Synthesis and antioxidant activities of benzylic bromophenols inclusive of natural products

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Abstract: The synthesis of natural products 2-(2,3-dibromo-4,5-dihydroxyphenyl)acetic acid (**1**) and 2-(2,6-dibromo-3,5-dihydroxyphenyl)acetic acid (**2**) and as well as their derivatives **25** and **26** were carried out by substitution, hydrolysis and demethylation reactions of the corresponding four benzyl bromides. The antioxidant potentials of benzylic acid-derived bromophenols were, for the first time, appraised by several outstanding bioanalytical methods. Besides these, we estimated the antioxidant effects which were studied using the methods of DPPH·, ABTS·+ scavenging activities, ferric (Fe^{3+}) and cupric (Cu^{2+}) ions and Fe^{3+}-TPTZ reducing capacities. Benzylic acid-derived bromophenols were found as effective DPPH·, and ABTS·+ scavengers. The potential antioxidant activities of bromophenol derivatives **1**, **2** and **17–28** were compared to standard antioxidants including BHA, BHT, α-Tocopherol, and Trolox, which is a water-soluble analog of vitamin E. We expect that this innovative work will direct future studies exploring the antioxidant properties of food, medicinal, and industrial applications.

Key words: Antioxidant activity, bromination, bromophenol, radical scavenging, natural product, benzylic acid

1. Introduction

Natural bromophenols are commonly found in marine life [1]. Many biological functions including antioxidant [2–5], antimicrobial [6,7], and carbonic anhydrase (CA) inhibition have been investigated for them and their derivatives [8–14].

Bromophenols **1-9** seen in Figure 1 are natural bromophenols [2,15–18]. Natural bromophenols 1 and 2 were isolated from the *Halopitys* incurvus algae of the Rhodomelaceae [15] and the red algae of *Rhodomelaceae confervoides* [16], respectively. From these natural bromophenols **1–9**, we reported the first synthesis as well as various biological activities such as carbonic anhydrase of **3–9** in our previous studies (Figure 1) [5,11,13].

Oxidative stress is associated with an imbalance between the antioxidants and the reactive oxygen species (ROS) in the body. This situation is known to cause the development of many chronic diseases [19–21]. The excess formation of free radicals and ROS cause degenerative damage to vital cellular molecules including carbohydrates, proteins, lipids, and nucleic acids [22–24]. As a result, oxidative stress and ROS are known as important environmental factors leading to many chronic diseases such as cancer, cardiovascular diseases, immunodeficiency syndrome, obesity, age-related pathologies, arteriosclerosis, and diabetes mellitus [25,26]. Even at low concentrations, antioxidants are quite effective at counteracting the detrimental effects of both oxidative stress and ROS. Phenolic compounds obtained from natural sources, especially those found in plants, exhibit a wide range of biological activity. They have also been intensely investigated due to their possible antioxidant and biological abilities [27–29]. Antioxidants are chemicals that are preferentially oxidized, preventing or completely inhibiting the oxidation of other oxidizable compounds which, once oxidized, may be hazardous effects on food or pharmacological products [30,31]. The most common synthetic antioxidants approved for use in food today are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), tertiary-butylhydroquinone, and propyl gallate [32,33]. On account of the serious safety concerns regarding petrochemical antioxidants, there is an increasing demand for natural or naturally derived antioxidants due to their positive effects on human health [34]. In this way, natural antioxidants and their derivatives play an important role in living systems and in human health. Moreover, antioxidants also have an important role as therapeutic effects in many chronic diseases [35].

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The synthesis of the bromophenols **1** and **2** is desirable because they are natural products that are potentially vital compounds for many biological processes. For this reason, the synthesis of compounds **1** and **2**, as well as their derivatives, was performed and the resulting compounds were investigated for their antioxidant properties using various bioanalytical methods.

2. Result and discussion

2.1. Chemistry

Natural bromophenols **1** and **2** both contain a benzylic acid group, as well as two Br and two ⁻OH groups at varied positions around the ring (Figure 1). To obtain these products and derivatives, vanillin (**10**), 3,5-dimethoxybenzaldehyde (**13**) and (3,4-dimethoxyphenyl)methanol (**14**) were chosen as starting compounds. Benzyl bromides **11**, **12, 15,** and **16** are already known and were synthesized by established methods in the literature (Scheme 1) [36–40].

According to the known procedure [41], substitution nitrile substituted compounds **17–20** were obtained from their corresponding bromides **11**, **12**, **15,** and **16** (Scheme 2). As expected, in ^{1}H-NMR spectra, an upfield shift is shown in the benzylic hydrogens of compounds **17–20** owing to the decreased electron-withdrawing nature of the nitrile group. Also, all data belonging to them suggested structures (Scheme 2).

The nitrile groups in the **17–20** were then hydrolyzed under basic conditions (NaOH in the EtOH/H$_2$O) (Scheme 2). The obtained compounds are carboxylic acids (**21–24**), as is evidenced by the appearance of carbonyl groups ranging between 170–177 ppm in their ^{13}C-NMR spectra. Furthermore, the appearance of peaks around 10.5 ppm in the ^{1}H-NMR spectra of compounds **21** and **24** is indicative of the conversion of these nitrile groups into the corresponding carboxylic acid groups (Scheme 2).

Bromophenols are important compounds because of biologically active [1–16]. Bromophenols 1 and 2 are more important because they are both biologically active and natural products [15,16]. The compounds 22 and 23 are precursor compounds for natural bromophenol compounds 1 and 2.
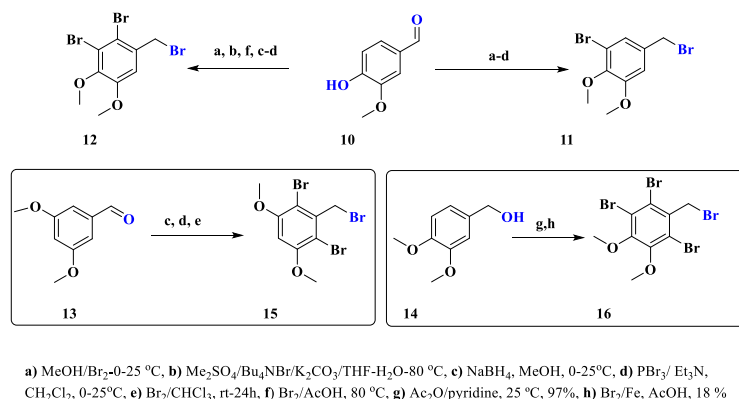
Finally, the synthesis of bromophenols 1, 2 was performed by treating 22 and 23 with BBr3. Similarly, the reactions of the compounds 21 and 24 with BBr3 yielded bromophenol derivatives 25 and 26 (Scheme 2).

In addition to the benzylic acid-containing bromophenols **1**, **2**, **25,** and **26**, benzyl nitrile containing bromophenols **27** and **28** were also sought after due to their important biologic activities. Therefore, their synthesis was carried out by treating compounds **18** and **20** with BBr$_3$ (Scheme 3). NMR data of bromophenol derivatives **1**, **2**, and **25–28** are consistent with their structures.

2.2. Biological activities

2.2.1. Reducing power results

Reducing power can donate the capacity of bioactive biological compounds that act as reductants and inactivate ROS and oxidant agents [42]. Fe^{3+} reduction ability assay measures the reducing potential of the compounds. Fe^{3+} ions addition to bromophenol derivatives **1, 2,** and **17–28** occur in blue colored complex of Fe$_4$[Fe(CN-)$_6$]$_3$. This complex demonstrated absorbance at 700 nm [43,44]. As a result of the complex, the yellow color of samples varies from green to blue according to the effectiveness of test compounds [45]. In this sense, bromophenol derivatives **1, 2** and **17–28** demonstrated potent


a) MeOH/Br$_2$-0-25 ºC, b) Me$_2$SO$_4$/Bu$_4$NBr/K$_2$CO$_3$/THF-H$_2$O-80 ºC, c) NaBH$_4$, MeOH, 0-25ºC, d) PBr$_3$/ Et$_3$N, CH$_2$Cl$_2$, 0-25ºC, e) Br$_2$/CHCl$_3$, rt-24h, f) Br$_2$/AcOH, 80 ºC, g) Ac$_2$O/pyridine, 25 ºC, 97%, h) Br$_2$/Fe, AcOH, 18 %

Scheme 1. Synthesis of benzyl bromides **11**, **12, 15** and **16**.

Scheme 1. Synthesis of benzyl bromides **11**, **12, 15,** and **16**.
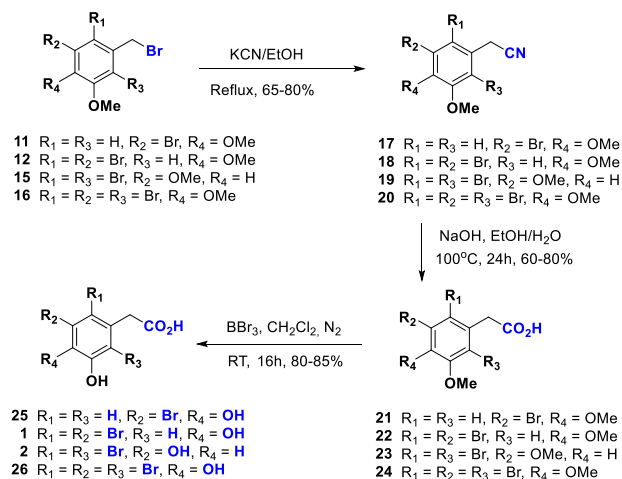
Scheme 2. As bromophenols, synthesis of 2-phenylacetic acid derivatives from the corresponding bromides via their nitriles.
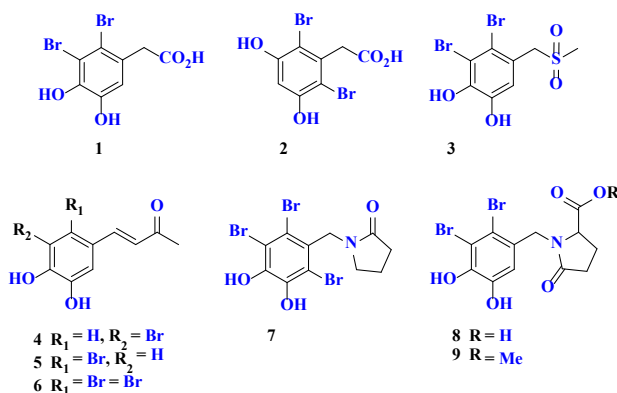
Figure 1. Some natural bromophenols.

and efficient reducing ability by using the $Fe[Fe(CN-)_6]_3$, Cu^{2+} and Fe^{3+}-TPTZ reduction abilities. For measuring of reduction ability of bromophenol derivatives **1, 2,** and **17–28**, Fe^{3+}-Fe^{2+} transformation was realized according to Oyaizu's method [46]. As given in Table 1 and Figure 1A, bromophenol derivatives **1, 2,** and **17–28**, demonstrated efficient Fe^{3+} reducing capability. Since the reducing potencies and radical scavenging capacities of bromophenol derivatives **17–24** are relatively lower due to the structure-activity relationship, they will not be considered much in the discussion part. The other bromophenol derivatives **17–24** demonstrated moderate Fe^{3+} reducing ability ranging from 0.253 ± 0.004–0.463 ± 0.010 when compared to standards. Generally, the antioxidant properties of the test sample are compared with standard antioxidants. Many compounds are used as standards for this purpose. In addition, the standard selection and criteria are also related to the stability, price, and solubility of the standard antioxidant in the solvent environment [47]. The increased absorbance shows the complex formation and increased enhanced reducing effect (Figure 2A). The results clearly show that bromophenol derivatives **1, 2,** and **17–28** have strong Fe^{3+} reducing effects with e^- donating properties for neutralizing free radicals and ROS. They can apply in biochemical and biological systems to reduce oxidative stress or damage.

Aside Fe^{3+}-TPTZ reduction abilities, Cu^{2+} reducing of bromophenol derivatives **1, 2,** and **17–28** are given in Table 1 and Figure 2B. A positive correlation was observed between the Cu^{2+} reducing and bromophenol derivatives **1, 2,** and **17–28** as concentration-dependently (20–60 μg/mL). At the concentration of 60 μg/mL, Cu^{2+} reducing capability of bromophenol derivatives **1, 2,** and **17–28** and standards were declined as following orders (Table 1 and Figure 2B): **1** (1.896 ± 0.002, r^2: 0.6828) > BHA (1.864 ± 0.015, r^2: 0.9854) ≈ **25** (1.853 ± 0.003, r^2: 0.6573) > Trolox (1.829 ± 0.004, r^2: 0.6061) > α-Tocopherol (1.795 ± 0.003, r^2: 0.9747) > BHT (1.744 ± 0.003, r^2: 0.7642) > **26** (1.642 ± 0.002, r^2: 0.9589) > **27** (1.538 ± 0.110, r^2: 0.9015) > **28** (1.609 ± 0.12, r^2: 0.9589) > **2** (1.345 ± 0.002, r^2: 0.9507). The Fe^{3+} reducing effects of bromophenol derivatives **1, 2,** and **17–28** and standards declined as following orders: **28** (1.856 ± 0.002, r^2: 0.8206) > **1** (1.775 ± 0.003, r^2: 0.8992) > BHA (1.744 ± 0.004, r^2: 0.7114) ≈ **25** (1.743 ± 0.002, r^2: 0.6555) > **26** (1.704 ± 0.002, r^2: 0.7336) > **27** (1.663 ± 0.003, r^2: 0.8012) > Trolox (1.648 ± 0.007, r^2: 0.8992) > BHT (1.563 ± 0.003, r^2: 0.8358) > α-Tocopherol (1.473 ± 0.003, r^2: 0.9499) > **2** (0.877 ± 0.020, r^2: 0.9463). The other bromophenol derivatives **17–24** exhibited weaker Cu^{2+} reducing capability between in 0.253 ± 0.004-0.445 ± 0.001 when compared to standard reducing agents. The CUPRAC test had low-cost and is a rapid, stable and selective assay for different antioxidants, regardless of chemical type and hydrophobicity [48].

Aside Fe^{3+} and Cu^{2+} reduction properties of bromophenol derivatives **1, 2,** and **17–28**, they had powerful reducing potentials in FRAP assay (Figure 1C and Table 2). Reducing ability of bromophenol derivatives **1, 2,** and **17–28** was found to be in descending order of **25** (2.455 ± 0.004, r^2: 0.8362) > BHA (2.254 ± 0.004, r^2: 0.7435) > BHT (2.146 ± 0.002, r^2: 0.8599) > **26** (2.016 ± 0.002, r^2: 0.7929) > **1** (1.996 ± 0.002, r^2: 0.7367) ≈ Trolox (1.993 ± 0.004, r^2: 0.9494) > **27** (1.894 ± 0.002, r^2: 0.8755) > **28** (1.605 ± 0.001, r^2: 0.8889) > α-Tocopherol (1.497 ± 0.002, r^2: 0.8531) > **2** (0.744 ± 0.002, r^2: 0.8929). The other bromophenol derivatives **17–24** showed relatively weaker Fe^{3+}-TPTZ reducing ability between in 0.304 ± 0.004-0.667 ± 0.002 when compared to standard reducing compounds. As mentioned in prior reduction assay, high reducing absorbance shows high reducing ability of the complex. The FRAP method is realized in an acidic medium to protect iron ions solubility [49].

Table 1. Fe^{3+}, Cu^{2+} and Fe^{3+}-TPTZ reducing ability of bromophenol derivatives **1, 2,** and **17–28** and standards at 60 µg/mL concentration

Antioxidants	Fe^{3+} reducing		Cu^{2+} reducing		Fe^{3+}-TPTZ reducing	
	λ_{700}	r^2	λ_{450}	r^2	λ_{593}	r^2
BHA	1.744 ± 0.004	0.7114	1.864 ± 0.015	0.9854	2.254 ± 0.004	0.7435
BHT	1.563 ± 0.003	0.8358	1.774 ± 0.003	0.7642	2.146 ± 0.002	0.8599
α-Tocopherol	1.473 ± 0.003	0.9499	1.795 ± 0.003	0.9747	1.497 ± 0.002	0.8531
Trolox	1.648 ± 0.007	0.8992	1.829 ± 0.004	0.6061	1.993 ± 0.004	0.9494
1	1.775 ± 0.003	0.6791	1.896 ± 0.002	0.6828	1.996 ± 0.002	0.7367
2	0.877 ± 0.020	0.9463	1.345 ± 0.005	0.9507	0.744 ± 0.002	0.8929
17	0.312 ± 0.009	0.9023	0.303 ± 0.002	0.7484	0.453 ± 0.002	0.8902
18	0.445 ± 0.001	0.9557	0.506 ± 0.010	0.5767	0.404 ± 0,004	0.9627
19	0.377 ± 0.003	0.9544	0.430 ± 0.009	0.8159	0.407 ± 0.002	0.7252
20	0.355 ± 0.001	0.9542	0.716 ± 0.002	0.7199	0.344 ± 0.002	0.9844
21	0.355 ± 0.003	0.9290	0.666 ± 0.002	0.8792	0.667 ± 0.002	0.8175
22	0.253 ± 0.004	0.9321	0.265 ± 0.001	0.9579	0.304 ± 0.004	0.9683
23	0.302 ± 0.001	0.9460	0.726 ± 0.002	0.8705	0.403 ± 0.003	0.9321
24	0.463 ± 0.010	0.9264	0.665 ± 0.003	0.9843	0.578 ± 0.010	0.8693
25	1.743 ± 0.002	0.6555	1.853 ± 0.003	0.6573	2.455 ± 0.004	0.8362
26	1.704 ± 0.002	0.7336	1.642 ± 0.002	0,9589	2.016 ± 0.002	0.7929
27	1.663 ± 0.003	0.8012	1.538 ± 0.110	0.9015	1.894 ± 0.002	0.8755
28	1.856 ± 0.002	0.8206	1.609 ± 0.120	0.9589	1.605 ± 0.001	0.8889

2.2.2. Radicals scavenging results

DPPH· and ABTS·+ scavenging assays are among the most convenient spectrophotometric scavenging methods. Both assays were used to determine the antioxidant abilities and radical scavenging capacities of plants, foods, and beverages [50]. The percent inhibition depends on the concentrations of oxidizers such as antioxidants and radicals, the ratios of solvent and reagent used, incubation time, temperature, and also the presence of hydrogen, metal, and water in the antioxidant test systems [51]. On the other hand, IC_{50} is called the effective concentration that causes 50% removal of oxidants such as radicals in antioxidant studies. It is often used to assess the antioxidant activity [47]. For DPPH radical scavenging were found to be in following order: BHT (IC_{50}: 4.12 µg/mL, r^2: 0.9690) > **25** (IC_{50}: 4.27 µg/mL, r^2: 0.9016) > **1** (IC_{50}: 6.41 µg/mL, r^2: 0.9961) > **27** (IC_{50}: 6.86 µg/mL, r^2: 0.9018) > **28** (IC_{50}: 10.66 µg/mL, r^2: 0.9652) > BHA (IC_{50}: 11.17 µg/mL, r^2: 0.9030) > Trolox (IC_{50}: 11.75 µg/mL, r^2: 0.8513) > α-Tocopherol (IC_{50}: 23.89 µg/mL, r^2: 0.9732) > **2** (IC_{50}: 30.13 µg/mL, r^2: 0.9457) > **26** (IC_{50}: 231.00 µg/mL, r^2: 0.9652). On the other hand, the other bromophenol derivatives **17–24** demonstrated relatively weak DPPH radical scavenging ability and found IC_{50} values between 17.32–346.50 µg/mL when compared to standard radical scavengers. A lower IC_{50} value demonstrates a higher DPPH· scavenging ability (Table 2 and Figure 1D). In another study, DPPH radical scavenging activity of nineteen bromophenols from *Rhodomela confervoides* was realized. Among these bromophenols, bromophenols **1** was also studied. It was shown that bromophenols **1** had IC_{50} value of 19.84 µM for DPPH radical scavenging activity. Also, it was demonstrated that the metabolites with *ortho*-dihydroxy groups on the aromatic ring generally display higher activity than the compounds having a single free hydroxyl group on the ring [16,52]. The bromophenol derivatives **1, 2,** and **17–28** exhibited effective ABTS·+ removing ability. As given in Table 2 and Figure 1E, bromophenol derivatives **1, 2,** and **17–28** effectively scavenged ABTS radicals as concentration-dependently (20–60 µg/mL, p < 0.001). EC_{50} values of bromophenol-derivatives **1, 2,** and **17–28** in ABTS·+ scavenging assay were found to be in descending order of **25** (IC_{50}: 9.36 µg/mL, r^2: 0.6059) > Trolox (IC_{50}: 9.36 µg/mL, r^2: 0.6119) ≈ **26** (IC_{50}: 9.49 µg/mL, r^2: 0.8680) > **1** (IC_{50}: 9.90 µg/mL, r^2: 0.6119) > **28** (IC_{50}: 10.19 µg/mL, r^2: 0.6496) > **27** (IC_{50}: 10.28 µg/mL, r^2: 0.6654) > **2** (IC_{50}: 10.66 µg/mL, r^2: 0.8511) > BHA (IC_{50}: 14.74 µg/mL, r^2: 0.7129) > BHT (IC_{50}: 15.75 µg/mL, r^2: 0.9986) > α-Tocopherol (IC_{50}: 12.15 µg/mL, r^2: 0.7950). On the other hand, the other

bromophenol derivatives **17–24** demonstrated relatively weak DPPH radical scavenging ability and found IC_{50} values when compared to standard radical scavengers. As DPPH in radical scavenging, a lower IC_{50} value shows higher $ABTS^{+}$ scavenging ability. In a previous study, it was shown that 2.87 mM TEAC $ABTS^{+}$ scavenging ability. In this study, the isolated nineteen bromophenols were found effective radical scavenging potential against ABTS cation radicals [16]. Already, it is well-known that ABTS radical scavenging properties of antioxidants can attribute H-donating effect [53].

3. Conclusion

As a result, after bromides **11**, **12**, **15**, and **16** were synthesized, the acids including natural products were obtained via the corresponding nitriles **17–20**. Chronic diseases, mutagenesis, DNA damage, carcinogenesis, and inhibition of pathogenic bacterial growth are generally associated with the scavenging of ROS and free radical propagation in living systems. Antioxidant activity is used as an effective and common parameter for medicinal bioactive components and newly

Table 2. Half maximal scavenging concentration (IC_{50}, µg/mL) for DPPH$^{\bullet}$ scavenging and $ABTS^{\bullet+}$ scavenging effects of bromophenol derivatives **1**, **2**, and **17–28** and standards.

Compounds	DPPH• scavenging		$ABTS^{\bullet+}$ scavenging	
	IC_{50}*	r^2	IC_{50}*	r^2
BHA	14.74	0.7129	11.17	0.9030
BHT	15.75	0.9986	4.12	0.9690
α-Tocopherol	12.15	0.7950	23.89	0.9732
Trolox	9.36	0.6575	11.75	0.8513
1	9.90	0.6119	6.41	0.9961
2	10.66	0.8511	30.13	0.9457
17	173.25	0.9145	173.25	0.8727
18	231.00	0.7611	346.50	0.9371
19	17.32	0.9865	231.00	0.9134
20	346.50	0.9735	115.50	0.8320
21	231.00	0.8642	173.25	0.9078
22	138.60	0.9851	173.25	0.9722
23	33.00	0.8860	231.00	0.9675
24	138.60	0.9810	138.60	0.8109
25	9.36	0.6059	4.27	0.9016
26	9.49	0.8680	231.00	0.9652
27	10.28	0.6654	6.86	0.9018
28	10.19	0.6496	10.66	0.9652
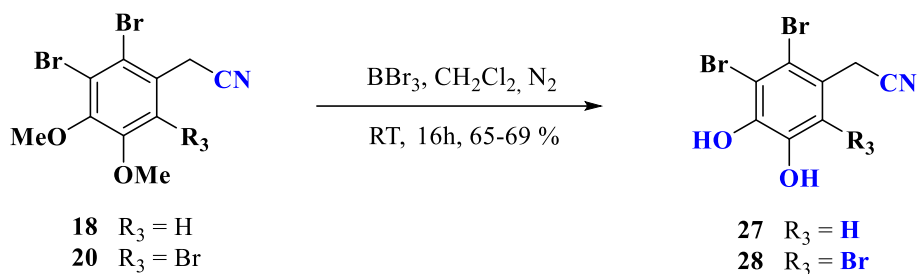
Scheme 3. Synthesis of bromophenol derivatives **27** and **28** from the compounds **18** and **20**.
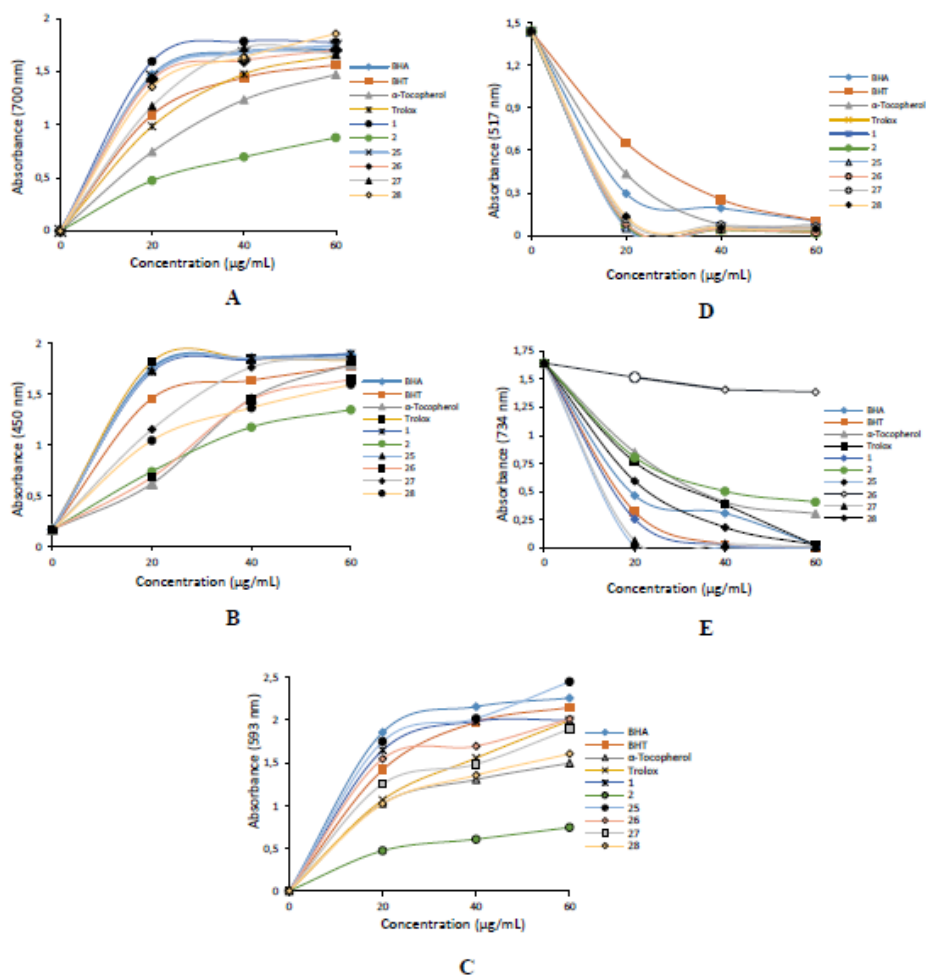
Figure 2. Different antioxidant assay for bromophenol derivatives **1, 2,** and **17–28**: **A**. Fe^{3+} reducing method, **B**. Cu^{2+} reducing method, **C**. Fe^{3+}-TPTZ reducing method, **D**. DPPH· scavenging method, **E**. ABTS$^{·+}$ scavenging method.

synthesized biologically important molecules. For this reason, natural products **1** and **2**, including their derivatives **25** and **26** were synthesized through the conversion of benzyl bromides **11–16** into the corresponding nitriles **17–20** followed by base catalyzed hydrolysis and demethylation. In addition to these products, benzyl nitriles **27** and **28** were also synthesized through direct demethylation of **18** and **20**. Once these bromophenol-derivatives were obtained **1, 2,** and **17–28** they were assessed for their antioxidant ability and compared with established antioxidants including, BHA, α-tocopherol, BHT, and Trolox.

4. Experimental section

4.1. General procedures

Chemicals (including solvents) used in the experiments and data of the synthesized compounds (such as NMR and HRMS) were performed as previously stated. [14,54]. Reagent benzyl bromides **11**, **12**, **15,** and **16** were prepared in known ways in the literature [36–40].

4.2. Synthesis

4.2.1. Synthesis of 2-(3-bromo-4,5-dimethoxyphenyl)acetonitrile (17): Standard procedure for the substitution reaction with KCN

KCN (315 mg, 4.84 mmol) was added to the stirred solution of bromide **11** (500 mg, 1.61 mmol) in EtOH (20 mL). After refluxing of the solution at 80 °C for 16 h, termination of the reaction, removal of the solvent under vacuum and

extraction of reside with EtOAc (2 × 20 mL) were performed, respectively. Then, combination and drying over Na_2SO_4 of organic phases and evaporation of the solvent under vacuum were carried out. After purification of the residue by column chromatography on silica gel (10 g) using EtOAc:hexane (1:9) eluent, the product **17** (270 mg, 65%, white solid) was obtained. Mp: 76–77°C; ^{1}H NMR (400 MHz, CDCl$_3$): 7.08 (s, 1H, ArH), 6.80 (s, 1H, ArH), 3.88 (s, OMe, 3H), 3.84 (s, OMe, 3H), 3.68 (s, CH$_2$, 2H); ^{13}C NMR (100 MHz, CDCl$_3$): 154.26, 146.58, 127.04, 124.31 (CH), 118.31, 117.61 (C). 111.54 (CH), 60.84 (OMe), 56.43 (OMe), 23.28 (CH$_2$); IR (CH$_2$Cl$_2$, cm^{-1}): 2939, 2251, 1570, 1491, 1416, 1274, 1141, 1046 cm^{-1}; HRMS (m:z): calcd. for $[C_{10}H_{10}^{79}BrNO_2]^+$: 254.9895; found 254.9896, R$_f$: 0.51; EtOAc:hexane: (3:7).

4.2.2. Synthesis of 2-(2,3-dibromo-4,5-dimethoxyphenyl)acetonitrile (18)
Using standard procedure written in 4.2.1, the product **18** (280 mg, 65%, yellow solid) was obtained. Mp: 121–122 °C; ^{1}H NMR (400 MHz, CDCl$_3$): 7.08 (s, 1H, ArH), 3.91 (s, OCH$_3$, 3H), 3.87 (s, CH$_2$, 2H), 3.86 (s, OCH$_3$, 3H); ^{13}C NMR (100 MHz, CDCl$_3$): 153.09, 147.83, 127.10, 122.78, 117.00, 117.06, 112.51 (CH), 60.83 (OCH$_3$), 56.60 (OCH$_3$), 26.72 (CH$_2$); IR (CH$_2$Cl$_2$, cm^{-1}): 2930, 2246, 1652, 1477, 1412, 1378, 1322, 1265, 1210, 1058, 1002; HRMS (m:z): calcd. for $[C_{10}H_9^{79}Br_2NO_2]^+$: 332.9000; found 332.9003; R$_f$: 0.5; EtOAc:hexane: (15:85)

4.2.3. Synthesis of 2-(2,6-dibromo-3,5-dimethoxyphenyl)acetonitrile (19)
Using standard procedure written in 4.2.1, the product **19** (650 mg, 75%, yellow solid) was obtained. Mp: 198–199 °C; ^{1}H NMR (400 MHz, CDCl$_3$): 6.53 (s, ArH, 1H), 4.18 (s, 2H), 3.92 (s, OCH$_3$, 6H); ^{13}C NMR (100 MHz, CDCl$_3$): 156.48, 131.62, 115.76, 105.50, 96.96 (CH), 56.73 (2 OCH$_3$), 26.25 (CH$_2$); IR (CH$_2$Cl$_2$, cm^{-1}): 2935, 2246, 1574, 1453, 1339, 1220, 1080, 1068 cm^{-1}; HRMS (m:z): calcd. for: $[C_{10}H_9^{79}Br_2NO_2]^+$: 332.9000; found 332.9001; R$_f$: 0.46; EtOAc:hexane: (3:7).

4.2.4. Synthesis of 2-(2,3,6-tribromo-4,5-dimethoxyphenyl)acetonitrile (20)
Using standard procedure written in 4.2.1, the product **20** (530.00 mg, 80%, white solid) was obtained. Mp: 161–162°C; ^{1}H NMR (400 MHz, CDCl$_3$): 4.22 (s, CH$_2$, 2H), 3.92 (s, OCH$_3$, 3H), 3.91 (s, OCH$_3$, 3H); ^{13}C NMR (100 MHz, CDCl$_3$): 152.60, 151.28, 127.88, 122.88, 122.45, 120.76, 115.63, 61.24 (OCH$_3$), 61.20 (OCH$_3$), 28.22 (CH$_2$); IR (CH$_2$Cl$_2$, cm^{-1}): 2935, 2246, 1372, 1092, 1047, 1008 cm^{-1}; HRMS (m:z): calcd. for: $[C_{10}H_8^{79}Br_3NO_2]^+$: 410.8105; found: 410.8108; R$_f$: 0.74; EtOAc:hexane: (3:7).

4.2.5. Synthesis of 2-(3-bromo-4,5-dimethoxyphenyl)acetic acid (21): Standard procedure for the hydrolysis reaction
A solution of **17** (500 mg, 1.95 mmol) in EtOH (12 mL) and H$_2$O (8 mL) was added to NaOH (780 mg, 19.52 mmol). After the reaction was allowed to stir for 24 h at 100 °C and was cooled to RT, removal of the solvent under vacuum, and then acidification of the reaction mixture with addition of cold HCl solution (1.0 M, 0 °C) until pH of the solution adjusted to 2.0 were performed. Respectively, extraction of the mixture with EtOAc (2 × 20 mL), combination and drying over Na$_2$SO$_4$ of organic phases and evaporation of the under vacuum, removal of the solvent under vacuum, and the product **21** (430 mg, 80%, yellow solid) was obtained. Mp: 106–107 °C; ^{1}H NMR (400 MHz, CDCl$_3$): 10.68 (s, CO$_2$H, 1H), 7.05 (s, ArH, 1H), 6.77 (s, ArH, 1H), 3.85 (s, OCH$_3$, 3H), 3.83 (s, OCH$_3$, 3H), 3.56 (s, CH$_2$, 2H); ^{13}C NMR (100 MHz, CDCl$_3$): 177.63 (CO), 153.83 (C), 145.97 (C), 130.41 (C), 125.72 (CH), 117.85 (C), 113.09 (CH), 60.80 (OCH$_3$), 56.33 (OCH$_3$), 40.65 (CH$_2$); IR (CH$_2$Cl$_2$, cm^{-1}): 3446, 1646, 1569, 1490, 1273, 1142, 1046 cm^{-1}; HRMS (m:z): calcd. for: $[C_{10}H_{11}^{79}BrO_4]^+$: 273.9841 found 273.9842, R$_f$: 0.23; EtOAc:hexane: (3/7).

4.2.6. Synthesis of 2-(2,3-dibromo-4,5-dimethoxyphenyl)acetic acid (22)
Using standard procedure written in 4.2.5, the product **22** (420 mg, 80%, yellow solid) was obtained. Mp: 140–141°C; ^{1}H-NMR (400 MHz, CDCl$_3$): 11.40-10.00 (m, COOH), 6.84 (s, ArH, 1H), 3.86 (s, CH$_2$, 2H), 3.86 (s, OCH$_3$, 3H), 3.84 (s, OCH$_3$, 3H); ^{13}C-NMR (100 MHz, CDCl$_3$): 176.68 (CO), 152.69 (C), 147.29 (C), 130.91(C), 122.05 (C), 118.51 (C), 114.40 (CH), 60.74 (OCH$_3$), 56.45 (OCH$_3$), 43,26 (CH$_2$); IR (CH$_2$Cl$_2$, cm^{-1}): 3447, 1634, 1472, 1424, 1381, 1310, 1263, 1202, 1163, 1060, 1004; HRMS (m:z): calcd. for: $[C_{10}H_{10}^{79}Br_2O_4]^+$: 351.8945, found 351.8955; R$_f$: 0.17, EtOAc:hexane: (3:7).

4.2.7. Synthesis of 2-(2,6-dibromo-3,5-dimethoxyphenyl)acetic acid (23)
Using standard procedure written in 4.2.5, the product **23** (495 mg, 78%, white solid) was obtained. Mp: 235–236 °C (231–232 °C) [55]; ^{1}H-NMR (400 MHz, acetone-d$_6$): 6.71 (s, ArH, 1H) 4.02 (s, CH$_2$, 2H), 3.82 (s, OCH$_3$, 6H); ^{13}C-NMR (100 MHz, acetone-d$_6$): 170.29 (CO), 157.11 (2 C), 136.82 (C), 106.52 (2 C), 97.46 (CH), 57.04 (2 OCH$_3$), 42.93 (CH$_2$); IR (CH$_2$Cl$_2$, cm^{-1}): 3330, 1702,1574, 1427, 1330, 1217, 1096 cm^{-1}; HRMS (m:z): calcd. for: $[C_{10}H_{10}^{79}Br_2O_4]^+$: 351.8946; found: 351.8951; R$_f$: 0.45; MeOH:CH$_2$Cl$_2$:(5:95).

4.2.8. Synthesis of 2-(2,3,6-tribromo-4,5-dimethoxyphenyl)acetic acid (24)
Using standard procedure written in 4.2.5, the product **24** (250 mg, 60%, white solid) was obtained. Mp: 162–163 °C; ^{1}H-NMR (400 MHz, CDCl$_3$): 10.80–10.30 (m, CO$_2$H, 1H), 4.27 (s, CH$_2$, 2H), 3.90 (s, OCH$_3$, 3H), 3.90 (s, OCH$_3$, 3H); ^{13}C-NMR (100 MHz, CDCl$_3$): 175.62 (CO), 151.43 (C), 150.65 (C), 131.37 (C), 123.29 (C), 121.55 (C), 121.21 (C), 60.89

(OCH$_3$), 60.86 (OCH$_3$), 44.10 (CH$_2$); IR (CH$_2$Cl$_2$, cm^{-1}): 3444, 2935, 1703, 1651, 1395, 1285, 1093, 1010; HRMS (m/z): calcd. for: [C$_{10}$H$_9$^{79}Br$_3$O$_4$]$^+$: 429.8051, found 429.8057, R$_f$: 0.30; EtOAc/Hexane: (3/7).

4.2.9. Synthesis of 2-(3-bromo-4,5-dihydroxyphenyl)acetic acid (25): Standard procedure for the demethylation reaction with BBr$_3$

A solution of BBr$_3$ (728 mg, 2.91 mmol) in CH$_2$Cl$_2$ (10 mL) was added to a stirring solution of the compound **21** (400 mg, 1.45 mmol) CH$_2$Cl$_2$ (5 mL) under N$_2$ (g) at RT, and then the mixture was stirred at the same condition for 16 h. Termination of the reaction mixture and then slow addition of H$_2$O (3 mL) over 15 min at 0 °C, removal of the solvent under vacuum, the addition of H$_2$O (15 mL) again and extraction of the mixture with EtOAc (2 × 25 mL) were done, respectively. After combination and drying over Na$_2$SO$_4$ of organic phases and evaporation of the solvent under vacuum were performed, the bromophenol **25** (290 mg 85%, brown solid) was obtained.

Mp: 166–167°C; ^{1}H-NMR (400 MHz, acetone-d$_6$): 8.70–8.50 (m, OH, 1H), 8.00–7.85 (m, OH, 1H), 6.96 (d, J = 2.0 Hz, ArH, 1H)), 6.83 (d, J = 2.0 Hz, ArH, 1H)), 3.48 (s, CH$_2$, 2H); ^{13}C-NMR (100 MHz, acetone-d$_6$): 172.24 (CO), 145.79 (C), 142.02 (C), 127.69 (C), 124.39 (CH), 115.90 (CH), 109.13 (C), 39.48 (CH$_2$); IR (CH$_2$Cl$_2$): 3523, 3309, 3204, 1702, 1496, 1434, 1289, 1093 cm^{-1}; R$_f$: 0.25, MeOH:CH$_2$Cl$_2$: (5:95). HRMS (m:z): calcd. for: [C$_8$H$_7$^{79}BrO$_4$-H]$^+$: 244.9449; found 244.9454;

4.2.10. Synthesis of the natural product 2-(2,3-dibromo-4,5-dihydroxyphenyl)acetic acid (1)

Using standard procedure written in 4.2.9, the natural product **1** (310 mg, 85%, brown solid) purified from EtOAc:hexane was obtained. Mp: 157–158 °C, (156–157 °C) [15]; ^{1}H-NMR (400 MHz, acetone-d$_6$): 6.97 (s, ArH, 1H), 3.75 (s, CH$_2$, 2H); ^{13}C-NMR (100 MHz, acetone-d$_6$): 170.94 (CO), 144.59 (C), 127.86 (C), 117.36 (C), 117.24 (CH), 116.57 (C), 112.77 (C), 42.01 (CH$_2$); IR (CH$_2$Cl$_2$): 3275, 1471, 1403, 1277, 1217, 1192, 1066, 1023; R$_f$: 0.40, MeOH:CH$_2$Cl$_2$: (15:85). HRMS (m:z): calcd. for: [C$_8$H$_7$^{79}Br^{81}BrO$_4$-H]$^+$: 324.8534; found 324.8549.

4.2.11. Synthesis of 2-(2,6-dibromo-3,5-dihydroxyphenyl)acetic acid (2)

Using standard procedure written in 4.2.9, the natural product **2** (148 mg, 80%, brown solid) was obtained. Mp: 151–152 °C (191 °C) [16]; ^{1}H-NMR (400 MHz, acetone-d$_6$): 9.10-8.65 (s, OH, 2H), 6.60 (s, ArH, 2H), 3.98 (s, CH$_2$, 2H); ^{13}C-NMR (100 MHz, acetone-d$_6$): 170.38 (CO), 154.70 (2 C), 136.65 (CH), 104.66 (2 C), 103.28 (C), 42.99 (CH$_2$); IR (CH$_2$Cl$_2$): 3616, 3445, 3199, 1706, 1432, 1200, 1093 cm^{-1}; R$_f$: 0.42; MeOH:CH$_2$Cl$_2$: (15:85); HRMS (m:z) calcd for [C$_8$H$_7$^{79}Br$_2$O$_4$ - H]$^+$: 322.85546; found 322.85543.

4.2.12. Synthesis of 2-(2,3,6-tribromo-4,5-dihydroxyphenyl)acetic acid (26)

Using standard procedure written in 4.2.9, the bromophenol **26** (230 mg, 82%, white solid) was obtained. Mp: 190–191 °C; ^{1}H-NMR (400 MHz, acetone-d$_6$): 7.00–6.60 (m, OH, 3H), 4.16 (s, CH$_2$, 2H); ^{13}C-NMR (100 MHz, acetone-d$_6$): 170.76 (CO), 144.51 (C), 144.01 (C), 128.49 (C), 118.31 (C), 113.99 (C), 113.57 (C), 44.22 (CH$_2$); IR (CH$_2$Cl$_2$): 3525, 3305, 3212, 1652, 1401, 1197, 1093 cm^{-1}; R$_f$: 0.17, MeOH:CH$_2$Cl$_2$: (15:85); HRMS (APCI–TOF) (m:z) calcd for [C$_8$H$_4$N^{79}Br$_2$^{81}BrO$_2$ -H]$^-$: 402.76392; found 402.76672.

4.2.13. Synthesis of 2-(2,3-dibromo-4,5-dihydroxyphenyl)acetonitrile (27)

Using standard procedure written in 4.2.9, the bromophenol **27** (95.0 mg, 69%, brown solid) was obtained. Mp 158–159 °C; ^{1}H-NMR (400 MHz, acetone-d$_6$): 9.18 (bs, OH, 1H), 8.60 (bs, OH, 1H), 7.14 (s, ArH, 1H), 3.95 (CH$_2$, 2H); ^{13}C-NMR (100 MHz, acetone-d$_6$): 145.27 (CN), 144.67 (C), 123.52 (C), 117.62 (C), 115.71 (CH), 115.47 (C), 113.67 (C), 25.32 (CH$_2$); IR (CH$_2$Cl$_2$): 3517, 3445, 3354, 1647, 1628, 1413, 1093; HRMS (m:z) calcd for [C$_8$H$_5$^{79}Br^{81}BrNO$_2$ + H$_2$O + H]$^+$: 325.8850; found: 325.87671.

4.2.14. Synthesis of 2-(2,3,6-tribromo-4,5-dihydroxyphenyl)acetonitrile (28)

Using standard procedure written in 4.2.9, the bromophenol **28** (120.0 mg, 65%, brown solid) was obtained. M.p: 193–194 °C; ^{1}H-NMR (400 MHz, acetone-d$_6$): 4.15 (s, CH$_2$, 2H); ^{13}C-NMR (100 MHz, acetone-d$_6$): 145.50, 144.43, 124.28, 117.61, 116.88, 114.04, 112.84, 28.06 (CH$_2$); IR (CH$_2$Cl$_2$): 3521, 3312, 2324, 1404, 1092 cm^{-1}; R$_f$: 0.42, MeOH:CH$_2$Cl$_2$: (5:95); HRMS (APCI – TOF) (m/z) calcd for [C$_8$H$_4$NBr$_3$O$_2$ + Na]$^+$: 405.76899; found: 405.78860.

4.3. Biological assay (Antioxidant activity)

Fe^{3+}-reducing effects of the compounds were realized by Fe^{3+}(CN-)$_6$ reducing [46] as given previously [56]. Cu^{2+} reducing effect of the bromophenol compounds was realized according to a prior study [57] as detailed given [58,59]. FRAP method of the bromophenol compounds is realized by reducing of Fe^{3+}-TPTZ complex in an acidic environment [60]. The DPPH radical removing effects of the bromophenol compounds were done according to the method of Blois [61] as given previously [62–64]. ABTS radical scavenging of bromophenol compounds is performed according to Gulcin's methods [65–67]. The radical scavenging capacities (RSC) of the bromophenol compounds were calculated as follows: RSC (%) =

(1-Ac/Ad) × 100 [4,68,69]. Where Ac and Ad are the absorbances of control and the compounds. IC_{50} was obtained from the graph, which plotted inhibition percentage against the bromophenol compounds concentrations (µg/mL) [35,70,71].

## Acknowledgment

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Supplementary material contains NMR and HRMS spectra of synthesized compounds.
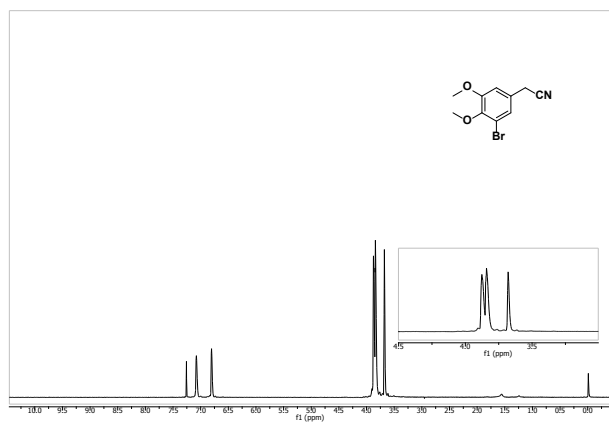
¹H-NMR spectrum of the compound **17** (400 MHz, CDCl₃)
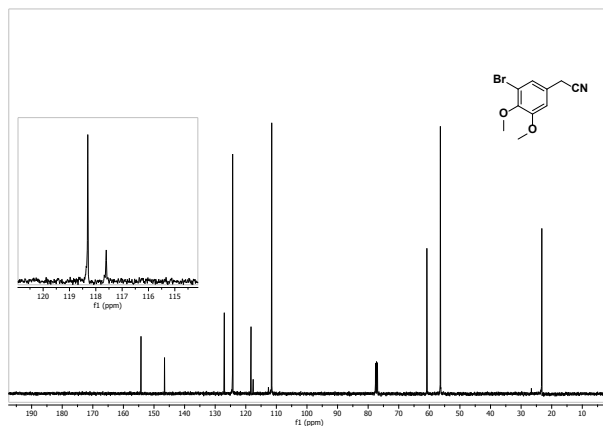
¹³C-NMR spectrum of the compound **17** (100 MHz, CDCl₃).
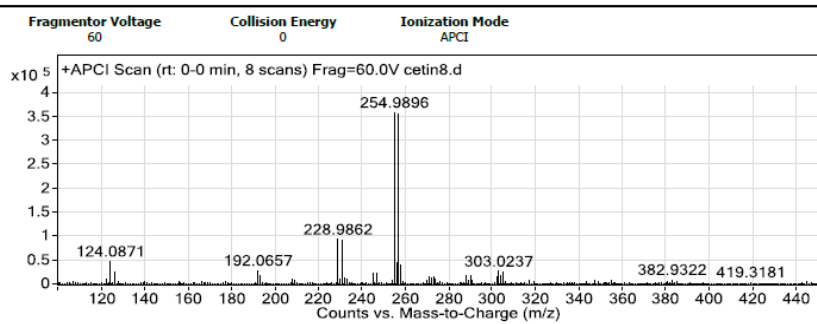
HRMS spectrum of the compound **17.**
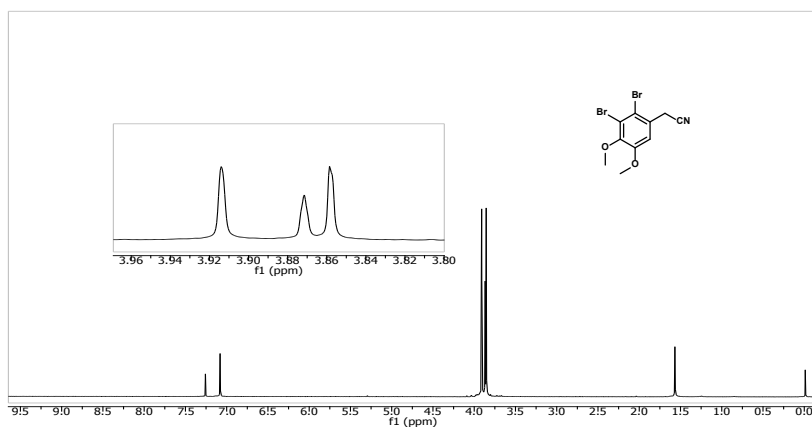
¹H-NMR spectrum of the compound **18** (400 MHz, CDCl₃).
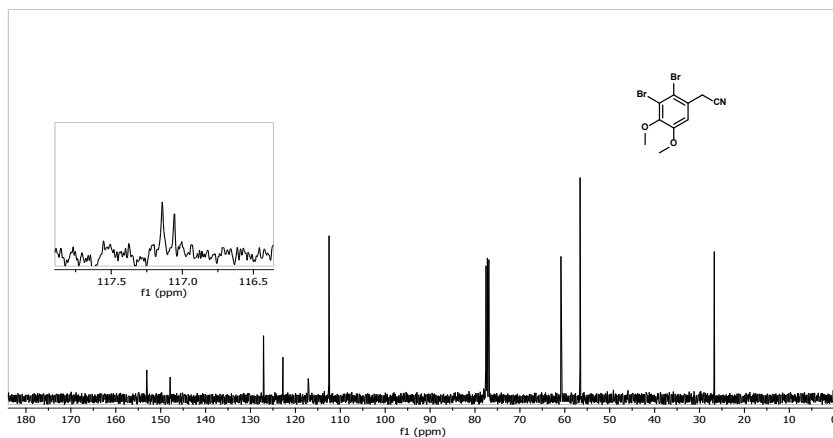
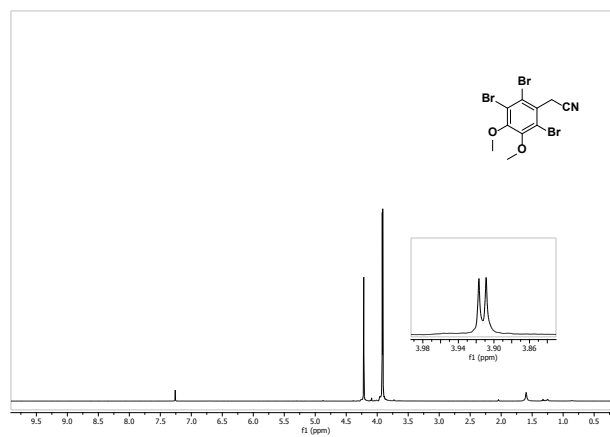^{13}C-NMR spectrum of the compound **18** (100 MHz, CDCl$_3$).
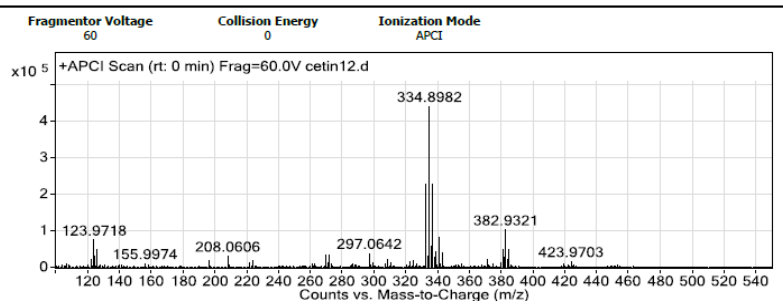
297.0642	1	37843.61
308.8941		22530.77
324.8872		17110.18
332.9003	1	227479.84
333.9016	1	29998.16
334.8982	1	439875.94
335.9009	1	56953.25
336.8964	1	227899.66
337.8985	1	27387.01
338.8878	1	41289.29

HRMS spectrum of the compound **18.**
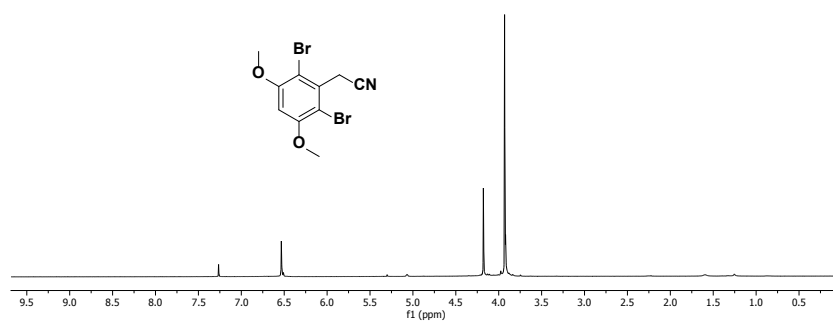
^{1}H-NMR spectrum of the compound **19** (400 MHz, CDCl$_3$).
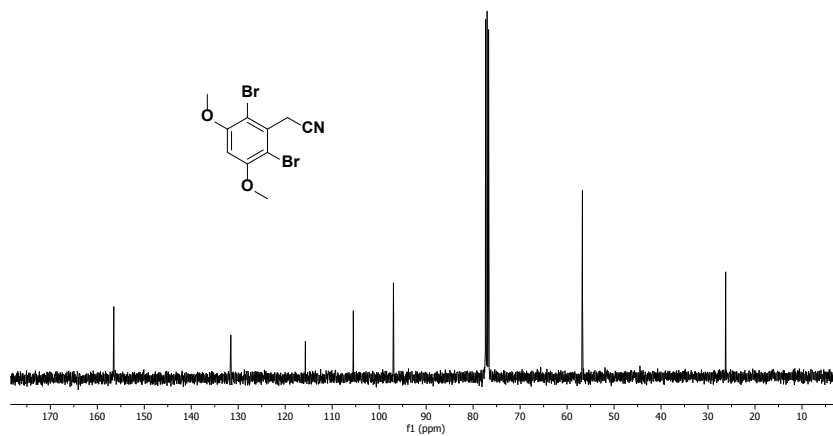
^{13}C-NMR spectrum of the compound **19** (100 MHz, CDCl$_3$).
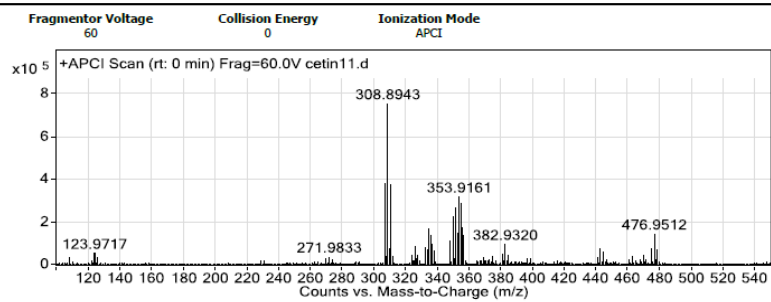
HRMS spectrum of the compound **19.**

^{1}H-NMR spectrum of the compound **20** (400 MHz, CDCl$_3$).
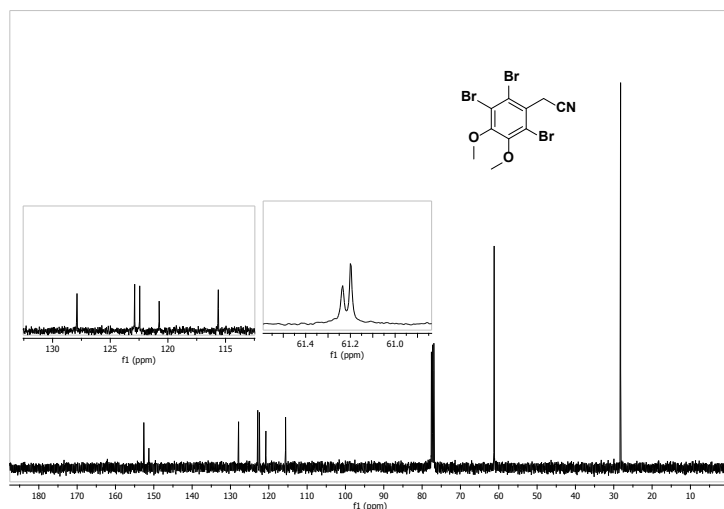
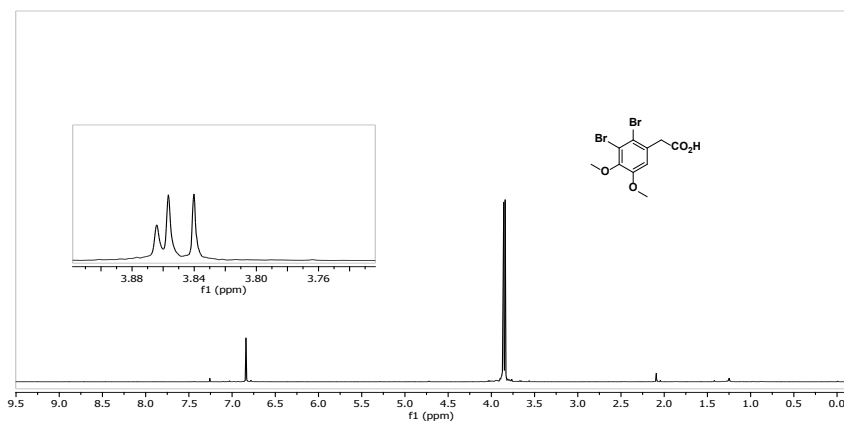^{13}C-NMR spectrum of the compound **20** (100 MHz, CDCl$_3$).
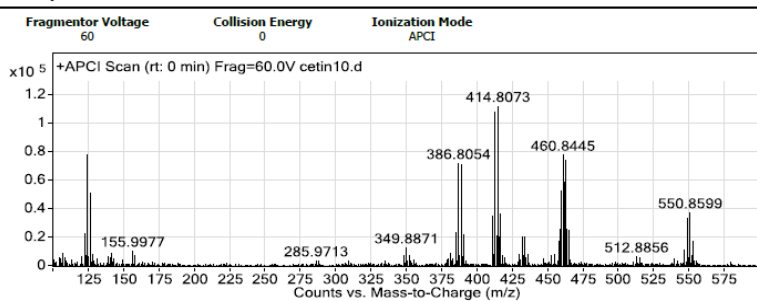
HRMS spectrum of the compound **20.**
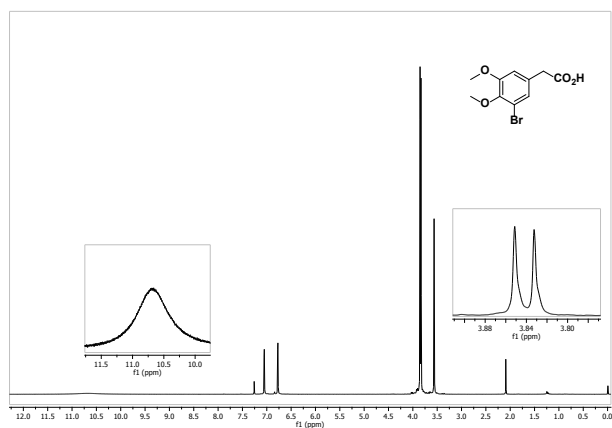
^{1}H-NMR spectrum of the compound **21** (400 MHz, CDCl$_3$).
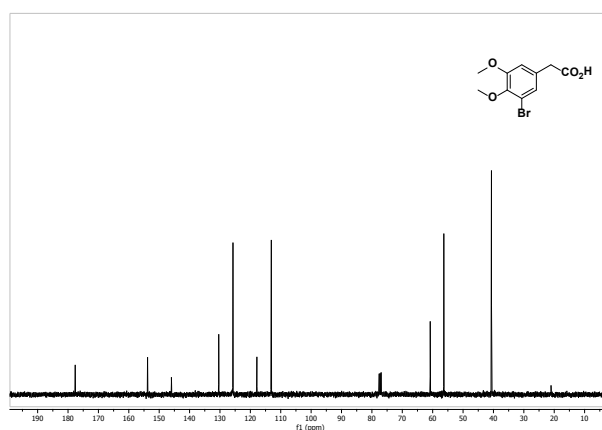
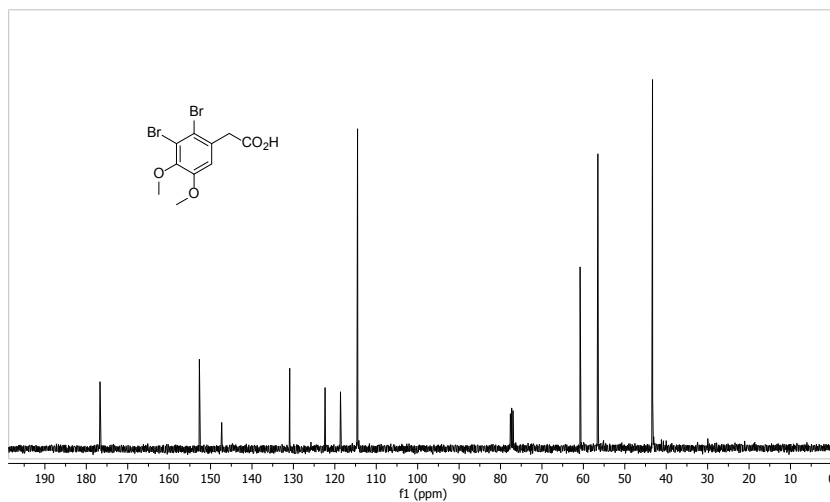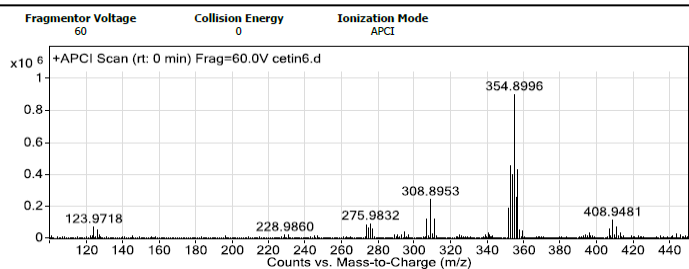^{13}C-NMR spectrum of the compound **21** (100 MHz, CDCl$_3$).

User Spectra

| Fragmentor Voltage	Collision Energy	Ionization Mode
60	0	APCI

x10 5 +APCI Scan (rt: 0 min) Frag=60.0V cetin5.d

274.9915

196.0734

576.0068

365.9933

663.4557

775.9527

Counts vs. Mass-to-Charge (m/z)
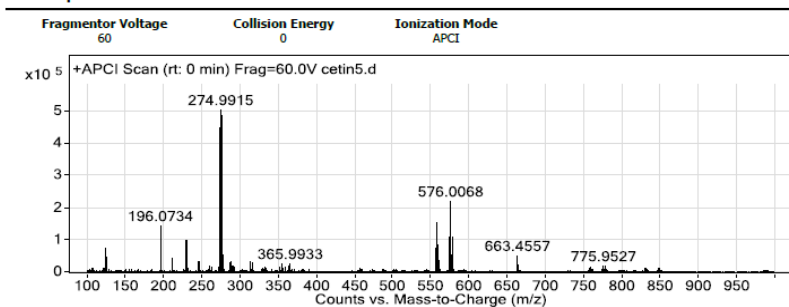
Peak List

m/z	z	Abund
196.0734	1	143059.23
228.9863	1	97426.02
273.9842		447348.72
274.9915		503826.59
275.9832		480836.72
276.9895	1	485298.09

HRMS spectrum of the compound **21.**

^{1}H-NMR spectrum of the compound **22** (400 MHz, CDCl$_3$).

^{13}C-NMR spectrum of the compound **22** (100 MHz, CDCl$_3$).

User Spectra

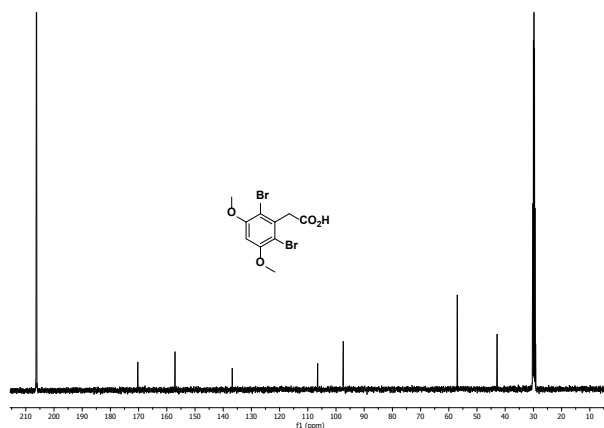| Fragmentor Voltage	Collision Energy	Ionization Mode
60	0	APCI

+APCI Scan (rt: 0 min) Frag=60.0V cetin6.d

x10 6

354.8996

308.8953

275.9832

123.9718 228.9860 408.9481

Counts vs. Mass-to-Charge (m/z)

Peak List

m/z	z	Abund
306.897	1	116720.84
308.8953	1	240420.28
310.8931	1	116388.96
351.895		184165.31
352.9025		451257.59
353.8948		396803.56
354.8996	1	899758.13
355.8961	1	252435.44
356.8991	1	430681

HRMS spectrum of the compound **22.**
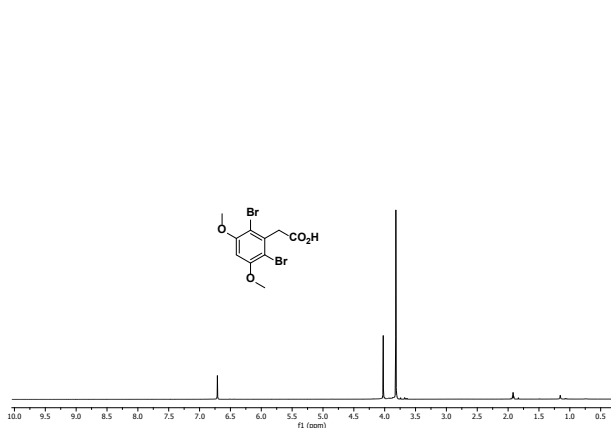
^{1}H-NMR spectrum of the compound **23** (400 MHz, acetone-d$_6$).

^{13}C-NMR spectrum of the compound **23** (100 MHz, acetone-d$_6$).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
60	0	APCI

+APCI Scan (rt: 0 min) Frag=60.0V cetin7.d

x10 5

124.0870

354.9005

193.0705 275.9838 395.9296

Counts vs. Mass-to-Charge (m/z)
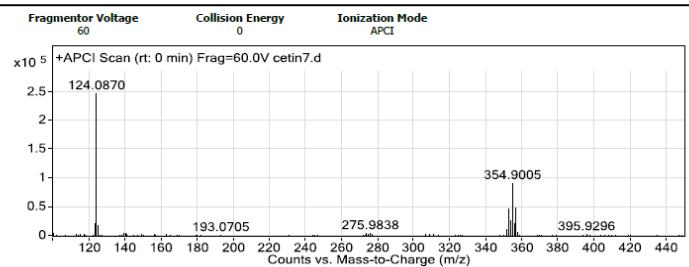
Peak List

| m/z	z	Abund
123.079		20386.04
124.087	1	246743.14
125.0893	1	17806.93
351.8951		10953.78
352.9025		46660.81
353.8953		25409.31
354.9005	1	90814.16
355.8977	1	19802.19
356.8982	1	47647.96

HRMS spectrum of the compound **23.**
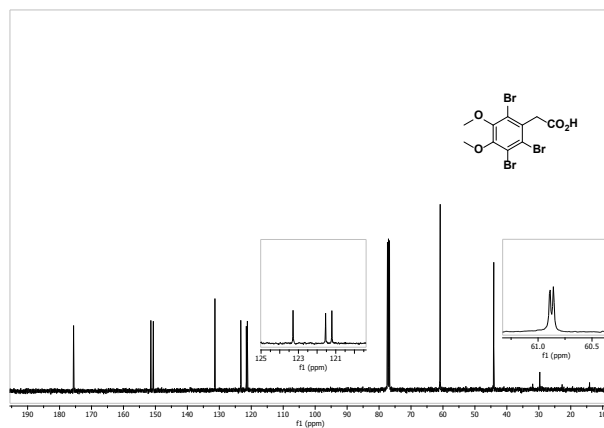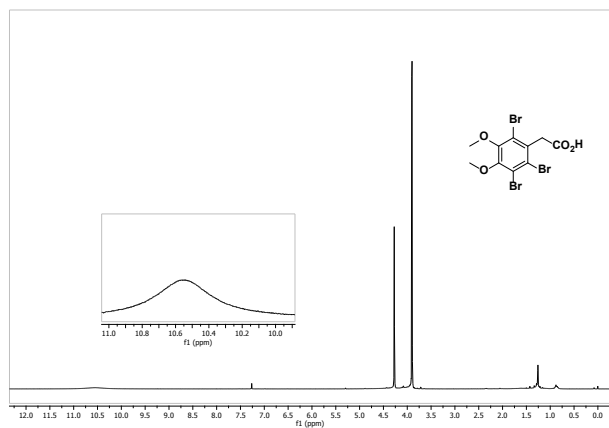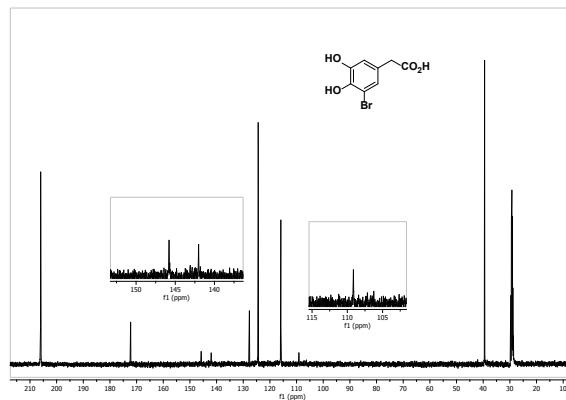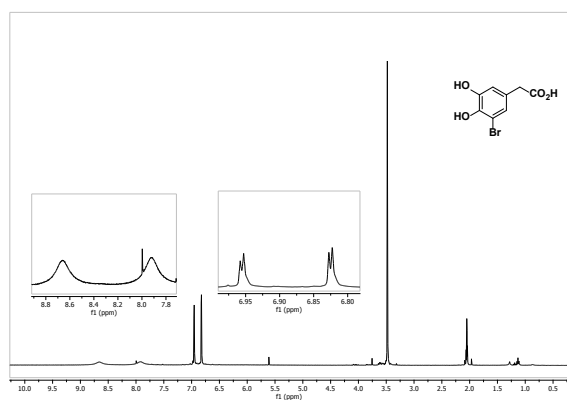
¹H-NMR spectrum of the compound **24** (400 MHz, CDCl₃).

¹³C-NMR spectrum of the compound **24** (100 MHz, CDCl₃).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
60	0	APCI
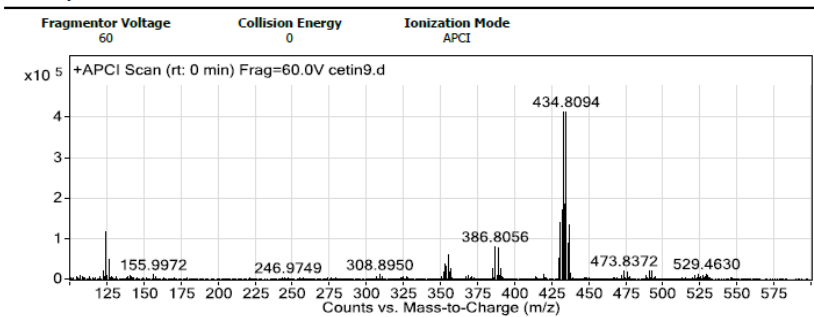
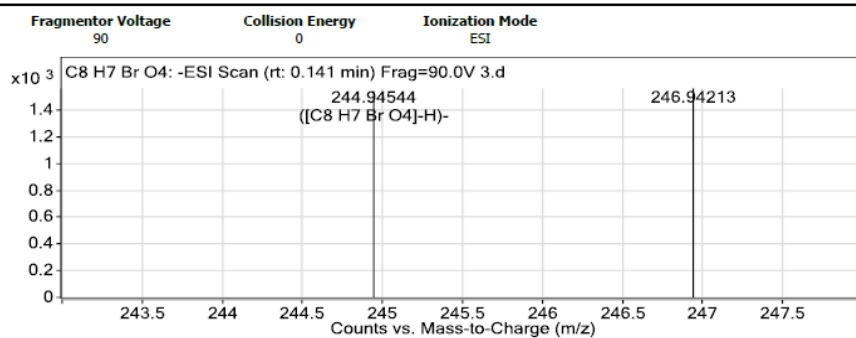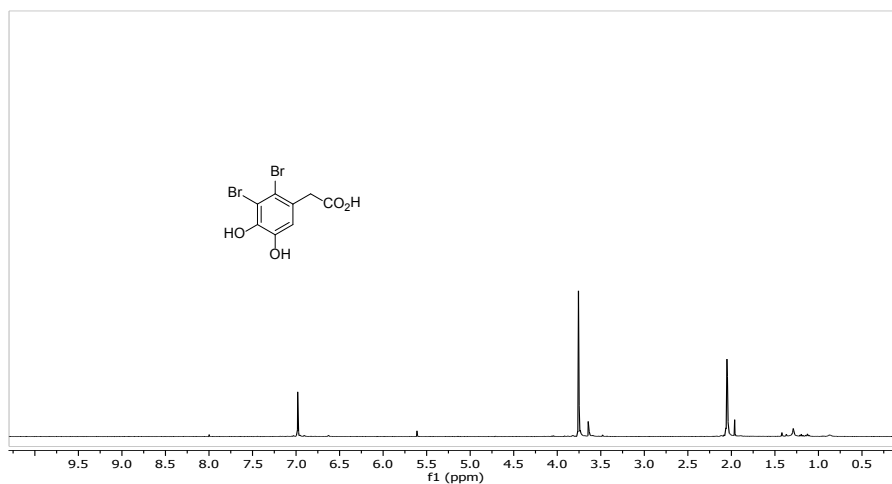|		
356.8983	1	25124.25
384.8075	1	26449.5
386.8056	1	79756.05
388.8034	1	77935.27
390.802	1	24647.34
419.3168	1	12886.86
429.8057		52382.87
430.8132		139661.25
431.8046		170433.81
432.8111	1	412906
433.8044	1	185079.81
434.8094	1	412978.28
435.8058	1	88696.3
436.8073	1	133981.95
437.8098	1	14030.51

HRMS spectrum of the compound **24.**

¹H-NMR spectrum of the compound **25** (400 MHz, acetone-d₆).

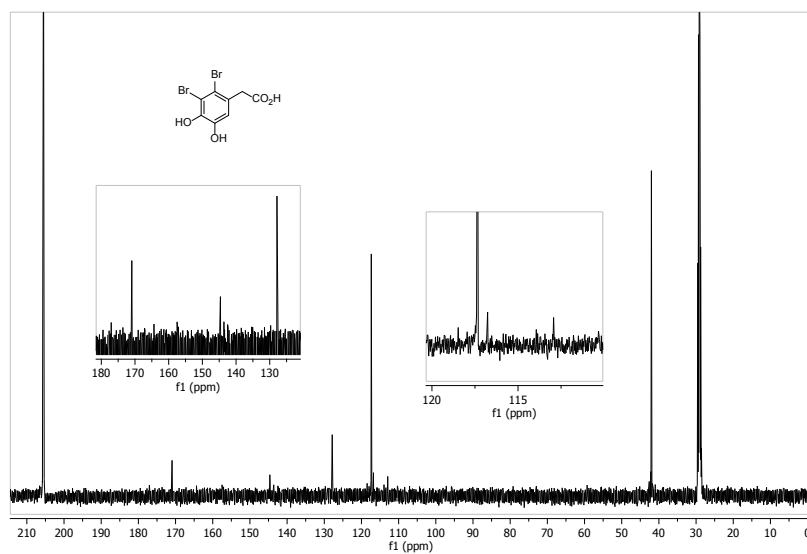¹³C-NMR spectrum of the compound **25** (100 MHz, acetone-d₆).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
90	0	ESI

x10³ C8 H7 Br O4: -ESI Scan (rt: 0.141 min) Frag=90.0V 3.d

244.94544
([C8 H7 Br O4]-H)-

246.94213

Counts vs. Mass-to-Charge (m/z)

HRMS spectrum of the compound **25.**

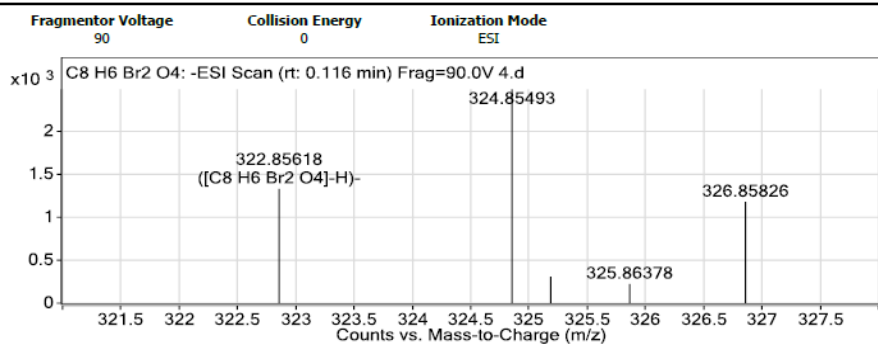¹H-NMR spectrum of the natural product **1** (400 MHz, acetone-d$_6$).

¹³C-NMR spectrum of the natural product **1** (100 MHz, acetone-d$_6$).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
90	0	ESI

x10 3 C8 H6 Br2 O4: -ESI Scan (rt: 0.116 min) Frag=90.0V 4.d

324.85493

322.85618
([C8 H6 Br2 O4]-H)-

326.85826

325.86378

Counts vs. Mass-to-Charge (m/z)

HRMS spectrum of the compound **1.**
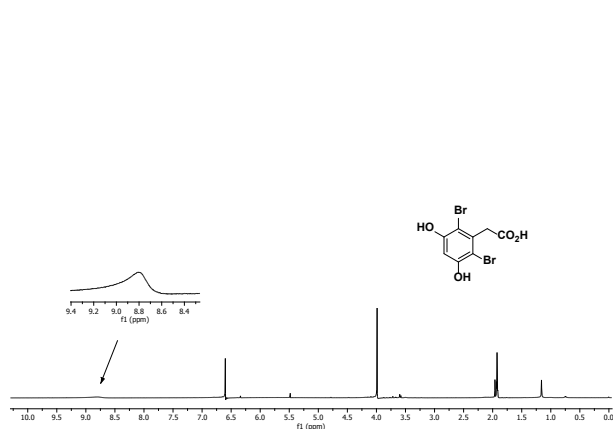
^{1}H-NMR spectrum of the natural product **2** (400 MHz, acetone-d$_6$).
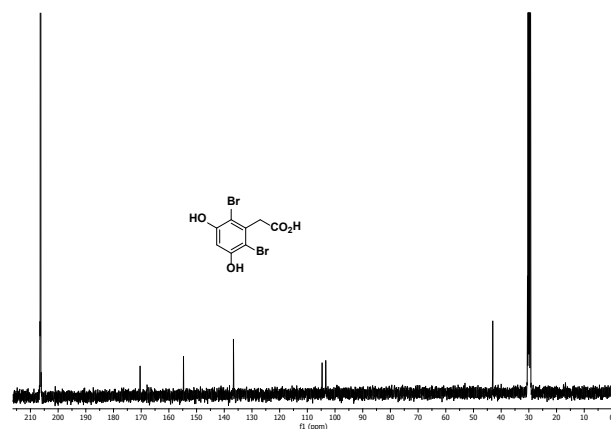
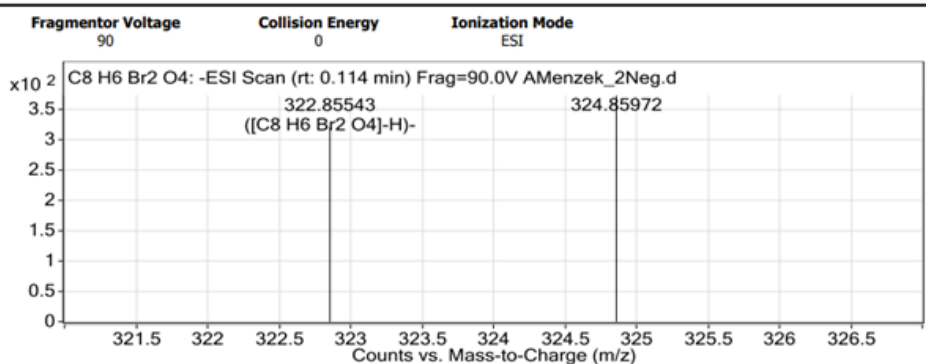^{13}C-NMR spectrum of the natural product **2** (100 MHz, acetone-d$_6$).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
90	0	ESI

x10 2 C8 H6 Br2 O4: -ESI Scan (rt: 0.114 min) Frag=90.0V AMenzek_2Neg.d

322.85543
([C8 H6 Br2 O4]-H)-

324.85972

Counts vs. Mass-to-Charge (m/z)

Peak List

m/z	z	Abund	Formula	Ion
112.98642		1518.68		
132.8701		1035.65		
134.86615		1288.44		

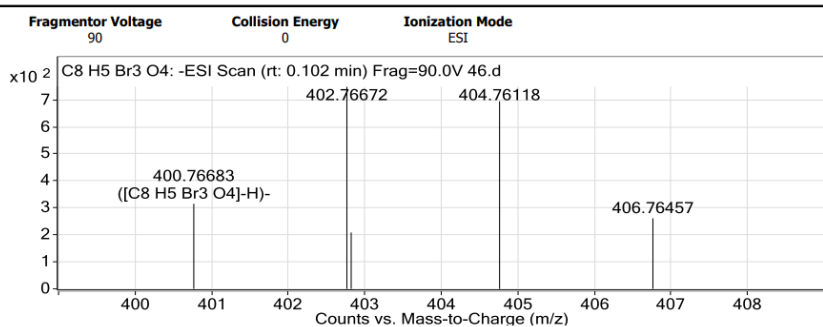| 307.86633	293.94		
322.85543	321.04	C8 H6 Br2 O4	(M-H)-
324.85972	373.45		
358.7778	274.34		

HRMS spectrum of the compound **2.**
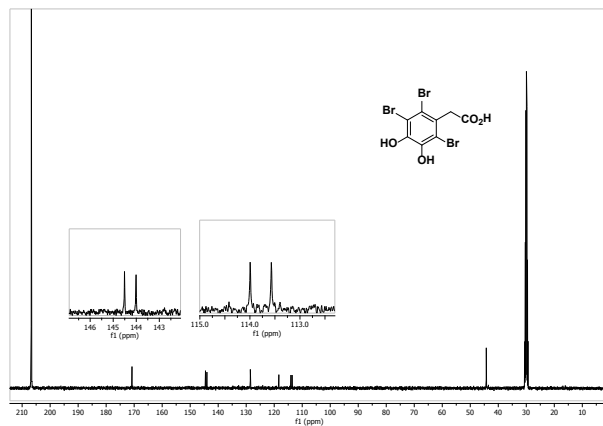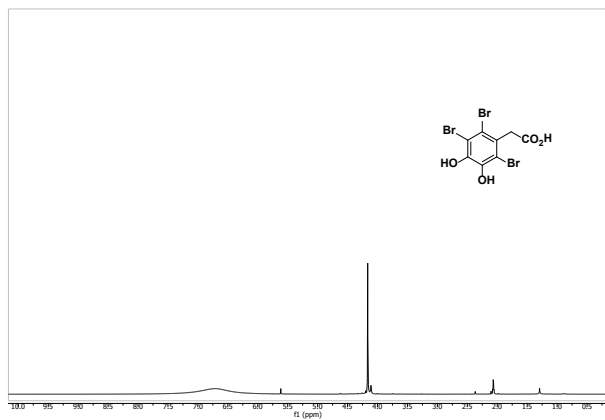
¹H-NMR spectrum of the compound **26** (400 MHz, acetone-d₆).

¹³C-NMR spectrum of the compound **26** (100 MHz, acetone-d₆).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
90	0	ESI

x10 2   C8 H5 Br3 O4: -ESI Scan (rt: 0.102 min) Frag=90.0V 46.d

Peak List

m/z	z	Abund	Formula	Ion
112.9884		652.99		
400.76683		315.11	C8 H5 Br3 O4	(M-H)-
402.76672		748.82		
402.82288		208.7		
404.76118		694.41		

HRMS spectrum of the compound **26.**

¹H-NMR spectrum of the compound **27** (400 MHz, acetone-d₆).

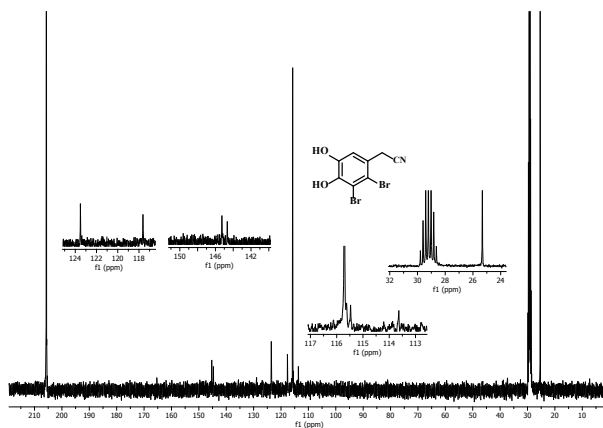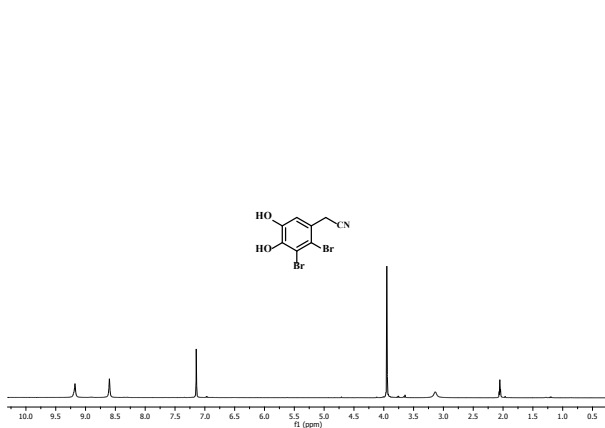¹³C-NMR spectrum of the compound **27** (100 MHz, acetone-d₆).

User Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
90	0	ESI

x10 3 +ESI Scan (rt: 0.106-0.299 min, 118 scans) Frag=90.0V AM27.d

437.18374

359.89693

230.24128

325.87671

415.20194

191.15841

Counts vs. Mass-to-Charge (m/z)

Peak List

m/z	z	Abund
102.12434	1	22182.62
102.15192		762.7

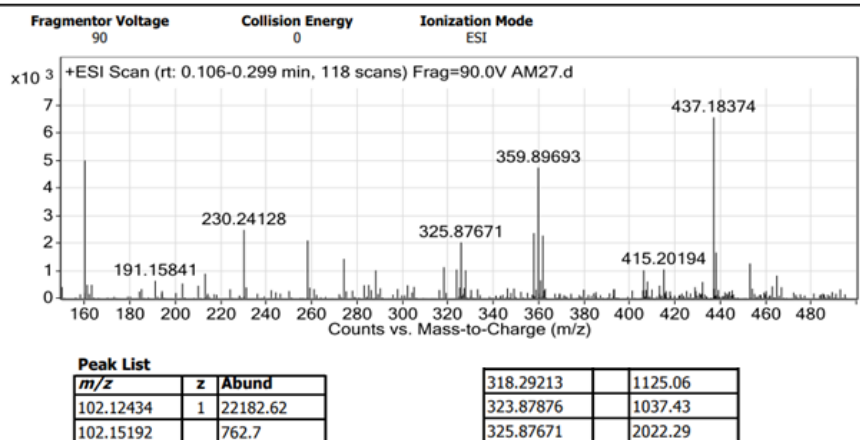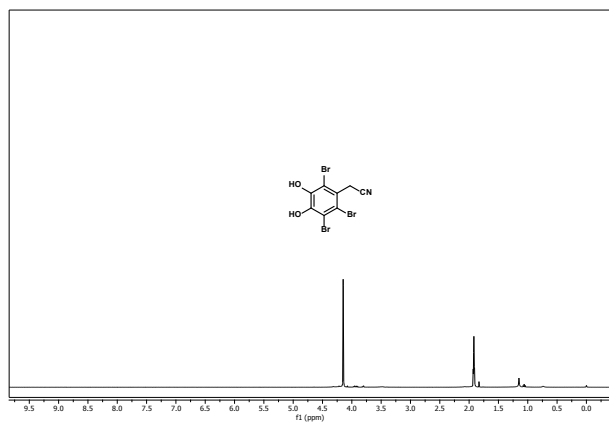|	
318.29213	1125.06
323.87876	1037.43
325.87671	2022.29

HRMS spectrum of the compound **27.**

¹H-NMR spectrum of the compound **28** (400 MHz, acetone-d₆).

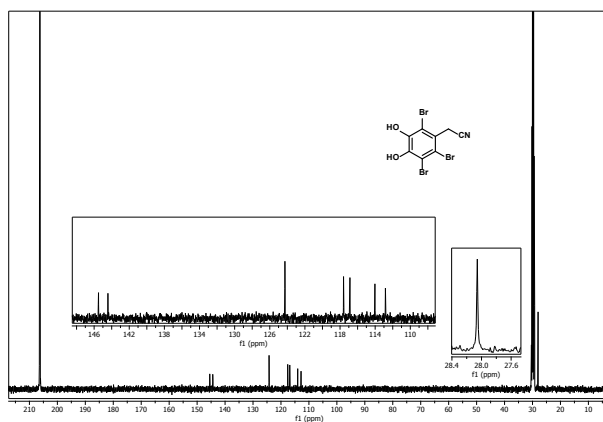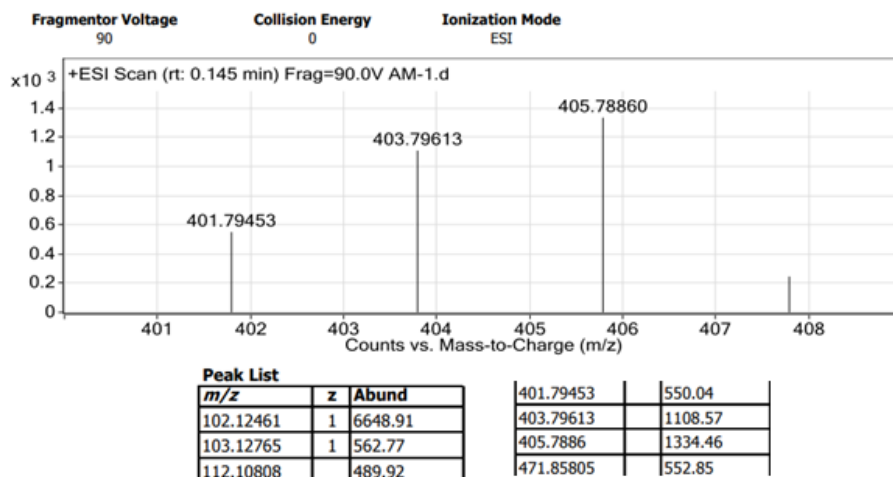¹³C-NMR spectrum of the compound **28** (100 MHz, acetone-d₆).

Fragmentor Voltage	Collision Energy	Ionization Mode
90	0	ESI

x10 3 +ESI Scan (rt: 0.145 min) Frag=90.0V AM-1.d

405.78860

403.79613

401.79453

Counts vs. Mass-to-Charge (m/z)

Peak List

m/z	z	Abund
102.12461	1	6648.91
103.12765	1	562.77
112.10808		489.92

401.79453	550.04
403.79613	1108.57
405.7886	1334.46
471.85805	552.85

HRMS spectrum of the compound **28.**