Full Paper

Synthesis and Biological Evaluation of Novel Bromophenol Derivatives as Carbonic Anhydrase Inhibitors

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Here, we provide an alternative synthesis of the natural bromophenol 3,4-dibromo-5-(2,3-dibromo-4,5-dihydroxybenzyl)-6-(ethoxymethyl)benzene-1,2-diol (**3**) and the first synthesis of (4,5-dihydroxy-2-methylphenyl)(3,4-dihydroxyphenyl)methanone (**18**) and its brominated derivatives **19**–**21**. The compounds were characterized and tested against the two most studied members of the pH regulatory enzyme family, carbonic anhydrase (CA). The inhibitory potencies of the novel compounds and two natural bromophenols **2**, **3** were analyzed at the human isoforms hCA I and hCA II as targets and the K_I values were calculated. The K_I values of the novel compounds were measured in the range of 13.7–32.7 μM for the hCA I isozyme and 0.65–1.26 μM for the hCA II isozyme. The structurally related compound **14** was also tested in order to understand the structure–activity relationship, and the clinically used sulfonamide acetazolamide (AZA) was tested for comparison reasons. All of the compounds exhibited competitive inhibition with 4-nitrophenylacetate as substrate. The compounds showed strong inhibitory activity against hCA I, being more effective as compared to the clinically used AZA (K_I: 36.2 μM), but rather less activity against hCA II.

Keywords: Bromination / Bromophenols / Carbonic anhydrase inhibitors / Natural products

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Introduction

Among natural organohalogen compounds, bromophenols are abundant in marine life. These compounds are mostly isolated from red algae of the family Rhodomelaceae [1, 2]. Bromophenols are important compounds in the field of functional food and pharmaceuticals. They also exhibit a wide spectrum of useful biological activities [3]. The enzyme inhibition [4], feeding deterrent [5], and anti-microbial [6] activities of natural bromophenols **1** and **2**; cytotoxicity [7] and anticancer [8] activities of **1**–**3** have been reported. The anti-microbial activity of the natural bromophenol **4** has been informed by Oh et al. [9]. Aldose reductase inhibitory activity of natural products **4** and **5** has also been declared by Wang et al. [10]. Radical-scavenging [11] and antioxidant [12] activities of **5** have also been clarified. Recently we have performed the synthesis and biological evaluation of **1**, **4**, **5** and some novel bromophenols **6**–**10**. In these studies, the antioxidant activities of **1**, **6**–**9** [13], and carbonic anhydrase (CA) inhibitory activities of **4**, **5**, **10** and some other phenolic compounds [14–17] were investigated (Fig. 1).

CA enzymes play important roles in several physiological and pathological processes. Sixteen CA isoforms have been identified in mammals that differ in subcellular localization and catalytic activity [18]. CA isoforms take part in several vital biological processes such as acid-base balance, electrolyte secretion, carbon dioxide and ion transport, bone resorption,

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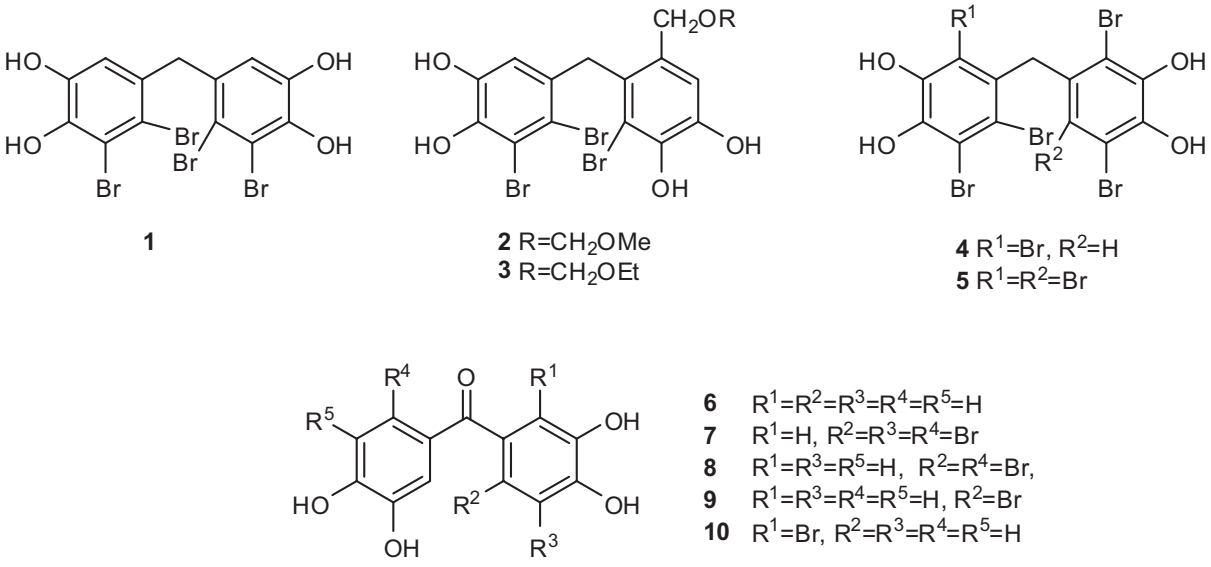


Figure 1. Structures of the compounds **1–10**.

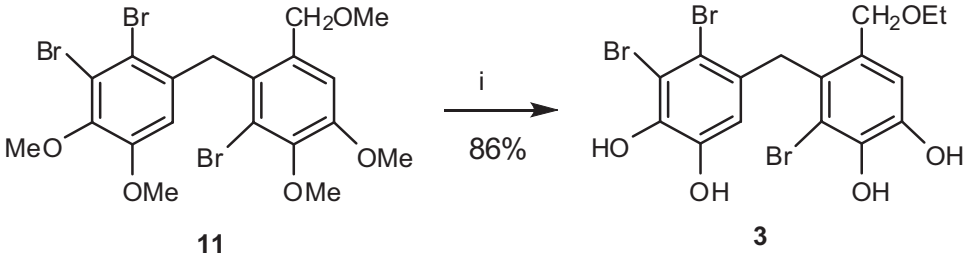
respiration, ureagenesis, gluconeogenesis, and lipogenesis [19, 20]. Inhibitors or activators of these enzymes have several medical applications, such as diuretics, in the treatment of glaucoma, in the management of several neurological disorders, including epilepsy, possibly in the treatment of Alzheimer's disease. Moreover, several agents are in clinical evaluations as antiobesity or antitumor drugs/diagnostic tools. However, it is relatively difficult to design agents (inhibitors or activators) with specificity or selectivity for any of these isoforms, and many pharmacological agents belonging to the class of the CA inhibitors (CAIs) or CA activators (CAAs) act as promiscuous inhibitors/activators of most isozymes with physiological/pathological relevance resulting in undesired side effects. So far inhibitory effects of different sulfonamide derivatives, metal ions, phenols, anions, and drugs have been investigated against many CAs [21–23]. However, it is still important to discover further classes of potential CA inhibitors in order to develop novel compounds with distinct inhibition profiles as compared to the known molecules. Therefore, in the present study we focused on the synthesis, characterization, and CA inhibitory properties of four novel and two natural bromophenols.

Results and discussion

Chemistry

In our previous study, we achieved the first synthesis of the natural bromophenol **2** from demethylation of 2,3-dibromo-1-(2-bromo-3,4-dimethoxy-6-(methoxymethyl)benzyl)-4,5-dimethoxybenzene (**11**) with BBr_3 [24]. By a similar approach, we performed an alternative synthesis of the natural product **3** from **11**. Compound **11** was treated with BBr_3 at 0–25°C under N_2 in CH_2Cl_2 for 24 h. After the reaction was completed, quenching of excess BBr_3 with EtOH at 0–60°C gave **3** with a yield of 86% (Scheme 1). The first synthesis of **3** has been reported by Shuju et al. [25] lately.

Alkylation or acylation of aromatic compounds with polyphosphoric acid (PPA) has previously been given clearly in the literature [26]. By applying the same procedure, acylation of 3,4-dimethoxytoluene (**12**) with 3,4-dimethoxybenzoic acid



Scheme 1. (i) BBr_3, 0°C to RT, 1 day; then addition of EtOH, 60°C, 3 h.

(**13**) in PPA at 80°C gave (4,5-dimethoxy-2-methylphenyl)(3,4-dimethoxyphenyl)methanone (**14**) with a yield of 85%. *N*-Bromosuccinimide (NBS) [27], LiBr/ceric ammonium nitrate (CAN) [13], or molecular bromine (Br_2) [28] can be used for the bromination of aromatic compounds. Here, Br_2 and LiBr/CAN were chosen for the bromination of **14**. Bromination of **14** with 3 eq Br_2 at 60°C in $CHCl_3$ afforded (3-bromo-4,5-dimethoxy-2-methylphenyl)(3,4-dimethoxyphenyl)methanone (**15**) and (3-bromo-4,5-dimethoxy-2-methylphenyl)(2-bromo-4,5-dimethoxyphenyl)methanone (**16**) with yields of 56 and 20% respectively (Scheme 2). On the other hand, bromination of **14** with LiBr (3 eq) and CAN (3 eq) in CH_3CN at reflux temperature under N_2 gave **15**, **16** and (2-bromo-4,5-dimethoxyphenyl)(4,5-dimethoxy-2-methylphenyl)methanone (**17**) with 16, 8, and 38% yields, respectively (Scheme 2).

Since crystallization of the novel compounds was challenging, we used their precursor forms **15, 16**, and **17** to identify the structures. NMR analysis of compounds **15** and **16** did not allow us to determine their exact structures. Therefore, the structures of these compounds were determined by X-ray diffraction analysis (Fig. 2). The structure of compound **17** was determined by comparison of its NMR data with data of starting compound **13**, brominated compounds **15** and **16.**

Cleavage of alkyl aryl ethers can be achieved with HBr [29, 30], or BBr_3 [31, 32]. Here, *0*-demethylation of aryl methyl ethers was carried out according to the procedure described by Talaz et al. [31]. Reaction of **14**–**17** with BBr_3 in CH_2Cl_2 at 0–25°C under N_2 followed by addition of H_2O or MeOH provided the novel phenolic compound (4,5-dihydroxy-2-methylphenyl)(3,4-dihydroxyphenyl)methanone (**18**), synthetic bromophenols (3-bromo-4,5-dihydroxy-2-methylphenyl)(3,4-dihydroxyphenyl)methanone (**19**), (3-bromo-4,5-dihydroxy-2-methylphenyl)(2-bromo-4,5-dihydroxyphenyl)methanone (**20**),

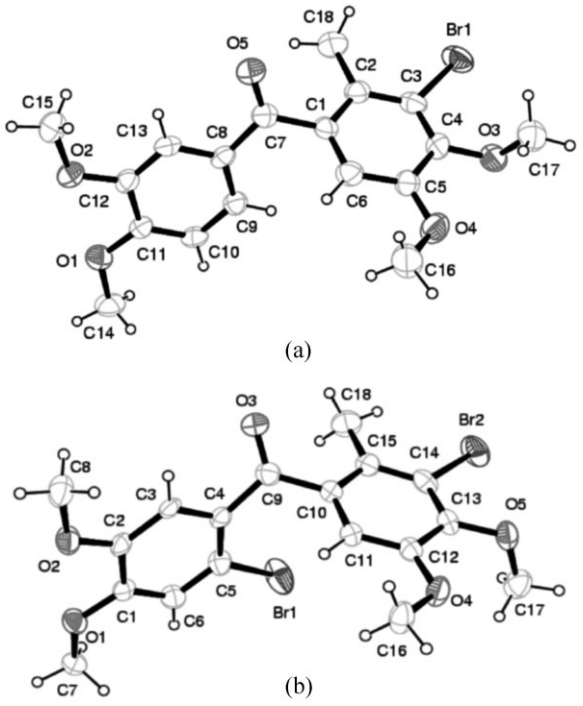
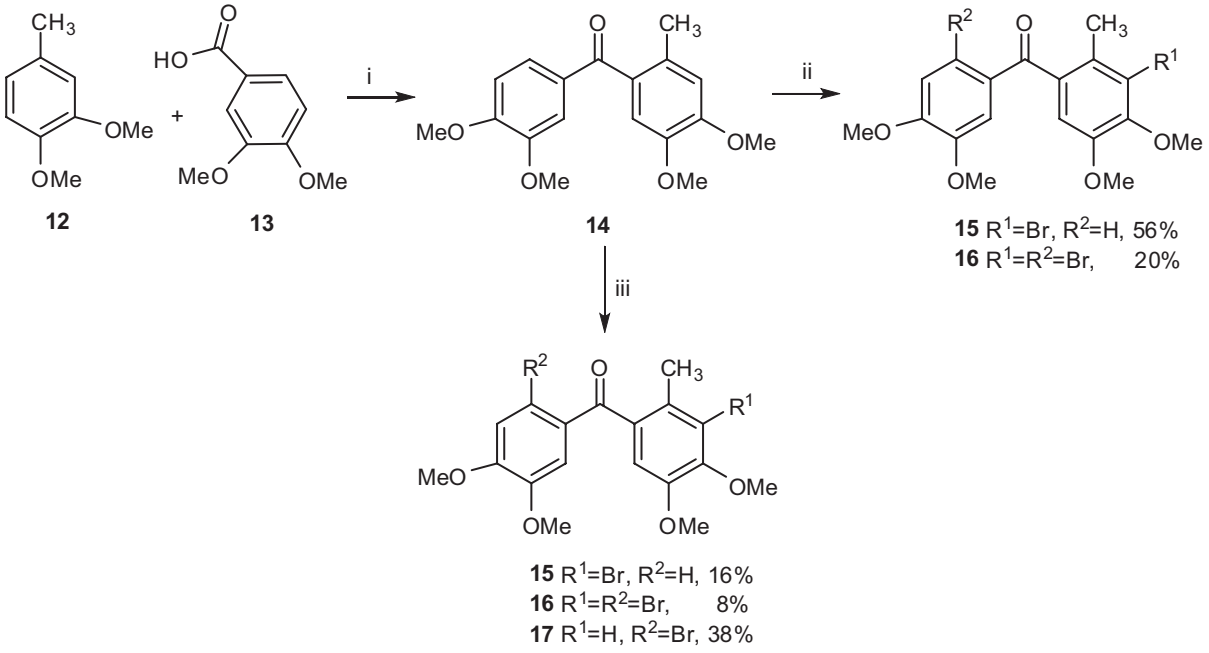


Figure 2. View of the X-Ray structures of **15** (a) and **16** (b).



Scheme 2. (i) PPA, 80°C, 45 min, 75%; (ii) Br_2 (3 eq), RT, 30 h; (iii) LiBr/CAN (3 eq), RT, 12 h.

and (2-bromo-4,5-dihydroxyphenyl)(4,5-dihydroxy-2-methylphenyl)methanone (**21**) with yields of 91, 91, 94, and 94%, respectively (Scheme 3).

Biological evaluation of the synthesized and reference compounds for CA inhibitory activity

Compounds **2, 3, 14, 18–21,** as well as the standard, clinically used CAI acetazolamide (AZA), have been tested for the inhibition of two cytosolic ubiquitous isozymes of human origin, that is, hCA I and hCA II (Table 1).

The following should be noted regarding the CA inhibitory data of Table 1:

(i) The bromophenol derivatives investigated here showed moderate to strong inhibitory properties against the slow cytosolic isoform hCA I. Compound **14** exhibited the lowest inhibition of this isoform, with K_I-s value of 341.8 μM. The compounds **2**, **3**, **18–21** were much more effective inhibitors against hCA I, with K_I-s in the range of 13.7–32.7 μM. These results demonstrate the contribution of the hydroxyl groups to the inhibition efficacy. Interestingly, bromide containing compounds **2**, **3**, **19–21** were less effective than compound **18**. This trend also shows the attenuator potency of the bromide moiety. As these compounds do not possess any of the zinc-anchoring groups present in known CAIs, presumably such compounds may bind in the coumarin/phenol binding site.

(ii) Similar to hCA I inhibition data, compound **14** acted as the weakest inhibitor against the ubiquitous and dominant rapid cytosolic isozyme hCA II. However, **2**, **3**, **18–21** derivatives acted as strong hCA II inhibitors with a comparable potency as the reference compound AZA (K_I: 0.37 μM). The most potent inhibitor was compound **18** (K_I: 0.65 μM). This trend again demonstrates the attenuator potency of the bromide moiety. Also, hydroxyl group containing compounds **18–21** were much more effective compared to hydrophobic group containing compounds **2**, **3**, and **14**.

Table 1. hCAs I and II inhibition data with studied compounds and acetazolamide, by an esterase assay with 4-nitrophenylacetate as substrate.

Compound	K_I[a] (μM)	
	hCA I	hCA II
2	24.3	0.98
3	32.7	1.26
14	341.8	33.8
18	13.7	0.65
19	18.5	0.74
20	22.6	0.83
21	28.5	0.92
AZA	36.2	0.37

[a] Mean from at least three determinations. Errors in the range of ~3% of the reported value (data not shown).

Conclusion

In summary, an alternative synthesis of natural bromophenol **3** and the first synthesis of **18–21** have been achieved. In addition, several bromophenol compounds including novel derivatives have been assayed for the inhibition of the physiologically relevant human CA isozymes hCA I and II. These compounds showed inhibition constants in the range of 13.7–341.8 μM for hCA I and 0.65–33.8 μM for hCA II, respectively. In general, the compounds had comparable inhibitory activity with the clinically used sulfonamide AZA. 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. Here, we extend these earlier investigations to a novel series of bromophenols. The novel

14 R^1=R^2=H
15 R^1=Br, R^2=H
16 R^1=R^2=Br,
17 R^1=H, R^2=Br

18 R^1=R^2=H, 91%
19 R^1=Br, R^2=H, 91%
20 R^1=R^2=Br, 94%
21 R^1=H, R^2=Br, 94%

Scheme 3. BBr_3, 0°C (2 h) to RT (1 day); then addition of MeOH, 0°C (15 min).

bromophenols represent a promising class of CA inhibitors and the results discussed in this study may help medicinal chemists to design new analogs with enhanced activity and other tailored properties.

Experimental

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). Preparative thick layer chromatography: 1 mm of SiO_2 60 PF (Merck) on glass plates. Mp: cap. melting-point apparatus (BUCHI 530); uncorrected. IR spectra: solns. in 0.1 mm cells with a Mattson 1000 FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra: 400 (100)-MHz Varian spectrometer; δ in ppm; Me_4Si as the internal standard. Elemental analyses: Leco CHNS-932 apparatus.

Chemistry

*3,4-Dibromo-5-(2,3-dibromo-4,5-dihydroxybenzyl)-6-(ethoxymethyl)benzene-1,2-diol (**3**)*

To a solution of 2,3-dibromo-1-(2-bromo-3,4-dimethoxy-6-(methoxymethyl)benzyl)-4,5-dimethoxybenzene (**11**) (0.41 g, 0.72 mmol) in CH_2Cl_2 (10 mL) was added a solution of BBr_3 (0.58 mL, 1.53 g, 6.1 mmol) in CH_2Cl_2 (6.5 mL) at 0°C under N_2. After the addition of BBr_3 was completed, the mixture was stirred at room temperature for 24 h. The reaction mixture was cooled to 0°C, and EtOH (10 mL) was added to this mixture dropwise for 10 min. The mixture was heated at 60°C for 3 h. After evaporation of the solvent, AcOEt (40 mL) and H_2O (30 mL) were added to the residue. The organic layer was separated, and the H_2O phase was extracted with AcOEt (2 × 25 mL). The organic layers were combined and dried over Na_2SO_4. Evaporation of the solvent gave the natural product **3** (0.33 g, 86%). Brown solid. Mp 199–201°C (197–198°C in the literature [33]). ^1H NMR (400 MHz, acetone-d_6): δ 8.66 (bs, OH, 1H), 8.10 (bs, OH, 1H), 7.01 (s, 1H), 6.09 (s, 1H), 4.26 (s, CH_2O, 1H), 4.13 (s, CH_2, 2H), 3.41 (q, J = 6.97 Hz, CH_2, 2H), 1.06 (t, J = 6.97 Hz, CH_3, 3H). ^{13}C NMR (100 MHz, acetone-d_6): δ 144.74 (C), 144.18 (C), 142.80 (C), 142.72 (C), 131.86 (C), 130.59 (C), 128.86 (C), 115.64 (2C), 114.35 (C), 114.35 (CH), 112.99 (CH), 70.73 (CH_2O), 65.53 (OCH_2), 38.81 (CH_2), 14.73 (CH_3).

*(4,5-Dimethoxy-2-methylphenyl)(3,4-dimethoxyphenyl)-methanone (**14**)*

PPA, prepared from conc. H_3PO_4 (85%, 11.25 g) and P_2O_5 (20.23 g, 142.5 mmol), was heated to 80°C in a beaker (100 mL). 3,4-Dimethoxytoluene **12** (3.00 g, 19.7 mmol) and 3,4-dimethoxybenzoic acid (**13**) (3.59 g, 19.7 mmol) were added to this mixture 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 (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. Crystallization of the residue with CH_2Cl_2/hexane gave (4,5-dimethoxy-2-methylphenyl)(3,4-dimethoxyphenyl) methanone (**14**) as white crystals (4.65 g, 75% yield). Mp 134–136°C (123–124°C in the literature [34]). IR (CH_2Cl_2, cm^{-1}): 3079, 3000, 2935, 2841, 2605, 1730, 1649, 1594, 1583, 1514, 1464, 1416, 1343, 1267, 1231, 1211, 1159, 1141, 1097, 1023. ^1H NMR (400 MHz, $CDCl_3$): δ 7.51 (d, J = 1.97, 1H), 7.27 (dd, A part of AB system, J = 8.32, 1.97 Hz, 1H), 6.86 (s, 1H), 6.85 (d, B part of AB system, J = 8.32 Hz, 1H), 6.75 (s, 1H), 3.94 (s, OCH_3, 3H), 3.93 (s, 2 OCH_3, 6H), 3.81 (s, OCH_3, 3H), 2.27 (s, CH_3, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 198.57 (CO), 155.30 (C), 152.32 (C), 151.10 (C), 148.22 (C), 133.22, 132.87, 132.35 (CH), 127.58 (CH), 115.83 (CH), 114.39, 113.62, 111.94 (CH), 58.13 (OCH_3), 58.05 (2 OCH_3), 57.94 (2OCH_3), 21.71 (CH_3). Anal. calcd. for $C_{18}H_{20}O_5$: C 68.34, H 6.37; found: C 68.35, H 6.47.

*Bromination of (4,5-dimethoxy-2-methylphenyl)(3,4-dimethoxyphenyl)methanone (**14**) with 3 eq Br_2*

To a stirred solution of the ketone **14** (0.38 g, 1.2 mmol) in $CHCl_3$ (25 mL) was added a solution of bromine (0.58 g, 3 eq, 3.6 mmol) in $CHCl_3$ (20 mL) dropwise at room temperature for 5 min. After the addition of Br_2 was completed, the mixture was stirred at reflux temperature for 30 h. Evaporation of solvent and chromatography of the residue on the silica gel (SiO_2, 50 g) with ethyl acetate/hexane (1:9) gave monobromide **15** (0.27 g, 56%) and dibromide **16** (0.11 g, 20%), respectively.

*(3-Bromo-4,5-dimethoxy-2-methylphenyl)(3,4-dimethoxyphenyl)methanone (**15**)*

Recrystallized from CH_2Cl_2/hexane. Yellow crystals. Mp 112–113°C. IR (CH_2Cl_2, cm^{-1}): 3081, 3002, 2937, 2840, 1655, 1593, 1512, 1484, 1464, 1431, 1418, 1317, 1267, 1232, 1207, 1163, 1143, 1095, 1039, 1021. ^1H NMR (200 MHz, $CDCl_3$): δ 7.55 (d, J = 2.00, 1H), 7.22 (dd, A part of AB system, J = 8.35, 2.00 Hz, 1H), 6.82 (d, B part of AB system, J = 8.35 Hz, 1H), 6.80 (s, 1H), 3.94 (s, 2 OCH_3, 6H), 3.90 (s, OCH_3, 3H), 3.82 (s, OCH_3, 3H), 2.23 (CH_3, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 197.97 (CO), 156.00 (C), 152.91 (C), 151.40 (C), 149.67 (C), 137.90, 132.18, 130.66, 128.16 (CH), 124.11, 113.17, 112.93, 112.10 (CH), 62.42 (OCH_3), 58.25 (OCH_3), 58.09 (2 OCH_3), 22.07 (CH_3). Anal. calcd. for $C_{18}H_{19}O_5Br$: C 54.70, H 4.85; found: C 54.74, H 4.72.

*(3-Bromo-4,5-dimethoxy-2-methylphenyl)(2-bromo-4,5-dimethoxyphenyl)methanone (**16**)*

Recrystallized from EtOAc/hexane. White crystals. Mp 152–154°C. IR (CH_2Cl_2, cm^{-1}); 3002, 2937, 2842, 1665, 1591, 1561, 1505, 1482, 1453, 1488, 1375, 1334, 1314, 1261, 1215, 1204, 1164, 1108, 1043, 1019. ^1H NMR (200 MHz, $CDCl_3$): δ 7.06 (s, 1H), 7.05 (s, 1H), 6.84 (s, 1H), 3.93 (s, OCH_3, 3H), 3.91 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 3.77 (s, OCH_3, 3H), 2.40 (CH_3, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 197.52 (CO), 154.19 (C), 152.81 (C), 151.43 (C), 150.51 (C), 137.12 (C), 134.30 (C), 133.65 (C), 124.82 (C), 118.78 (CH), 116.18 (CH), 115.63 (CH), 115.08 (C), 62.44 (OCH_3), 58.44 (OCH_3), 58.33 (2 OCH_3), 21.98 (CH_3). Anal. calcd. for $C_{18}H_{18}O_5Br_2$: C 45.60, H 3.83; found: C 45.66, H 3.73.

*Bromination of (4,5-dimethoxy-2-methylphenyl)(3,4-dimethoxyphenyl)methanone (**14**) with 3 eq LiBr/Cerium(IV) ammonium nitrate (CAN)*

To a solution of **14** (0.45 g, 1.42 mmol) and LiBr (0.37 g, 4.26 mmol) in CH_3CN (17 mL), was added a solution of CAN (2.34 g, 4.26 mmol) in CH_3CN (17 mL) dropwise at room temperature under N_2 for 15 min. After the solution was stirred at RT and under N_2 for 12 h, water (25 mL) was added and the mixture was extracted with ethyl acetate (3 × 50 mL).

The organic phase was washed with solutions of $NaHCO_3$ (5%, 2 × 40 mL), H_2O (2 × 40 mL), and brine (40 mL), then dried over Na_2SO_4. Evaporation of the solvent afforded **15** (0.090 g, 16% yield), **16** (0.054 g, 8% yield) and (2-bromo-4,5-dimethoxyphenyl)(4,5-dimethoxy-2-methylphenyl)methanone (**17**) (0.213 g, 38% yield).

*(2-Bromo-4,5-dimethoxyphenyl)(4,5-dimethoxy-2-methylphenyl)methanone (**17**)*

Recrystallized from EtOAc/hexane. Yellow crystals. Mp 152–153°C. IR (CH_2Cl_2, cm^{-1}): 3058, 3003, 2961, 2936, 2844, 1656, 1596, 1567, 1518, 1505, 1464, 1441, 1375, 1344, 1263, 1207, 1162, 1112, 1040, 1028, 1008. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.03 (s, 1H), 6.92 (s, 1H), 6.90 (s, 1H), 6.72 (s, 1H), 3.91 (s, OCH_3, 3H), 3.90 (s, OCH_3, 3H), 3.82 (s, OCH_3, 3H), 3.72 (s, OCH_3, 3H), 2.43 (CH_3, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 197.94 (CO), 154.04 (C), 153.08 (C), 150.33 (C), 148.43 (C), 136.26 (CH), 135.87 (C), 131.04 (C), 118.08 (CH), 117.07 (C), 116.48 (CH), 114.90 (CH), 113.62 (C), 58.27 (OCH_3), 58.20 (2 OCH_3), 57.92 (OCH_3), 22.90 (CH_3). Anal. calcd. for $C_{18}H_{19}O_5Br$: C 54.70, H 4.85; found: C 54.68, H 4.81.

*Synthesis of (4,5-dihydroxy-2-methylphenyl)(3,4-dihydroxyphenyl)methanone (**18**)*

General procedure for *O*-demethylation of arylmethyl ethers: To a stirred solution of diarylmethanone **14** (0.40 g, 1.01 mmol) in CH_2Cl_2 (10 mL) at 0°C was added a solution of BBr_3 (0.65 mL) in CH_2Cl_2 (7 mL) dropwise under N_2(g) for 5–10 min. The reaction mixture was stirred at the same temperature for 2 h and then at room temperature for 1 day under N_2. The reaction was monitored by TLC and after completion, methanol (40 mL) was slowly added for 15 min to the mixture at 0°C. The solvent was evaporated, water (45 mL) and EtOAc (50 mL) were added to the residue. The organic layer was separated and the water phase was extracted with EtOAc (2 × 30 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. An oily (4,5-dihydroxy-2-methylphenyl)(3,4-dihydroxyphenyl)methanone (**18**) was obtained (0.24 g, 91% yield). IR (acetone, cm^{-1}): 3247, 2975, 2930, 1695, 1633, 1587, 1518, 1441, 1354, 1293, 1217, 1192, 1155, 1119, 1080, 971. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.37 (bs, 4H, OH), 7.31 (s, 1H), 7.30 (d, J = 2.2 Hz, 1H), 7.17 (dd, J = 8.2, 2.2 Hz, 1H), 6.90 (d, J = 8.2 Hz, 1H), 6.81 (s, 1H), 6.76 (s, 1H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, acetone-d_6): δ 195.61 (CO), 150.10 (C), 146.95 (C), 144.92 (C), 142.06 (C), 131.17 (C), 130.75 (C), 129.26 (C), 123.89 (CH), 117.88 (CH), 116.75 (CH), 116.58 (CH), 114.91 (CH), 18.82 (CH_3). Anal. calcd. for $C_{14}H_{12}O_5$: C 64.61, H 4.65; found: C 64.60, H 4.64.

*(3-Bromo-4,5-dihydroxy-2-methylphenyl)(3,4-dihydroxyphenyl)methanone (**19**)*

The procedure described for **18** was applied to **15** (0.41 g, 1.04 mmol) to give bromopehol **19** (0.32 g, 91%). Solidified. Yellow solid. Mp 208–210°C. IR (acetone, cm^{-1}): 3386, 3254, 2974, 1628, 1587, 1518, 1480, 1421, 1359, 1294, 1217, 1117, 1077, 1029. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.74 (bs,2H, OH), δ 8.41 (bs, 1H, OH), δ 8.32 (bs, 1H, OH), δ 7.31 (d, J = 2.2 Hz, 1H), δ 7.18 (dd, J = 8.3 Hz, J = 2.2 Hz, 1H), δ 6.91 (d, J = 8.3 Hz, 1H), 6.79 (s, 1H), 2.20 (CH_3, 3H). ^{13}C NMR (100 MHz, acetone-d_6): δ 195.09 (CO), 150.68 (C), 145.11 (C), 144.54 (C), 142.68 (C), 132.17 (C), 130.48 (C), 127.51 (C), 124.15 (CH), 116.64 (CH), 115.14 (CH), 114.08 (CH), 113.64 (C), 19.43 (CH_3). Anal. calcd. for $C_{14}H_{11}O_5Br$: C 49.58, H 3.27; found: C 49.56, H 3.27.

*(3-Bromo-4,5-dihydroxy-2-methylphenyl)(2-bromo-4,5-dihydroxyphenyl)methanone (**20**)*

O-Demethylation of **16** (0.50 g, 1.05 mmol) with BBr_3 gave **20** (0.41 g, 94% yield). Solidified. Yellow solid. Mp 183–185°C. IR (acetone, cm^{-1}): 3339, 1698, 1640, 1590, 1501, 1419, 1361, 1283, 1097. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.87 (bs, OH, 1H),8.71 (bs, OH, 1H), 8.60 (bs, OH,1H), 8.54 (bs, OH, 1H), 7.10 (s, 1H), 6.94 (s, 1H), 6.82 (s, 1H), 2.45 (s, CH_3, 3H). ^{13}C NMR (100 MHz, acetone-d_6): δ 195.07 (CO), 148.64 (C), 146.26 (C), 144.65 (C), 142.44 (C), 132.91 (C), 130.87 (C), 120.16 (CH), 117.78 (CH), 116.90 (CH), 116.82 (C), 114.65 (C), 110.00 (C), 19.43 (CH_3). Anal. calcd. for $C_{14}H_{10}O_5Br_2$: C 40.22, H 2.41; found C 40.34, H 2.54.

*(2-Bromo-4,5-dihydroxyphenyl)(4,5-dihydroxy-2-methylphenyl)methanone (**21**)*

Demethylation reaction of **17** (0.41 g, 1.04 mmol) with BBr_3 afforded bromophenol **21** (0.33 g, 94% yield). Solidified. Brownish solid. Mp 221–223°C. IR (acetone, cm^{-1}): 3426, 2974, 1631, 1591, 1513, 1497, 1454, 1418, 1366, 1287, 1214, 1183, 1155. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.76 (bs, 1H, OH), δ 8.52 (bs, 2H, OH), δ 8.06 (bs, 1H, OH), δ 7.09 (s, 1H), 6.86 (s, 1H), 6.84 (s, 1H), 6.78 (s, 1H), 2.40 (CH_3, 3H). ^{13}C NMR (100 MHz, acetone-d_6): δ 195.36 (CO), 149.08 (C), 147.78 (C), 144.65 (C), 142.23 (C), 134.13 (CH), 133.13 (CH), 128.65 (C), 119.74 (CH), 119.68 (CH), 118.86 (C), 116.73 (CH), 109.09 (C), 20.27 (CH_3). Anal. calcd. for $C_{14}H_{11}O_5Br$: C 49.58, H 3.27; found: C 49.58, H 3.29.

X-ray diffraction analysis

For the crystal structure determination, the single-crystal of the complex [Ru(Ph_2PNHCH_2-C_4H_3S)(η^6-benzene)Cl_2] was 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 $\Delta\omega = 5^\circ$ 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 $F^2 > 2\sigma(F^2)$. H atoms were positioned geometrically and refined using a riding model. Integration of the intensities, correction for Lorentz and polarization effects and cell refinement was performed using CrystalClear (Rigaku/MSC Inc., 2005) software [35]. The structures were solved by direct methods using SHELXS-97 and refined by a full-matrix least-squares procedure using the program SHELXL-97 [36]. The final difference Fourier maps showed no peaks of chemical significance. Crystal data for monobromide **15**: $C_{18}H_{19}O_5Br$, crystal system, space group: monoclinic, $P2_1/c$; (no:14); unit cell dimensions: a = 12.2185(2), b = 7.3752(1), c = 20.2548(4) Å, β = 105.93(2)°; volume: 1755.17(5) Å^3; Z = 4; calculated density: 1.496 g/cm^3; absorption coefficient: 2.366 mm^{-1}; F(000): 808; θ-range for data collection 2.3–30.67°; refinement method: full-matrix least-square on F^2; data/parameters: 3229/218; goodness-of-fit on F^2: 1.391; final R indices [$I > 2\sigma(I)$]: R_1 = 0.0853, wR_2 = 0.1457; largest diff. peak and hole: 0.341 and −0.418 e Å^{-3}; CCDC-873395. Crystal data for dibromide **16**: $C_{18}H_{18}Br_2O_5$, crystal system, space group: triclinic, P-1; (no:2); unit cell dimensions: a = 8.8103(2), b = 9.9903(3), c = 11.5582(5) Å, α = 86.13(3), β = 87.44(3), γ = 68.88(2)°;

volume: 946.63(6)Å^3; $Z = 2$; calculated density: 1.663 g cm^{-3}; absorption coefficient: 4.306 mm^{-1}; $F(000)$: 472; θ-range for data collection 2.5–30.55°; refinement method: full-matrix least-square on F^2; data/parameters: 4020/229; goodness-of-fit on F^2: 1.348; final R indices [$I > 2\sigma(I)$]: $R_1 = 0.078$, $wR_2 = 0.1985$; largest diff. peak and hole: 0.873 and −0.989 e Å^{-3}; CCDC-873651. Crystallographic data that were deposited in CSD under CCDC registration numbers contain the supplementary crystallographic data for this Letter. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre (CCDC) via www.ccdc.cam.ac.uk/data_request/cif and are available free of charge upon request to CCDC, 12 Union Road, Cambridge, UK (fax: +441223 336033, e-mail: deposit@ccdc.cam.ac.uk).

CA inhibition

Enzyme activity was determined spectrophotometrically by following the change in absorbance at 348 nm of 4-nitrophenylacetate to 4-nitrophenylate over a period of 3 min at 25°C [37]. The enzymatic reaction contained 1.4 mL 0.05 M Tris–SO_4 buffer (pH 7.4), 1 mL 3 mM 4-nitrophenylacetate, 0.5 mL H_2O and 0.1 mL enzyme solution, in a total volume of 3.0 mL [38]. Inhibitory effects of compounds **2**, **3**, **14**, **18**–**21** were compared with AZA. Different inhibitor concentrations were used and all compounds were tested in triplicate at each concentration used. Control cuvette activity was acknowledged as 100% in the absence of inhibitor. An Activity% – [Inhibitor] graph was drawn for each inhibitor [39–43]. The curve-fitting algorithm allowed to obtain the IC_{50} values, working at the lowest concentration of substrate of 0.15 mM, from which K_I values were calculated [44–48]. The catalytic activity of these enzymes was calculated from Lineweaver–Burk plots, as reported earlier [49], and represent the mean from at least three different determinations. The CAI and II isoenzymes used here were purified from human blood as described earlier [50–52].

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