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Synthesis and characterization of novel bromophenols: Determination of their anticholinergic, antidiabetic and antioxidant activities

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ABSTRACT

In this paper, a series of novel bromophenol derivatives were synthesized and evaluated for their acetylcholinesterase and α-glycosidase enzymes inhibition properties and antioxidant activity. Diarylmethanones were synthesized and their bromination was carried out. During bromination, some compounds gave new bromophenols via regioselective O-demethylation. Demethylation of brominated diarylmethanones was also performed with BBr_3 to give novel bromophenols. In addition, we examines the antioxidant capacity of novel bromophenol derivatives using several in vitro bioanalytical methodologies such as 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\cdot +}$) and 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH·) radical scavenging activity, Fe^{3+} and Cu^{2+} reducing activities and ferrous (Fe^{2+}) ions chelating activities. Also, novel bromophenols and methoxylated bromophenols derivatives were tested against acetylcholinesterase and α-glycosidase, which associated with some metabolic diseases. The novel bromophenols showed Ki values in range of 8.94 ± 0.73–59.45 ± 14.97 nM against AChE and 4.31 ± 1.93–44.14 ± 2.19 nM against α-glycosidase.

1. Introduction

Natural products are playing important role in design and the development of new drugs. Natural products and their semi-synthetic derivatives are important source of medicine and there are a large number of natural compounds used as drugs today [1]. Bromophenols, secondary metabolites of marine organisms, are also natural organic compounds [2]. Most of these compounds are generally isolated from marine red algae [3], brown algae [4], and green algae [5]. Compounds **1**–**5** are examples of naturally occurring bromophenols and they show various biological activities such as radical scavenging [6], antibacterial [3], anti-cancer [7], feeding deterrent [8], antidiabetic [9], aldose reductase inhibition [10], and tyrosine phosphatase properties [11] (Fig. 1).

Because of the diverse biological activities of bromophenols, in the last decade, our research group has also focused on the total synthesis of naturally occurring bromophenols and their synthetic derivatives. In these studies we reported an alternative synthesis of **1** [12] and the first total synthesis of **2–5** [13–15]. Together with the natural products, some phenolic brominated benzophenones were also synthesized. We well know from our early studies that diarylmethanones are also potent enzyme inhibitors as natural bromophenol derivatives. The acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and carbonic anhydrase inhibitory properties of **6–9** were reported in this journal [15] (Fig. 1). In addition, antioxidant activity [12], AChE [16], paraoxonase [17], aldose reductase, α-amylase and α-glycosidase [18] inhibitory properties of some bromophenols have been addressed by our research group. In continuation of our studies, here we report the synthesis, antioxidant activity, acetylcholinesterase and α-glycosidase inhibition properties of some novel bromophenols incorporating diarylmethanone skeleton.

Oxidation is an essential biological process to many living organisms a result of normal cellular metabolism for the production of energy [19,20]. Reactive oxygen species (ROS) can occur in the human body and have a strong effect of inducing lipid peroxidation (LP), which in turn induces oxidative imbalance and causes various diseases. LP is a major deteriorative process in pharmaceuticals and foods. ROS can cause a myriad of diseases such as ageing, cardiovascular ailments, cancer and deficiencies of immune system [21–23]. They are easily formed in the living metabolism and neutralized by antioxidant defenses. Also, excessive amount of ROS can damage cellular components including DNA, membrane lipids, proteins and monosaccharides, inducing many diseases including aging and cancer atherosclerosis, pulmonary dysfunction, skin lesions and inflammatory disorders [24–26]. On the other hand, antioxidants can block formation of ROS and free radical reactions by donating an electron or a hydrogen atom to

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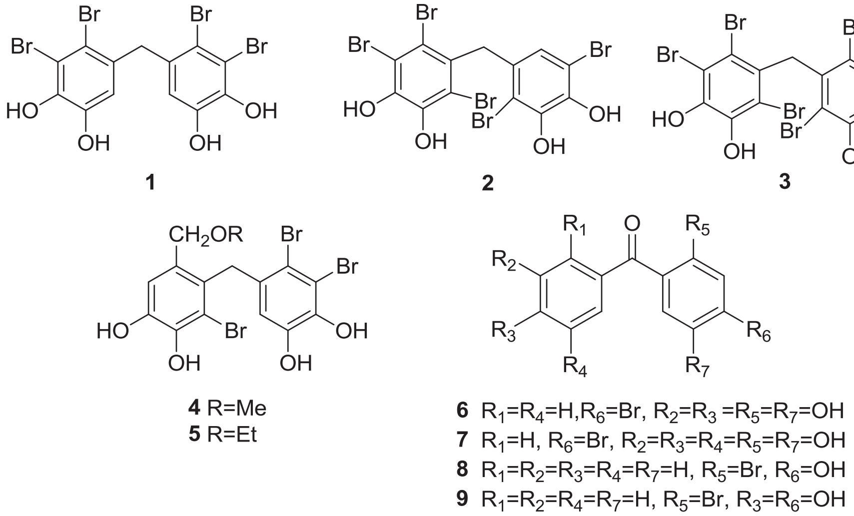


Fig. 1. Biologically active natural and synthetic bromophenols.

reactive species and free radicals [27]. Antioxidants can retard the production of toxic oxidation compound, and increase the shelf life of food products [28,29]. The phenolic compounds are one of the most abundant secondary metabolites and well-studied antioxidants in vitro and in vivo [30–32]. They are major groups of secondary metabolites, and their biosynthetic precursors are metabolites of the shikimate, pentose phosphate and phenylpropanoid pathways [33]. Thus, there was a need to identify the alternative and safer antioxidants. Recently, these demands have increased significantly [34].

Alzheimer's disease (AD) affects mostly the aged people and above resulting in impaired memory and behavior. This disorder clinically involves the progressive degeneration of brain tissue that is influenced by the deficit in acetylcholine (ACh) [35–37]. AChE, a major element of the cholinergic system in the peripheral and central nervous system (CNS), is able to convert acetylcholine (ACh) to acetate and choline (Ch) [38–40]. It is caused majorly by environmental and genetic influences. Cerebral amyloid-β aggregation, a deficit in ACh and a deficit in cholinergic neurotransmission, was observed in patients with AD [41–43]. Because of serious side effects of the available AChE, there is the need to search for newer effective and safe AChE to treat neurodegenerative damages. AChE inhibitors (AChEIs) or anti-cholinesterases inhibit cholinesterase, increasing the level and length of ACh action. A variety of usages of AChEIs are common in medicine. As a result, a number of AChEIs have been thought for the treatment of AD. They have been used in clinical trials, including natural substances. It was reported that terpenoids, flavonoids and phenolic compounds have been recognized as AChEIs and promising lead compounds for AD [41,44].

α-Glycosidase enzyme (E.C.3.2.1.20) release from intestine cells and hydrolyzes oligosaccharides and polysaccharide to monosaccharide units, such as glucose and fructose in small intestine. In human, α-glycosidase inhibitors (α-GIs) had a great importance for controlling of type-2 diabetes (T2DM) and hyperglycemia [45]. Recently, two main chemical classes of *N*-comprising α-GIs contain carbasugar-based inhibitors like acarbose and voglibose and iminosugar-based inhibitors like miglitol have been developed [46]. α-GIs can reduce the uptake of dietary carbohydrates and repress postprandial hyperglycemia and T2DM. Thus, these α-GIs are endowed with sugar molecule such as compete and moieties with the oligosaccharides for binding to the active site of the enzyme, hence effectively reducing the postprandial glucose amounts in T2DM [47]. On the other hand, bromophenols as natural compounds are secondary plant and marine organisms metabolites and consist of brominated and hydroxylated benzyl unit. There is a large number recent study on the synthesis, biological activities and isolation of bromophenols. Also, their antioxidant activities and AChE, BChE and hCA inhibitory effectiveness were well established [47–49].

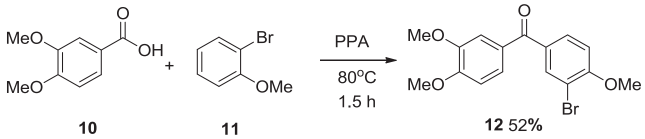
In this regards, we synthesized novel bromophenols **16**, **17**, **20** and **21** and methoxylated bromophenols derivatives **12**, **18** and **19.** We also verified the antioxidant activities of the resultant compounds using fife different bioanalytical methods, namely, ferrous ions (Fe^{2+}) chelating, cupric ions (Cu^{2+}) and ferric ions (Fe^{3+}) reducing antioxidant power, 1,1- diphenyl-2-picrylhydrazyl (DPPH·) and the 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{+}$) radicals scavenging assays. These novel compounds were tested against acetylcholinesterase, and α-glycosidase as crucial metabolic enzymes either. Furthermore, another goal of this study is to demonstrate antioxidant activities of novel bromophenols and methoxylated bromophenols derivatives as compared to putative commercial and standard compounds.

2. Results and discussion

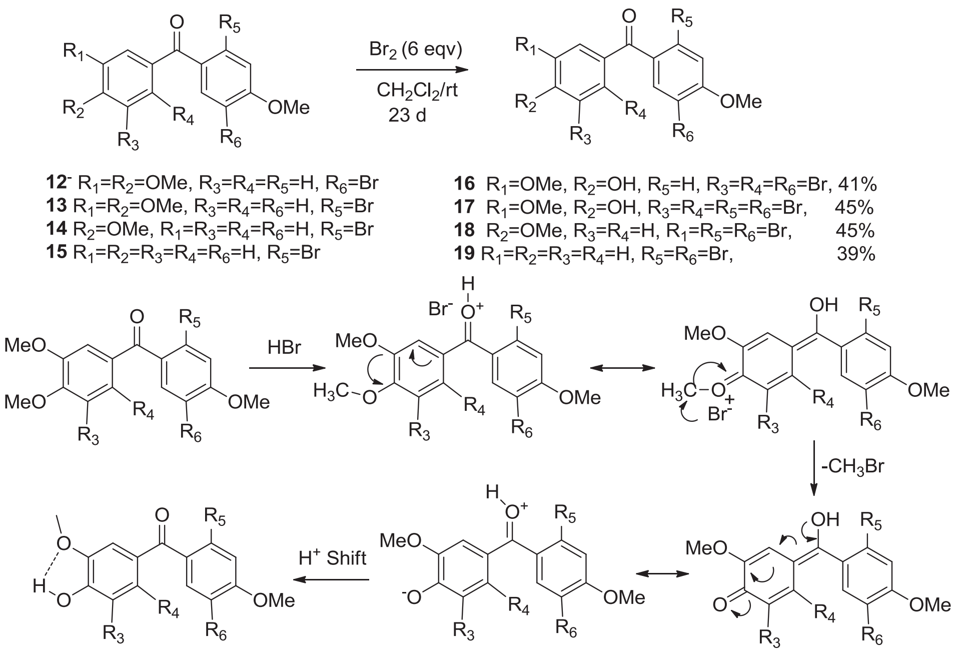
2.1. Chemistry

Benzene derivatives are easily giving acylation reaction with benzoic acid derivatives in the presence of polyphosphoric acid (PPA) [15]. The synthesis of diarylmethanone **12** was accomplished by a similar approach. Therefore, the reaction of 2-bromoanisole (**11**) with 3,4-dimethoxybenzoic acid (**10**) in the presence of PPA newly prepared from conc. H_3PO_4 and P_2O_5 gave compound **12** in a moderate yield (Scheme 1). Compounds **13–15** were synthesized as described above and as reported in a previous paper [15].

The bromination of diarylmethanones **12–15** with 6 equiv. Br_2 in CH_2Cl_2 at rt. for 23 days afforded **16–19** with the yields of 41, 45, 45 and 39% respectively. As seen in the structures of compounds **16** and **17**, one OMe shown as R_2 at each compounds have been converted into OH groups by a regioselective O-demethylation. This is due to the occurrence of HBr during the bromination reaction. Because of the closed flask, the HBr is collected at the reaction ambient and this cause to demethylation (Scheme 2). When the literature checked carefully, it can be seen that these kinds of reactions are possible. Regioselective O-



Scheme 1. Synthesis of compound **12.**



12 $R_1=R_2=OMe, R_3=R_4=R_5=H, R_6=Br$
13 $R_1=R_2=OMe, R_3=R_4=R_6=H, R_5=Br$
14 $R_2=OMe, R_1=R_3=R_4=R_6=H, R_5=Br$
15 $R_1=R_2=R_3=R_4=R_6=H, R_5=Br$

16 $R_1=OMe, R_2=OH, R_5=H, R_3=R_4=R_6=Br$, 41%
17 $R_1=OMe, R_2=OH, R_3=R_4=R_5=R_6=Br$, 45%
18 $R_2=OMe, R_3=R_4=H, R_1=R_5=R_6=Br$, 45%
19 $R_1=R_2=R_3=R_4=H, R_5=R_6=Br$, 39%

Scheme 2. Synthesis of **16–19** and the possible reaction mechanism for the occurrence of **16** and **17**.

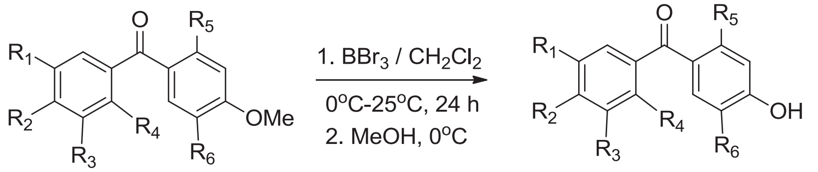
demethylation of 3,4-dimethoxy substituted benzene derivatives including ketone groups with Lewis acids easily take place from 4-position [50]. Cleavage of OMe at C-4 most probably proceeds due to carbonyl group. Lewis acid or protons of HX are readily bound to carbonyl oxygen to give an intermediate as shown at Scheme 2. Positive charge on carbonyl group especially affects C-4 of benzene ring on which there is an OMe group. Thus, O-Me bond is weakening and easily cleavage by attacking of bromide ion. We suppose that OMe group at C-3 has supporting effect thermodynamically because of the formation of an internal hydrogen bond between hydrogen and methoxy groups (O−⊖ H···OMe) (Scheme 2).

On the other hand, transformation of the functional groups to phenolic derivatives is very important on the synthesis of biologically active compounds. There are some excellent procedures described for O-demethylation of arylmethyl ethers to phenols by using BBr_3 [14], $AlCl_3$ [50] or HBr [51]. In some of our early studies we found out that BBr_3 is working very well as a reagent for O-demethylation of arylmethyl ethers. Therefore, demethylation of compounds **12** and **19** was carried out by using BBr_3. In this context, the reactions of diarylmethanones **12** and **19** with BBr_3 under N_2 at 0–25 °C for 24 h were performed. Then, MeOH was added to reaction mixture to react with excess BBr_3 and to give compounds **20** and **21** with high yields (Scheme 3).

The structures of compounds **17** and **18** were performed by X-ray crystallographic analysis. The structure of **16** was assigned by comparison of its ^1H- and ^{13}C NMR with that of **17**. When the structures of compounds **16** and **17** are checked carefully, it is very clear that each of the compounds contains same 3-methoxy, 4-hydroxy and 5,6-dibromo groups in one phenyl ring. Therefore, H_1 of compound **16** resonates as a singlet at 7.00 ppm. Similarly H_1 of **17** gives singlet at 6.82 ppm. Again, dimethoxy groups of **16** resonate at 3.98 and 3.91 ppm as singlets and dimethoxy of **17** resonates as singlets at 3.96 and 3.93 ppm. ^{13}C NMR spectrum of the two compounds is in agreement with the demethylated and dibrominated one phenyl rings in each compound. While C_1 of **16** resonates at 111.2 ppm, C_1 of **17** resonates at 111.7 ppm. In a similar way, ^{13}C NMR analysis of compound **16** shows two signals of OCH_3 at 56.6 and 56.5 ppm. Signals of two OCH_3 groups belonging to **17** resonate at 56.8 and 56.7 ppm. On the other hand, in the structures of starting compound **12** and its derivative **16**, one phenyl ring did not change during the reaction. Therefore, H_6, H_7 and H_{10} of compounds **12** and **16** give the same chemical shift. H_{10} of compounds **12** and **16** give d at 8.04 and 8.05 ppm respectively ($J = 2.1$ Hz). H_6 and H_7 protons at each compound give AB system. H_6 of **12** resonates at 7.77 ppm as dd ($J = 8.6$, 2.1 Hz), and H_6 of **16** also gives dd at 7.72 ppm ($J = 8.6$, 2.1 Hz). While H_7 of **12** resonates at 6.97 ppm as d ($J = 8.6$ Hz), H_7 of **16** gives d at 6.93 ppm ($J = 8.6$ Hz). In the ^{13}C NMR spectrum of the compounds **12** and **16,** same phenyl rings show similar chemical shifts. Therefore, all findings support the proposed structure of **16** as shown in Fig. 2.

In addition, the structure of **19** was also designated by ^1H and ^{13}C NMR analysis. H_2 and H_5 of **19** give singlets at 7.56 and 7.14 ppm. When the ^{13}C NMR of **19** was carefully checked, it can be seen that there are five quaternary carbons at 157.5, 136.3, 133.8, 119.9 and 110.7 ppm. This data support the formation of **19** from **15** by a substitution with Br_2 (Fig. 2).

X-ray analysis of (2,3-dibromo-4-hydroxy-5-methoxyphenyl)(2,5-



12 $R_1=R_2=OMe, R_3=R_4=R_5=H, R_6=Br$
19 $R_1=R_2=R_3=R_4=H, R_5=R_6=Br$

20 $R_1=R_2=OH, R_3=R_4=R_5=H, R_6=Br$, 94%
21 $R_1=R_2=R_3=R_4=H, R_5=R_6=Br$, 95%

Scheme 3. The synthesis of bromophenols **20** and **21**.

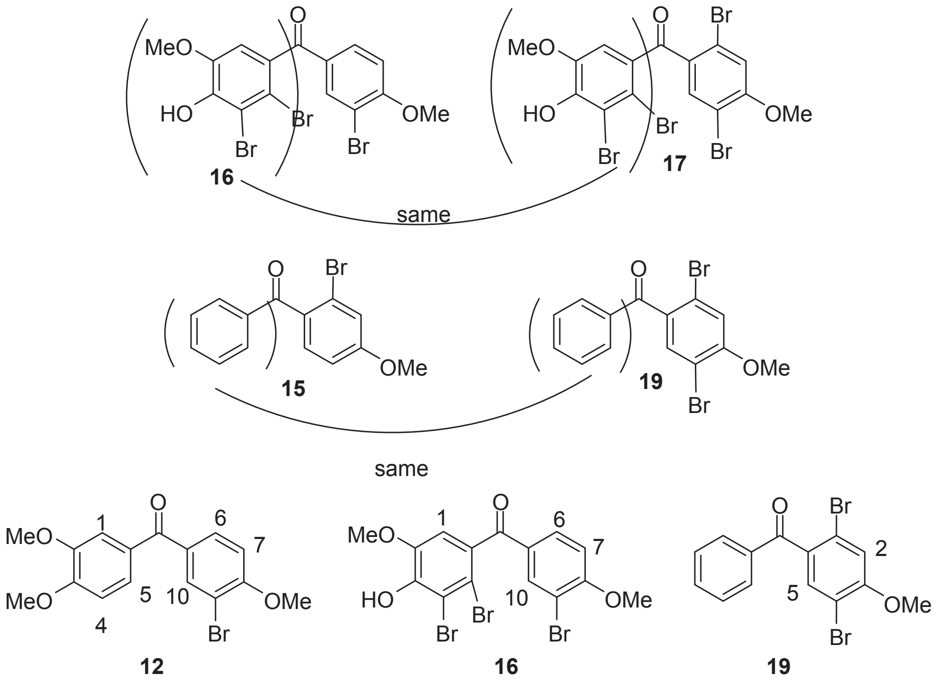


Fig. 2. The compression of the structures and numbering of H for compounds.

dibromo-4-methoxyphenyl)methanone (**17**) and (3-bromo-4-methoxyphenyl)(2,5-dibromo-4-methoxyphenyl)methanone (**18**) was performed to verify the structures, configurations and to see the possible intra and intermolecular interactions. Tetramer and dimer units with the atom labeling and the stacking of the molecules with the unit cells are shown in Figs. 3 and 4. Bromophenols **17** and **18** crystallize in the centrocymetric $P2_1/n$ (monoclinic) and P-1 (triclinic) space groups, respectively. Compounds have isomorphous crystal structures, despite one having an extra bromine and OH group, namely geometries of the molecules are very similar. C-Br distances are in the range of 1.869(3)-1.902(3) Å. C=O (carbonyl) distances are 1.208(3) and 1.238(3) Å for the structure **17** and **18,** respectively and both have double bond character. The halogen bond is similar to the hydrogen bond, which had great importance noncovalent interaction and plays important roles in diverse chemistry-related fields. There are bromine-based halogen-bonding interactions between two bromophenol derivatives. Br···O halogen interactions (sum of van der Waals radii = rO + rBr = 3.35 Å.) are responsible for the rearrangement of tetramer and dimer units in crystals (Figs. 3 and 4). Br2···O1 [3.229(3) Å] and Br1···O4 [3.140(3) Å] distances are significantly smaller than 3.35 Å. Also, it should be noted that there is a significant O4-H···O3 [3.140(3) Å] hydrogen bond, which constitutes the tetramer structure.

2.2. Antioxidant activities

It is well known that phenol and especially bromophenol compounds are biological active components [52–56]. They are also useful for chemical purposes for example such as precursors in synthetic and organic chemistry [57,58]. The antioxidant properties of novel bromophenols and methoxylated bromophenols derivatives were determined by different bioanalytical assays. The ferric ions (Fe^{3+}) reducing ability of novel bromophenol derivatives recorded by reduction of Fe^{3+} to Fe^{2+}, which resulted from the occurrence of the Perl's Prussian blue complex [59,60]. This method is relying on the reducing of ferricyanide excess relative to the antioxidants [61,62]. The $Fe^{3+}(CN^{-})_6$ reducing method measures the antioxidant ability of ordinary antioxidant molecule. Novel synthesized bromophenols and their methoxylated derivatives had the most influential reducing effects using ferric ions reducing capacity when classed with the putative standards (BHA, trolox, BHT, and α-tocopherol). Also, as seen in Fig. 5A and Table 1, same concentration (30 µg/mL) of novel synthesized bromophenols and their methoxylated derivatives showed effective Fe^{3+} reducing capability, which had absorbance in ranging of 0.152–1.471. These diversities were statistically found as important ($p < 0.01$). On the other hand, absorbance values for Fe^{3+} reducing capacity of standard antioxidant found as 0.980 for trolox (r^2: 0.9943), 1.054 for α-tocopherol (r^2: 0.9987), 1.671 for BHA (r^2: 0.9967), and 1.185 for BHT (r^2: 0.9976) at the concentration of 30 µg/mL. The results obtained from this assay proved that (3-bromo-4-hydroxyphenyl)(3,4-dihydroxyphenyl)methanone (**20**) had effective Fe^{3+} reducing capability (1.471, r^2: 0.9943) when compared to standard antioxidants. Also, this compound (**20**) had electron-donating properties for neutralizing ROS and free radicals by creating stable products Table 2.

In order to determine the cupric ions (Cu^{2+}) reducing ability of novel bromophenols and methoxylated bromophenols derivatives, the previous proposed method was used [63,64]. Cu^{2+} reducing power of cynarin and positive controls is shown in Fig. 5B and Table 1. A positive relationship was found between Cu^{2+} reducing power and different concentrations of novel bromophenols and methoxylated bromophenols derivatives. It was determined that cupric ions reducing capacity of novel bromophenols and methoxylated bromophenols derivatives was addicted to the concentration (10–30 µg/mL) and found between absorbance of 0.332–0.845. On the other hand, the absorbance values of Cu^{2+} reducing capability of standard antioxidants at the indicated concentration range (30 mg/mL) demonstrated the following order: BHA > BHT > α-Tocopherol > Trolox.

The radical scavenging capacities of novel bromophenols and methoxylated bromophenols derivatives were determined using the DPPH· and ABTS·$^+$ radicals scavenging methods [65]. The DPPH· solution had a deep violet color and can be detected spectrophotometrically at 517 nm. In this assay, when an electron or a hydrogen atom was transferred to DPPH·, the quantity of absorbance (517 nm) reduced proportional to the DPPH [66]. This free radical scavenging test is generally used for recording of antioxidant capacities [67]. It is based on the reduction of DPPH· in alcoholic medium in the source of hydrogen donating antioxidant capacity, because of

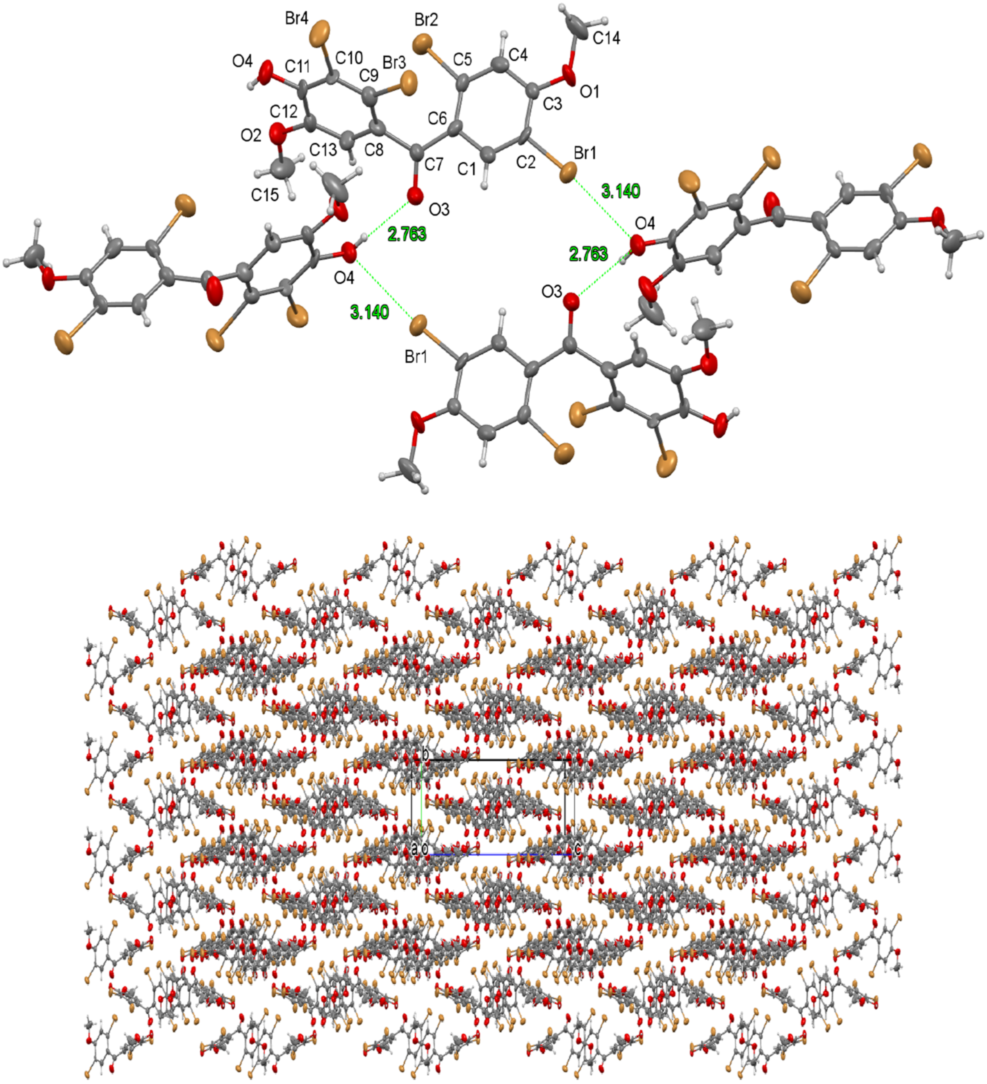


Fig. 3. (Up) Molecular structure of bromophenol (**17**) with the tetramer building. Thermal ellipsoids are drawn at the 40% probability level. (Down) Stacking of the tetramer units with the unit cell along the *a*-axis.

occurrence of $DPPH_2$ [68]. Fig. 5A defines a crucial decrement ($p < 0.01$) in the concentration of DPPH radical owing to the scavenging capability of novel bromophenols and methoxylated bromophenols derivatives and references like trolox, α-tocopherol, BHA, and BHT. As seen in Table 1and Fig. 5C, IC_{50} values were found between 23.10 and 34.65 μg/mL. On the other hand, IC_{50} values were calculated as 13.86 μg/mL for trolox (r^2: 0.9890), 9.93 μg/mL for α-tocopherol (r^2:0.9891), 9.90 μg/mL for BHA (r^2:0.9700), and 10.66 μg/mL for BHT (r^2:0.9680). A lower IC_{50} value indicates a higher DPPH$\cdot$ scavenging effect.

In ABTS$\cdot^{+}$ scavenging assay, a radical scavenger is added to a preformed ABTS$\cdot^{+}$ solution, and after a period of time the remaining ABTS $\cdot^{+}$ is spectrophotometrically recorded at 734 nm in a spectrophotometer [69,70]. As shown in Fig. 5D and Table 1, novel bromophenols and methoxylated bromophenols derivatives had powerful ABTS$\cdot^{+}$ scavenging effects in a quantity-dependent manner (10–30 mg/mL). IC_{50} values for novel bromophenols and methoxylated bromophenols derivatives were found in ranging of 69.3 and 231.0 μg/mL. It is seen that novel bromophenols and methoxylated bromophenols derivatives had weaker ABTS$\cdot^{+}$ scavenging effects when compared with trolox (17.33 μg/mL, r^2: 0.9720) and α-tocopherol (13.86 μg/mL, r^2: 0.9900), BHA (6.86 μg/mL, r^2: 0.9630) and BHT (11.55 μg/mL, r^2: 0.9350). As can be in DPPH$\cdot$ method, a lower EC_{50} value indicates a higher ABTS radical scavenging efficiency [71].

Metal binding activity of novel bromophenols and methoxylated bromophenols derivatives was recorded by blocking the occurrence of Fe^{2+}-2,2′-Bipyrdyl complex after addition of the novel bromophenols and methoxylated bromophenols derivatives with Fe^{2+} [72]. Finally, novel bromophenols and methoxylated bromophenols derivatives showed effective Fe^{2+} chelating efficiency. The difference between novel bromophenols and methoxylated bromophenols derivatives (10–30 μg/mL) and the control sample was statistically found as important ($p < 0.01$). Also, IC_{50} values for novel bromophenols and methoxylated bromophenols derivatives are in the ranging of 6.93–17.33 μg/mL (Fig. 5E, Table 1). The most metal chelating effects was found in (2,5-dibromo-4-hydroxyphenyl)(phenyl)methanone (**21**) (6.93–17.33 μg/mL, r^2: 0.9460), which possessed dibromide (Br), carbonyl (C=O) and phenolic hydroxyl (OH) groups. It was proved that the substance including some functional groups like C=O and C–OH can easily chelate ferrous (Fe^{2+}) ions. Also, the compounds, which possess two or more of functional groups of –O–, –S–, –OH, –SH, $-NR_2$, C=O, $-H_2PO_3$ and –COOH had structure-function relationship [72–74]. IC_{50} values belong to metal chelating effects of standards like trolox, α-tocopherol, BHT, and BHA were calculated as 17.3, 23.1, 69.3 and 34.7 μg/mL, respectively. A lower IC_{50} value shows a higher Fe^{2+} binding affinity. The results obtained this assay clearly demonstrated

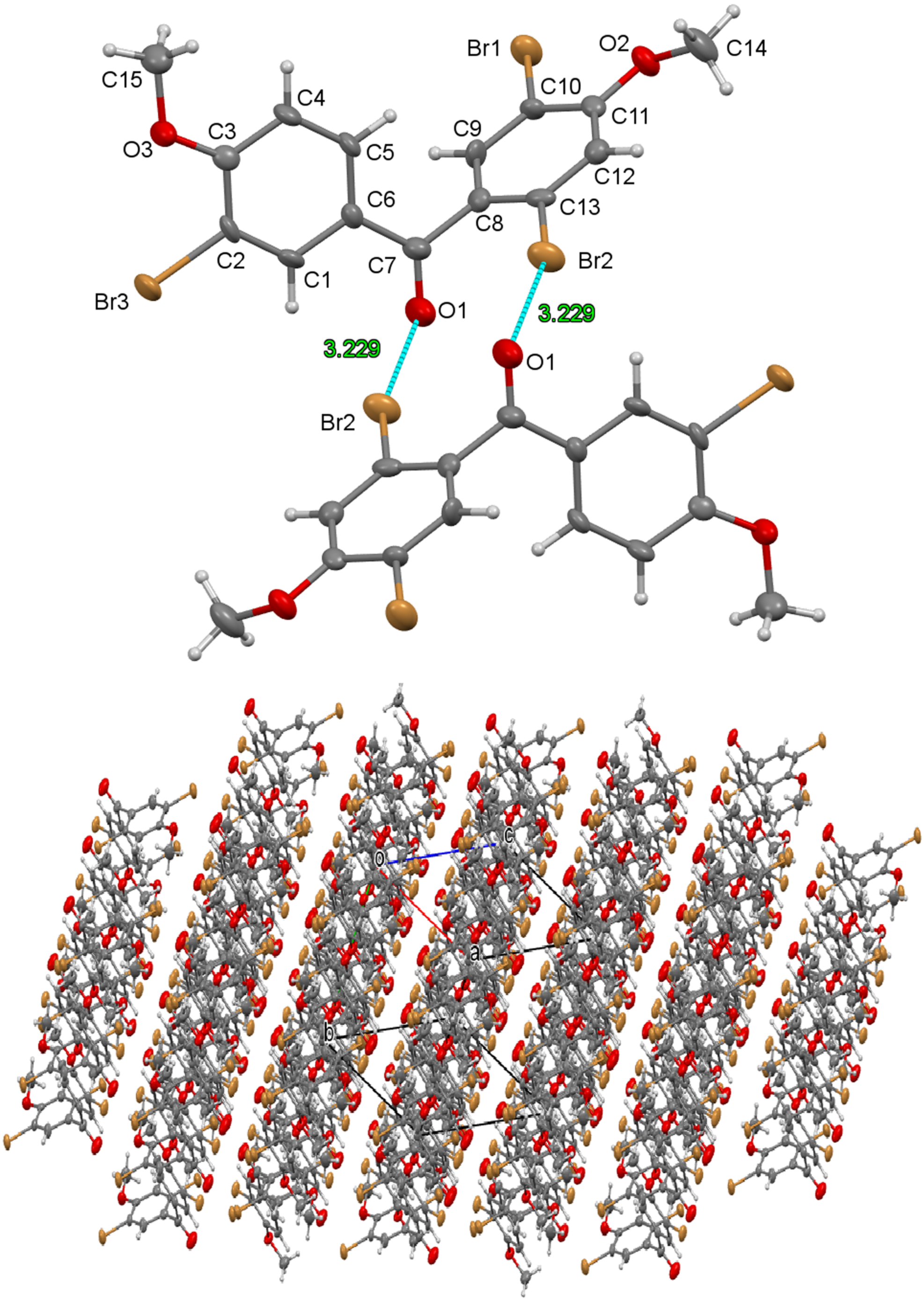


Fig. 4. (Up) Molecular structure of bromophenol **18** with the dimeric unit. Thermal ellipsoids are drawn at the 40% probability level. (Down) Stacking of the dimers with the unit cell viewed down along the diagonal axis.

that metal binding effect of novel bromophenols and methoxylated bromophenols derivatives was better than that of standard antioxidant compounds [75–77].

2.3. Enzymes inhibition studies

Another aim of biochemical estimation is the AChE and α-glycosidase inhibition effects of novel bromophenols **16**, **17**, **20** and **21** and methoxylated bromophenols derivatives **12**, **18** and **19**. AChE inhibition properties were recorded according to the procedure of Ellman et al. [78] and described previously [79]. Novel bromophenols incorporating diarylmethanone skeleton had Ki values in ranging from 8.94 ± 0.73 to 59.45 ± 14.97 nM for AChE. On the other hand, tacrine had Ki value of 5.99 ± 1.79 nM toward cholinergic AChE. All evaluated novel bromophenols and methoxylated bromophenols derivatives showed effective inhibition against AChE enzyme, but (2,5-dibromo-4-methoxyphenyl)(phenyl)methanone (**19**), which posses dibromide (-Br) in *para-* position and one methoxy (–OCH_3), showed perfect inhibition effect against AChE (Ki values of 8.94 ± 0.73 nM), followed by (2,3-dibromo-4-hydroxy-5-methoxyphenyl)(2,5-dibromo-4-methoxyphenyl) methanone (**17**) with Ki values of 14.23 ± 1.99 nM. In the same context, (3-bromo-4-hydroxyphenyl)(3,4-dihydroxyphenyl) methanone (**20**) led to weaker AChE inhibition in this study with remarkable decrease in activity (Ki: 59.45 ± 14.97 nM). The order of

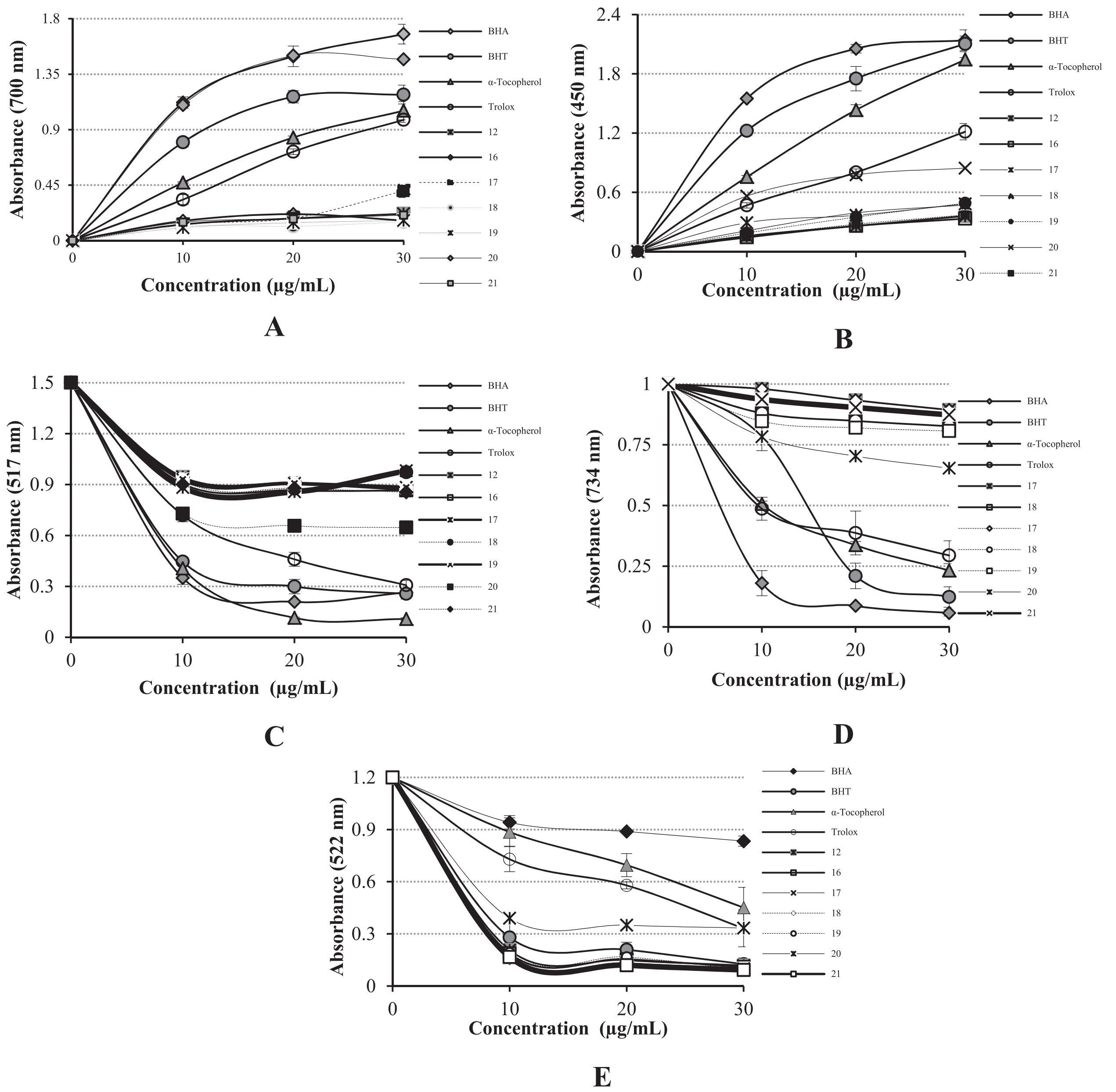


Fig. 5. Determination of antioxidant activity of novel bromophenols and their methoxylated bromophenols derivatives. **A.** Ferric ions (Fe^{3+}) reducing activity, **B.** Cupric ions (Cu^{2+}) reducing capacity by CUPRAC method, **C.** 1,1-Diphenyl-2-picryl-hydrazyl free radical (DPPH·) activity, **D.** 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS\cdot^{+}$) radical scavenging activity, **E.** Ferrous ions (Fe^{2+}) chelating activities.

activities of the novel bromophenols and methoxylated bromophenols derivatives was decreased in the order of Tacrine > **19** > **17** > **18** > **21** > **16** > **12** > **20**.

On the other hand, for the α-glycosidase, the novel bromophenols and methoxylated bromophenols derivatives had IC_{50} and Ki values ranging between 8.73 and 24.93 and 4.31 ± 1.93–44.14 ± 2.19 nM, respectively (Table 1). The results obtained from α-glycosidase assay showed that all novel bromophenols and methoxylated bromophenols derivatives had effective α-glycosidase inhibition profiles than that of acarbose (IC_{50}: 22.800 mM) as standard α-GI. Also, highly effective Ki values were obtained (2,3-dibromo-4-hydroxy-5-methoxyphenyl)(2,5-dibromo-4-methoxyphenyl) methanone (**17**) with Ki values of 4.31 ± 1.93 nM. The ClogP of the most active α-glycosidase inhibitor is 5.37 and its molecular weight is 574 g/mol. Thus, it violates 2 rules of Lipinski's Ro5, and probably has poor absorption. This is a plus for a compound meant to act locally. The inhibition of diggestive enzyme of α-glycosidase had great importanve due treating and preventing diabetes, postprandial glucose amounts pand hyperglycemia [80–82].

3. Conclusion

In conclusion, diarylmethanone **12** was synthesized for the first time. The bromination of **12** and the known diarylmethanones **13–15** were investigated in this study. Interestingly regioselective O-demethylation was observed during bromination of compounds **12** and **13**. The structures of brominated products **16–19** were characterized. The synthesized compounds **12** and **16–21** were evaluated for their biological assays like antioxidant activity, AChE and α-glycosidase inhibition properties. Novel bromophenols and methoxylated bromophenol derivatives were found as powerful antiradical, antioxidant, and anticholinergic in different bioanalytical assays when compared with standard compounds. These bromophenols and methoxylated bromophenol derivatives can be minimized and preventing LP in food and pharmaceuticals. Also, α-glycosidase inhibition in human can be an

Table 1
Determination of reducing power, radical scavenging and metal chelating effects of novel bromophenols (**16**, **17**, **20** and **21**) and methoxylated bromophenols derivatives (**12,18** and **19**) (BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene).

Antioxidants	Fe^{3+} reducing (700 nm)[a]	Cu^{2+} reducing (450 nm)[a]	DPPH· scavenging		ABTS·$^{+}$ scavenging		Metal chelating	
			IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
BHA	1.675 ± 0.079	2.141 ± 0.105	9.90	0.9700	6.86	0.9630	69.30	0.972
BHT	1.185 ± 0.076	2.104 ± 0.079	10.66	0.9680	11.55	0.9350	34.65	0.95
α-Tocopherol	1.054 ± 0.074	1.945 ± 0.019	6.93	0.9890	13.86	0.9900	23.10	0.994
Trolox	0.980 ± 0.020	1.214 ± 0.082	13.86	0.9890	17.33	0.9720	17.33	0.978
12	0.152 ± 0.044	0.359 ± 0.027	31.50	0.9490	198.00	0.9820	9.90	0.949
16	0.163 ± 0.023	0.332 ± 0.014	28.87	0.9540	231.00	0.9840	7.70	0.94
17	0.218 ± 0.038	0.483 ± 0.006	34.65	0.9640	173.25	0.9910	7.70	0.94
18	0.163 ± 0.016	0.471 ± 0.056	28.88	0.9690	138.60	0.9820	6.93	0.939
19	0.203 ± 0.037	0.490 ± 0.099	26.65	0.9430	231.00	0.9790	7.70	0.975
20	1.471 ± 0.026	0.845 ± 0.005	23.10	0.9600	69.30	0.9910	13.86	0.947
21	0.206 ± 0.019	0.370 ± 0.009	33.00	0.9420	115.50	0.9960	6.93	0.946

[a] They were expressed as absorbance values.

efficient act to control hyperglycemia in T2DM. Novel bromophenols and their derivatives had significantly effective AChE inhibition and promise drugs candidates for treat to the symptomatic therapy of both Alzheimer and Parkinson's diseases.

4. Experimental

4.1. General

All chemicals and solvents are commercially available. All solvents were distilled and dried as describes in the standard procedures. Silica gel (SiO_2, 60 mesh) was used for column chromatography. A total of 1 mm of SiO_2 60 PF on glass plates was used for preparative thick layer chromatography. Melting point of all compounds was determined with capillary melting-point apparatus (BUCHI 530), which are uncorrected. IR spectra were recorded as solutions in 0.1 mm cells with a Mattson 1000 FT-IR. The ^1H and **^{13}C NMR** spectra were obtained on a 400 (1 0 0)-MHz Varian and 400 (1 0 0)-MHz Bruker spectrometer (δ in ppm). Elemental analyses were measured on Leco CHNS-932 apparatus. Antioxidant activity, AChE, and α-Glycosidase inhibitory properties of samples were determined spectrophotometrically (Evolution 201 UV–visible spectrophotometers). The original spectra of the investigated compounds are provided as Supporting Information.

4.2. Chemistry

Compounds **13–15** were synthesized as described in one of our early reported paper [15].

4.2.1. Synthesis of (3-bromo-4-methoxyphenyl)(3,4-dimethoxyphenyl) methanone (12).

Firstly, PPA was prepared by addition of P_2O_5 (11.5 g, 81.24 mmol) to H_3PO_4 (6.4 g, 64.77 mmol) in a 50 mL glass balloon. To this mixture were added 1-bromo-2-methoxybenzene (**11**) (3.08 g, 16.47 mmol) and 3,4-dimetoxybenzoic acid (**10**) (2 g, 10.98 mmol) and it was stirred for 1.5 h at 80 °C. The reaction mixture was poured into ice, basified with dilute NaOH and extracted with CH_2Cl_2 (3x30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. Precipitation from EtOAc-*n*-hexane gave **12** (2.32 g) as a white solid with 52% yield. Mp: 159–161 °C.

^1H **NMR (400 MHz, CDCl$_3$), δ (ppm)**: 8.04 (1H, d, *J* = 2.1 Hz, Ar—H), 7.77 (1H, dd, *J* = 8.6, 2.1 Hz Ar—H), 7.43 (1H, d, *J* = 1.9 Hz, Ar—H), 7.35 (1H, dd, *J* = 8.4, 1.9 Hz, Ar—H), 6.97 (1H d, *J* = 8.6 Hz, Ar—H), 6.91 (1H, d, *J* = 8.4 Hz, Ar—H), 3.99 (3H, s, OCH_3), 3.97 (3H, s, OCH_3), 3.95 (3H, s, OCH_3). **^{13}C NMR (100 MHz, CDCl$_3$) δ (ppm)**: *δ* 193.1(C═O), 158.9 (CO), 152.9 (CO), 149.1 (CO), 135.2 (CH), 131.8 (C), 131.0 (CH), 130.2 (C), 124.9 (CH), 112.1(CH), 111.5 (CBr), 110.9 (CH), 109.8 (CH), 56.47 (CH_3O), 56.10 (OCH_3), 56.07 (CH_3O). **IR (cm^{-1}, CH_2Cl_2):** 3423, 2934, 2840, 1643, 1593, 1513, 1415, 1265, 1131, 1020, 767. Anal. calcd for: ($C_{16}H_{15}BrO_4$) :C, 54.72; H, 4.31, found: C, 54.15, H, 5.26.

4.2.2. General procedure for the bromination of diarylmethanones

4.2.2.1. Synthesis of (3-bromo-4-methoxyphenyl) (2,3-dibromo-4-hydroxy-5-methoxyphenyl) methanone (16). (3-Bromo-4-methoxyphenyl)(3,4-dimethoxyphenyl)methanone (**12**) (3 g, 8.54 mmol) was dissolved in CH_2Cl_2 (50 mL). Br_2 (8.19 g, 2.64 mL) was added to this stirred solution drop wise and the mixture was stirred for 23 days by keeping in a dark place at room temperature. The solvent was evaporated. Recrystallization

Table 2
The enzyme inhibition results of novel bromophenols (**16**, **17**, **20** and **21**) and methoxylated bromophenols derivatives (**12**, **18** and **19**) against acetylcholinesterase (AChE), and α-glycosidase enzymes.

Antioxidant	IC_{50} (nM)				K_i (nM)	
	AChE	r^2	α-Glycosidase	r^2	AChE	α-Glycosidase
12	49.5	0.9824	11.55	0.9840	45.72 ± 3.30	20.34 ± 1.10
16	27.83	0.9756	17.77	0.9784	37.29 ± 0.25	41.13 ± 2.91
17	13.67	0.9784	8.73	0.9946	14.23 ± 1.99	4.31 ± 1.93
18	17.82	0.9887	12.62	0.9697	21.96 ± 7.60	12.55 ± 3.70
19	38.08	0.9826	26.15	0.9628	8.94 ± 0.73	44.14 ± 2.19
20	49.5	0.9725	19.52	0.9749	59.45 ± 14.97	34.45 ± 5.73
21	54.14	0.9798	24.93	0.9873	27.55 ± 9.73	37.48 ± 6.39
TAC[*]	5.97	0.9706	–	–	5.99 ± 1.79	–
ACR[**]	–	–	22.800	–	–	12600 ± 78

[*] TAC (Tacrine) was used as a positive control for acetylcholinesterase (AChE) enzyme.

[**] ACR (Acarbose) was used as a positive control for α-glycosidase enzyme and taken from the reference of 100.

from $CHCl_3$-CH_2Cl_2 gave **16** (2 g, 3.49 mmol) 41%. Brown crystals. Mp: 226–228 °C.

^1H **NMR (400 MHz, $CDCl_3$), δ (ppm)**: 8.05 (1H, d, $J = 2.1$ Hz, Ar—H), 7.72 (1H, dd, $J = 8.6$ Hz, $J = 2.1$ Hz Ar—H), 6.93 (1H, d, $J = 8.6$ Hz, Ar—H), 6.83 (1H, s, Ar—H), 6.31(1H s, Ar—OH), 3.98 (3H, s, OCH_3), 3.92 (3H, s, OCH_3). ^{13}C **NMR (100 MHz, $CDCl_3$) δ (ppm)**: δ 192.6 (C═O), 160.2 (CO), 146.0 (CO), 145.6 (CO), 135.2 (CH), 133.1 (C), 131.7 (CH), 129.9 (C), 113.7 (CBr), 112.9 (CBr), 112.2 (CBr), 111.2 (CH), 109.8 (CH), 56.6 (CH_3O), 56.5 (CH_3O). **IR (cm^{-1}, CH_2Cl_2):** 3414, 2934, 2842, 1658, 1589, 1448, 1383, 1269, 11221, 11049, 1015. Anal. calcd for: ($C_{15}H_{11}Br_3O_4$) :C, 36.40; H, 2.24, found: C, 36.35; H, 2.32.

4.2.3. *(2,3-Dibromo-4-hydroxy-5-methoxyphenyl)(2,5-dibromo-4-methoxyphenyl) methanone (17)*

Yield 45%, dirty white crystals. Mp: 196–198 °C.

^1H **NMR (400 MHz, CDCl3), δ (ppm)**: 7.67 (1H, s, Ar—H), 7.13 (1H, s, Ar—H), 7.00 (1H, s, Ar—H), 6.45 (1H, s, Ar—OH), 3.97 (3H, s, OCH_3), 3.94 (3H, s, OCH_3). ^{13}C **NMR (100 MHz, $CDCl_3$) δ (ppm)**: 192.6 (C═O), 158.5 (CO), 146.9 (CO), 146.0 (CO), 136.0 (CH), 132.8 (C), 132.1 (C), 121.9 (CBr), 117.3 (CH), 115.8 (CBr), 113.5 (CBr), 111.7 (CH), 110.7 (C), 56.8 (CH_3O), 56.7 (CH_3O). **IR (cm^{-1}, CH_2Cl_2):** 3428, 2941, 2842, 1656, 1576, 1435, 1384, 1219, 1057, 1017. Anal. calcd for: ($C_{15}H_{10}Br_4O_4$):C, 31.39; H, 1.76, found: C, 31.29, H, 1.97.

4.2.4. *(3-bromo-4-methoxyphenyl)(2,5-dibromo-4-methoxyphenyl) methanone (18)*

Yield 45%, dirty white crystals. Mp: 164–166 °C.

^1H **NMR (400 MHz, $CDCl_3$), δ (ppm)**: 8.02 (1H, d, $J = 2.0$ Hz, Ar—H), 7.72 (1H, dd, $J = 8.6$ Hz, 2.0 Hz, Ar—H), 7.53 (1H, s, Ar—H), 7.13 (1H, s, Ar—H), 6.94 (1H, d, $J = 8.6$ Hz, Ar—H), 3.97 (6H, s, OCH_3). ^{13}C **NMR (100 MHz, $CDCl_3$) δ (ppm)**: 191.7 (C═O), 160.1 (CO), 157.5 (CO), 135.3 (CH), 133.5 (CH), 133.4 (C), 131.6 (CH), 130.2 (C), 119.6 (CBr), 116.5 (CH), 112.1 (CBr), 111.1 (CH), 110.8 (CBr), 56.7 (CH_3O), 56.5 (CH_3O). **IR (cm^{-1}, CH_2Cl_2):** 3435, 3095, 2941, 2842, 1663, 1588, 1494, 1128, 1267, 1156, 1065, 1017. Anal. calcd for: ($C_{15}H_{11}Br_3O_3$) : C, 37.62; H, 2.31, found: C, 38.01; H, 2.28.

4.2.5. *(2,5-dibromo-4-methoxyphenyl)(phenyl)methanone (19)*

Yield 39%, cream color crystals. Mp: 139–141 °C.

^1H **NMR (400 MHz, $CDCl_3$), δ (ppm)**: 7.80 (2H, d, $J = 7.8$ Hz, Ar—H), 7.61 (1H, t, $J = 7.8$ Hz, Ar—H), 7.56 (1H, s, Ar—H), 7.47 (2H, t, $J = 7.8$ Hz, Ar—H), 7.14(1H s, Ar—H), 3.97 (3H, s, OCH_3). ^{13}C **NMR (100 MHz, $CDCl_3$) δ (ppm)**: δ 194.1 (C═O), 157.5 (CO), 136.3 (C), 133.8 (C), 133.7 (CH), 133.6 (CH), 130.2 (2CH), 128.6 (2CH), 119.8 (CBr), 116.6 (CH), 110.7 (CBr), 56.7 (CH_3O). **IR (cm^{-1}, CH_2Cl_2):** 3058, 2937, 2841, 1668, 1584, 1442, 1313, 1295, 1242, 1065, 1024. Anal. calcd for: ($C_{14}H_{10}Br_2O_2$) :C, 45.44; H, 2.72; found: C, 45.10; H, 2.99.

4.2.6. *General procedure for the demethylation of arylmethyl ethers.*

4.2.6.1. Synthesis of (3-bromo-4-hydroxyphenyl)(3,4-dihydroxyphenyl) methanone (20). To a stirred solution of (3-bromo-4-methoxyphenyl) (3,4-dimethoxyphenyl)methanone (**3**) (0.5 g, 1.43 mmol) in anhydrous CH_2Cl_2 (50 mL) at 0 °C under N_2 was added BBr_3 (2.63 g, 10.49 mmol). The reaction mixture was stirred at the same temperature for 30 min and then the reaction mixture was allowed to warm to room temperature. The mixture was stirred for 24 h. at rt. After the reaction mixture was cooled to 0 °C, MeOH (30 mL) was added to the mixture drop wise. The reaction mixture was stirred at the same temperature for 30 min. The solvent was evaporated and the residue was dissolved in EtOAc (30 mL) and H_2O (20 mL) was added to the solution. The organic layer was separated and aqueous phase was extracted with EtOAc (2x30 mL). The organic layers were combined and dried over Na_2SO_4. Removal of the solvent by evaporation and crystallization of the crude product with EtOAc-*n*-hexane afforded **11** (0.4 g, 94% yield) as brown crystals. Mp: 225–227 °C.

^1H **NMR (400 MHz, $CDCl_3$), δ (ppm)**: 9.72 (1H, s, Ar—OH), 8.72 (1H, s, Ar—OH), 8.43 (1H, s, Ar—OH), 7.94 (1H, d, $J = 2.0$ Hz, Ar—H), 7.66 (1H, dd, $J = 8.3$, 2.0 Hz Ar—H), 7.35 (1H, d, $J = 2.0$ Hz, Ar—H), 7.22 (1H, dd, $J = 8.3$, 2.0 Hz, Ar—H), 7.14 (1H, d, $J = 8.3$ Hz, Ar—H), 6.96 (1H, d, $J = 8.3$ Hz, Ar—H). ^{13}C **NMR (100 MHz, $CDCl_3$) δ (ppm)**: δ 193.3 (C═O), 158.2 (CO), 150.6 (CO), 145.7 (CO), 135.7 (CH), 132.4 (C), 131.8 (CH), 130.5 (C), 124.3 (CH), 117.6 (CH), 116.6 (CBr), 115.6 (CH), 110.2 (CH). **IR (cm, $^{-1}$ CH_2Cl_2):** 3413, 2934, 2845, 1640, 1573, 1523, 1410, 1265, 1131, 1025. Anal. calcd for: ($C_{13}H_9BrO_4$) :C, 50.51; H, 2.93, found: C, 50.43.15, H, 3.12.

4.2.7. *(2,5-Dibromo-4-hydroxyphenyl)(phenyl)methanone (21)*

Yield 95%, cream color crystals. Mp: 185–187 °C.

^1H **NMR (400 MHz, $CDCl_3$), δ (ppm)**: 7.80 (2H, d, $J = 7.5$ Hz, Ar—H), 7.62 (1H, t, $J = 7.8$ Hz, Ar—H), 7.51 (1H, s, Ar—H), 7.47 (2H, t, $J = 7.8$ Hz, Ar—H), 7.33(1H s, Ar—H), 5.88 (1H, s, Ar—OH). ^{13}C **NMR (100 MHz, $CDCl_3$) δ (ppm)**: δ 194.1 (C═O), 154.2 (CO), 136.4 (C), 134.0 (C), 133.7 (CH), 132.7 (CH), 130.2 (2CH), 128.6 (2CH), 120.8 (CH), 120.3 (CBr), 109.1 (CBr). **IR (cm^{-1}, CH_2Cl_2):** 3361, 2935, 2841, 1665, 1581, 1432, 1310, 1275, 1232, 1055, 1020. Anal. calcd for: ($C_{13}H_8Br_2O_2$): C, 43.86; H, 2.26; found: C, 43.62; H, 2.48.

4.3. *X-ray analysis*

For the crystal structure determination, single-crystal of molecules **17** and **18** were used for data collection on a four-circle Rigaku R-Axis Radid S diffractometer (equipped with a two-dimensional area IP detector). Plane graphite-monochromated Mo-K_α radiation ($\lambda = 0.71073$ Å) and rotation scans technique for one image were used for data collection. The lattice parameters were determined by the least-squares methods on the basis of all reflections with $F^2 > 2\sigma$ (F^2). Integration of the intensities, correction for Lorentz and polarization effects and cell refinement was performed using CrystalClear software [83]. The structures were solved by direct methods using SHELXS-97 and refined by a full-matrix least-squares procedure using the program SHELXL-97 [84]. H atoms were positioned geometrically and refined using a riding model. The final difference Fourier maps showed no peaks of chemical significance. *Crystal data for **17***: $C_{15}H_{10}O_4Br_4$, crystal system, space group: monoclinic, $P2_1/n$; (no: 14); unit cell dimensions: $a = 10.932(2)$, $b = 7.746(2)$, $c = 20.911(4)$ Å, $\alpha = 90$, $\beta = 99.131(7)$, $\gamma = 90°$; volume: 1757.0(6) Å^3; Z = 4; calculated density: 1.372 g/cm^3; absorption coefficient: 9.177 mm^{-1}; $F(0\,0\,0) = 1088$; θ-range for data collection 2.0–26.48° ; refinement method: full matrix least-square on F^2; data/parameters: 2839/211; goodness-of-fit on F^2: 1.372; final R-indices [I > 2σ(I)]: $R_1 = 0.086$, w$R_2 = 0.154$; largest diff. peak and hole: 0.210and −0.240 e Å^{-3}. *Crystal data for **18***: $C_{15}H_{11}O_3Br_3$, crystal system, space group: triclinic, P-1; (no: 2); unit cell dimensions: $a = 7.5972$ (6), $b = 9.0571(7)$, $c = 11.8156(9)$ Å, $\alpha = 89.218(4)$, $\beta = 72.716(3)$, $\gamma = 89.525(2)°$; volume: 776.2 (1) Å^3; Z = 2; calculated density: 2.049 g/cm^3; absorption coefficient: 7.801 mm^{-1}; F (0 0 0) = 460; θ-range for data collection 2.4–28.4°; refinement method: full matrix least-square on F^2; data/parameters: 3783/192; goodness-of-fit on F^2: 1.416; final R-indices [I > 2σ(I)]: $R_1 = 0.085$, w$R_2 = 0.191$; largest diff. peak and hole: 0.760 and −0970 e Å^{-3}. Crystallographic data for all the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No's. CCDC-1880412 (17) and 1880757 (18). Copies of these data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; FAX: (+44) 1223 336033, or online via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk.

4.4. *Biochemical studies*

4.4.1. *Antioxidant assays*

The reducing ability of bromophenols and derivatives was performed in accordance with method of Oyaizu [85]. Briefly, 1 mL different concentrations of samples (10–30 μg/mL), which diluted in distilled water was added to phosphate buffer (2.5 mL, pH 6.6, 0.2 M) and $K_3Fe(CN)_6$ (2.5 mL, 1%). The solution was incubated at 50 °C for 20 min. Then, trichloroacetic acid (2.5 mL, 10%) and $FeCl_3$ (0.5 mL, 0.1%) were added to the reaction mixture. The absorbance was spectrophotometrically recorded at 700 nm. Increased absorbance was indicates increased reducing capacity.

The reducing ability of bromophenols and derivatives was also measured according to the method developed by Apak et al. [86] as described in a previous study [87]. In this assay, $CuCl_2$ solution (0.25 mL, 10 mM), ethanolic neocuproine solution (0.25 mL, 7.5 mM) and acetate buffer (0.25 mL, 1.0 M) were mixed and bromophenols and derivatives were added to this mixture. Then, absorbances were spectrophotometrically recorded at 450 nm [88].

The metal chelating effects of novel bromophenols and methoxylated bromophenols derivatives were monitored at 522 nm [89]. For this purpose, different concentrations of sample was mixed with Tris-HCl buffer (1 mL, pH 7.4), $FeSO_4$ solution (0.25 mL, 2 mM), ethanol (2.5 mL) and 2,2′-bipyridine solution (1 mL, 0.2% in 0.2 M HCl). The absorbances were recorded spectrophotometrically at 522 nm [90–92].

The DPPH$\cdot$ scavenging property of bromophenols and derivatives was spectrophotometrically recorded at 450 nm [93]. Ethanolic DPPH radical solution (1.0 mM) was prepared before the assay and of DPPH$\cdot$ solution (1.0 mL) was mixed with 3 mL of samples at different concentrations (10–30 μg/mL). Decreased absorbance of medium indicates DPPH$\cdot$ scavenging ability [94].

ABTS$\cdot^{+}$ scavenging effects of bromophenols and derivatives was performed using the spectroscopic method of Re et al. [95]. ABTS$\cdot^{+}$ was acquired by reacting with ABTS solution (7 mM) with $K_2S_2O_8$2.45 (mM). ABTS$\cdot^{+}$ was diluted in ethanol for obtaining of 0.750 ± 0.05 at 734 nm [96].

Percentage of metal chelating, and radicals scavenging of bromophenols and derivatives were calculated as following equation: $S\ (\%) = [1 - (As/Ac)] \times 100$. Where S is scavenging effects, Ac and As are the absorbances of control and samples, respectively [97].

4.4.2. AChE inhibition studies

The inhibitory effect of novel bromophenol derivatives on AChE enzyme activity was performed according to the spectrophotometric method of Ellman et al [78] as described previously [98]. Acetylthiocholine iodide (AChI) was used as substrate for cholinergic reactions [38]. In brief, an aliquot (100 μL) of Tris/HCl buffer (pH 8.0, 1.0 M) and different concentration of sample solutions (10–30 μg/mL) were added to 50 μL of AChE enzyme solution (5.32×10^{-3} EU). The solution was incubated at 20 °C during 10 min. An aliquot (50 μL, 0.5 mM) of 5,5′-dithio-bis(2-nitro-benzoic)acid (DTNB) and AChI were added to incubated mixture and enzymatic reaction was initiated. AChE activity was spectrophotometrically determined at 412 nm [99].

4.4.3. α-Glycosidase inhibition studies

α-Glycosidase inhibition effect of novel bromophenols and methoxylated bromophenols derivatives was evaluated according to the method of Tao et al. [100]. Firstly, phosphate buffer (pH 7.4, 75 μL) was mixed with of 5 μL of the sample and α-glycosidase enzyme solution (20 μL), which prepared in phosphate buffer (0.15 U/mL, pH 7.4). After preincubation 50 μL of *p*-Nitrophenyl-D-glycopyranoside (*p*-NPG) in phosphate buffer (5 mM, pH 7.4) was added and solution was re-incubated at 37 °C. The absorbance o mixtures were recorded at 405 nm [98]. For the determination of Ki values, three different novel bromophenols **16**, **17**, **20** and **21** and methoxylated bromophenols derivatives **12**, **18** and **19** concentrations were used. Then, the Lineweaver-Burk graphs were drawn [99].

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Declaration of interest

The authors report no conflicts of interests.

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