tract [74]. Novel ureas **10–14** and phenolic derivatives **15–17** had low nanomolar K_i values in ranging of 0.307–0.432 nM against the cytosolic isoenzyme hCA I, (Table 3). Phenolic derivatives **15**, which possess a phenolic hydroxyl group demonstrated the most powerful CA I inhibitory profile with K_i value of 0.307 ± 0.063 nM. Phenolic compounds are slightly acidic and have weak tendencies to lose the proton (H^+) ion from the hydroxyl group (–OH), resulting in the highly water-soluble phenolate anion. Phenols effectively inhibit CA isoenzymes [75]. Phenolics are not active antioxidants unless substitution at either the *ortho*- or *para*-positions has increased the electron density at the hydroxyl group and lowered the oxygen–hydrogen bond energy, in effect increasing the reactivity toward the lipid free radicals [76]. On the other hand, AZA, which is extensively used as positive control for both CA isoenzymes, demonstrated K_i value of 143.95 ± 18.11 nM.

The physiologically predominant hCA II plays very fundamental roles in human physiology and pathology. It is essential in keeping the adequate balance between CO_2 and HCO_3^- and thus controlling the pH level in cells. Mutations of hCA II have been found to cause CA deficiency syndrome leading to diseases such as osteoporosis, renal tubular acidosis [77], and cerebral calcification. On the other hand, overexpression of hCA II was found to favor glaucoma [78]. As novel inhibitors of hCA II, ureas **10–14** and phenolic derivatives **15–17** were found to bind hCA II with an inhibition constant in the range of 0.149 ± 0.023 to 0.278 ± 0.056 nM. In contrast, AZA, a well-known example of a clinically established CA inhibitor, showed a K_i value of 49.60 ± 1.52 nM against cytosolic hCA II. It is well-known that the inhibition of CA II is brought about by an inhibitor's ability to bind to the catalytic Zn^{2+} in the CA active site and mimic the tetrahedral transition state [79–81].

The compounds possessing AChE inhibitory effects are used for the treatment of AD. However, these drugs have many undesired side effects. We also determined the effect of novel ureas **10–14** and phenolic derivatives **15–17** against AChE that catalyses the breakdown of acetylcholine and of some other choline esters that function as neurotransmitters. AChE was very strongly inhibited by novel ureas **10–14** and phenolic derivatives **15–17** (Table 3). These new compounds had K_i

Table 3. The inhibition profiles of novel ureas 10–14 and phenolic derivatives 15–17 on human carbonic anhydrase isoenzymes (hCA I and II), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes.

Compounds	IC_{50} (nM)								K_i (nM)			
	hCA I	r^2	hCA II	r^2	AChE	r^2	BChE	r^2	hCA I	hCA II	AChE	BChE
10	0.427	0.9836	0.238	0.9981	0.685	0.9858	0.531	0.9344	0.330 ± 0.093	0.238 ± 0.061	0.434 ± 0.013	0.207 ± 0.051
11	0.440	0.9663	0.251	0.9916	0.267	0.9547	0.343	0.9471	0.432 ± 0.161	0.219 ± 0.025	0.129 ± 0.049	0.171 ± 0.055
12	0.307	0.9711	0.249	0.9874	0.277	0.9375	0.291	0.9901	0.308 ± 0.066	0.223 ± 0.067	0.195 ± 0.090	0.137 ± 0.050
13	0.355	0.9715	0.295	0.9845	0.332	0.9814	0.332	0.9673	0.353 ± 0.090	0.278 ± 0.056	0.214 ± 0.037	0.095 ± 0.030
14	0.385	0.9786	0.232	0.9877	0.433	0.9648	0.377	0.9793	0.315 ± 0.043	0.178 ± 0. 037	0.162 ± 0.025	0.124 ± 0.023
15	0.374	0.9903	0.242	0.9944	0.590	0.9915	0.577	0.9382	0.307 ± 0.063	0.149 ± 0.023	0.399 ± 0.084	0.158 ± 0.073
16	0.423	0.9885	0.226	0.9945	0.348	0.9726	0.321	0.9470	0.404 ± 0.090	0.174 ± 0.011	0.188 ± 0.037	0.119 ± 0.017
17	0.412	0.9908	0.241	0.9903	0.396	0.9941	0.338	0.9677	0.371 ± 0.089	0.215 ± 0.049	0.219 ± 0.016	0.157 ± 0.074
AZA[a)]	163.80	9710	59.42	0.9799	–	–			143.95 ± 18.11	49.60 ± 1.52	–	–
TAC[b)]	–	–	–	–	80.83	0.9248	75.52	0.9779	–	–	46.41 ± 2.36	394.01 ± 0.176

[a)] Acetazolamide (AZA) was used as a standard inhibitor for both carbonic anhydrase I and II isoenzymes (hCA I and II).
[b)] Tacrine (TAC) was used as a standard inhibitor for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes.

value ranging from 0.129 ± 0.049 to 0.434 ± 0.013 nM. On the other hand, tacrine, which is used for the treatment of mild-to-moderate AD and various other memory impairments, had been shown to lower AChE inhibitory activity (K_i: 46.41 ± 2.36 nM). It is used for reducing of dementia symptoms in patients with Alzheimer disease [81]. BChE is a nonspecific ChE enzyme that hydrolyses many different choline-based esters. In humans, it is encoded by the BChE gene, occurred in the liver, and found mainly in blood plasma [82, 83]. Finally, BChE was very effectively inhibited by novel ureas **10–14** and phenolic derivatives **15–17**. It was found that K_i values were in the range of 0.095 ± 0.030 to 0.207 ± 0.051 nM for BChE (Table 3). On the other hand, tacrine (TAC), used as standard inhibitor, had K_i value of 394.01 ± 0.176 nM.

Conclusion

In conclusion, we synthesized a series of ureas **10–14** starting from phenethylamines. Some of the synthesized ureas were converted to their phenolic derivatives **15–17**. All synthesized compounds were investigated for their biological activities such as AChE, BChE, hCA I and hCA II inhibitory and antioxidant properties. Especially, phenolic derivatives **15–17** were found to be powerful antioxidant and antiradical compounds in different antioxidant assays when compared to standard antioxidant compounds. Also, they demonstrated unique inhibition profiles against hCA I and II isoforms, AChE and BChE enzymes. The inhibition profiles of hCA I and hCA II demonstrated that in the light of the high homology between these two CAs, they exhibit similar activity. Additionally, novel ureas **10–14** and phenolic derivatives **15–17** had effective AChE and BChE inhibitory properties. Therefore, the synthesized compounds described here can be good candidates for the treatment of mild-to-moderate AD and various other memory impairments.

Experimental

Chemistry

General methods

All chemicals and solvents are commercially available and were used without purification or after distillation and treatment with drying agents. Melting points are uncorrected and they were determined on a capillary melting apparatus (Buechi 530). IR spectra were obtained from solutions in 0.1 mm cells with a PerkinElmer 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. All biologically evaluated compounds were demonstrated to exist in >95% purity by elemental analysis.

The H NMR and C NMR spectra as well as the InChI codes of the investigated compounds are provided as Supporting Information.

*Synthesis of 3-(4-methoxyphenethyl)-1,1-dimethylurea (**10**)*

3-(4-Methoxyphenethyl)-1,1-dimethylurea **10** [84] was synthesized as described previously.

*General procedure for the synthesis of ureas: 3-(3,4-dimethoxyphenethyl)-1,1-dimethylurea (**11**)*

3,4-Dimethoxyphenethylamine **5** (1.00 g, 5.52 mmol) was dissolved in CH_2Cl_2 (15 mL). NEt_3 (0.93 mL, 6.62 mmol) was added to this solution and it was stirred in a salt-ice bath for 30 min. Then, *N*,*N*-dimethyl carbamoylchloride (0.41 mL, 5.52 mmol) was added to this mixture and stirred for 24 h at room temperature. At the end of the reaction time, a solution of 0.1 N HCl (40 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (2×30 mL). Combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by column chromatography (hexane/EtOAc, 1:1) to give 3-(3,4-dimethoxyphenethyl)-1,1-dimethylurea **11** (1.03 g, 74%) as a white solid. M.p. 89–91°C. IR (CH_2Cl_2, cm^{-1}): 3340; 2939; 1682; 1583; 1357; 1160; 1039. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.81–6.77 (m, 1H, ArH), 6.74–6.70 (m, 2H, ArH), 4.38 (bs, 1H, NH), 3.85 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.47–3.42 (m, 2H, CH_2), 2.84 (s, 6H, $2CH_3$), 2.77 (t, 2H, CH_2, $J = 6.9$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.6 (CO), 149.2 (C), 147.7 (C), 132.2 (C), 120.9 (CH), 112.2 (CH), 111.5 (CH), 56.12 (OCH_3), 56.05 (OCH_3), 42.5 (CH_2), 36.34 (CH_2), 36.30 ($2CH_3$). Anal. calcd. for ($C_{13}H_{20}N_2O_3$): C, 61.88; H, 7.99; N, 11.10. Found: C, 61.90; H, 7.97; N, 11.12.

*3-(2,4-Dimethoxyphenethyl)-1,1-dimethylurea (**12**)*

3-(2,4-Dimethoxyphenethyl)-1,1-dimethylurea **12** was synthesized with a yield of 80% (0.84 g) according to the general procedure described for **11**. White solid. M.p. 110–112°C. IR (CH_2Cl_2, cm^{-1}): 3341; 2934; 1678; 1588; 1364; 1159; 1045. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.03 (d, 1H, ArH, $J = 8.0$ Hz), 6.44–6.43 (m, 1H, ArH), 6.41 (d, 1H, ArH, $J = 2.4$ Hz), 4.61 (bs, 1H, NH), 3.79 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.41–3.37 (m, 2H, CH_2), 2.83 (s, 6H, $2CH_3$), 2.75 (t, 2H, CH_2, $J = 6.6$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.8 (C), 158.8 (CO), 158.5 (C), 131.1 (CH), 120.5 (C), 104.3 (CH), 98.8 (CH), 55.6 (OCH_3), 55.5 (OCH_3), 41.8 (CH_2), 36.3 (CH_2), 30.2 ($2CH_3$). Anal. calcd. for ($C_{13}H_{20}N_2O_3$): C, 61.88; H, 7.99; N, 11.10. Found: C, 61.85; H, 7.98; N, 11.09.

*3-(2,3-Dimethoxyphenethyl)-1,1-dimethylurea (**13**)*

3-(2,3-Dimethoxyphenethyl)-1,1-dimethylurea **13** was synthesized with a yield of 60% (0.63 g) according to the general procedure described for **11.** Pale yellow solid. M.p. 105–107°C. IR (CH_2Cl_2, cm^{-1}): 3350; 2941; 1676; 1568; 1355; 1167; 1051. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00 (t, $J = 7.9$ Hz, 1H, ArH),

6.83–6.75 (m, 2H, ArH), 4.84 (bs, 1H, NH), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.46–3.42 (m, 2H, CH_2), 2.85–2.82 (m, 8H, CH_2, and $2CH_3$). APT ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.8 (CO), 152.9 (C), 147.2 (C), 133.7 (C), 124.4 (CH), 122.6 (CH), 110.9 (CH), 60.81 (OCH_3), 60.79 (OCH_3), 42.3 (CH_2), 36.2 ($2CH_3$), 30.6 (CH_2). Anal. calcd. for ($C_{13}H_{20}N_2O_3$): C, 61.88; H, 7.99; N, 11.10. Found: C, 61.87; H, 7.96; N, 11.13.

*3-(3,4,5-Trimethoxyphenethyl)-1,1-dimethylurea (**14**)*
3-(3,4,5-Trimethoxyphenethyl)-1,1-dimethylurea **14** was synthesized with a yield of 67% (0.67 g) according to the general procedure described for **11**. Pale brown solid. M.p. 120–122°C. IR (CH_2Cl_2, cm^{-1}): 3360; 2929; 1739; 1465; 1339; 1113; 1025. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.34 (s, 2H, ArH), 4.57 (t, 1H, NH, $J = 5.3$ Hz), 3.75 (s, 6H, OCH_3), 3.73 (s, 3H, OCH_3), 3.39–3.34 (m, 2H, CH_2), 2.79 (s, 6H, $2CH_3$), 2.68 (t, 2H, CH_2, $J = 7.1$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.6 (CO), 153.4 (2C), 136.4 (C), 135.5 (C), 105.8 (2CH), 61.0 (OCH_3), 56.2 ($2OCH_3$), 42.4 (CH_2), 37.2 ($2CH_3$), 36.3 (CH_2). Anal. calcd. for ($C_{14}H_{22}N_2O_4$): C, 59.56; H, 7.85; N, 9.92. Found: C, 59.58; H, 7.86; N, 9.90.

*General procedure for the synthesis of phenolic ureas: 3-(4-hydroxyphenethyl)-1,1-dimethylurea (**15**)*
3-(4-Methoxyphenethyl)-1,1-dimethylurea **10** (0.50 g, 2.25 mmol) was dissolved in CH_2Cl_2 (15 mL) in a salt-ice bath. BBr_3 (0.85 mL, 9.00 mmol) was added to this solution under N_2 gas and stirred in a salt-ice bath for 1 h. After this time, the reaction mixture was allowed to warm up to room temperature and stirred for an additional 23 h. At the end of this time, the reaction mixture was quenched with ice (20 g) and extracted with CH_2Cl_2 (2×20 mL). Organic phase was separated and the aqueous phase was extracted with EtOAc (3×10 mL). Combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. 3-(4-Hydroxyphenethyl)-1,1-dimethylurea **15** was synthesized with a yield of 73% (0.34 g). White solid. M.p. 175–176°C. IR (CH_2Cl_2, cm^{-1}): 3629; 3355; 2873; 1788; 1574; 1392; 1122; 1034. ^{1}H NMR (400 MHz, CD_3OD): δ 7.01 (d, 2H, ArH, $J = 8.5$ Hz), 6.69 (d, 2H, ArH, $J = 8.5$ Hz), 4.88 (bs, 1H, OH, and 1H, NH, overlapping with OH coming from CD_3OD), 3.34–3.26 (m, 2H, CH_2), 2.86 (s, 6H, $2CH_3$), 2.67 (t, 2H, CH_2, $J = 7.2$ Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 159.9 (CO), 155.6 (C), 130.5 (C), 129.6 (2CH), 115.0 (2CH), 42.7 (CH_2), 35.6 ($2CH_3$), 35.2 (CH_2). Anal. calcd. for ($C_{11}H_{16}N_2O_2$): C, 63.44; H, 7.74; N, 13.45. Found: C, 63.45; H, 7.73; N, 13.48.

*3-(3,4-Dihydroxyphenethyl)-1,1-dimethylurea (**16**)*
Phenolic urea **16** was synthesized with a yield of 69% (0.29 g) according to the same procedure given for **15**. Brown solid. M.p. 173–174°C. IR (CH_2Cl_2, cm^{-1}): 3645; 3591; 3314; 2873; 1781; 1599; 1383; 1105. ^{1}H NMR (400 MHz, CD_3OD): δ 6.67 (d, 1H, ArH, $J = 8.0$ Hz), 6.64 (d, 1H, ArH, $J = 2.0$ Hz), 6.51 (dd, 1H, ArH, $J = 8.0$, 2.0 Hz), 4.88 (bs, 2H, OH, and 1H, NH, overlapping with OH coming from CD_3OD), 3.29–3.25 (m, 2H, CH_2), 2.85 (s, 6H, $2CH_3$), 2.61 (t, 2H, CH_2, $J = 7.1$ Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 159.9 (CO), 145.0 (C), 143.4 (C), 131.3 (C), 119.9 (CH), 115.8 (CH), 115.1 (CH), 42.6 (CH_2), 35.8 ($2CH_3$), 35.2 (CH_2). Anal. calcd. for ($C_{11}H_{16}N_2O_3$): C, 58.91; H, 7.19; N, 12.49. Found: C, 58.89; H, 7.21; N, 12.53.

*3-(2,4-Dihydroxyphenethyl)-1,1-dimethylurea (**17**)*
Phenolic urea **17** was synthesized with a yield of 59% (0.26 g) according to the same procedure given for **15**. Pale yellow solid. M.p. 180–181°C. IR (CH_2Cl_2, cm^{-1}): 3614; 3573; 3321; 2832; 1699; 1515; 1322; 1119. ^{1}H NMR (400 MHz, CD_3OD): δ 6.88 (d, 1H, ArH, $J = 4.1$ Hz), 6.31 (bs, 1H, ArH), 6.25 (d, 1H, ArH, $J = 8.2$ Hz), 4.88 (bs, 2H, OH and 1H, NH, overlapping with OH coming from CD_3OD), 3.36–3.29 (m, 2H, CH_2), 2.87 (s, 6H, $2CH_3$), 2.71 (t, 2H, CH_2, $J = 7.0$ Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 161.3 (C), 157.8 (CO), 157.2 (C), 132.0 (CH), 118.5 (CH), 107.6 (C), 103.5 (CH), 43.1 (CH_2), 36.4 ($2CH_3$), 31.2 (CH_2). Anal. calcd. for ($C_{11}H_{16}N_2O_3$): C, 58.91; H, 7.19; N, 12.49. Found: C, 58.93; H, 7.20; N, 12.51.

Biochemical studies

Antioxidant studies
For determination of reducing ability of novel ureas **10–14** and phenolic derivatives **15–17,** $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$ reduction method was used [85]. In brief, different concentrations of novel ureas **10–14** and phenolic derivatives **15–17** (10–30 μg/mL) in 0.75 mL of deionized H_2O were added with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of $K_3Fe(CN)_6$ (1%). Then, the solution was incubated at 50°C during 20 min. After this period, trichloroacetic acid (TCA) was added (1.25 mL, 10%) to solution. Lastly, a portion of $FeCl_3$ (0.5 mL, 0.1%) was transferred to this mixture and the absorbance value was enrolled at 700 nm in a spectrophotometer. According to the obtained results, when reduction capability increases, absorbance indicates greater value [86, 87].

Cupric ions (Cu^{2+}) reducing power was used as a second reducing ability method for novel ureas **10–14** and phenolic derivatives **15–17**. Cu^{2+} reducing capability was performed according to the method of Apak et al. [88] with slight modification [89]. For this purpose, aliquots of $CuCl_2$ solution (0.25 mL, 0.01 M), ethanolic neocuproine solution (0.25 mL, 7.5×10^{-3} M), and NH_4Ac buffer solution (0.25 mL, 1.0 M) were transferred to a test tube, which contains novel ureas **10–14** and phenolic derivatives **15–17** at different concentrations (10–30 μg/mL). Total volume was completed with distilled H_2O to 2 mL and shaken vigorously. Absorbance of samples was recorded at 450 nm after 30 min.

FRAP assay is based upon reduction of Fe^{3+}-TPTZ complex under acidic medium and conditions. Increased absorbance of blue-colored ferrous form (Fe^{2+}-TPTZ complex) is recorded at 593 nm [90]. TPTZ solution (2.25 mL, 10 mM TPTZ in 40 mM HCl) was freshly prepared, then transferred to acetate buffer (25 mL, 0.3 M, pH 3.6), and $FeCl_3$ solution (2.25 mL, 20 mM) in water. Then, different concentrations of novel ureas **10–14** and phenolic derivatives **15–17** (10–30 μg/mL) were dissolved in 5 mL of appropriate buffer solvent, stirred, and incubated at 37°C for 30 min. Finally, the absorbance of mixture was measured at 593 nm.

Fe^{2+} chelating ability of novel ureas **10–14** and phenolic derivatives **15–17** was performed according to Dinis et al. [91] with slight modification [92]. Fe^{2+}-binding capacity of novel ureas **10–14** and phenolic derivatives **15–17** was spectrophotometrically recorded at 562 nm. In brief, to a mixture of $FeCl_2$ (0.1 mL, 0.6 mM) novel ureas **10–14** and phenolic derivatives **15–17** were added at three different concentrations (10–20 μg/mL) in methanol (0.4 mL). The reactions were started by pipyrdyl solution addition (0.1 mL, 5 mM). After that, the solution was mixed and incubated at room temperature for 10 min. Finally, absorbance value of the mixture was quantified spectrophotometrically at 562 nm versus blank sample.

$DPPH^{\bullet}$ scavenging activity of novel ureas **10–14** and phenolic derivatives **15–17** was performed according to the previously described method [93]. The solution of $DPPH^{\bullet}$ was daily prepared, stored in a flask coated with aluminum foil and kept in the dark at 4°C. In brief, fresh solution of $DPPH^{\bullet}$ (0.1 mM) was prepared in ethanol. Then, 1.5 mL of each novel ureas **10–14** and phenolic derivatives **15–17** in ethanol was added an aliquot (0.5 mL) of this solution (10–30 μg/mL). These mixtures were mixed vigorously and incubated in the dark for 30 min. Finally the absorbance value was recorded at 517 nm in a spectrophotometer [94].

ABTS radical scavenging activity of novel ureas **10–14** and phenolic derivatives **15–17** was performed using the spectroscopic method described by Re et al. [95]. The ABTS radical cation ($ABTS^{\bullet+}$) was acquired by reacting 7 mM solution of ABTS with 2.45 mM $K_2S_2O_8$. Prior to assay, the ABTS radical cation solution was diluted with ethyl alcohol to an absorbance of 0.750 ± 0.05 at 734 nm. Then, 1 mL of $ABTS^{\bullet+}$ solution was supplement to 3 mL of each novel ureas **10–14** and phenolic derivatives **15–17** and control solutions. The extent of decolorization is calculated as percentage reduction of absorbance.

Percentage of metal chelating, and radicals scavenging of each novel ureas **10–14** and phenolic derivatives **15–17** was computed using the following equation:

$$\mathrm{RSE}(\%) = [1 - (A_S/A_C)] \times 100$$

where RSE is radical scavenging effects, A_C is the absorbance value of the control and A_S is the absorbance value of the sample [96].

Enzymes studies

Inhibition effects of novel ureas **10–14** and phenolic derivatives **15–17** on AChE/BChE activities were measured according to Ellman's method [97]. AChI/BChI and DTNB were used for the determination of the AChE/BChE activities. Namely, 100 mL of buffer (Tris/HCl, 1 M, pH 8.0), 10 mL of sample solution dissolved in deionised water at different concentrations. Then, 50 mL AChE/BChE (5.3210^{-3} EU) solution was added and incubated for 10 min at 25°C. After incubation, a portion of DTNB (50 mL 0.5 mM) was added. Finally, the reaction was started by the addition of 50 mL of AChI/BChI. The enzymatic hydrolysis of these substrates was determined spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine at a wavelength of 412 nm. For determination of the inhibition effect of novel ureas **10–14** and phenolic derivatives **15–17** on AChE/BChE, different concentrations of these compounds were added to the reaction mixture. Then, AChE/BChE activities were measured. IC_{50} values were obtained from activity (%) versus compounds plots [98].

For determination of the effects of novel ureas **10–14** and phenolic derivatives **15–17** on CA isoenzymes, both isoenzymes were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography in a single purification step [99]. Sepharose-4B-L-tyrosine-sulfanilamide was prepared according to a reported method [100]. Thus, pH of the solution was adjusted to 8.7, using solid Tris. Then, supernatant was transferred to the previously prepared Sepharose-4B-L-tyrosine-sulphanilamide affinity column [101]. Subsequently, the proteins from the column were spectrophotometrically determined at 280 nm [102]. For determination of the purity of the hCA isoenzymes, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE), having 10 and 3% acrylamide as an eluent and packing gel, respectively, with 0.1% SDS [103] was performed, through which a single band was observed for each isoenzyme.

CA isoenzymes activities were determined following the methods described by Verpoorte et al. [104] and the methods reported previously [105]. Absorbance change at 348 nm from *p*-nitrophenylacetate (NPA) to *p*-nitrophenolate (NP) was recorded by 3 min intervals at the room temperature (25°C) using a spectrophotometer (Shimadzu, UV-VIS spectrophotometer, UVmini-1240, Kyoto, Japan). Quantity of the protein was measured spectrophotometrically at 595 nm during the purification steps according to the Bradford method [106] as reported previously [107]. Bovine serum albumin was used as a standard protein. An activity (%)-[novel ureas or phenolic derivatives] graph was depicted to determine the inhibitory effect of each novel ureas **10–14** and phenolic derivatives **15–17**. For K_i values, five different novel ureas **10–14** and three phenolic derivatives **15–17** were tested. NPA was used as a substrate at five different concentrations, and Lineweaver–Burk curves [108] were drawn as described previously [109].

The authors have declared no conflict of interest.

References

[1] S. Liu, C. Tang, B. Ho, M. Ankersen, C. E. Stidsen, A. M. Crider, *J. Med. Chem.* **1998**, *41*, 4693–4705.
[2] F. Bigi, E. Zambonin, *Chem. Commun.* **1998**, *4*, 513–514.
[3] J. Pochampally, A. Valeru, R. Macha, A. Kishorekumar, P. Tigulla, B. Gandu, A. Gangagnirao, *Der Pharma Chem.* **2014**, *6*, 269–282.
[4] R. K. Zeldin, R. A. Petruschke, *J. Antimicrob. Chemoth.* **2004**, *53*, 4–9.

[5] A. Wakil, A. S. Rigby, A. L. Clark, A. Kallvikbacka-Bennett, S. L. Atkin, *Eur. J. Endocrinol*. **2008**, *159*, R11–R14.
[6] B. Byrne, R. Rotchild, *Chirality* **1999**, *11*, 529–535.
[7] W. H. Waugh, *Brit. UK Pat. Appl*, **1995**, GB 2290235 A 19951220.
[8] K. Aksu, M. Nar, M. Tanç, D. Vullo, İ. Gülçin, S. Göksu, F. Tümer, C. T. Supuran, *Bioorg. Med. Chem*. **2013**, *21*, 2925–2931.
[9] B. Özgeriş, S. Göksu, L.P. Köse, İ. Gülçin, R. E. Salmas, S. Durdagi, F. Tümer, C. T. Supuran, *Bioorg. Med. Chem*. **2016**, *24*, 2318–2329.
[10] A. Akincioglu, A. Göçer, İ. Gülçin, S. Durdağı, C. T. Supuran, S. Göksu, *Bioorg. Med. Chem*. **2015**, *23*, 3592–3602.
[11] K. Aksu, F. Topal, İ. Gulcin, F. Tümer, S. Göksu, *Arch. Pharm*. **2015**, *348*, 446–455.
[12] M. Küçük, İ. Gulcin, *Environ. Toxicol. Pharm*. **2016**, *44*, 134–139.
[13] T. Artunç, Y. Çetinkaya, H. Göçer, İ. Gülçin, A. Menzek, E. Sahin, C. T. Supuran, *Chem. Biol. Drug Des*. **2016**, *87*, 594–607.
[14] P. Taslimi, İ. Gülçin, N. Öztaşkın, Y. Çetinkaya, S. Göksu, S. H. Alwasel, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 603–607.
[15] N. M. Abdel Gawad, N. H. Amin, M. T. Elsaadi, F. M. M. Mohamed, A. Angeli, V. De Luca, C. Capasso, C. T. Supuran, *Bioorg. Med. Chem*. **2016**, *24*, 3043–3051.
[16] H. İ. Gül, K. Kucukoglu, C. Yamali, S. Bilginer, H. Yuca, İ. Ozturk, P. Taslimi, İ. Gülçin, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 568–573.
[17] H. Gocer, A. Aslan, İ. Gülçin, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 503–507.
[18] H. Gocer, F. Topal, M. Topal, M. Küçük, D. Teke, İ. Gülçin, S. H. Alwasel, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 441–447.
[19] C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 345–360.
[20] P. Taslimi, İ. Gulcin, B. Ozgeris, S. Goksu, F. Tumer, S. H. Alwasel, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 152–157.
[21] A. Scozzafava, P. Kalın, C. T. Supuran, İ. Gülçin, S. Alwasel, *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 941–946.
[22] C. T. Supuran, A. Scozzafava, A. Casini, *Med. Res. Rev*. **2003**, *23*, 146–89.
[23] A. Yıldırım, U. Atmaca, A. Keskin, M. Topal, M. Çelik, İ. Gülçin, C. T. Supuran, *Bioorg. Med. Chem*. **2015**, *23*, 2598–2605.
[24] C. T. Supuran, *Bioorg. Med. Chem. Lett*. **2010**, *20*, 3467–74.
[25] A. Scozzafava, M. Passaponti, C. T. Supuran, İ. Gülçin, *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 586–591.
[26] M. Boztaş, Y.Çetinkaya, M. Topal, İ. Gülçin, A. Menzek, E. Şahin, M. Tanc, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2015**, *58*, 640–650.
[27] M. Işık, M. Korkmaz, E. Bursal, İ. Gülçin E. Köksal, H. Tohma, *Int. J. Pharm*. **2015**, *11*, 366–371.
[28] N. Öztaşkın, Y. Çetinkaya, P. Taslimi, S. Göksu, İ. Gülçin, *Bioorg. Chem*. **2015**, *60*, 49–57.
[29] L. Polat Köse, İ. Gülçin, A. C. Gören, J. Namiesnik, A. L. Martinez-Ayala, S. Gorinstein, *Ind. Crops Prod*. **2015**, *74*, 712–721.
[30] H. D. Scheibmeir, K. Christensen, S. H. Whitaker, J. Jegaethesan, R. Clancy, J. D. Pierce, *Intensive Crit. Care Nurs*. **2005**, *21*, 24–28.
[31] M. Topal, H. Gocer, F. Topal, P. Kalin, P. Polat Köse, İ. Gülçin, K. C. Çakmak, M. Küçük, L. Durmaz, A. C. Gören, S. H. Alwasel, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 266–275.
[32] F. Topal, M. Topal, H. Gocer, P. Kalın, U. M. Koçyiğit, İ. Gülçin, S. H. Alwasel, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 674–683.
[33] J. H. Bae, Y. J. Park, J. Namiesnik, İ. Gulcin, T. C. Kim, H. C. Kim, B. G. Heo, S. Gorinstein, Y. G. Ku, *Int. J. Food Sci. Tech*. **2016**, *51*, 1378–1385.
[34] P. Kalın, İ. Gülçin, A. C. Gören, *Rec. Nat. Prod*. **2015**, *9*, 496–502.
[35] M. H. Sehitoglu, H. Han, P. Kalin, İ. Gülçin, A. Ozkan, H. Y. Aboul-Enein, *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 264–269.
[36] O. I. Aruoma, *J. Am. Oil Chem. Soc*. **1998**, *75*, 199–212.
[37] S. Çakmakçı, E. F. Topdaş, P. Kalın, H. Han, P. Şekerci, L. Polat Kose, İ. Gülçin, *Int. J. Food Sci. Tech*. **2015**, *50*, 472–481.
[38] T. Wang, R. Jónsdóttir, G. Ólafsdóttir, *Food Chem*. **2009**, *116*, 240–248.
[39] H. P. Wichi, *Food Chem. Toxicol*. **1988**, *26*, 717–723.
[40] H. Göçer, A. Akıncıoğlu, N. Öztaşkın, S. Göksu, İ. Gülçin, *Arch. Pharm. (Weinheim)*, **2013**, *346*, 783–792.
[41] E. R. Sherwin, A. L. Brancn, P. M. Davidson, S. Salmincn, *Food Additives*. Marvel Dekker Inc., New York **1990**, pp. 139–193.
[42] E. Bursal, E. Köksal, İ. Gülçin, G. Bilsel, A. C. Gören, *Food Res. Int*. **2013**, *51*, 66–74.
[43] P. Muniandy, A. B. Shorib, A. S. Baba, *Food Packaging Shelf Life* **2016**, *8*, 1–8.
[44] H. Göçer, A. Akıncıoğlu, S. Göksu, İ. Gülçin, C. T. Supuran, *J. Enzyme Inhib. Med. Chem*. **2015**, *30*, 316–320.
[45] A. Akıncıoğlu, M. Topal, İ. Gülçin, S. Göksu, *Arch. Pharm. (Weinheim)*, **2014**, *347*, 68–76.
[46] M. S. Mathew, A. Baksi, T. Pradeep, K. Joseph, *Biosens. Bioelectron*. **2016**, *81* 68–74.
[47] K. R. Dave, A. R. Syal, S. S. Katyare, *Z. Naturforsch*. **2000**, *55*, 100–108.
[48] D. O. Kennedy, F. L. Dodd, B. C. Robertson, E. J. Okello, J. L. Reay, A. B. Scholey, C. F. Haskell, *J. Psychopharmacology* **2011**, *25*, 1088–1100.
[49] M. B. Colovic, D. Z. Krstic, T. D. Lazarevic-Pasti, A. M. Bondzic, V. M. Vasic, *Curr. Neuropharmacol*. **2013**, *11*, 315–335.

[50] İ. Gülçin, A. Scozzafava, C. T. Supuran, H. Akıncıoğlu, Z. Koksal, F. Turkan, S. Alwasel, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 1095–1101.
[51] Z. Huyut, S. Beydemir, İ. Gulcin, *Enzyme Inhib. Med. Chem*. **2016**, *31*, 1234–1240.
[52] İ. Gülçin, A. Scozzafava, C. T. Supuran, Z. Koksal, F. Turkan, S. Çetinkaya, Z. Bingöl, Z. Huyut, S. H. Alwasel, *J. Enzyme Inhib. Med. Chem*. **2016**, *31*, 1698–1702.
[53] S. Goksu, H. Secen, Y. Sutbeyaz, *Helv. Chim. Acta* **2006**, *89*, 270–273.
[54] S. Goksu, C. Kazaz, Y. Sutbeyaz, H. Secen, *Helv. Chim. Acta* **2003**, *86*, 3310–3313.
[55] Y. Akbaba, H. T. Balaydin, A. Menzek, S. Göksu, E. Sahin, D. Ekinci, *Arch. Pharm. (Weinheim)* **2013**, *346*, 447–454.
[56] H. T. Balaydin, Y. Akbaba, A. Menzek, E. Sahin, S. Goksu, *Arkivoc* **2009**, *14*, 75–87.
[57] İ. Gülçin, M. Elmastaş, H. Y. Aboul-Enein, *Arab. J. Chem*. **2012**, *5*, 489–499.
[58] İ. Gülçin, *Arch. Toxicol*. **2012**, *86*, 345–391.
[59] Y. Çetinkaya, H. Göçer, A. Menzek, İ. Gülçin, *Arch. Pharm. (Weinheim)* **2012**, *345*, 323–334.
[60] M. Oyaizu, *Jpn. J. Nutr*. **1986**, *44*, 307.
[61] İ. Gülçin, S. Beydemir, F. Topal, N. Gagua, A. Bakuridze, R. Bayram, A. Gepdiremen, *J. Enzyme Inhib. Med. Chem*. **2012**, *27*, 587–594.
[62] H. Göçer, İ. Gülçin, *Int. J. Food Sci. Nutr*. **2011**, *62*, 821–825.
[63] E. Bursal, İ. Gülçin, *Food Res. Int*. **2011**, *44*, 1482–1489.
[64] İ. Gülçin, *J. Med. Food* **2011**, *14*, 975–985.
[65] İ. Gülçin, *Innov. Food Sci. Emerg*. **2010**, *11*, 210–218.
[66] O. Talaz, İ. Gülçin, S. Göksu, N. Saracoglu, *Bioorg. Med. Chem*. **2009**, *17*, 6583–6589.
[67] İ. Gülçin, *Chem. Biol. Interact*. **2009**, *179*, 71–80.
[68] T. Ak, İ. Gülçin, *Chem. Biol. Interact*. **2008**, *174*, 27–37.
[69] İ. Gülçin, R. Elias, A. Gepdiremen, K. Taoubi, E. Köksal, *Wood Sci. Technol*. **2009**, *43*, 195–212.
[70] İ. Gülçin, A. Z. Tel, E. Kirecci, *Int. J. Food Propert*. **2008**, *11*, 450–471.
[71] J. S. Wright, E. R. Johnson, G. A. DiLabio, *J. Am. Chem. Soc*. **2001**, *123*, 1173–1183.
[72] İ. Gülçin, F. Topal, S. B. Ozturk Sarikaya, E. Bursal, A. C. Gören, M. Bilsel, *Rec. Nat. Prod*. **2011**, *5*, 158–175.
[73] İ. Gülçin, F. Topal, R. Çakmakçı, A. C. Gören, M. Bilsel, U. Erdoğan, *J. Food Sci*. **2011**, *76*, C585–C593.
[74] S. B. Öztürk Sarıkaya, F. Topal, M. Şentürk, İ. Gülçin, C. T. Supuran, *Bioorg. Med. Chem. Lett*. **2011**, *21*, 4259–4262.
[75] Y. Akbaba, E. Bastem, F. Topal, İ. Gülçin, A. Maraş, S. Göksu, *Arch. Pharm. (Weinheim)* **2014**, *347*, 950–957.
[76] İ. Gülçin, S. Beydemir, *Mini Rev. Med. Chem*. **2013**, *13*, 408–430.
[77] G. N. Shah, G. Bonapace, P. Y. Hu, P. Strisciuglio, W. S. Sly, *Hum. Mutat*. **2004**, *24*, 272.
[78] T. O. Wambo, L. Y. Chen, F. Stanton McHardy, A. T. Tsin, *Biophysical Chem*. **2016**, *214–215*, 54–60.
[79] C. T. Supuran, *Bioorg. Med. Chem. Lett*. **2010**, *20*, 3467–3474.
[80] A. Akıncıoğlu, Y. Akbaba, H. Göçer, S. Göksu, İ. Gülçin, C. T. Supuran, *Bioorg. Med. Chem*. **2013**, *21*, 1379–1385.
[81] C. T. Supuran, A. Scozzafava, *Expert Opin. Ther. Pat*. **2000**, *10*, 575–600.
[82] J. M. Ogilvie, K. K. Ohlemiller, G. N. Shah, B. Ulmasov, T. A. Becker, A. Waheed, A. K. Hennig, P. D. Lukasiewicz, W. S. Sly, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 8514–8519.
[83] P. W. Allderdice, H. A. Gardner, D. Galutira, O. Lockridge, B. N. LaDu, P. J. McAlpine, *Genomics* **1991**, *11*, 452–454.
[84] K. Smith, G. A. El-Hiti, M. B. Alshammari, *Synthesis* **2014**, *46*, 394–402.
[85] İ. Gülçin, Z. Huyut, M. Elmastaş, H. Y. Aboul-Enein, *Arab J. Chem*. **2010**, *3*, 43–53.
[86] İ. Gülçin, E. Bursal, H. M. Şehitoğlu, M. Bilsel, A. C. Gören, *Food Chem. Toxicol*. **2010**, *48*, 2227–2238.
[87] İ. Gülçin, E. Kirecci, E. Akkemik, F. Topal, O. Hisar, *Turk. J. Biol*. **2010**, *34*, 175–188.
[88] R. Apak, K. Guclu, M. Ozyurek, S. E. Karademir, M. Altun, *Free Radical Res*. **2005**, *39*, 949–961.
[89] H. Şerbetçi Tohma, İ. Gülçin, *Int. J. Food Prop*. **2010**, *13*, 657–671.
[90] H. T. Balaydın, İ. Gülçin, A. Menzek, S. Göksu, E. Şahin, *J. Enzyme Inhib. Med. Chem*. **2010**, *25*, 685–695.
[91] T. C. P. Dinis, V. M. C. Madeira, L. M. Almeida, *Arch. Biochem. Biophys*. **1994**, *315*, 161–169.
[92] İ. Gülçin, R. Elias, A. Gepdiremen, L. Boyer, *Eur. Food Res. Technol*. **2006**, *223*, 759–767.
[93] İ. Gülçin, *Life Sci*. **2006**, *78*, 803–811.
[94] İ. Gülçin, *Toxicology* **2006**, *217*, 213–220.
[95] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free Radical Biol. Med*. **1999**, *26*, 1231–1237.
[96] İ. Gülçin, V. Mshvildadze, A. Gepdiremen, R. Elias, *Phytomedicine* **2006**, *13*, 343–351.
[97] G. L. Ellman, K. D. Courtney, V. Andres, R. M. Featherston, *Biochem. Pharmacol*. **1961**, *7*, 88–95.
[98] T. A. Çoban, Ş. Beydemir, İ. Gülçin, D. Ekinci, *Biol. Pharm. Bull*. **2007**, *30*, 2257–2261.
[99] S. Göksu, A. Naderi, Y. Akbaba, P. Kalın, A. Akıncıoğlu, İ. Gulcin, S. Durdağı, R. E. Salmas, *Bioorg. Chem*. **2014**, *56*, 75–82.
[100] M. Güney, A. Coşkun, F. Topal, A. Daştan, İ. Gülçin, C. T. Supuran, *Bioorg. Med. Chem*. **2014**, *22*, 3537–3543.
[101] M. Topal, İ. Gülçin, *Turk. J. Chem*. **2014**, *38*, 894–902.
[102] Y. Çetinkaya, H. Göçer, İ. Gülçin, A. Menzek, *Arch. Pharm. (Weinheim)* **2014**, *347*, 354–359.

[103] A. Atasever, H. Özdemir, İ. Gülçin, Ö. İ. Küfrevioğlu, *Food Chem*. **2013**, *136*, 864–870.
[104] J. A. Verpoorte, S. Mehta, J. T. Edsall, *J. Biol. Chem*. **1967**, *242*, 4221–4229.
[105] M. Şentürk, İ. Gülçin, A. Daştan, Ö. İ. Küfrevioğlu, C. T. Supuran, *Bioorg. Med. Chem*. **2009**, *17*, 3207–3211.
[106] M. M. Bradford, *Anal. Biochem*. **1976**, *72*, 248–251.
[107] B. Aydin, I. Gülcin, S. H. Alwasel, *Int. J. Food Propert*. **2015**, *18*, 2735–2745.
[108] H. Lineweaver, D. Burk, *J. Am. Chem. Soc*. **1934**, *56*, 658–666.
[109] B. Arabaci, İ. Gülçin, S. Alwasel, *Molecules* **2014**, *19*, 10103–10114.