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RESEARCH ARTICLE

Synthesis and carbonic anhydrase inhibitory properties of novel bromophenols including natural products

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Abstract
(2-Bromo-3,4-dimethoxyphenyl) (3,4-dimethoxyphenyl)methanone (**10**) and its derivatives with Br, one dibromide and isomeric three tribromides, were synthesized. Demethylation of these compounds afforded a series of new bromophenols. Inhibition of human cytosolic carbonic anhydrase II (hCA II) isozyme by these new bromophenols and naturally occurring 3,4,6-tribromo-5-(2,5-dibromo-3,4-dihydroxybenzyl)benzene-1,2-diol (**3**), and 5,5′-methylenebis(3,4,6-tribromo-benzene-1,2-diol) (**4**) was investigated. The synthesized compounds showed carbonic anhydrase inhibitory capacities with IC_{50} values in the range of 0.7–372 μM against hCA II. Some bromophenols investigated here showed effective hCA II inhibitory activity and might be used as leads for generating novel carbonic anhydrase inhibitors which are valuable drug candidates for the treatment of glaucoma, epilepsy, gastric and duodenal ulcers, neurological disorders, or osteoporosis.

Keywords: Bromophenols, diphenylmethane, carbonic anhydrase, glaucoma, enzyme inhibition

Introduction

Naturally occurring bromophenols, frequently isolated from red algae of the family Rhodomelaceae, have prominent biological activities[1,2]. Of these natural compounds, 5,5′-methylenebis(3,4-dibromobenzene-1,2-diol) (**1**) and 3,4-dibromo-5-(2-bromo-3,4-dihydroxy-6-(methoxymethyl)benzyl)benzene-1,2-diol (**2**) exhibit enzyme inhibition, e.g. isocitrate lyase[3] cytotoxicity[4], feeding deterrent[5], and microbial[6,7] activities, while 3,4,6-tribromo-5-(2,5-dibromo-3,4-dihydroxybenzyl) benzene-1,2-diol (**3**) and 5,5′-methylenebis(3,4,6-tribromo-benzene-1,2-diol) (**4**) exhibit significant aldose reductase inhibitory activity[8]. Additionally, it was reported that bromophenol **1** is an inhibitor of protein tyrosine phosphatase[9]. Antioxidant activities of **1** and **4** have also been reported[10,11]. Recently, we have achieved an alternative synthesis of **1**[10], first total synthesis of **2, 3, 4**[12,13], and a series of diphenylmethanone like bromophenols **5**[10]. We have reported that compound **1** and a series of **5** show high antioxidant and radical scavenging activities[10]. Compound **6** and its derivatives with different number of bromines are also diphenylmethanone like compounds which are similar to **5** (Figure 1).

The carbonic anhydrases (CA; Carbonate hydrolyase, EC 4.2.1.1) are a ubiquitous family of zinc-containing enzymes that classically participate in the maintenance of pH homeostasis in human body, catalyzing the reversible hydration of carbon dioxide in a two-step reaction to yield bicarbonate and protons[14]. Sixteen isozymes have been described so far, that differ in their subcellular

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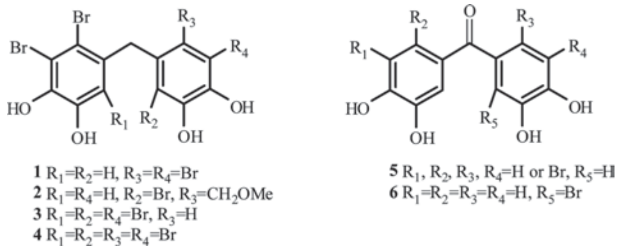


Figure 1. Some naturally occurring bromophenols.

localization, catalytic activity and susceptibility to different classes of inhibitors. Some of these isozymes are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), others are membrane-bound (CA IV, CA IX, CA XII and CA XIV), two are mitochondrial (CA VA and CA VB), and one is secreted in saliva (CA VI). It has been reported that CA XV isoform is not expressed in humans or in other primates, but it is abundant in rodents and other higher vertebrates[15–17]. CAs are produced in a variety of tissues where they participate in several important biological processes such as acid-base balance, respiration, carbon dioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis and body fluid generation[15,18,19]. The two major CA isozymes (CA I and CA II) are present at high concentrations in the cytosol in erythrocytes, and CA II has the highest turnover rate among all CAs. Many of the CA isozymes involved in these processes are important therapeutic targets with the potential to be inhibited to treat a range of disorders including oedema, glaucoma, obesity, cancer, epilepsy and osteoporosis[18–20].

Interaction of most CA isozymes with several types of phenols, such as simple phenol and its substituted derivatives, clioquinol, salicyclates and some of their derivatives, has been recently investigated[20–23]. Here, we extend these earlier investigations to a novel series of bromophenols.

Chemicals are generally known to activate or inhibit several enzymes *in vivo* and affect metabolic pathways. Inhibitory effects of different anions, metal ions, drugs, phenols and sulfonamides, which are specific inhibitors, have been so far investigated against many CAs[21,24–28]. CA II inhibitors are used for several purposes, in particular for the treatment of glaucoma, epilepsy, and as diuretics or antitumor agents/diagnostic tools[18,19,29].

Many chemical substances and synthesized drugs affect metabolisms by changing enzyme activities[30–33]. As CA II inhibitors are valuable molecules for therapeutical and pharmacological applications, we have synthesized novel bormophenols in the current research and evaluated their potency to be novel carbonic anhydrase inhibitors.

Materials and methods

All chemicals and solvents are commercially available and were used after distillation or treatment with drying agents. Column chromatography (CC): silica gel (SiO_2; 60 mesh, Merck, Darmstadt, Germany). Preparative thick layer chromatography: 1 mm of SiO_2 60 PF (Merck) on glass plates. Mp: cap. melting-point apparatus (BUCHI 530: Flawil, Switzerland); uncorrected. IR Spectra: solns. in 0.1 mm cells with a Mattson 1000 FT-IR spectrophotometer (Cambridge, England). ^{1}H- and ^{13}C- NMR spectra: 200 (50) and 400 (100)-MHz Varian spectrometer (Danbury, CT); δ in ppm; Me4Si as the internal standard. Elemental analyses: Leco CHNS-932 apparatus (MI, USA). Antioxidant activities of samples were determined in a spectrophotometer (UV-1208, Shimadzu, Japan).

Synthesis of (2-bromo-3,4-dimethoxyphenyl)(3,4-dimethoxyphenyl)methanone (10)

Polyphosphoric acid (PPA), prepared from conc. H_3PO_4 (85%, 2.63 g) and P_2O_5 (4.72 g, 33.2 mmol), was heated to 80°C in a beaker (100 mL). To this mixture were added **8** (0.84 g, 4.6 mmol) and **9**[36] (1.0 g, 4.6 mmol) quickly. The mixture was stirred with a glass stick at 80°C for 45 min and was then carefully poured onto 35 mL of ice/water. The organic phase was extracted with EtOAc (2 × 125 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. Monobromide **10** (85%) was the sole product and was crystallized from ethyl acetate as white crystals. Mp 166–167°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.54 (d, J = 2.2 Hz, 1 H), 7.23 (dd, A part of AB-system, J = 8.3 Hz, 2.2 Hz, 1 H), 7.07 (d, A part of AB-system, J = 8.4 Hz, 1 H), 6.93 (d, B part of AB-system, J = 8.4 Hz, 1 H), 6.82 (d, B part of AB-system, J = 8.4 Hz, 1 H), 3.93 (s, methoixde, 6 H), 3.92 (s, methoxide, 3 H), 3.88 (s, methoixde, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 194.24 (CO), 154.89 (C), 154.05 (C), 149.45 (C), 146.95 (C), 134.35 (C), 129.95 (C), 126.51 (CH), 124.78 (CH), 116.18 (C), 111.46 (CH), 111.18 (CH), 110.14 (CH), 60.84 (OCH_3), 56.41 (OCH_3), 56.31 (OCH_3), 56.25 (OCH_3); IR (CH_2Cl_2, cm^{-1}): 3003, 2938, 2839, 1657, 1586, 1512, 1487, 1463, 1417, 1394, 1341, 1294, 1274, 1240, 1217, 1171, 1135, 1032, 991, 904, 879, 813, 796, 759, 729, 636, 569; Anal. Calcd for $C_{17}H_{17}BrO_5$: C, 53.56; H 4.49. found: C, 53.52; H 4.40.

Bromination of compound 10

To a stirring solution of monobromide **10** (2.0 g, 5.2 mmol) in $CHCl_3$ (50 mL) was added a solution of bromine (5.0 g, 31.2 mmol, 6 eq.) in $CHCl_3$ (30 mL) drop wise at room temperature (RT) over 10 min. After the reaction mixture was stirred at RT for 3 days, the solvent was evaporated. Chromatography of the residue (2.71 g) on silica gel (SiO_2, 100 g) with ethyl acetate/hexane (5:95) gave dibromide **11** (0.68 g, 28%), **14** (0.32 g, 12%), **13** (0.71 g, 26%) and **12** (0.76 g, 27%), respectively.

(2-Bromo-3,4-dimethoxyphenyl)(2-bromo-4,5-dimethoxyphenyl)methanone (11)

Mp 122–123°C as white crystals; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.21 (d, A part of AB-system, J = 8.4 Hz, 1 H), 7.06 (s, 1 H), 7.03 (s, 1 H) 6.88 (d, B part of AB-system, J = 8.4 Hz, 1 H) 3.93 (s, methoxide, 3 H), 3.92 (s, methoxide, 3 H), 3.86 (s, methoxide, 3 H), 3.85 (s, methoxide, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 194.19 (CO), 156.31 (C), 152.18 (C), 148.49 (C), 147.26 (C), 133.38 (C), 131.96 (C), 127.67 (CH), 117.97 (C), 116.56 (CH), 114.19 (CH), 113.61 (C), 110.83 (CH), 60.80 (OCH_3), 56.56 (OCH_3), 56.45 (OCH_3), 56.37 (OCH_3); IR (CH_2Cl_2, cm^{-1}): 3005, 2964, 2842, 2591, 1668, 1584, 1505, 1486, 1463, 1445, 1399, 1375, 1336, 1271, 1211, 1171, 1159, 1059, 1030, 994, 919, 867, 820, 785, 735, 702, 647, 584; Anal. Calcd for $C_{17}H_{16}Br_2O_5$: C, 44.38, H 3.51 found: C, 44.38; H 3.52.

(2-Bromo-3,4-dimethoxyphenyl)(2,3-dibromo-4,5-dimethoxyphenyl)methanone (12)

Mp 100–101°C as pale yellow crystals; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.24 (d, A part of AB-system, J = 8.8 Hz, 1 H), 6.98 (s, 1 H), 6.86 (d, part of AB-system, J = 8.8 Hz, 1 H), 3.93 (s, methoxide, 3 H), 3.91 (s, methoxide, 3 H), 3.87 (s, methoxide, 3 H), 3.86 (s, methoxide, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 193.60 (CO), 157.03 (C), 152.76 (C), 147.67 (C), 138.11 (C), 131.33 (C), 129.07 (CH), 126.18 (C), 123.27 (C), 118.75 (C), 114.70 (C), 113.20 (CH), 110.54, (CH), 60.93 (OCH_3), 60.81 (OCH_3), 56.62 (OCH_3), 56.41 (OCH_3); IR (CH_2Cl_2, cm^{-1}): 3003, 2938, 1673, 1588, 1564, 1507, 1464, 1403, 1337, 280, 1262, 1217, 1166, 1141, 1070, 1032, 996, 924, 865, 837, 790, 733, 681, 609. Anal. Calcd for $C_{17}H_{16}Br_3O_5$: C, 37.88, H 2.80 found: C, 37.93; H 2.85.

(2-Bromo-4,5-dimethoxyphenyl)(2,6-dibromo-3,4-dimethoxyphenyl)methanone (13)

Mp 115–117°C as pale yellow crystals; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 1 H), 7.11 (s, 1 H), 7.02 (s, 1 H) 3.94 (s, methoxide, 3 H), 3.91 (s, methoxide, 3 H), 3.87 (s, methoxide, 3 H), 3.84 (s, methoxide, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 192.88 (CO), 153.66 (C), 152.80 (C), 152.14 (C), 148.59 (C), 137.82 (C), 130.58 (C), 129.52 (CH), 117.00 (C), 116.81 (C), 116.74 (CH), 114.36 (CH), 114.23 (C), 61.39 (OCH_3), 61.25 (OCH_3), 56.62 (OCH_3), 56.50 (OCH_3); IR (CH_2Cl_2, cm^{-1}): 2938, 2841, 1671, 1579, 1541, 1512, 1464, 1419, 1399, 1366, 1300, 1270, 1212, 1185, 1142, 1080, 1032, 1004, 918, 804, 775, 734, 665; Anal. Calcd for $C_{17}H_{16}Br_3O_5$: C, 37.88, H 2.80 found: C, 37.86; H 2.84.

(2-Bromo-4,5-dimethoxyphenyl)(2,5-dibromo-3,4-dimethoxyphenyl)methanone (14)

Mp 138–139°C as colourless crystals; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1 H), 7.10 (s, 1 H), 7.09 (s, 1 H) 3.86 (s, methoxide, 3 H), 3.85 (s, methoxide, 3 H), 3.85 (s, methoxide, 3 H), 3.84 (s, methoxide, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 191.38 (CO), 154.50 (C), 153.46 (C), 148.58 (C), 146.65 (C), 134.96 (C), 128.23 (C), 117.55 (CH), 116.61 (C), 116.29 (CH), 115.80 (C), 115.02 (CH), 114.60 (C), 60.97 (OCH_3), 56.63 (2 OCH_3), 56.43 (OCH_3); IR (CH_2Cl_2, cm^{-1}): 3003, 2936, 2841, 1679, 1655, 1586, 1508, 1476, 1442, 1380, 1338, 1298, 1262, 1212, 1158, 1065, 1027, 992, 931, 844, 815, 785, 756, 733, 701, 588; Anal. Calcd for $C_{17}H_{16}Br_3O_5$: C, 37.88, H 2.80 found: C, 37.66; H 2.82.

Standard procedure for demethylation of compounds with OMe by ether cleavage (2-bromo-3,4-dihydroxyphenyl)(3,4-dihydroxyphenyl)methanone (6)

A solution of monobromide **10** (0.43 g, 1.32 mmol) in CH_2Cl_2 (15 mL) was cooled to 0°C and then a solution of BBr_3 (0.9 mL) in CH_2Cl_2 (10.0 mL) was added drop wise under N_2(g) over 5 min. After the cold bath was removed, the mixture was stirred at RT and under N_2 for 1 day. Methanol (35 mL) was slowly added over 15 min and then the solvent was evaporated. After water (45 mL) and EtOAc (2 × 40 mL) were added, the mixture was shaken. The organic phase was separated and the water phase was extracted with EtOAc (2 × 30 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was evaporated. Bromophenol **6** (0.40 g, 93%) was obtained as pale yellow amorphous. Mp 77–78°C; ^{1}H-NMR (400 MHz, CD_3COCD_3) δ 9.06 (m, 1 OH), 8.77 (m, 1 OH), 8.43 (m, 1 OH) 8.28 (m, 1 OH), 7.32 (d, J = 2.2 Hz, 1 H), 7.17 (dd, A part of AB-system, J = 8.1, 2.2 Hz, 1 H), 6.94 (d, A part of AB-system, J = 8.1 Hz, 1 H), 6.90 (d, B part of AB-system, J = 8.1 Hz, 1 H), 6.74 (d, B part of AB-system, J = 8.1 Hz, 1 H); ^{13}C-NMR (100 MHz, CD_3COCD_3) δ 193.59 (CO), 150.79 (C), 146.86 (C), 145.12 (C), 143.33 (C), 133.88 (C), 129.86 (C), 124.26 (CH), 120.21 (CH), 116.69 (CH), 115.08 (CH), 113.89 (CH), 107.53 (C); IR (CH_2Cl_2, cm^{-1}): 3434, 2967, 2075, 1638, 1595, 1524, 1442, 1388, 1300, 1201, 1120, 1032, 1015, 943, 816, 782, 763; Anal. Calcd for $C_{13}H_9BrO_5$: C, C, 48.03; H 2.79 found: C, 48.01; H 2.80.

Synthesis of bromophenols **15–18** from the corresponding compounds **11–14**, respectively.

The standard procedure[10,12,13,35] described above for the synthesis of **6** with BBr_3 was applied. From these reactions, bromophenols **15–18** were obtained.

(2-Bromo-3,4-dihydroxyphenyl)(2-bromo-4,5-dihydroxyphenyl)methanone (15)

It was crystallized from ethyl acetate/hexane as pale yellow crystals (0.382 g, 85%); mp 186–187°C; ^{1}H-NMR (400 MHz, CD_3COCD_3) δ 9.29 (s, 1 OH), 8.96 (s, 1 OH), 8.53 (s, 1 OH) 8.26 (s, 1 OH), 7.11 (s, 1H), 6.96 (s, 1H), 6.91 (d, A part of AB-system, J = 8.2 Hz, 1 H), 6.86 (d, B part of AB-system, J = 8,2 Hz, 1 H); ^{13}C-NMR (100 MHz, CD_3COCD_3) δ 193.29 (CO), 149.15 (C), 148.58 (C), 144.52 (C), 143.70 (C), 132.57 (C), 131.49 (C), 123.31 (CH), 120.58 (CH), 118.66 (CH), 113.64 (CH), 111.01 (C), 108.98 (C); IR (CH_2Cl_2, cm^{-1}): 3368, 2947, 2834, 2526, 2041, 1655, 1594, 1452, 1419, 1295, 1115, 1032, 668; Anal. Calcd for $C_{13}H_8Br_2O_5$: C, 38.65; H 2.00. found: C, 38.64; H 2.01.

(2-Bromo-3,4-dihydroxyphenyl)(2,3-dibromo-4,5-dihydroxyphenyl)methanone (16)

Yellow amorphous (0.34 g, 95%); mp 121–123°C; ^{1}H-NMR (400 MHz, CD_3COCD_3) δ 6.96 (s, 1 H), 6.91 (s, 1 H), 6.90 (s, 1

H); ^{13}C-NMR (100 MHz, CD_3COCD_3), δ 193.05 (CO), 149.24 (C), 146.94 (C), 144.55 (C), 143.96 (C), 133.79 (C), 131.19 (C), 124.47 (CH), 116.19 (CH), 114.38 (C), 113.54 (CH), 113.37 (C), 109.43 (C); IR (CH_2Cl_2, cm^{-1}): 3400, 2950, 2839, 2076, 1648, 1452, 1396, 1295, 1114, 1019, 667; Anal. Calcd for $C_{13}H_7Br_3O_5$: C, 32.33; H 1.46. found: C, 32.11; H 1.45.

(2-Bromo-4,5-dihydroxyphenyl)(2,6-dibromo-3,4-dimethoxyphenyl)methanone (17)

Red amorphous; (0.47 g, 87%); mp 240–242°C′(its color was changed at ≥ 180°C); ^{1}H-NMR (400 MHz, CD_3COCD_3) δ 7.20 (s, 1 H), 7.15 (s, 2 H); ^{13}C-NMR (100 MHz, CD_3COCD_3) δ 190.55 (CO), 150.60 (C), 147.04 (C), 144.49 (C), 143.42 (C), 133.90 (C), 122.01 (CH), 120.16 (CH), 118.75 (C), 118.29 (CH), 113.62 (C), 112.90 (C), 108.33 (C); IR (CH_2Cl_2, cm^{-1}): 3681, 2973, 2863, 2071, 1654, 1587, 1495, 1476, 1454, 1286, 1213, 1144, 1054, 1033; Anal. Calcd for $C_{13}H_7Br_3O_5$: C, 32.33; H 1.46. found: C, 32.33; H 1.45.

(2-Bromo-4,5-dihydroxyphenyl)(2,5-dibromo-3,4-dihydroxyphenyl)methanone (18)

It was crystallized from ethyl acetate/hexane as pale yellow crystals (0.382 g, 85%); mp 186–188°C (its color changed at ≥ 160°C); ^{1}H-NMR (400 MHz, CD_3COCD_3), 9.09 (bs, 1 OH), 9.05 (bs, 1 OH), 8.80 (bs, 1 OH) 8.58 (bs, 1 OH), δ 7.16 (s, 1 H), 7.13 (s, 1 H), 7.02 (s, 1 H); ^{13}C-NMR (100 MHz, CD_3COCD_3), δ 192.02 (CO), 149.66 (C), 146.28 (C), 144.65 (C), 144.30 (C), 133.35 (C), 130.49 (C), 125.70 (CH), 120.78 (CH), 118.94 (CH), 111.33 (C), 108.45 (C), 108.15 (C); IR (CH_2Cl_2, cm^{-1}): 3436, 3225, 2076, 1638, 1285, 1033, 720; Anal. Calcd for $C_{13}H_7Br_3O_5$: C, 32.33; H 1.46. found: C, 32.33; H 1.49.

CA purification assay

The purification of the CA II isozyme was performed in a simple single-step method by means of Sepharose-4B-aniline-sulfanilamide affinity column chromatoghrapy[36]. hCA II was purified 311-fold with a specific activity of 2500 EU mg^{-1} and an overall yield of 16%. Erythrocytes were purified from fresh human blood obtained from the Blood Centre of the Research Hospital at Atatürk University. The blood samples were centrifuged at 1500 rpm for 15 min and the plasma and buffy coat were removed. The red cells were isolated and washed twice with 0.9% NaCl and hemolyzed with 1.5 volumes of ice-cold water. The ghost and intact cells were removed by centrifugation at 20,000 rpm for 30 min at 4°C. The pH of the hemolysate was adjusted to 8.7 with solid Tris. Firstly, Sepharose-4B was oxidized by $KMnO_4$ and subsequently activated by $SOCl_2$. Subsequently, aniline was attached to the activated gel as a spacer arm and finally diazotized sulfanilamide was clamped to the *para* position of aniline molecule as ligand. The hemolysate was applied to the prepared Sepharose 4B-aniline-sulfanylamide affinity column which had been equilibrated with 25 mM Tris-HCl/0.1 M Na_2SO_4 (pH 8.7). The affinity gel was washed with 25 mM Tris-HCl/22 mM Na_2SO_4 (pH 8.7). The human carbonic anhydrase II (hCA II) isozyme was eluted with 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6). All procedures were performed at 4°C.

Hydratase activity assay

Carbonic anhydrase activity was assayed by following the hydration of CO_2 according to our previous studies[37,38]. CO_2-hydratase activity as an enzyme unit was calculated by using the equation (t_0-tc/tc) where t_0 and tc are the times for pH change of the non-enzymatic and the enzymatic reactions, respectively.

Protein determination

Protein quantity was determined spectrophotometrically at 595 nm according to the Bradford method during the purification steps, using bovine serum albumin as the standard[39].

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was performed after purification of the enzymes. It was carried out in 10 and 3% acrylamide for the running and the stacking gel, respectively, containing 0.1% SDS according to Laemmli procedure. A 20-μg sample was applied to the electrophoresis medium. Gels were stained for 1.5 h in 0.1% Coomassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid, then destained with several changes of the same solvent without the dye[40].

Crystal structure determination

For the crystal structure determination, the single-crystal of **13** and **14** were used for data collection on a four-circle Rigaku R-AXIS RAPID-S diffractometer (equipped with a two-dimensional area IP detector). The graphite-monochromatized Mo K_α radiation (λ = 0.71073 Å) and oscillation scans technique with Δω = 5° for one image were used for data collection. The lattice parameters were determined by the least-squares method 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 were performed using CrystalClear (Rigaku/MSC Inc., 2005) software[41]. The structures were solved by direct methods using SHELXS-97[42] and refined by a full-matrix least-squares procedure using the program SHELXL-97. 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* **13**: $C_{17}H_{15}O_5Br_3$, crystal system, space group: triclinic, *P*-1; (no:2); unit cell dimensions: $a = 8.2823(2)$, $b = 10.2487(2)$, $c = 12.7924(3)$ Å, $\alpha = 72.529(5)$, $\beta = 68.923(5)$, $\gamma = 87.664(7)$°; volume: 963.71(6) Å^3; $Z = 2$; calculated density: 1.86 mg/m^3; absorption coefficient: 6.302 mm^{-1}; $F(000)$: 524; θ range for data collection 2.6–30.5°; refinement method: full-matrix least-square on F^2; data/parameters: 3936/228; goodness-of-fit on F^2: 1.267; final R indices [I > 2σ(I)]: $R_1 = 0.089$, $wR_2 = 0.103$; R indices (all data): $R_1 = 0.137$, $wR_2 = 0.115$; largest diff. peak and hole: 0.391 and –0.556 e Å^{-3}; CCDC: 774839. *Crystal data for* **14**: $C_{17}H_{15}O_5Br_3$, crystal system, space group: triclinic, *P*-1; (no:2); unit cell dimensions: $a = 8.3610(5)$, $b = 8.3972(5)$, $c = 14.7542(7)$ Å, $\alpha = 98.097(2)$, $\beta = 95.915(3)$, $\gamma = 107.200(2)°$; volume: 968.2(2) Å^3; $Z = 2$; calculated density: 1.85 mg/m^3; absorption coefficient: 6.273 mm^{-1}; $F(000)$: 524; θ range for data collection 2.6–30.5°; refinement method: full-matrix least-square on F^2; data/parameters: 4293/230; goodness-of-fit on F^2: 1.333; final R indices [I > 2σ(I)]: $R_1 = 0.086$, $wR_2 = 0.135$; R indices (all data): $R_1 = 0.139$, $wR_2 = 0.146$; largest diff. peak and hole: 0.367 and −0.662 e Å^{-3}; crystallographic data were deposited in CSD under CCDC registration number 774807.

Results and discussion

We have synthesized natural product bromophenols **3** and **4** from corresponding materials by the known method as shown in Scheme 1[13]. Reactions of compound **7** with 1,4-dibromo-2,3-dimethoxybenzene and 1,2,5-tribromo-3,4-dimethoxybenzene in the presence of PPA at 80°C gave methylether substituted diarylmethanes in high yields as sole product. The ether cleavage reaction of these diarylmethanes with BBr_3 under mild conditions afforded naturally occurring bromophenols **3** and **4**[13].

(2-Bromo-3,4-dimethoxyphenyl)(3,4-dimethoxyphenyl)methanone (**10**) was synthesized from the reaction of 3,4-dimethoxybenzoic acid (**8**) and 3-bromoveratrole (**9**) with PPA in 85% yield as sole product (Scheme 1). Bromination of monobromide **10** (in $CHCl_3$) with Br_2 (6 eq.) at RT for 3 days followed by CC allowed us to isolate four products **11–14** (Scheme 1). The NMR analysis of **13** and **14** did not allow determination of their structures. Therefore, the exact structures of them were determined by X-ray diffraction analysis (Figure 2).

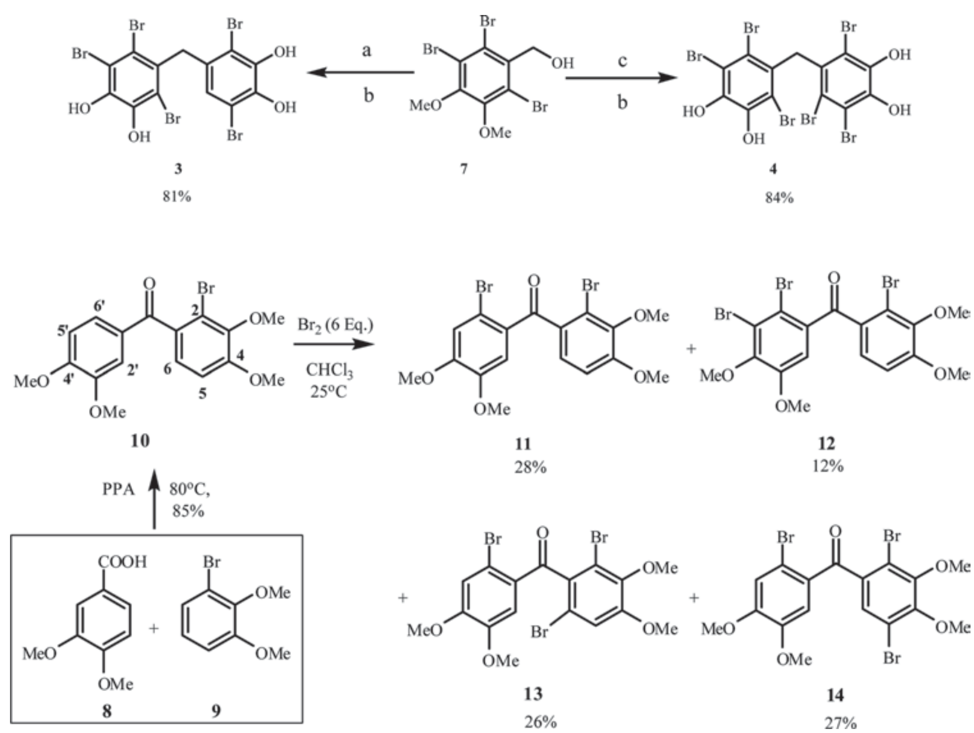
Bromophenols derived from compounds **10–14** may be potential biologically active compounds, because they are similar to **5** with high antioxidant and radical scavenging activities[10]. Therefore, bromophenols **6** and **15–18** were synthesized from compounds **10–14** by ether cleavage reaction with BBr_3 in high yields (Figure 3). Spectroscopic data of **6** and **15–18** are consistent with the proposed structures.

Inhibitory effects of the compounds on CA II catalytic activity were tested under *in vitro* conditions; IC_{50} values were calculated and are given in Table 1.

We report here the first study on the inhibitory effects of the bromophenols derivatives **3–6** and **10–18** on the hydratase activity of hCA II. The data in Table 1 show the following regarding the inhibition of hCA II by bromophenol derivatives.

The strongest inhibitory activity has been observed with compounds **11**, **15–18**, (Table 1). Three derivatives, **3, 10, 13**, showed weak hCA II inhibitory activity with IC_{50}-s in the range of 86.4–372 μM, (Table 1), whereas the remaining four derivatives were quite effective hCA II inhibitors, with IC_{50}-s in the range of 26.4–58 μM, (Table 1). The best hCA II inhibitor in this series of derivatives was the bulky, (2-Bromo-3,4-dihyroxyphenyl)(2,3-dibromo-4,5-dihyroxyphenyl)methanone **(16)**, with a IC_{50} of 0.7 μM.

As revealed by a comparison of the inhibition ranges of molecules, **13** has a higher IC_{50} value than those of its isomers **12** and **14**, and, likewise, **17** has a higher IC_{50} than those of **16** and **18**.



Scheme 1. (A) 1,4-dibromo-2,3-dimethoxybenzene, PPA/80°C, (B) BBr_3/CH_2Cl_2, 0–25°C, (C) 1,2,5-tribromo-3,4-dimethoxybenzene, PPA/80°C.

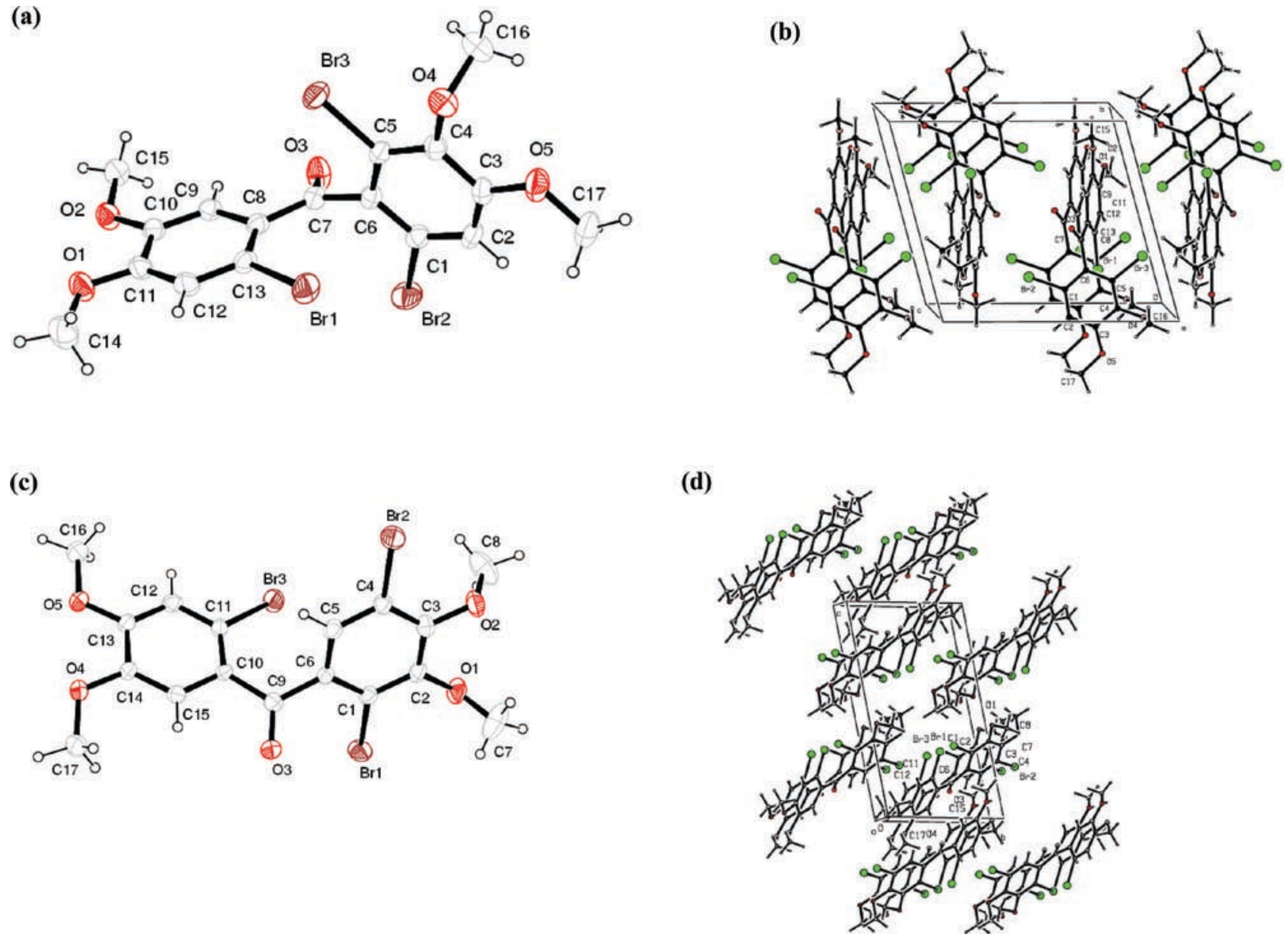


Figure 2. (A) The molecular structure of tribromide **13** showing the atom numbering scheme. (B) Packing diagram for **13**. (C) The molecular structure of tribromide **14** showing the atom numbering scheme. (D) Packing diagram for **14**.

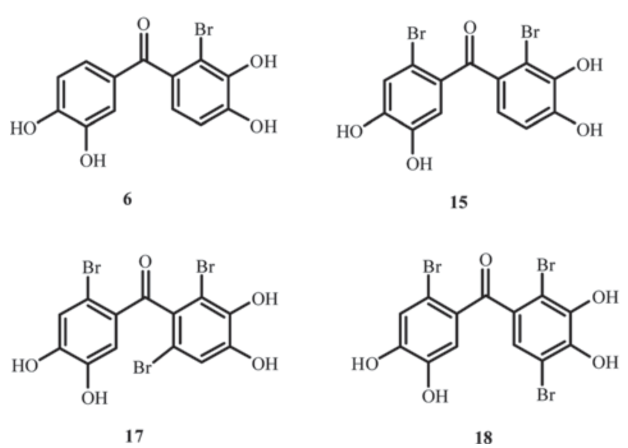


Figure 3. The new synthesized bromophenols.

The phenolic compounds have been investigated as CA inhibitors (CAIs) in this study. The rationale of investigating these compounds as CAIs lies in the fact that phenol has been shown to be the only competitive inhibitor with CO_2 as the substrate for the main isoform of CA, i.e., human CA II (hCA II)[20]. In a very sound study, Christianson and colleagues reported on the X-ray crystal structure for the adduct of hCA II with phenol[20], showing this compound to bind to CA by anchoring its OH moiety to the zinc-bound water/hydroxide ion of the enzyme active site through a hydrogen bond as well as to the NH amide of Thr199, an amino acid conserved in all α-CAs and critically important for the catalytic cycle of these enzymes[18,19].

CAIs are a class of pharmaceuticals used as antiglaucoma agents, diuretics, antiepileptics, in the management of mountain sickness, gastric and duodenal ulcers, neurological disorders, or osteoporosis. Thus, discovery

Table 1. IC_{50} values (concentration that causes 50% inhibition of the enzyme activity) for the molecules.

Inhibitor	IC_{50} (μM)
3	86.4
4	38.29
6	26.4
10	372
11	11.7
12	49.5
13	126
14	58
15	1.65
16	0.7
17	9.23
18	1.36

of novel CAIs is of great importance for pharmacological and medicinal approaches, and many inhibitors have been designed and synthesized in the literature. However, it is critically important to explore further classes of potent CAIs in order to detect compounds with a different inhibition profile when compared to sulfonamides and their bioisosteres, and to find novel applications for the inhibitors of these widespread enzymes.

Conclusions

Diphenylmethanone derivative **10** was obtained and its bromination gave dibromide **11** and tribromides **12–14**. From these compounds, potential biological active bromophenols **6** and **15–18** were synthesized in high yields. The structures of the products were determined and characterized by spectroscopic methods. Bromophenol derivatives **1–13** used in this study affect the activity of CA II isozyme due to the presence of the different functional groups (OH, OCH_3) in their aromatic scaffold. It has been determined in our study that compounds **15**, **16** and **18** are effective inhibitors for CA II when compared to Acetazolamide, which is used as the reference inhibitor for carbonic anhydrase. Our findings here indicate thus another class of possible CAIs of interest, in addition to the well-known sulfonamides/sulfamates/sulfamides. These findings point out that substituted phenolic compounds may be used for generation of potent CAIs.

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Declaration of interest

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