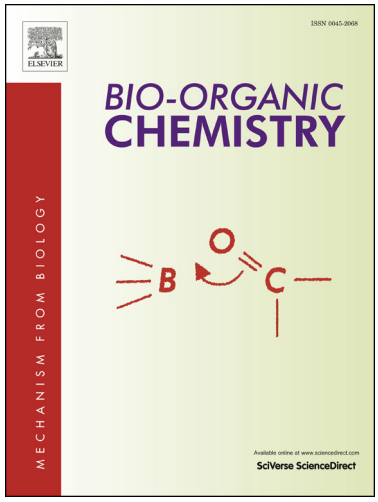

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Antioxidant and Acetylcholinesterase Inhibition Properties of Novel Bromophenol Derivatives

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Running title: Biological activities of novel bromophenol derivatives

ABSTRACT

In this study, series of novel bromophenol derivatives were synthesized and investigated for their antioxidant and AChE inhibition properties. Novel brominated diarylmethanones were obtained from the acylation reactions of benzoic acids with substituted benzenes. One of the bromodiarylmethanone was synthesized from the bromination of diarylmethanone with molecular bromine. All diarylmethanones were converted into their bromophenol derivatives with BBr_3. The antioxidant activities of all synthesized compounds were elucidated by using various bioanalytical assays. Radical scavenging activities of compounds **10-24** were evaluated by means of DPPH$^{\cdot}$ and ABTS$^{\bullet+}$ scavenging activities. In addition, reducing ability of **10-24** were determined by Fe^{3+}, Cu^{2+}, and $[Fe^{3+}\text{-}(TPTZ)_2]^3$ reducing activities. α-Tocopherol, trolox, BHA, and BHT were used as positive antioxidant and radical scavenger molecules. On the other hand, IC_{50} values were calculated for DPPH$^{\cdot}$, ABTS$^{\bullet+}$scavenging, and AChE inhibition effects of novel compounds. The results obtained from the current studies clearly show that novel bromophenol derivatives **20**-**24** have considerable antioxidant, antiradical, and AChE inhibition effects.

Keywords: Acetylcholinesterase; Antioxidant activity; Bromophenols; Diarylmethanones; Demethylation; Enzyme inhibition

1. INTRODUCTION

Naturally occurring bromophenols are abundant in marine life [1]. They are mostly isolated from the marine algae as a very large and diverse group of eukaryotic organisms [2]. Recently, the total syntheses of these compounds have also attracted the synthetic community's attention [3], because it has been demonstrated that natural and synthetic bromophenols have diverse biological activities [4]. For instance, Vidalol A (**1**) and Vidalol B (**2**), which are isolated from the Caribbean marine red alga *Vidalia*, were shown to have anti-inflammatory properties [5]. On the other hand, bromophenols **3-5** isolated from the brown alga *Leathesia nana* showed cytotoxicity [6] and anticancer [7] activities. In another study, Wang et al. has reported the aldose reductase inhibitory activity of the natural product **6** [8]. Furthermore, radical-scavenging properties of **6** [9], antioxidant activities of **1** [10] and **6** [11] have also been investigated. Recently, the total synthesis and the biological activities of some natural bromophenols have been performed by our group [12-15]. In these studies, the human carbonic anhydrase inhibitory actions of **2** [12], **4-6** [13a,b], their synthetic derivatives [14] and paroxonase inhibitory properties [15] of some related compounds were evaluated (Figure 1).

Figure 1 here

The oxidative stress has been a major research interest due to its implicated role in human diseases such as cancer, atherosclerosis, and brain dysfunction [16]. Free radicals, especially reactive oxygen species (ROS), such as superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical (HO·), and hydrogen peroxide (H_2O_2) generated during metabolism can damage macromolecules including fatty acids, proteins, DNA, and other macromolecules, which cause various diseases including neurodegenerative diseases, cardiovascular diseases, cancers and aging-related disorders [17]. Many degenerative disorders are initiated by the reaction of ROS with human body cell constituents. All aerobic organisms have antioxidant defenses, including both antioxidant enzymes and antioxidant food constituents to remove or repair the damaged biomolecules [18a-c]. Antioxidants are bioactive compounds commonly used to preserve the quality of food products by protecting them against oxidative rancidity [19]. In the case of oxidative stress, the most susceptible molecules for ROS attack are lipids, proteins, and nucleic acids [20]. The most injurious effects on the viability of the cells and human health are oxidative changes in nucleic acids, which cause mutagenic processes and cancer [21]. Antioxidant compounds protect the human body from free radicals and many chronic cardiovascular diseases, cancers, and ageing by capturing free radicals [22]. Also, these

bioactive compounds can scavenge free radicals or ROS and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food and pharmaceutical products during processing and storage [23a,b]. They retard the progress of many chronic diseases as well as lipid peroxidation. Recently, there is a growing interest in safer antioxidants for food and pharmaceutical applications, and a growing trend in consumer preferences toward safer antioxidants [24a-d].

Alzheimer's disease (AD) is a fatal and chronic neurodegenerative disease that usually starts slowly and gets worse over time. It was reported that the disease process is associated with tangles and plaques in the brain. There were 35 million people worldwide with AD, and there has been no effective treatment developed for this disease up to now. The use of acetylcholinesterase inhibitors (AChEIs) to block the cholinergic degradation of acetylcholine (Ach) is therefore considered to be a promising approach for the treatment of AD [25a-c]. At the present, the main clinical treatment strategy for AD is the use of AChEIs such as Donepezil, Rivastigmine, and Galantamine. These drugs modestly improve the memory and cognitive function but do not prevent progressive neurodegenerative effects because of the complexity of AD pathogenesis. Based on these discoveries, many different approaches to treat AD have been developed. In particular, the use of AChEIs for this multifaceted disease has received much attraction [25a-c].

As it can be seen from the brief description given above, bromophenols show useful biological activities. In our previous studies, we synthesized bromophenols containing polybromides in their structures. The synthesis and biological evaluation of novel bromophenols having only a single bromine atom will be important for synthetic and biological features. In this context, here we report the synthesis and determination of antioxidant, antiradical and AChE inhibition effects of five novel bromophenols.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The reactions of acids or alcohols with benzene related compounds in the presence of polyphosphoric acid (PPA) afford diarylmethanones [26a,b]. It is obvious from the structure of **9** that an electrophilic aromatic substitution carried out on this compound can lead to two different products. However, methoxy group has more steric hindrance than a bromine atom.

For this reason, an electrophilic aromatic substitution of these kinds of compounds react at the *ortho*- position of −Br substituent and at the *para*- position of an −OMe group [26b]. Therefore, benzoic acids **7** and **8** were reacted with commercially available 3-bromoanisole (**9**) in the presence of PPA to give diarylmethanons **10** and **11** in moderate yields. On the other hand**,** 2-substituted 1,4-dimethoxy benzenes (especially Br or Me substituted ones) undergo electrophilic aromatic substitution at the *ortho*- position of the OMe group and the *para*- position of the substituent [27]. Similarly, the reactions of acids **12** and **13** with a known 2-bromo-1,4-dimethoxy benzene (**14**) [28] afforded diarylmethanones **15** and **16** [29] in 76% and 50% yield, as expected (Scheme 1).

Scheme 1 here

Again the reaction of carboxylic acid **12** with 1,3-dimethoxy benzene (**17**) in the presence of PPA produced the compound **18** [30]. Selective bromination of aromatic compounds is an important route for the synthesis of novel target compounds. There are several reagents used for this purpose. Molecular bromine (Br_2) is one of these reagents mostly used for the bromination of activated aromatic compounds [31]. To extend our research on the synthesis of another mono brominated compound, the bromination of **18** with 1.01 equiv. of Br_2 at room temperature (rt) for 12 hour (h) afforded mono brominated diarylmethanone **19** with a yield of 62% (Scheme 2).

Scheme 2 here

Demethylation of aryl methyl ethers is one of the most convenient methods for the synthesis of biologically active phenolic compounds [32a,b]. BBr_3 is one of the most widely-used reagents for the *O*-demethylation of aryl methyl ethers [33]. Hence, demethylation reactions of diarylmethanones **10**, **11**, **15**, **16,** and **19** with BBr_3 at 0-25 ^{o}C under N_2 for 24 h furnished novel bromophenols **20**-**24** in 91, 80, 92, 93, and 83% yields, respectively (Scheme 3).

The structures of the synthesized compounds were elucidated with ^{1}H-, ^{13}C-NMR, IR and elemental analysis.

Scheme 3 here

2.1. Biochemistry-Antioxidant Properties

Lipid peroxidation in foods has become a major concern with the increased use of polyunsaturated vegetable, fish, or microbial oils for health benefits. It not only produces undesirable off-flavours, but also decreases the nutritional quality and safety of food products [34a,b], which are unacceptable to consumers. Among the methods employed to retard or inhibit oxidation of lipids, addition of antioxidants is the most effective way [35a,b]. On the other hand, phenolic compounds have attracted wide range of interest because of their health benefits, biological features and impact on multiple sensory attributes of food including colour, flavour and astringency of foods [36a,b]. They are thought to be an integral part of both human and animal diets and frequently used for pharmacological applications [37a-c]. Phenolic compounds are secondary plant metabolites, structure of which includes one or more aromatic rings substituted by one or more hydroxyl groups [36a]. They are secondary plant metabolites and naturally present in almost all plant materials, including food and pharmaceutical products of plant origin [38a-d]. The antioxidant capacities of novel bromophenols are determined by using several antioxidant assays. For this purpose, $[Fe(CN)_6]^{-3}$-$[Fe(CN)_6]^{-4}$ reduction method is the first used antioxidant evaluation of novel bromophenols [39a,b]. Table 1 shows that novel bromophenols have effective $[Fe(CN)_6]^{-3}$ reducing ability and these differences were statistically very significant ($p<0.01$). Increasing $[Fe(CN)_6]^{-4}$ absorbance of the reaction mixture indicates increased reducing capacity, due to an increase in the complex formation. Reducing ability of novel bromophenols and positive controls showed the following order: **23** (2.563±0.003, r^2: 0.9895) > BHA (2.354±0.003, r^2: 0.9575) > **21** (2.123±0.011, r^2: 0.9999) ≈ **20** (2.094±0.004, r^2: 0.9711) > **22** (1.947±0.003, r^2: 0.9757) > **24** (1.163±0.002, r^2: 0.9700) > BHT (1.405±0.003, r^2: 0.9630) > α-tocopherol (0.927±0.008, r^2: 0.9819) > trolox (0.893±0.002, r^2: 0.9930). On the other hand, compounds **10**-**19** as precursors of novel bromophenols **20**-**24** not having any phenolic groups in the aromatic rings are located in the *para*- position. These compounds demonstrated to have weak

antioxidant activities. The results clearly showed that novel bromophenol **23** had the most powerful Fe^{3+} ability and changed yellow colour of the test solution to green or blue, depending on their reducing properties. In this molecule, both hydroxyl groups (-OH) are located in aromatic ring at the *para-* position (Table 1). It was reported that phenolic compounds are not active antioxidants, unless they contain substituents at either *ortho-* or *para-* positions. Both positions have increased the electron density at the -OH groups and lowered the oxygen-hydrogen bond energy, in effect increasing the reactivity towards the lipid free radicals. Substituents of phenolic compounds at the *meta-* positions have rather limited effects. Electronic and steric effects are responsible for the antioxidant activities and stoichiometric factors of the chain-breaking phenolic antioxidant compounds [38c,40]. For elucidation of the hydrogen abstraction mechanism and reducing ability of phenolic antioxidant compounds in the chain process of autoxidation, molecular orbital theory has been applied [41].

Cupric ions (Cu^{2+}) reducing assays are based on reduction the reduction of Cu^{2+} to Cu^{+}. Cu^{+}-neocuproine complexes occur in the presence of neocuproine. This complex had maximum absorption at 450 nm [42]. Also, this assay is cost-effective, selective, stable, rapid and suitable for antioxidants regardless of hydrophilicity or chemical type [40a]. The Cu^{2+} reducing capacities of novel bromophenols and standard compounds were shown in Table 1. Cu^{2+} reducing ability of novel bromophenols and standard compounds at the same concentration (20 μg/mL) exhibited the following order: **23** (2.018±0.005, r^2: 0.9997) ≈ **21** (2.014±0.004, r^2: 0.9984) > **22** (1.966±0.002, r^2: 0.9707) ≈ BHA (1.962±0.002, r^2: 0.9999) > **20** (1.602±0.001, r^2: 0.9430) > BHT (1.407±0,004, r^2: 0.9972) > trolox (1.005±0.002, r^2: 0.9992) > α-tocopherol (0.854±0.003, r^2: 0.9998) > **24** (0.655±0.003, r^2: 0.9692). As in $Fe[(CN)_6]^3$ reducing ability, the most powerful cupric ions (Cu^{2+}) reducing ability was observed in novel bromophenol **23**. Additionally, buried thiols groups (-SH) of proteins release in urea buffer at near of pH 7.0 and react with reagent. So, this method can be used for measurement of reducing activity of antioxidants, which include thiol groups such as glutathione [43].

Table 1 here

The FRAP assay measures the ability of phenolic antioxidant compounds to reduce the ferric 2,4,6-tripyridyl-s-triazine complex $[Fe^{3+}\text{-}(TPTZ)_2]^{3+}$ to the intensely blue coloured ferrous complex $[Fe^{2+}\text{-}(TPTZ)_2]^{2+}$ in acidic medium [44a,b]. FRAP assay is one of the widely used, simple and convenient method for determining the antioxidant activity [45].

Traditionally, the assay is conducted at acidic pH (3.6) to maintain iron solubility. The results, in terms of the reducing abilities of the novel bromophenols and standard antioxidant compounds, were as follows: **23** (2.093±0.004, r^2: 0.9709) ≈ **20** (2.012±0.001, r^2: 0.9591) ≥ **21** (1.986±0.004, r^2: 0.9916) ≥ **22** (1.974±0.005, r^2: 0.9546) ≥ BHA (1.869±0.001, r^2: 0.9806) > trolox (1.742±0.002, r^2: 0.9997) > BHT (1.328±0.004, r^2: 0.9986) > α-tocopherol (1.123±0.002, r^2: 0.9986) > **24** (0.401±0.002, r^2: 0.9404) (Table 1). These results emphasized that novel bromophenols had notable Fe^{3+} reducing ability and electron donor properties for neutralizing free radicals.

The novel bromophenol **23** has two phenolic groups at *para*- positions. Thereby, this novel bromophenol is expected to have a strong reducing activity. The presence of different substituents in the phenol backbone structure modulates their antioxidant property, in particular their hydrogen-donating capacity. In addition, the presence of a carbonyl group, such as aromatic acid, ester, or lactone, enhanced its antioxidant activity [40a]. Moreover, it is well known that steric and electronic effects are responsible for the antioxidant activities and stoichiometric factors of the chain-breaking phenolic antioxidants [38c]. The activity of an antioxidant molecule also increases when its carbonyl group is separated from the aromatic ring. For example, cinnamic acid is more effective antioxidant than benzoic acid. Steric hindrance of the phenolic hydroxyls by a neighbouring inert group, such as methoxy groups, enhanced its antioxidant activity [40a,38c].

The radical scavenging properties of novel bromophenols were investigated by DPPH· and $ABTS^{\bullet+}$ scavenging assays [46a-c]. DPPH· is a stable nitrogen-centred free radial and has been well used to employ the ability of free-radical scavenging properties or hydrogen donation of compounds in food, medicinal, and pharmaceutical materials [47a-c]. This assay is a fast and simple radical scavenging method. DPPH radical scavenging was broadly accepted for the in vitro evaluation of natural substance antioxidant potential due to its simplicity and low cost [48a-c]. In this study, DPPH· scavenging assay was used for the primary screening of free radical scavenging activity of novel bromophenols. Consequently, novel bromophenols exhibit a remarkable DPPH free radical scavenging activity. As it can be seen by Table 2, IC_{50} values of DPPH· scavenging effects of novel bromophenols and standard radical scavengers increased in the order: **23** (14.15 μg/mL) ≈ **22** (14.74 μg/mL) ≥ **21** (15.40 μg/mL) ≈ **20** (15.75 μg/mL) > **24** (28.92 μg/mL). A lower IC_{50} value indicates powerful DPPH· scavenging activity of novel bromophenols. On the other hand, BHA, BHT, α-tocopherol and trolox demonstrated IC_{50} values of 22.35, 34.65, 21.65 and 16.11 μg/mL, respectively. Also, IC_{50} values of precursors (**10**-**19**) used for the synthesis of (**20**-**24**) are

ranging of 221.37-346.50 μg/mL. These results clearly revealed that bromophenols (**20**-**24**) had notable DPPH· scavenging ability and electron donor properties for neutralizing free radicals.

Table 2 here

In $ABTS^{\bullet+}$ scavenging assay, ABTS is oxidized by oxidants like $K_2S_2O_8$ and MnO_2 to its radical cation ($ABTS^{\bullet+}$), which is bright green-blue in colour and has an absorption maximum of 734 nm [49a-c]. The absorbance of the $ABTS^{\bullet+}$ solution decreases when an electron is donated by an antioxidant to quench the free radical. This assay has been used for both hydrophilic and lipophilic antioxidants such as tocopherols and carotenoids [50]. $ABTS^{\bullet+}$ radicals are more reactive than DPPH radicals and applicable for both hydrophilic and lipophilic compounds [51a-c]. As it can be seen by Table 2, novel bromophenols (**20**-**24**) show important $ABTS^{\bullet+}$ scavenging properties. IC_{50} values of $ABTS^{\bullet+}$ scavenging of novel bromophenols (**20**-**24**) increased in the following order: **23** (2.23 μg/mL) ≈ **20** (2.55 μg/mL) ≈ **21** (2.58 μg/mL) ≈ **22** (2.61 μg/mL) > **24** (22.35 μg/mL). A lower IC_{50} value indicates powerful $ABTS^{\bullet+}$ scavenging activity. Also, IC_{50} values of precursors (**10**-**19**) of novel bromophenols (**20**-**24**) are ranging of 138.60-138.60 μg/mL. In addition, BHA (2.72 μg/mL), BHT (2.88 μg/mL), α-tocopherol (4.81 μg/mL) and trolox (2.86 μg/mL) were used as positive controls for $ABTS^{\bullet+}$ scavenging activity.

AChE is found in the brain and in erythrocytes with high concentrations and it is a crucial enzyme for the nervous system. AChE inhibitors are used in the treatment of several neuromuscular diseases, and in the treatment of AD [25a]. The activity (%)–[Bromophenols] graphs were drawn and the half maximal inhibitory concentration (IC_{50}) values of novel bromophenols demonstrated 50% inhibition of AChE isoenzymes were calculated after suitable dilutions. Ki values for novel bromophenols were determined for AChE. Ki is defined as the binding affinity constant of the novel bromophenols to AChE. To determine the Ki values, each novel bromophenols was tested at three different concentrations [52a-d]. Lineweaver–Burk curves were drawn in detail as described previously [53a-c]. For descriptions of inhibitory effects, researchers often used an IC_{50} value; however, Ki constant is a more suitable parameter. Ki values were calculated from Lineweaver–Burk graphs (Figures 2). In this study, both the IC_{50} and Ki parameters of novel bromophenols were calculated (Table 2). The most powerful AChE inhibition ability observed for novel bromophenol **22**. Figure 2 shows IC_{50} and Ki graphs of novel bromophenol **22.**

Figure 2 here

We are very experienced by our previous studies that the Ki values of phenolic dopamine-related compounds were generally found in the range of 33.04-131.68 nM [54a]. In contrast to the phenolic compounds, arylmethyl ethers such as sulphonamides incorporating the tetralin scaffold demonstrated Ki values in the range of 56.04-537.3 μM [54b]. Furthermore, it was reported that Galantamine, which is used for the treatment of AD and various other memory impairments, has been shown the lower AChE inhibition activity (EC_{50}: 3.70 mM) [54b]. In the last part of the study, AChE was also highly inhibited by novel bromophenol derivatives (**10**-**24**) at the low nanomolar inhibition with Ki values in the range of 2.78-5.88 nM (Table 2). These results clearly showed that all novel synthesized compounds (**10-24**) demonstrated powerful AChE inhibition properties. The most powerful inhibition was observed for novel bromophenol **23** with a Ki value of 2.78 nM. All the remaining newly synthesized compounds reported here were also showed highly efficient inhibition constants against AChE. On the other hand, tacrine, which first centrally acting cholinesterase inhibitor approved for the treatment of AD demonstrated Ki value of 7.64 nM.

3. CONCLUSION

In conclusion, five diarylmethanones incorporating bromine and methoxy functional groups were synthesized by starting from benzoic acids. Five novel bromophenols (**20-24)** were synthesized from these compounds via *O*-demethylation with BBr_3. The antioxidant activities and AChE inhibition properties of all synthesized compounds were evaluated. Novel bromophenols were found to be effective AChE inhibitors. Different assays including reducing power, DPPH· and $ABTS^{\bullet+}$ radical scavenging activities showed that the synthesized bromophenols were also effective antioxidants. When compared with the standard antioxidants such as popular synthetic antioxidants BHA, BHT, α-tocopherol and trolox, the synthesized compounds reported in this paper are most potent antioxidants. Based on the information given above, the synthesized novel bromophenols can be used for minimizing or preventing lipid peroxidation in food, medical and pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of pharmaceuticals.

4. EXPERIMENTAL

4.1. General

All chemicals and solvents are commercially provided and were used after distillation or treatment with drying agents. IR spectra were obtained from solutions in 0.1 mm cells with a Perkin-Elmer spectrophotometer. Melting points were determined on a capillary melting apparatus (BUCHI 530) and are uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on a 400 (100) MHz Varian 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 with silica gel (60-mesh, Merck). TLC is preparative thin layer chromatography prepared from 1 mm of silica gel 60 PF (Merck) on glass plates.

4.2. Chemistry

The synthesis of 2-bromo-1,4-dimethoxy benzene (**14**) was carried out according to the literature procedure [28].

4.2.1. General procedure for the synthesis of diarylmethanones [Synthesis of (2-bromo-4-methoxyphenyl)(3,4-dimethoxyphenyl)methanone **(10)]**

In order to prepare PPA (polyphosphoric acid), a mixture of H_3PO_4 (6.4 g) and P_2O_5 (11.5 g) in 150 mL beaker was stirred at 80 ^{o}C for 15 min. 1-Bromo-3-methoxybenzene (**9**) (3.08 g, 16.47 mmol) and 3,4-dimethoxybenzoic acid (**7**) (2 g, 10.98 mmol) were added to prepared PPA solution and the mixture was stirred with a glass stick at 80 ^{o}C for 1.5 h. After the completion of the reaction, cold water was added to the reaction mixture and then the organic layer was extracted with EtOAc (3x150 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography with silica gel (30 g) by eluting with hexane:ethyl acetate (9:1). Recrystallization of the solid with hexane-ethyl acetate gave 2-bromo-4-methoxyphenyl)(3,4-dimethoxyphenyl)methanone (**10**) (2.32 g) as a white solid with 60% yield. Mp: 140-142 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (d, 1H, J = 1.8 Hz), 7.28 (d, 1H, A part of AB system, J = 8.5 Hz,), 7.23 (dd, 1H, A part of AB system, J = 8.4, 1.8 Hz), 7.17 (d, 1H, J = 2.3 Hz), 6.91 (dd, 1H, B part of AB system, J = 8.5, 2.3 Hz), 6.83 (d, 1H, B part of AB system, J = 8.4 Hz),

3.93 (s, 6H), 3.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.5, 161.2, 153.9, 149.40, 133.2, 130.7, 130.0, 126.4, 121.1, 118.8, 113.2, 111.4, 110.1, 77.6, 77.3, 76.9, 56.3, 56.2, 55.9. IR (cm^{-1}, CH_2Cl_2): 3082, 3006, 2938, 2839, 1657, 1597, 1559, 1512, 1491, 1463, 1417, 1341, 1298, 1267, 1232, 1183, 1130, 1031, 984, 914, 864. Anal. Calcd. for $C_{16}H_{15}BrO_4$: C 54.72; H 4.31, Found: C 54.76; H 4.28.

4.2.2. 2-Bromo-4-methoxyphenyl)(2,3-dimethoxyphenyl)methanone (**11**)

The reaction of 1-bromo-3-methoxybenzene (**9**) (3.08 g, 16.47 mmol) with 2,3-dimethoxy benzoic acid (**8**) (2.0 g, 10.98 mmol) according to the general procedure described at 4.2.1 afforded (2-bromo-4-methoxyphenyl)(2,3-dimethoxyphenyl)methanone (**11**) (2.13 g, 55% yield) in 1.5 h. Yellow solid. Mp: 88-90 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (d, 1H, A part of AB system, J = 8.6 Hz), 7.15 (d, 1H, J = 2.4 Hz), 7.13 – 7.03 (m, 3H), 6.83 (dd, 1H, B part of AB system, J = 8.6, 2.4 Hz), 3.87 (s, 3H), 3.83 (s, 3H), 3.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.0, 161.9, 153.1, 148.4, 134.2, 133.6, 132.7, 124.2, 121.9, 119.4, 115.8, 112.9, 61.7, 56.2, 55.9. IR (cm^{-1}, CH_2Cl_2): 3082, 3004, 2940, 2838, 1668, 1594, 1559, 1478, 1440, 1426, 1390, 1314, 1266, 1231, 1199, 1184, 1077, 1033, 1003, 964,863. Anal. Calcld. for $C_{16}H_{15}BrO_4$: C 54.72; H 4.31, Found: C 54.59; H 4.28.

4.2.3. (4-Bromo-2,5-dimethoxyphenyl)(4-methoxyphenyl)methanone (**15**)

The general procedure described above at 4.2.1 was applied to 2-bromo-1,4-dimethoxybenzene (**14**) (1.07 g, 4,93 mmol) and 4-methoxybenzoic acid (**12**) (0.5 g, 3,29 mmol) to give (4-bromo-2,5-dimethoxyphenyl)(4-methoxyphenyl)methanone (**15**) (0.88 g, 76%) in 1.5 h. White solid. Mp: 130-132 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, 2H, J = 8.9 Hz), 7.20 (s, 1H), 6.92-6.90 (m, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.68 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 194.1, 164.0, 151.4, 150.40, 132.5, 130.5, 129.1, 117.6, 114.27, 113.8, 113.0, 57.1, 56.8, 55.7. IR (cm^{-1}, CH_2Cl_2): 3074, 3006, 2940, 2842, 1659, 1599, 1574, 1509, 1490, 1464, 1441, 1380, 1316, 1216, 1170, 1128, 1049, 1030, 982, 912. Anal. Calcld. for $C_{16}H_{15}BrO_4$: C, 54.72; H, 4.31; Found: C, 54.68; H, 4.36.

4.2.4. (4-Bromo-2,5-dimethoxyphenyl)(phenyl)methanone (**16**)

Applying of the procedure given at 4.2.1 was applied to 2-bromo-1,4-dimethoxybenzene (**14**) (3.02 g,13.92 mmol) and benzoic acid (**13**) (1.7 g, 13,92 mmol) to afford (4-bromo-2,5-dimethoxyphenyl) (phenyl)methanone (**16**) [28] (2.25 g 50% yield) in 1.5 h. White solid. Mp: 137-139 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (d, 2H, J = 7.2 Hz), 7.55 (t, 1H, J = 7.4 Hz), 7.43 (t, 2H, J = 7.7 Hz,), 7.2 (s, 1H), 6.95 (s, 1H), 3.84 (s, 3H), 3.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.3, 151.5, 150.2, 137.5, 133.2, 129.7, 128.4, 128.3, 117.4, 114.8, 112.9, 56.9, 56.5. IR (cm^{-1}, CH_2Cl_2): 3102, 3071, 3002, 2930, 2897, 2843, 1644, 1593, 1570, 1488, 1454, 1439, 1375, 1312, 1248, 1212, 1174, 1151, 1050, 1017, 982. Anal. Calcld. for $C_{15}H_{13}BrO_3$: C 56.10; H 4.08, Found: C 56.05; H 4.12.

4.2.5. Synthesis of (2,4-dimethoxyphenyl)(4-methoxyphenyl)methanone (**18**)

The synthesis of compound **18** was achieved from the reaction of acid **12** with 1,3-dimethoxy benzene (**17**) according to the procedure described above at 4.2.1.

^{1}H- and ^{13}C-NMR data of (2,4-dimethoxyphenyl)(4-methoxyphenyl)methanone (**18**) is in agreement with data given in the literature [30].

4.2.6. Synthesis of (5-bromo-2,4-dimethoxyphenyl)(4-methoxyphenyl)methanone (**19**)

To a solution of (2,4-dimethoxyphenyl)(4-methoxyphenyl)methanone (**18**) (0.5 g, 1.84 mmol) in $CHCl_3$ (50 mL) was added a solution of Br_2 (0.29 g, 1.85 mmol) in $CHCl_3$ (40 mL) at rt. The reaction mixture was stirred at rt for 12 h. After completion of the reaction, the solvent was evaporated under reduced pressure. The crystallization of the residue with ethyl acetate-hexane furnished (5-bromo-2,4-dimethoxyphenyl)(4-methoxyphenyl)methanone (**19**) (0.4 g, 62% yield) as a white solid. Mp: 135-137 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, 2H, A part of AB system, J = 8.9 Hz), 7.53 (s, 1H), 6.90 (d, 2H, B part of AB system, J = 8.9 Hz), 6.52 (s, 1H), 3.96 (s, 3H), 3.86 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.0, 163.7, 158.7, 158.6, 134.3, 132.4, 131.1, 123.0, 113.7, 102.3, 96.9, 56.6, 56.3, 55.7. IR (cm^{-1}, CH_2Cl_2): 3003, 2966, 2933, 2837, 1632, 1587, 1507, 1458, 1433, 1387, 1310, 1282, 1253, 1210, 1169, 1018, 969, 902. Anal. Calcld. for $C_{16}H_{15}BrO_4$: C 54.72; H 4.31; Found: C 54.67; H 4.38.

4.2.7. General procedure for the synthesis of bromophenols [Synthesis of (2-bromo-4-hydroxyphenyl)(3,4-dihydroxyphenyl)methanone (**20**)]

To a solution of 2-bromo-4-methoxyphenyl)(3,4-dimethoxyphenyl)methanone (**10**) (0.5 g, 1.42 mmol) in CH_2Cl_2, a solution of BBr_3 (2.14 g, 8.54 mmol) in CH_2Cl_2 (8 mL) was added drop wise within 5 min. under N_2 atmosphere at 0 ^oC. After the mixture was allowed to warm to room temperature, it was stirred at rt for 24 h. Methanol (30 mL) was added to the reaction mixture drop wise at 0 ^oC within 10 min. The solvent was evaporated under reduced pressure. To the residue were added EtOAc (50 mL) and H_2O (50 mL). The organic layer was separated and the H_2O layer was extracted with EtOAc (2x30 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. (2-Bromo-4-hydroxyphenyl)(3,4-dihydroxyphenyl)methanone (**20**) (0.40 g, 91% yield) was obtained as a black viscous liquid. ^1H NMR (400 MHz, CD_3COCD_3): δ 9.19 (s, 1H, OH), 8.77 (s, 1H, OH), 8.42 (s, 1H, OH), 7.33 (d, 1H, J = 1.9 Hz), 7.24 (d, 1H, A part of AB system, J = 8.3 Hz), 7.17 - 7.13 (m, 2H), 6.95 (dd, 1H, J = 8.2, 2.2 Hz), 6.91 (d, 1H, B part of AB system, J = 8.3 Hz). ^{13}C NMR (100 MHz, CD_3COCD_3): δ 193.5, 159.3, 150.9, 145.2, 132.8, 130.7, 129.7, 124.3, 120.1, 119.8, 116.6, 115.1, 114.5. IR (cm^{-1}, CH_2Cl_2): 3208, 2923, 2845, 1641, 1589, 1513, 1468, 1419, 1350, 1273, 1209, 1104, 1030, 985, 952. Anal. Calcld. for $C_{13}H_9BrO_4$: C 50.51; H 2.93, Found: C 50.48; H 2.30.

4.2.8. (2-Bromo-4-hydroxyphenyl)(2,3-dihydroxyphenyl)methanone (**21**)

General demethylation procedure described above at 4.2.8 with BBr_3 (2.14 g, 8.54 mmol) was applied to **11** (0.5 g, 1.42 mmol) to give (2-bromo-4-hydroxyphenyl)(2,3-dihydroxyphenyl) methanone (**21**) (0.35 g, 80% yield) as a brown solid. Mp: 254-256 ^oC. ^1H NMR (400 MHz, CD_3COCD_3): δ 12.05 (s, 1H, OH), 9.33 (s, 1H, OH), 8.13 (s, 1H, OH), 7.35 (d, 1H, A part of AB system, J = 8.4 Hz), 7.21 (d, 1H, J = 2.2 Hz), 7.14 (dd, 1H), 7.01 (dd, 1H, B part of AB system, J = 8.4, 2.2 Hz), 6.84-6.76 (m, 2H). ^{13}C NMR (100 MHz, CD_3COCD_3): δ 201.8, 159.9, 151.4, 146.6, 131.1, 130.7, 124.2, 121.9, 120.1, 120.0, 119.9, 119.1, 114.7. IR (cm^{-1}, CH_2Cl_2): 3231, 3072, 1618, 1581, 1481, 1445, 1423, 1368, 1327, 1267, 1208, 1046, 1010, 890. Anal. Calcld. for $C_{13}H_9BrO_4$: C 50.51; H 2.93 Found: C 50.45; H 3.04.

4.2.9. (4-Bromo-2,5-dihydroxyphenyl)(4-hydroxyphenyl)methanone (**22**)

Demethylation of (4-bromo-2,5-dimethoxyphenyl)(4-methoxyphenyl)methanone (**15**) (0.70 g, 1.99 mmol) with BBr_3 (3.00 g, 11.96 mmol) for 24 h afforded (4-bromo-2,5-dihydroxyphenyl)(4-hydroxyphenyl)methanone (**22**) (0.57 g, 92%) as an orange solid. Mp: 201-203 ^oC. ^1H NMR (400 MHz, CD_3COCD_3): δ 11.19 (s, 1H, OH), 9.32 (s, 1H, OH), 8.61 (s, 1H, OH), 7.72 (d, 2H, A part of AB system, J = 8.6 Hz), 7.29 (s, 1H), 7.24 (s, 1H), 7.02 (d, 2H, B part of AB system, J = 8.6 Hz). ^{13}C NMR (100 MHz, CD_3COCD_3): δ 199.4, 162.6, 156.1, 147.0, 133.0, 130.0, 122.6, 120.5, 119.3, 118.8, 116.1. IR (cm^{-1}, CH_2Cl_2): 3242, 3177, 3046, 2855, 1710, 1677, 1605, 1576, 1507, 1485, 1439, 1396, 1317, 1266, 1233, 1163, 998, 891. Anal. Calcld. for $C_{13}H_9BrO_4$: C 50.51; H 2.93, Found: C 50.48; H 3.01.

4.2.10. (4-Bromo-2,5-dihydroxyphenyl)(phenyl)methanone (**23**)

Demethylation of (4-bromo-2,5-dimethoxyphenyl)(phenyl)methanone (**16**) (0.3 g, 0.93 mmol) with BBr_3 (1.4 g, 5.6 mmol) for 24 h yielded (4-bromo-2,5-dihydroxyphenyl)(phenyl) methanone (**23**) (0.25 g, 93% yield) as an orange solid. Mp: 130-132 ^oC. ^1H NMR (400 MHz, CD_3OD): δ 7.61 (d, 2H, J = 7.1 Hz), 7.53 (t, 1H, J = 7.4 Hz), 7.44 (t, 2H, J = 7.5 Hz), 7.10 (s, 1H), 6.97 (s, 1H), 4.78 (bs, OH of CD_3OD and 2OH of ArOH). ^{13}C NMR (100 MHz, CD_3OD): δ 201.4, 155.9, 147.9, 139.2, 133.4, 130.2, 129.5, 122.9, 121.2, 119.9, 119.1. IR (cm^{-1}, CH_2Cl_2): 3327, 2965, 2833, 1648, 1593, 1575, 1491, 1456, 1440, 1377, 1276, 1260, 1214,1179, 1052, 1020, 985, 863. Anal. Calcld. for $C_{13}H_9BrO_3$: C 53.27; H 3.09, Found: C 53.20; H 3.14.

4.2.11. (5-Bromo-2,4-dihydroxyphenyl)(4-hydroxyphenyl)methanone (**24**)

Demethylation of (5-bromo-2,4-dimethoxyphenyl)(4-methoxyphenyl)methanone (**19**) (0.9 g, 2.56 mmol) with BBr_3 (3.85 g, 15.38 mmol) for 24h gave (5-bromo-2,4-dihydroxyphenyl)(4-hydroxyphenyl) methanone (**24**) (0.66 g, 83%) as a yellow solid. Mp: 218-220 ^oC. ^1H NMR (400 MHz, CD_3COCD_3): δ 12.47 (s, 1H, OH), 10.29 (s, 1H, OH), 9.36 (s, 1H, OH), 7.81 (s, 1H), 7.67 (d, 2H, A part of AB system, J = 8.6 Hz), 7.04 (d, 2H, B part of AB system, J = 8.6 Hz), 6.63 (s, 1H). ^{13}C NMR (100 MHz, CD_3COCD_3): δ 198.7, 165.6, 162.3, 161.4, 138.4, 132.6, 130.0, 116.2, 114.9, 104.9, 100.3. IR (cm^{-1}, CH_2Cl_2): 3417, 3057, 3016, 2876, 1579, 1510, 1448, 1422, 1384, 1334, 1241, 1208, 1168, 1147, 1114, 973, 926. Anal. Calcld. for $C_{13}H_9BrO_4$: C 50.51; H 2.93; Found: C 50.47; H 3.02.

4.3. Determination of Antioxidant Activity

For measurement of antioxidant activities of novel bromophenols, $DPPH^{\cdot}$, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$ and $O_2^{\bullet-}$ scavenging, metal chelating and AChE inhibition assays were used. The antioxidant activities were determined by the following bioanalytical assays:

4.3.1. Fe^{3+}-Fe^{2+} reducing assay

For determination of Fe^{3+} reducing ability of novel bromophenols $Fe^{3+}(CN^-)_6$-$Fe^{2+}(CN^-)_6$ reduction method was used [55a,b]. Briefly, different concentrations of novel bromophenols (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 potassium ferricyanide [$K_3Fe(CN)_6$] (1%). Then, the solution was incubated at 50°C during 20 min. After incubation period, 1.25 mL of trichloroacetic acid (TCA) was added (10%). Lastly, 0.5 mL of $FeCl_3$ (0.1%) was transferred to this mixture and the absorbance value was measured at 700 nm in a spectrophotometer [56].

4.3.2. CUPRAC Assay

This assay is based on utilising the Cu^{2+}- neocuproine reagent as the chromogenic oxidising agent. To the mixture of 1 mL of Cu^{2+}-neocuproine ($7.5x10^{-3}$ M) and CH_3COONH_4 (1 M) buffer solutions were transferred to a test tube, which contains different concentrations of novel bromophenols (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 [42,57].

4.3.3. FRAP assay

$[Fe^{3+}\text{-}(TPTZ)_2]^{3+}$ reducing values of novel bromophenols are determined by measuring the absorbance increase at 593 nm and relating it to a ferrous ions standard solution or to an antioxidant standard solution. The change in absorbance is proportional to the combined $[Fe^{3+}\text{-}(TPTZ)_2]^{3+}$ reducing value of novel bromophenols [40a,43b].

4.3.4. $ABTS^{\bullet+}$ scavenging assay

ABTS radical cation was generated by the interaction of ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM) [50] described previously [51a]. This solution was diluted with methanol until the absorbance in the samples reached 0.750±0.05 at 734 nm. Then, 1 mL of $ABTS^{\bullet+}$ solution was supplement to 3 mL of novel bromophenols and control solutions. The extent of decolorization is calculated as percentage reduction of absorbance.

4.3.5. $DMPD^{\bullet+}$ scavenging assay

$DMPD^{\bullet+}$ scavenging ability of novel bromophenols was performed according to the methods described previously [26a, 58]. The scavenging capability of $DMPD^{\bullet+}$ radical of the sample was monitored in spectrophotometer at 505 nm [40a].

4.3.6. $DPPH^{\bullet}$ scavenging assay

The DPPH radical scavenging activity of novel bromophenols was performed according to the method developed by Blois et al [59a,b]. Evaluation of the scavenging effect on DPPH radicals is described by Gülçin et al. [60a-c]. In this assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored $DPPH_2$. DPPH• scavenging assay uses the stable radical and this method was described previously in detailed [61a-c].

4.3.9. Acetylcholinesterase inhibition assay

The inhibition effects of novel bromophenols on AChE activities were measured according to the Ellman's method [25a, 62]. AChI and 5,5'-dithio-bis(2-nitro-benzoic)acid (DTNB) were used as substrate for this enzymatic reaction. To this end, 100 µL of Tris/HCl buffer (1 M, pH 8.0) and 10 µL of novel bromophenols solution at different concentrations and 50 µL AChE ($5.32x10^{-3}$ U) solution were mixed and incubated for 10 min at 25°C. Then 50 µL of DTNB (0.5 mM) was transferred. Then the reaction was initiated by the addition of 50 µL of AChI. The hydrolysis of AChI was recorded 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.

Declaration of interest

The authors report there is no conflict of interests.

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TABLES, FIGURES AND SCHEMES

Table 1. Determination of reducing power of same concentration (20 µg/mL) of novel bromophenols and their methylated precursors 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
BHA	2.354±0.003	0.9575	1.962±0.002	0.9999	1.869±0.001	0.9806
BHT	1.405±0.003	0.9630	1.407±0.004	0.9972	1.328±0.004	0.9739
α-Tocopherol	0.927±0.008	0.9819	0.854±0.003	0.9998	1.123±0.002	0.9986
Trolox	0.893±0.002	0.9930	1.005±0.002	0.9992	1.742±0.002	0.9997
10	0.136±0.003	0.9570	0.117±0.005	0.9438	0.387±0.002	0.9369
11	0.135±0.001	0.9589	0.083±0.002	0.9926	0.401±0.001	0.9510
14	0.145±0.004	0.9540	0.225±0.003	0.9748	0.389±0.001	0.9493
15	0.156±0.013	0.9976	0.074±0.003	0.9970	0.406±0.002	0.9427
16	0.182±0.002	0.9805	0.057±0.003	0.9913	0.404±0.003	0.9410
18	0.152±0.002	0.9990	0.086±0.002	0.9587	0.382±0.001	0.9373
19	0.252±0.002	0.9898	0.077±0.002	0.9896	0.421±0.001	0.9422
20	2.094±0.004	0.9711	1.602±0.001	0.9430	2.012±0.001	0.9591
21	2.123±0.011	0.9999	2.014±0.004	0.9984	1.986±0.004	0.9916
22	1.947±0.003	0.9757	1.966±0.002	0.9707	1.974±0.005	0.9546
23	2.563±0.003	0.9895	2.018±0.005	0.9997	2.093±0.004	0.9709
24	1.163±0.002	0.9700	0.655±0.003	0.9692	0.401±0.002	0.9404

[a] expressed as absorbance values.

Table 2. Determination of half maximal concentrations (IC_{50}) of novel bromophenols, their methylated precursors and standards for DPPH· and $ABTS^{\bullet+}$ radical scavenging and AChE inhibition assays

Antioxidant Compounds	AChE inhibition* IC_{50}	K_i	DPPH· scavenging§	$ABTS^{\bullet+}$ scavenging§
BHA	-	-	22.35	2.72
BHT	-	-	34.65	2.88
α-Tocopherol	-	-	21.65	4.81
Trolox	-	-	16.11	2.86
10	4.41	3.53	346.50	229.63
11	4.20	4.34	227.36	138.60
14	7.21	4.25	230.34	138.60
15	4.22	3.77	224.53	173.25
16	4.68	5.28	231.00	173.25
18	5.25	5.88	226.67	138.60
19	4.05	3.65	221.37	224.60
20	4.71	4.06	15.75	2.55
21	4.84	3.75	15.40	2.58
22	4.58	2.78	14.74	2.61
23	4.88	3.24	14.15	2.23
24	4.81	5.72	28.92	22.35
Tacrine#	7.53	7.64	-	-

*: They were determined as nM

§: They were determined as µg/mL

#: Tacrine was used as positive control for AChE inhibition

FIGURES LEGENDS

Figure 1. Some selected natural products **1**-**6**

Figure 2. Determination of the half maximal inhibitory concentration (IC_{50}) value (**A**) and inhibition constant (Ki) value (**B**) of novel bromophenol **22** for acetylcholinesterase enzyme (AChE) by using a Lineweaver-Burk graph

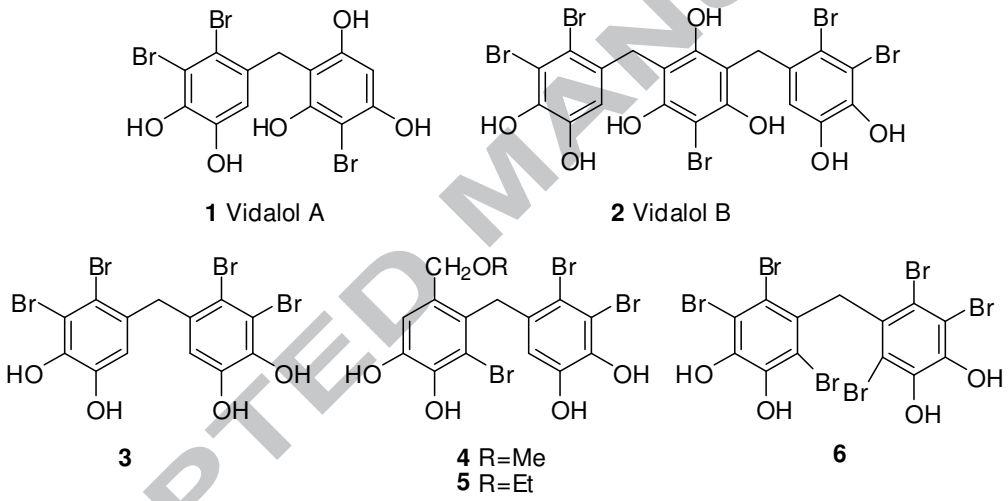
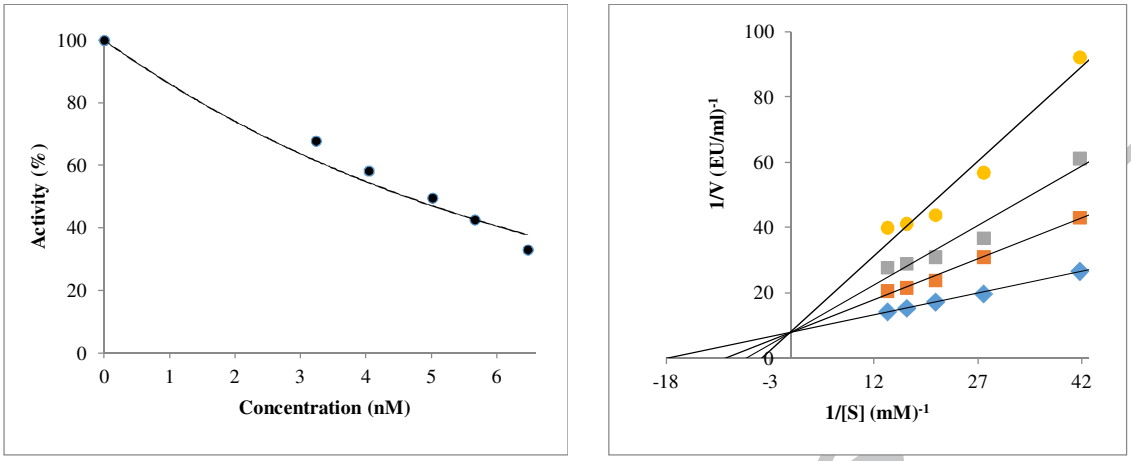


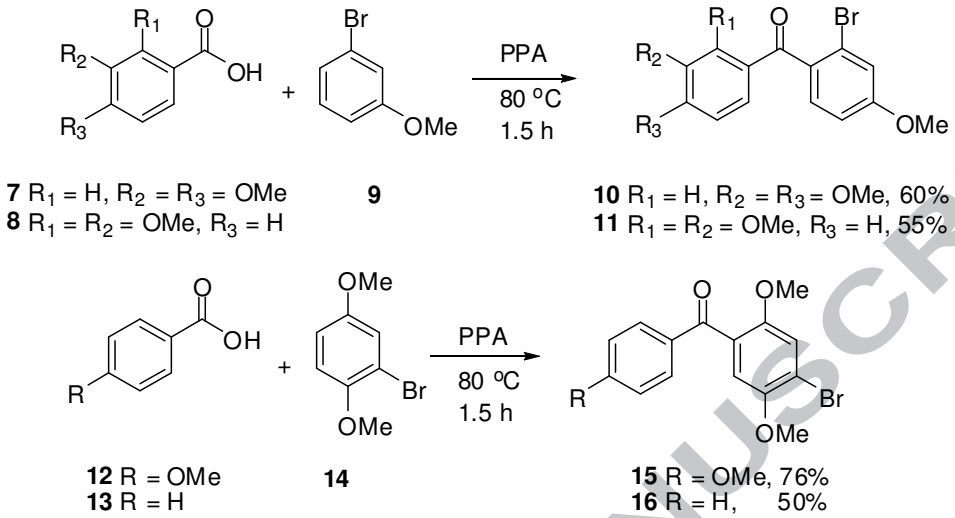
Figure 1



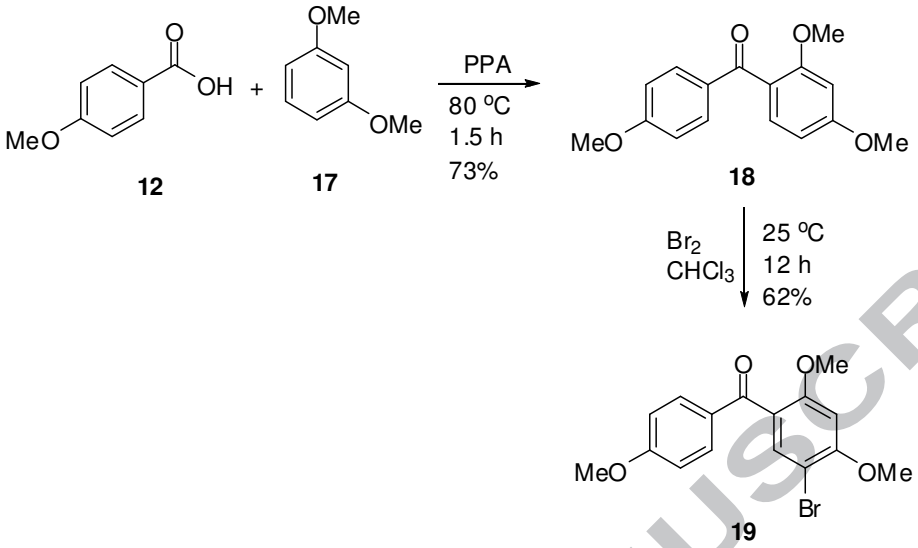
A B

Figure 2

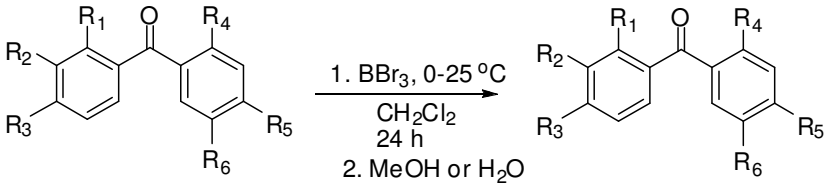
SCHEMES



Scheme 1. The synthesis of compounds **10**, **11**, **15** and **16**



Scheme 2. The synthesis of compound **19**



10 $R_1=R_6=H$, $R_4=Br$, $R_2=R_3=R_5=OMe$
11 $R_3=R_6=H$, $R_4=Br$, $R_1=R_2=R_5=OMe$
15 $R_1=R_2=H$, $R_5=Br$, $R_3=R_4=R_6=OMe$
16 $R_1=R_2=R_3=H$, $R_5=Br$, $R_4=R_6=OMe$
19 $R_1=R_2=H$, $R_6=Br$, $R_3=R_4=R_5=OMe$

20 $R_1=R_6=H$, $R_4=Br$, $R_2=R_3=R_5=OH$, 91%
21 $R_3=R_6=H$, $R_4=Br$, $R_1=R_2=R_5=OH$, 80%
22 $R_1=R_2=H$, $R_5=Br$, $R_3=R_4=R_6=OH$, 92%
23 $R_1=R_2=R_3=H$, $R_5=Br$, $R_4=R_6=OH$, 93%
24 $R_1=R_2=H$, $R_6=Br$, $R_3=R_4=R_5=OH$, 83%

Scheme 3. The synthesis of new bromophenols **20-24**

HIGHLIHTS

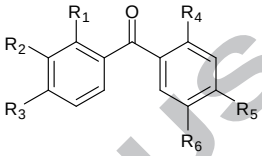
- Series of novel bromophenol derivatives were synthesized.
- Ferric ions (Fe^{3+}) and cupric ions (Cu^{2+}) reducing capacities were investigated.
- The synthesized compounds showed DPPH· and $ABTS^{\bullet+}$ scavenging activities.
- The bromophenols were found to be the most potent antioxidants.
- All compounds demonstrated low nanomolar inhibition against acetylcholinesterase.

Graphical Abstract

Antioxidant and Acetylcholinesterase Inhibition Properties of Novel Bromophenol Derivatives

Necla Öztaşkın, Yasin Çetinkaya, Parham Taslimi, Süleyman Göksu*, İlhami Gülçin*

A series of novel bromophenol derivatives were synthesized and evaluated for their antioxidant and acetylcholine esterase inhibitory properties. While all synthesized compounds showed potent acetylcholinesterase inhibition effects, bromophenols showed strong antioxidant properties.



R_1=R_6=H, R_4=Br, R_2=R_3 =R_5=OMe
R_3=R_6=H, R_4=Br, R_1=R_2=R_5=OMe
R_1=R_2= H, R_5=Br, R_3=R_4=R_6=OMe
R_1=R_2=R_3=H, R_5=Br, R_4=R_6=OMe
R_1=R_2=H, R_6=Br, R_3=R_4=R_5=OMe
R_1=R_6=H, R_4=Br, R_2=R_3 =R_5=OH
R_3=R_6=H, R_4=Br, R_1=R_2=R_5=OH
R_1=R_2= H, R_5=Br, R_3=R_4=R_6=OH
R_1=R_2=R_3=H, R_5=Br, R_4=R_6=OH
R_1=R_2=H, R_6=Br, R_3=R_4=R_5=OH