Bioorganic & Medicinal Chemistry Letters 22 (2012) 1352–1357

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and carbonic anhydrase inhibitory properties of novel cyclohexanonyl bromophenol derivatives

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ARTICLE INFO

Article history:
Received 11 October 2011
Revised 12 December 2011
Accepted 13 December 2011
Available online 21 December 2011

Keywords:
Carbonic anhydrase
Enzyme inhibition
Bromination
Bromophenols
Cyclohexanone with benzyl
Enamine
Esterification
Symphyoketone

ABSTRACT

The Naturally occurring novel cyclohexanonyl bromophenol 2(*R*)-2-(2,3,6-tribromo-4,5-dihydroxybenzyl)cyclohexanone (**4**) was synthesized as a racemic compound. Cyclohexylphenyl methane derivatives (**10–17**) with Br, OMe, CO, and OH were also obtained. Inhibition of four human carbonic anhydrase (hCA, EC 4.2.1.1) isozymes I, II, IV, and VI, with compounds **2–4, 8**, and **10–26** was investigated. These compounds were found to be promising carbonic anhydrase inhibitors and some of them showed interesting inhibitory activity. Some of the compounds investigated here showed effective hCA 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, and osteoporosis.

Natural bromophenols, frequently isolated from red algae of the family Rhodomelaceae, have prominent biological activities.[1] Among these natural compounds, **1** exhibits isocitrate lyase[1c] and microbial[1d–f] activities. The other natural compounds **2** and **3** show significant aldose reductase inhibitory activities (Fig. 1).[1f] Additionally, it was reported that phenolic natural[1g] and synthetic dopaminergic[2] compounds are inhibitors of human carbonic anhydrases. Antioxidant activities of **1** and **3** have also been reported.[1h,i] Choi and co-workers reported the isolation of natural bromophenol 2(*R*)-2-(2,3,6-tribromo-4,5-dihydroxybenzyl)cyclohexanone (**4**) and its antioxidant property.[1f]

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 the human body, catalyzing the reversible hydration of carbon dioxide in a two-step reaction to yield bicarbonate and protons.[2] The enzyme plays an important role in physiological anion exchange processes.[2a] At least 16 CA isozymes have been described to date in mammals, the most active ones as catalysts for carbon dioxide hydration being CA II.[3,4] CA II is found primarily in red blood cells but also in many other secretory tissues of the kidney, lung, eye, etc.[2,3] Carbonic anhydrase VI (CA VI) is a secretory enzyme that was initially described in the ovine parotid gland, and saliva, and normal human serum.[3b] Other CA isoforms are found 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, lipogenesis and electrolyte secretion.[2–6] Many such CA isozymes involved in these processes are important therapeutic targets with the potential to be inhibited/activated for the treatment of a range of disorders such as edema, glaucoma, obesity, cancer, epilepsy, and osteoporosis.[2,6]

Our group and Supuran's group recently investigated the interaction of CA I and II isozymes with several types of phenols, such as hydroxy-/methoxysubstituted benzoic acids as well as di-/trimethoxy benzenes, natural and unnatural bromophenols and several of its substituted derivatives, for example, salicyclates and some of their derivatives.[4–9] In the current research, the natural product **4** is also a diarylmethane derivative (Fig. 1) and an enantiomer. The syntheses of **4** and its racemic derivatives were important and potentially may have biological activities. Therefore, **4** and its derivatives were synthesized and their biological properties were investigated. As CA inhibitors (CAIs) are valuable molecules for therapeutic and pharmacologic applications, we have evaluated these bromophenol derivatives as novel CAIs.

We have purified human CA I, II, IV and VI (hCA I, hCA II, hCA IV and hCA VI) isoenzymes and examined the in vitro inhibition effects of some phenolic, bisphenol, methoxy and bromophenol

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doi:10.1016/j.bmcl.2011.12.069

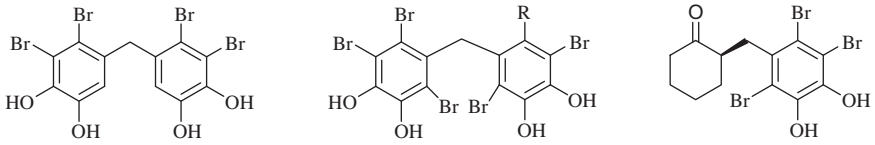
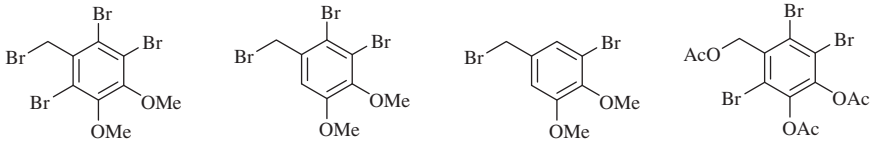


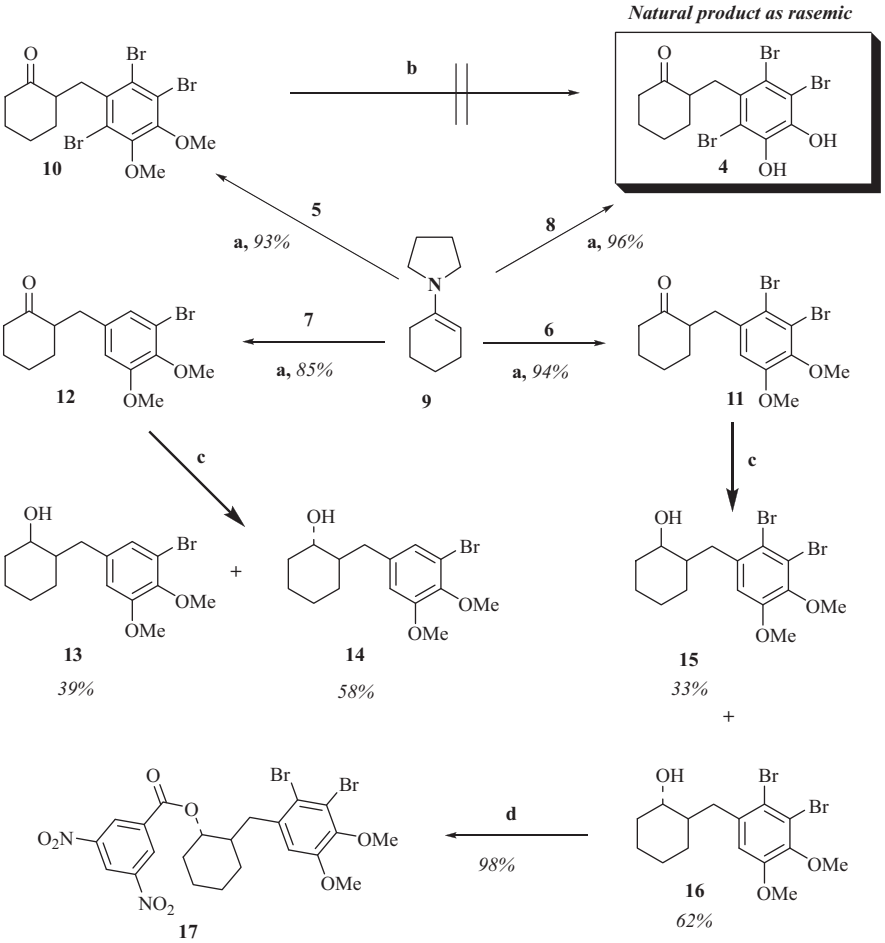
Figure 1. Some naturally occurring bromophenols.



Scheme 1. Prepared some reagents.[1g,14]

compounds mentioned above on these enzymes, using the esterase activity of hCA I, hCA II, hCA IV and hCA VI, with 4-nitrophenyl acetate as substrate.

Natural product **4** is a benzyl group substituted product at the α-carbon atom of cyclohexanone. Compound **4** and its derivatives may be synthesized by a substitution at this carbon of cyclohexanone. For this purpose, reagents tetrabromide **5**, tribromide **6** and dibromide **7** were prepared from their starting materials and related reactions (Scheme 1 and Scheme 2).[14] Enamine **9** was synthesized from cyclohexanone and pirrolydine because enamines can be



Scheme 2. Reagents and conditions: (a) (i) Reflux in dry dioxane, 18 h, under N_2, (ii) Water, additional 1 h; (b) BBr_3, in CH_2Cl_2, 0 °C to rt, under N_2; (c) $NaBH_4$, in MeOH/diethylether, 0 °C (15 min)–rt (1 (d); (d) 3,5-dintrobenzoylchloride, in CH_2Cl_2, rt, over night.

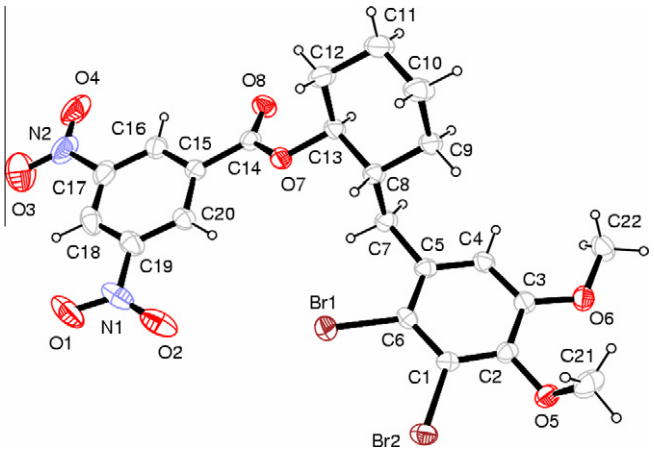


Figure 2. The molecular structure of **17**. Displacement ellipsoids are shown at the 40% probability level.

used as synthetic equivalents of aldehyde or ketone enolates.[15] The enamine **9** was subjected to reactions with bromides **5**, **6**, and **7**, respectively, in order that, in dry dioxane at reflux (Scheme 2). From these reactions, 2(*R*)*S*-2-(2,3,6-tribromo-4,5-dimethoxybenzyl) cyclohexanone (**10**), 2(*R*)*S*-2-(2,3-dibromo-4,5-dimethoxybenzyl) cyclohexanone (**11**), and 2(*R*)*S*-2-(3-bromo-4,5-dimethoxyben-zyl) cyclohexanone (**12**) were obtained as the sole products in high (85–94%) yields.[16]

The reaction of **10** with BBr_3 in CH_2Cl_2 at 0–25 °C did not give the natural bromophenol 2(*R*)*S*-2-(2,3,6-tribromo-4,5-dihydroxyb-enzyl)cyclohexanone (**4**) (Scheme 2). For use in the synthesis of 4′, another reagent 4-(acetoxymethyl)-3,5,6-tribromo-1,2-phenylene diacetate (**8**) was prepared by the known method (Scheme 1).[16,17] The reaction of **10** with BBr_3 in CH_2Cl_2 at 0–25 °C gave natural product bromophenol racemic **4** in high yields (Scheme 2).

It was thought that derivatives with OH groups of **11** and **12** may have more biological activity than **11** and **12** molecules, because OH is a more polar group than CO (carbonyl) groups. Therefore, compounds **11** and **12** were reduced with $NaBH_4$ (Scheme 2). From the chromatographies of these reaction residues, four alcohols, that is, 1*R*(*S*),2*R*(*S*)-2-(3-bromo-4,5-dimethoxybenzyl)cyclohexanol (**13**), 1(*R*)*S*,2*S*(*R*)-2-(3-bromo-4,5-dimethoxybenzyl)cyclohexanol (**14**), 1*R*(*S*),2*R*(*S*)-2-(2,3-dibromo-4,5-dimethoxybenzyl)cyclohexanol (**15**) and 1(*R*)*S*,2*S*(*R*)-2-(2,3-dibromo-4,5-dimethoxybenzyl)cyclo-hexanol (**16**) were isolated. As seen in Scheme 1, alcohols **13** and **14** are reduction products of **12** while **15** and **16** are reduction products of **11**.

Table 1
hCA I, II, IV and VI inhibition data with the tested compounds

Compound	K_i^*(μM)			
	hCA I	hCA II	hCA IV	hCA VI
2	34.41	21.16	27.48	17.43
3	35.12	21.47	28.04	17.83
4	1.67	0.56	1.08	0.59
8	187.13	58.91	102.4	765.3
10	13.27	4.42	7.68	5.59
11	12.92	4.33	7.42	5.24
12	10.54	3.97	7.03	4.81
13	1.04	0.38	0.85	0.48
14	1.03	0.38	0.87	0.47
15	1.12	0.41	0.93	0.51
16	1.10	0.39	0.88	0.47
17	2.45	0.84	1.12	0.96
18	292.109	104.4	163.36	919.182
19	193.240	94.35	104.41	158.592
20	4003[a]	9.9[a]	10.9[a]	606[a]
21	10.4[b]	0.50[b]	14.32	234.7
22	10.2[a]	5.50[a]	9.50[a]	208[a]
23	7.56	0.34	8.43	402.3
24	7.64	0.38	8.67	405.6
25	8.61	0.42	8.93	427.1
26	14.48	0.57	1.43	3.79
27	12.36	0.43	1.12	2.97
Ethoxzolamide	3.75	0.32	0.84	1.58
Zonisamide	14.8	1.07	38.45	2.42
Acetazolamide	36.2	0.37	0.578	0.34

* Mean from at least three determinations. Errors in the range of ~1% of the reported value (data not shown).
[a] Ref. 6a.
[b] Ref. 19b.

According to the NMR spectra, to determine the structures of alcohols is very difficult. Two in a mixture of them should be formed by reduction of **11** or **12** as *cis* and *trans* configurations. Each mixture's one product is more polar than the other because their R_f (40%, EtOAc/hexane) values are 0.50 and 0.66 for reduction products of **11** and 0.56 and 0.74 for those of **12**. Chemical shifts and appearances of peaks belonging to CHO, benzylic CH_2 and tertiary CH of more polar alcohols (**14** and **16**) and the others (**13** and **15**) are similar to each other. For example, OCH of **13–16** resonated at 3.79 (brs), 3.29–3.23 (m), 3.77 (brs) and 3.39–3.37 (m) ppm, respectively. On the basis of their polarities and NMR spectra, configurations of alcohols **13** with **15** and **14** with **16** should be *cis* and *trans*, respectively. To determine the exact structures of **15** and **16**, ester derivative 1(*R*)*S*,2*S*(*R*)-2-(2,3-dibromo-4,5-dimethoxy-benzyl)cyclohexyl 3,5-dinitrobenzoate (**17**)[16] of **16** was synthesized and its X-ray diffraction was analyzed (Scheme 2 and Fig. 3).[18] This analysis also supports the structures of **13** and **14**.

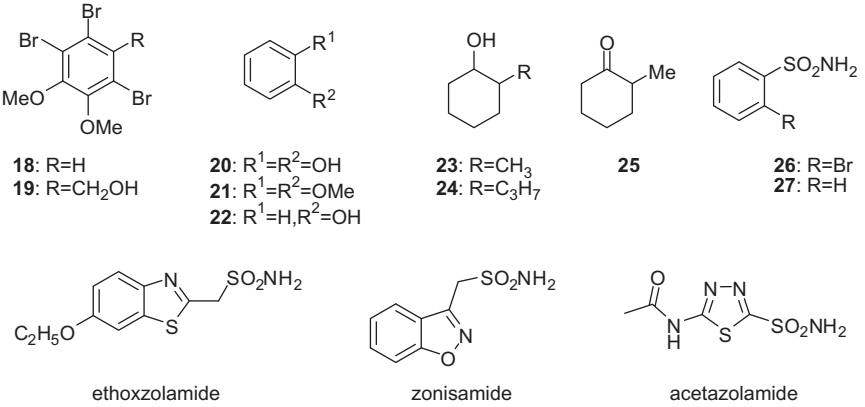


Figure 3. Structure of tested compounds **18–27** and some clinically used CA inhibitor sulfonamides.

Phenol **22** binds to CA in a different manner from the classical inhibitors of the sulfonamide type, for example ethoxzolamide (**EZA**), zonisamide (**ZNA**) and acetazolamide (**AZA**), which coordinate to the Zn(II) ion from the enzyme active site by substituting the fourth, non-protein ligand, a water molecule or hydroxide ion (Fig. 2).[6,9] Supuran's group and our group have recently investigated the interactions of phenol and some of its substituted derivatives with all mammalian CA enzymes,[6,9] demonstrating some low micromolar/submicromolar inhibitors as well as the possibility of designing isozyme selective CAIs. The inhibition profile of various CA isozymes with this class of agents is very variable, with inhibition constants ranging from the millimolar to the submicromolar range for many simple phenols.[9] Thus, it seemed reasonable to us to extend the previous studies,[6] including in our investigation phenolic, bisphenol, methoxy and bromophenol compounds with clinical and antioxidant applications as food additives, such as compounds **20**, **21**, **22**, and their derivatives.[1,3–5] Other structurally related derivatives such as **2–27**, were also included in our study (Figs. 1 and 3, Table 1 and Scheme 2).

We report here the first study on the inhibitory effects of these compounds on the esterase activity of hCA I, II, IV and VI. The sulfonamide CAI acetazolamide **AZA**[6b] has been used as a negative control in our experiments, and for comparison reasons (Fig. 4). The previous reports by Senturk et al.[6c,d] investigated other antioxidant phenol derivatives (including salicylic acid and propofol) by using an esterase assay. The data in Table 1 show the following regarding inhibition of hCA I, II, IV and VI, with these compounds, by an esterase assay,[6] with 4-nitrophenylacetate (4-NPA) as substrate:

(i) Against the slow cytosolic isozyme hCA I, compounds **8** and **18–20** behave as weak inhibitors, with K_i values in the range of 187.13–4003 μM.[6a] Catechol **22** was an ineffective hCA I inhibitor (K_i of 4003 μM). A second group of compounds **2** and **3** showed better inhibitory activity as compared to the previously mentioned compounds, with K_i values of 34.41 and 35.12 μM, (Table 1). Therefore, the nature of the groups in *ortho*-, *para*- and *meta*-positions to the phenolic OH and OMe moiety strongly influences hCA I inhibitory activity. **AZA** is also a medium CA I with this assay and substrate against hCA I (K_i of 36.2 μM). Kinetic investigations (Lineweaver Burk plots, data not shown) indicate that similarly to sulfonamides and inorganic anions,[9–19] all the investigated compounds act as noncompetitive inhibitors with 4-NPA as substrate, that is, they bind in different regions of the active site cavity as compared to the substrate. However the binding site of 4-NPA itself is unknown, but it is presumed to be in the same region as that of CO_2, the physiological substrate of this enzyme.[8]

(ii) Better inhibitory activity has been observed with compounds **4**, **13–17**, **21**, **23–27**, **EZA**, **ZNA**, and **AZA** (Fig. 4) for the inhibition of the rapid cytosolic isozyme hCA II (Table 1). Five derivatives, that is, **10–12**, **20**, and **22** showed moderate hCA II inhibitory activity with K_i-s in the range

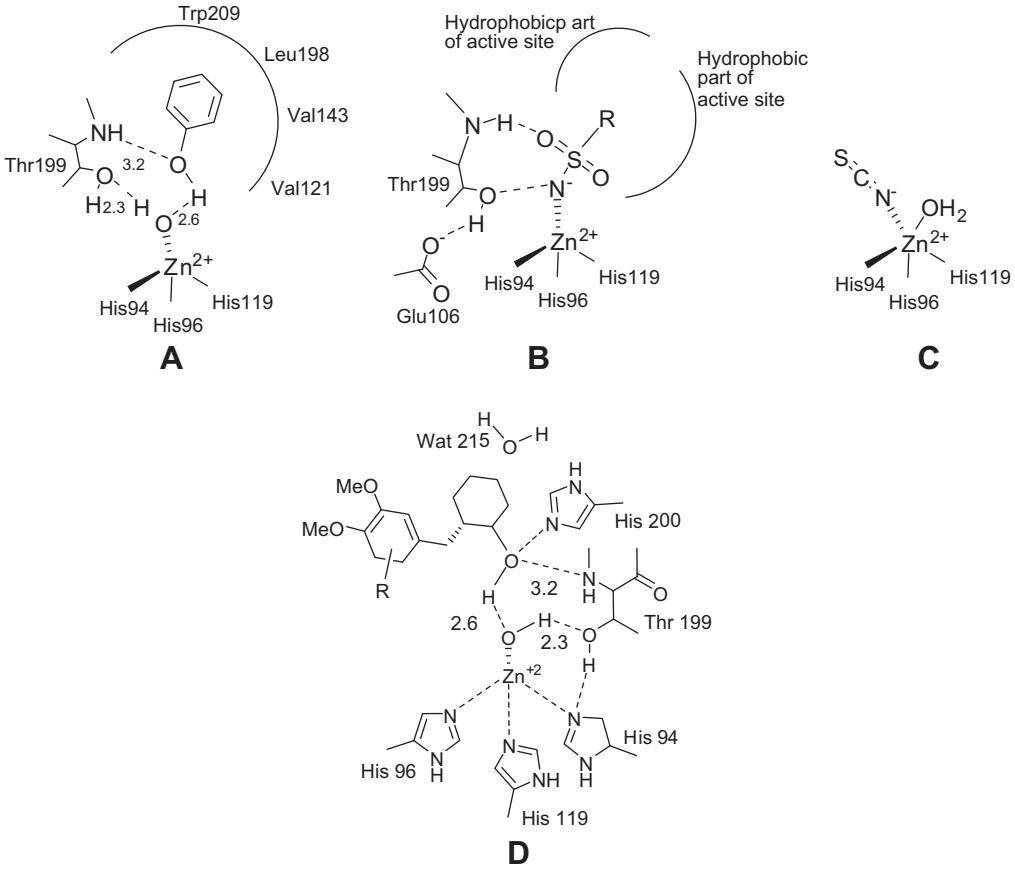


Figure 4. CA inhibition with: compounds anchoring to the zinc-bound water/hydroxide ion, such as phenol (A) zinc binders such as sulfonamides (B) and inorganic anions (C). Figures represent distances (in Å), as determined by X-ray crystallographic techniques.[6,8,19,20] Hydrogen bonds are represented as dashed lines. All these binding modes have been proven by means of X-ray crystallography on enzyme-inhibitor adducts.[2b,6,8,19] Putative binding mode of cyclitol derivatives **13–16**, **23**, and **24** to the CA active site (D), considering the X-ray crystal structure of the hCA II-phenol adduct reported earlier.[8a] Figures represent distances (in Å) and correspond to the hCA II-phenol adduct reported by Christianson's group.[8a]

of 3.97–9.9 μM (Table 1), whereas compounds **13–16** and **23–27** were quite effective hCA II inhibitors, with K_i-s in the range of 0.34–0.57 μM, (Table 1). It must be stressed that K_i-s measured with the esterase method is always in the micromolar range because hCA I and II are weak esterases[19,20]

(iii) Compound **18** is a weak inhibitor of hCA IV, with a K_i value of 163.36 μM. However, again compounds **2**, **3**, **10–12**, and **20–25** are medium potency inhibitors (K_i-s of 1.08–14.32 μM), and compounds **4**, **13–17**, **26**, and **27** show a higher affinity for this isozyme, with inhibition constants in the range of 0.85–1.43 μM, and **AZA** with K_i of 0.578 μM (Table 1).

(iv) Phenol **22** and some of its congeners such as **19–25**, and **8** are also weak inhibitors of the secreted isozyme hCA VI, with K_i-s of 158.592–919.182 μM.[6a] However, again the remaining derivatives **2**, **3**, **10–12**, **26**, **27** and **ZNA** are medium potency inhibitors (K_i of 2.42–17.83 μM), and derivative **10** and **16** show a higher affinity for this isozyme, with inhibition constant around 0.47 μM (Table 1).

The inhibition effect of halogenated sulfonamide derivatives has been investigated previously.[19a] In the current study it was demonstrated that halogenated derivatives of sulfonamide, bromosulfonamide **26**, are more effective as compared with the corresponding sulfonamides **27**.

In a recent study it was reported that derivatives of salicylic acid, some phenolic compounds and some benzoic acid derivatives,[6c,8b,21] a simple compound lacking the sulfonamide, sulfamate, or related functional groups that are typically found in all known CA inhibitors, acts as a CA I inhibitor, and could represent the starting point for a new class of inhibitors that may have advantages for patients with sulfonamide allergies.[11] The sulfonamide zinc-binding group is thus superior to the hydroxyl for generating CAIs with a varied and sometimes isozyme-selective inhibition profile against the mammalian enzymes.[20,21] However, it is critically important to explore further classes of potent CAIs in order to detect compounds with a different inhibition profile as compared to the sulfonamides and their bioisosteres and to find novel applications for the inhibitors of these widespread enzymes.[21]

In particular, phenolic, bisphenol, methoxy and bromophenol compounds influence the activity of hCA isozymes due to the presence of different functional groups (OH, OMe, COOH and Br) in their aromatic scaffold. Our findings thus indicate another class of possible CAIs of interest, in addition to the well-known sulfonamides/sulfamates/sulfamides, the phenols/biphenyl diphenols bearing bulky *ortho* moieties in their molecules. Compounds **20–22** investigated here showed effective hCA I and II inhibitory activity,[6a,20b] in the low micro molar range, by the esterase method which usually gives K_i-s an order of magnitude higher as compared to the CO_2 hydrase assay.[11] These findings indicate that substituted phenolic, bisphenol, methoxy and bromophenol compounds may be used as leads for generating potent CAIs eventually targeting other isoforms that have not been assayed yet for their interactions with such agents.

Acknowledgments

This study was financed by Turkish Republic Prime Ministry State Planning Organization (DPT), (Project No: 2010K120440) for (M.S.) and Artvin Çoruh University (BAP) (Project No: 2011-F30.02.21) for (H.T.B.). And the authors were indebted to their universities for financial support and using their laboratory equipments.

References and notes

1. (a) Gribble, G. W. *J. Nat. Prod.* **1992**, *55*, 1353; (b) Gribble, G. W. *Chem. Soc. Rev.* **1999**, *28*, 335; (c) Lee, H. S.; Lee, T. H.; Lee, J. H.; Chae, C. S.; Chung, S. C.; Shin, D. S.; Shin, J.; Oho, K. B. *J. Agric. Food Chem.* **2007**, *55*, 6923; (d) Oh, K. B.; Lee, J. H.; Chung, S. C.; Shin, J.; Shin, H. J.; Kim, H. K.; Lee, H. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 104; (e) Xu, N.; Fan, X.; Yan, X.; Li, X.; Niu, R.; Tseng, C. K. *Phytochemistry* **2003**, *62*, 1221; (f) Choi, J. S.; Park, H. J.; Jung, H. A.; Chung, H. Y.; Jung, J. H.; Choi, W. C. *J. Nat. Prod.* **2000**, *63*, 1705; (g) Balaydin, H. T.; Soyut, H.; Ekinci, D.; Goksu, S.; Beydemir, S.; Menzek, A.; Sahin, E. *J. Enzyme Inhib. Med. Chem.* **2011**. doi:10.3109/14756366.2011.574131; (h) Balaydin, H. T.; Gulcin, I.; Menzek, A.; Goksu, S.; Sahin, E. *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 685; (i) Duan, X. J.; Li, X. M.; Wang, B. G. *J. Nat. Prod.* **2007**, *70*, 1210.
2. (a) Maren, T. H. *Annu. Rev. Physiol.* **1988**, *50*, 695; (b) Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168.
3. (a) Hilvo, M.; Baranauskiene, L.; Salzano, A. M.; Scaloni, A.; Matulis, D.; Innocenti, A.; Scozzafava, A.; Monti, S. M.; Di Fiore, A.; De Simone, G.; Lindfors, M.; Jänis, J.; Valjakka, J.; Pastoreková, S.; Pastorek, J.; Kulomaa, M. S.; Nordlund, H. R.; Supuran, C. T.; Parkkila, S. *J. Biol. Chem.* **2008**, *283*, 27799; (b) Kivela, J.; Parkkila, S.; Waheed, A.; Parkkila, A. K.; Sly, W. S.; Rajaniemi, H. *Clin. Chem.* **1997**, *43*, 2318.
4. (a) Supuran, C. T.; Scozzafava, A. *Bioorg. Med. Chem.* **2007**, *15*, 4336; (b) Supuran, C. T.; Scozzafava, A. *Expert Opin. Ther. Pat.* **2002**, *12*, 217; (c) Casey, J. R. *Biochem. Cell Biol.* **2006**, *84*, 930; (d) Senturk, M.; Gulcin, I.; Dastan, A.; Kufrevioglu, O. I.; Supuran, C. T. *Bioorg. Med. Chem.* **2009**, *17*, 3207; (e) Senturk, M.; Gulcin, I.; Beydemir, S.; Kufrevioglu, O. I.; Supuran, C. T. *Chem. Biol. Drug Des.* **2011**, *77*, 494.
5. (a) Sly, W. S.; Hu, P. Y. *Annu. Rev. Biochem.* **1995**, *64*, 375; (b) Parkkila, S.; Parkkila, A. K. *Scand. J. Gastroenterol* **1996**, *31*, 305; (c) Pastorekova, S.; Parkkila, S.; Pastorek, J.; Supuran, C. T. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 199; (d) Ozturk Sarikaya, S. B.; Topal, F.; Senturk, M.; Gulcin, I.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4259.
6. (a) Innocenti, A.; Vullo, D.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1583; (b) Innocenti, A.; Hilvo, M.; Scozzafava, A.; Parkkila, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3593; (c) Bayram, E.; Senturk, M.; Kufrevioglu, O. I.; Supuran, C. T. *Bioorg. Med. Chem.* **2008**, *16*, 9101; (d) Ekinci, D.; Ceyhun, S. B.; Senturk, M.; Erdem, D.; Kufrevioglu, O. I.; Supuran, C. T. *Bioorg. Med. Chem.* **2011**, *19*, 744.
7. (a) Bradford, M. *Anal. Biochem.* **1976**, *72*, 248; (b) Laemmli, D. K. *Nature* **1970**, *227*, 680; (c) Lineweaver, H.; Burk, D. *J. Am. Chem. Soc.* **1934**, *57*, 685.
8. (a) Nair, S. K.; Ludwig, P. A.; Christianson, D. W. *J. Am. Chem. Soc.* **1994**, *116*, 3659; (b) Ekinci, D.; Cavdar, H.; Talaz, O.; Senturk, M.; Supuran, C. T. *Bioorg. Med. Chem.* **2010**, *18*, 3559.
9. (a) Wilbur, K. M.; Anderson, N. G. *J. Biol. Chem.* **1976**, *176*, 147; (b) Verpoorte, J. A.; Mehta, S.; Edsall, J. T. *J. Biol. Chem.* **1967**, *242*, 4221.
10. Detailed procedures for enzyme purification can be found in: (a) Parkkila, S.; Kaunisto, K.; Rajaniemi, L.; Kumpulainen, T.; Jokinen, K.; Rajaniemi, H. *J. Histochem. Cytochem.* **1990**, *38*, 941; (b) Alp, C.; Ekinci, D.; Gultekin, M. S.; Senturk, M.; Sahin, E.; Kufrevioglu, O. I. *Bioorg. Med. Chem.* **2010**, *18*, 4468.
11. (a) Senturk, M.; Talaz, O.; Ekinci, D.; Cavdar, H.; Kufrevioglu, O. I. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3661; (b) Ceyhun, S. B.; Senturk, M.; Yerlikaya, E.; Erdogan, O.; Kufrevioglu, O. I.; Ekinci, D. *Environ. Toxicol. Pharmacol.* **2011**, *32*, 69; (c) Cakmak, R.; Durdagi, S.; Ekinci, D.; Senturk, M.; Topal, G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5398.
12. (a) Ceyhun, S. B.; Senturk, M.; Erdogan, O.; Kufrevioglu, O. I. *Pestic. Biochem. Physiol.* **2010**, *97*, 177; (b) Ceyhun, S. B.; Senturk, M.; Ekinci, D.; Erdogan, O.; Ciltas, A.; Kocaman, M. *Comp. Biochem. Physiol. C* **2010**, *152*, 215; (c) Ekinci, D.; Senturk, M.; Beydemir, S.; Kufrevioglu, O. I.; Supuran, C. T. *Chem. Biol. Drug Des.* **2010**, *76*, 552.
13. Supuran, C. T.; Scozzafava, A. *J. Enzyme. Inhib. Med. Chem.* **1997**, *12*, 37.
14. (a) Mori, T.; Bando, H.; Kanaiwa, Y.; Amiya, T.; Kurata, K. *Chem. Pharm. Bull.* **1983**, *3*, 1754; (b) Akbaba, Y.; Balaydin, H. T.; Goksu, S.; Sahin, E.; Menzek, A. *Helv. Chim. Acta.* **2010**, *93*, 1127; (c) Balaydin, H. T.; Akbaba, Y.; Menzek, A.; Sahin, E.; Goksu, S. *Arkivoc* **2009**, *XIV*, 75; (d) Sahin, E.; Balaydin, H. T.; Goksu, S.; Menzek, A. *Acta Crystallogr.* **2010**, *E66*, o3029; (e) Cetinkaya, Y.; Menzek, A.; Sahin, E.; Balaydin, H. T. *Tetrahedron* **2011**, *67*, 3483.
15. Harvey, R. G.; Pataki, J.; Cortez, C.; Raddo, P. D.; Yang, C. X. *J. Org. Chem.* **1991**, *56*, 1210.
16. *Compounds **4** and **10–12** were synthesized as follow*: For **12**; **9** (1,27 g, 8,4 mmol) was added to a solution of **7** (2,61 g, 8,41 mmol) in dry dioxane (15 mL), and the solution was refluxed 18 h under N_2. Then, water was added and heating was continued for an additional hour. Then solvent was evaporated and the residue was extracted with ether (2 × 50 ml). The ether phase was washed with solution of HCl (5%, 20 mL), $NaHCO_3$ (5%, 50 mL) and water (50 mL), consecutively. Organic layer was dried over Na_2SO_4 and evaporated under vacuum. The residue was chromatographed on silicagel eluted with EtOAc/hexane (1/95) to provide pure product **12** (2.33 g, 85%). Liquid; R_f (40%, EtOAc/hexane) 0.83; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (d, J = 1.92 Hz, 1H), 6.68 (d, J = 1.89 Hz, 1H), 3.85 (s, OCH_3, 3H), 3.82 (s, OCH_3, 3H), 3.14 (dd, A part of AB-system, J = 14.00, 5.24 Hz, CH_2, 1H), 2.56–2.40 (m, OCH, 1H), 2.33 (dd, B part of AB-system, J = 13.93, 8.09 Hz, CH_2, 1H), 2.11–0.85 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.1 (CO), 153.4 (C), 144.6 (C), 137.8 (C), 124.9 (CH), 117.3 (C), 112.9 (CH), 60.5, 56.1, 52.4, 42.2, 35.1, 33.7, 28.0, 25.1; IR (CH_2Cl_2, cm^{-1}): 3612, 3397, 3053, 2940, 2862, 2720, 2665, 2588, 2469, 2410, 2262, 2089, 2004, 1914, 1740, 1714, 1596, 1566, 1489, 1415, 1363, 1331, 1273, 1235, 1183, 1143, 1047, 1006;

Anal. Calcd for ($C_{15}H_{19}BrO_3$): C 55.06, H 5.85; Found C 55.19, H 5.87.

For ***10***: Liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H), 3.54 (dd, A part of AB-system, J = 14.27, 3.66 Hz, CH_2, 1H), 3.27 (dd, B part of AB-system, J = 14.27, 10.61 Hz, CH_2, 1H), 2.81–2.78 (m, CHCO, 1H), 2.50–2.34 (m, 2H), 2.10–2.03 (m, 1H), 1.91–1.81 (m, 2H), 1.72–1.52 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.5 (CO), 150.8 (C), 137.7 (C), 122.9 (C), 121.9 (CH), 121.1 (C), 61.0, 60.9, 50.2, 42.2, 37.7, 33.0, 28.0, 25.5; IR (CH_2Cl_2, cm^{-1}): 3403, 2936, 2862, 1709, 1558, 1520, 1488, 1451, 1395, 1367, 1329, 1312, 1273, 1218, 1169, 1152, 128, 1074, 1047, 1010; Anal. Calcd for ($C_{15}H_{17}Br_3O_3$): C 37.15, H 3.53; Found C 37.17, H 3.55. For **11** (94%): Liquid; R_f (40%, EtOAc/hexane) 0.82; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.90 (s, 1H), 3,86 (s, OCH_3, 3H), 3.82 (s, OCH_3, 3H), 3,35 (dd, A part of AB-system, J = 13.64, 6.08 Hz, CH_2, 1H), 2.73–2.65 (m, OCH, 1H), 2.59 (dd, B part of AB-system, J = 13.64, 6.81 Hz, CH_2, 1H), 2.44–0.80 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.1 (CO), 152.1 (C), 146.0 (C), 137.5 (C), 121.7 (C), 117.5 (C), 114.8 (CH), 60.5, 56.2, 50.8, 42.4, 37.6, 34.3, 28.2, 25.4; IR (CH_2Cl_2, cm^{-1}): 3397, 2938, 2861, 2661, 2575, 2392, 2289, 2123, 2012, 1913, 1874, 1741, 1705, 1584, 1549, 1464, 1424, 1379, 1310, 1264, 1224, 1199, 1162, 1128, 1053, 1007; Anal. Calcd for ($C_{15}H_{18}Br_2O_3$): C 44.36, H 4.47; Found C 44.19, H 4.45.

For natural bromophenol ***4****′ or* ***4*** *(rasemic)*: waxy solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.00 (bs, 2OH), 3.48 (dd, A part of AB-system, J = 14.36, 3.85 Hz, CH_2, 1H), 3.17 (dd, B part of AB-system, J = 10.61, 14.36 Hz, CH_2, 1H), 2.82–2.74 (m, OCH, 1H), 2.50–2.30 (m, 2H), 2.18–2.04 (m, 1H), 1.90–1.47 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.0 (CO), 140.9 (C), 140.8 (C), 132.7 (C), 117.7 (C), 113.1 (C), 112.4 (C), 50.3 (CHCO), 50.1, 42.2, 37.1, 32.9, 29.9, 28.0, 25.5.

General procedure for reduction of ***11*** *and* ***12***: For **12**; To a cold (0 °C) solution (30 mL) of **12** (0.5 g, 1.53 mmol) in diethylether/methanol (1:1) $NaBH_4$ (0.018 g) was carefully added over 5 min and the resulting mixture was stirred at the same temperature for 15 min. After the cold bath was removed, it was stirred at rt for 1 day. Water (10 mL) was added and then the mixture was acidified with HCl (5%) until its pH was 3. The mixture was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layer was dried over Na_2SO_4 and the solvent was evaporated. The residue was subjected to column chromatography on silica gel (SiO_2, 50 g) and eluted using EtOAc/hexane (1:95) to give the cis alcohol **13** (0.196 g, 39%) and trans alcohol **14** (0.292 g, 58%), respectively.

For ***13***: Liquid; R_f (40%, EtOAc/hexane) 0.74; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (d, J = 0.46 Hz, 1H), 6.68 (d, J = 0.46 Hz, 1H), 3.84 (s, OCH_3, 3H), 3.82 (s, OCH_3, 3H), 3.79 (s, OCH, 1H), 2.64 (dd, A part of AB-system, J = 13.54, 7.68 Hz, CH_2, 1H), 2.45 (dd, B part of AB-system, J = 7.68, 13.54 Hz, CH_2, 1H), 1.78–1.21 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.6 (C), 144.7 (C), 138.6 (C), 125.2 (CH), 117.5 (C), 113.0 (CH), 68.5 (CHO), 60.8, 56.3, 43.6, 38.5, 33.5, 26.6, 25.4, 20.5; IR (CH_2Cl_2, cm^{-1}): 3445, 2997, 2932, 2857, 1722, 1596, 1566, 1489, 1464, 1451, 1429, 1413, 1302, 1272, 1235, 1183, 1141, 1102, 1039, 1004; Anal. Calcd for ($C_{15}H_{21}BrO_3$): C 54.72, H 6.43; Found C 54.42, H 6.40.

For ***14***: Liquid; R_f (40%, EtOAc/hexane) 0.56; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (d, J = 0.46 Hz, 1H), 6.66 (s, 1H), 3.84 (s, OCH_3, 3H), 3.82 (s, OCH_3, 3H), 3.29–3.23 (m, CHCO, 1H), 3.11 (dd, A part of AB-system, J = *1*3.54, 3.66 Hz, CH_2, 1H), 2.24 (dd, B part of AB-system, J = 9.33, 13.54 Hz, CH_2, 1H), 1.99–1.96 (m, 1H), 1.74–1.41 (m, 4H), 1.33–1.24 (m, 2H), 1.2–1.08 (m, 1H), 0.94–0.87 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 153.6 (C), 144.7 (C), 138.4 (C), 125.5 (CH), 117.4 (C), 113.1 (CH), 74.5 (CHO), 60.8, 56.3, 47.1, 38.8, 36.2, 30.2, 25.6, 25.1; IR (CH_2Cl_2, cm^{-1}): 3391, 2997, 2931, 2856, 2006, 1723, 1596, 1566, 1489, 1464, 1449, 1428, 1414, 1298, 1273, 1235, 1182, 1142, 1040, 1005; Anal. Calcd for ($C_{15}H_{21}BrO_3$): C 54.72, H 6.43; Found C 54.78, H 6.46.

For ***15***: Liquid; R_f (40%, EtOAc/hexane) 0.66; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (s, 1H), 3.84 (s, OCH_3, 3H), 3.82 (s, OCH_3, 3H), 3.77 (s, OCH, 1H), 2.87 (dd, A part of AB-system, J = 13.17, 7.68 Hz, CH_2, 1H), 2.70 (dd, B part of AB-system, J = 7.14, 13.17 Hz, CH_2, 1H), 1.78–1.24 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.3 (C), 146.1 (C), 138.1 (C), 122.0 (C), 117.8 (C), 114.5 (CH), 68.5 (CHO), 60.7, 56.4, 41.6, 41.0, 33.5, 26.5, 25.6, 20.3; IR (CH_2Cl_2, cm^{-1}): 3446, 2997, 2931, 2857, 1745, 1642, 1583, 1548, 1463, 1422, 1377, 1306, 1262, 1222, 1199, 1160, 1133, 1102, 1060, 1007; Anal. Calcd for ($C_{15}H_{20}Br_2O_3$): C 44.14, H 4.94; Found C 44.37, H 4.96.

For ***16***: Liquid; R_f (40%, EtOAc/hexane) 0.50; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80 (s, 1H), 3.89 (s, OCH_3, 3H), 3.86 (s, OCH_3, 3H), 3.49 (dd, A part of AB-system, J = 13.36, 3.24 Hz, CH_2, 1H), 3.39–3.37 (m, OCH, 1H), 2.52 (dd, B part of AB-system, J = 9.76, 13.36 Hz, CH_2, 1H), 2.08–2.00 (m, 1H), 1.78–1.62 (m, 4H), 1.38–1.27 (m, 2H), 1.16–0.91 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.4 (C), 146.1 (C), 138.2 (C), 122.0 (C), 118.0 (C), 114.5 (CH), 75.3 (CHO), 60.7, 56.4, 46.2, 41.1, 36.2, 30.2, 25.6, 25.1; IR (CH_2Cl_2, cm^{-1}): 3391, 2992, 2931, 2855, 1745, 1717, 1642, 1583, 1549, 1466, 1423, 1374, 1345, 1310, 1262, 1222, 1200, 1162, 1124, 1102, 1062, 1031, 1007; Anal. Calcd for ($C_{15}H_{20}Br_2O_3$): C 44.14, H 4.94; Found C 44.39, H 4.96.

Synthesis of ***17***: To a cold (0 °C) solution of **16** (0.250 g, 0.61 mmol) in CH_2Cl_2 (15 mL), triethylamine (5.0 equiv) and 3,5-dinitrobenzoylchloride (4.0 equiv) were added slowly under N_2, respectively. After the mixture was stirred at rt for 1 day, it was acidified with cold (0 °C) HCl (1%) and then neutralized with NaOH (1%) and washed with water (20 mL), respectively. Organic phase was dried over Na_2SO_4 and the solvent was evaporated. Ester **17** (0.360 g, 98%) was obtained and crystallized from CH_2Cl_2/hexane as yellow crystals. Mp 176–177 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.89 (s, OCH_3, 3H), 3.88 (s, OCH_3, 3H), 3.54 (dd, A part of AB-system, J = 14.27, 3.66 Hz, CH_2, 1H), 3.27 (dd, B part of AB-system, J = 14.27, 10.61 Hz, CH_2, 1H), 2.81–2.78 (m, OCH, 1H), 2.50–2.34 (m, 2H), 2.10–2.03 (m, 1H), 1.91–1.81 (m, 2H), 1.72–1.52 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.5 (CO), 150.8 (C), 137.7 (C), 122.9 (C), 121.9 (CH), 121.1 (C), 61.0, 60.9, 50.2, 42.2, 37.7, 33.0, 28.0, 25.5. IR (CH_2Cl_2, cm^{-1}): 3403, 2936, 2862, 1709, 1558, 1520, 1488, 1451, 1395, 1367, 1329, 1312, 1273, 1218, 1169, 1152, 128, 1074, 1047, 1010; Anal. Calcd for ($C_{15}H_{17}Br_3O_3$): C 37.15, H 3.53; Found C 37.17, H 3.55.

Compound **8** were prepared by known method[14a,17] and the method is similar to that of **17**. For **8**: mp 98–100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 (s, CH_2, 2H), 2.36 (s, CH_3, 6H), 2.10 (s, CH_3, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7 (CO), 167.0 (CO), 166.8 (CO), 143.5 (C), 142.0 (C), 135.0 (CH), 127.2 (C), 122.1 (C), 121.4 (C), 68.1 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 20.5 (CH_3); IR (CH_2Cl_2, cm^{-1}): 3176, 2924, 2857, 1783, 1743, 1645, 1561, 1463, 1418, 1372, 1278, 1235, 1187, 1148, 1050, 1027, 1011.

17. (a) Menzek, A.; Balci, M. *Tetrahedron* **1993**, *49*, 6071; (b) Menzek, A.; Kazaz, C.; Eryigit, F.; Cengiz, M. *J. Chem. Res.* **2004**, 210; (c) Menzek, A.; Sengül, M. E.; Cetinkaya, Y.; Ceylan, S. *J. Chem. Res.* **2005**, 209; (d) Sengül, M. E.; Menzek, A.; Menzek, A.; Balci, M. *Turk. J. Chem.* **2006**, *30*, 131; (e) Menzek, A.; Altundas, A. *Tetrahedron* **2006**, *62*, 12318; (f) Sengül, M. E.; Menzek, A.; Sahin, E.; Arik, M.; Saracoglu, N. *Tetrahedron* **2008**, *64*, 7289.
18. *X-Ray analysis*: For the crystal structure determination, the single-crystal of the compound **17** 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 Kα radiation (λ = 0.71073 Å) and oscillation scans technique with $\Delta\omega$ = 5° for one image were used for data collection. The lattice parameters were determined by the least-squares methods on the basis of all reflections with $F^2 > 2\sigma(F^2)$. Integration of the intensities, correction for Lorentz and polarization effects and cell refinement was performed using CrystalClear (Rigaku/MSC Inc., 2005) software.[22a] The structures were solved by direct methods using SHELXS-97[22b] and refined by a full-matrix least-squares procedure using the program SHELXL-97.[22b] *H* atoms were positioned geometrically and refined using a riding model. The final difference Fourier maps showed no peaks of chemical significance. *Crystal data for* ***17***: $C_{22}H_{22}N_2O_8Br_2$; crystal system, space group: triclinic, $P2_1/c$; (no:14); unit cell dimensions: a = 13.0170(4), b = 7.8501(3), c = 23.8484(7) Å, β = 91.94(3)°; volume: 2435.5(2) Å^3; Z = 4; calculated density: 1.642 g/cm^3; absorption coefficient: 3.378 mm^{-1}; F(000): 1208; θ range for data collection 3.0–26.6°; refinement method: full-matrix least-square on F^2; data/parameters: 5005/307; goodness-of-fit on F^2: 1.053; final R indices [$I > 2\sigma(I)$]: R_1 = 0.066, wR_2 = 0.128; R indices (all data): R_1 = 0.139, wR_2 = 0.158; largest diff. peak and hole: 0.458 and −0.656 e Å^{-3}; Crystallographic data were deposited in CSD under CCDC registration number **847309**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.
19. (a) Senturk, M.; Kufrevioglu, O. I.; Ciftci, M. *J. Enzyme Inhib. Med. Chem* **2009**, *24*, 420; (b) Durdagi, S.; Senturk, M.; Ekinci, D.; Balaydin, H. T.; Goksu, S.; Kufrevioglu, O. I.; Innocenti, A.; Scozzafava, A.; Supuran, C. T. *Bioorg. Med. Chem.* **2011**, *19*, 1381.
20. (a) Supuran, C. T.; Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G. *Eur. J. Med. Chem.* **1999**, *34*, 799; (b) Tas, M. *Afr. J. Biotechnol.* **2011**, *10*, 3849; (c) Balaydin, H. T.; Durdagi, S.; Ekinci, D.; Senturk, M.; Goksu, S.; Menzek, A. *J. Enzyme Inhib. Med. Chem.* **2011**. doi:10.3109/14756366.2011.596836.
21. Barrese, A. A.; Genis, C.; Fisher, S. Z.; Orwenyo, J. N.; Kumara, M. T.; Dutta, S. K.; Phillips, E.; Kiddle, J. J.; Tu, C.; Silverman, D. N.; Govindasamy, L.; Agbandje-McKenna, M.; McKenna, R.; Tripp, B. C. *Biochemistry* **2008**, *47*, 3174.
22. (a) Rigaku/MSC, Inc. 9009 new Trails Drive, The Woodlands, TX 77381.; (b) Sheldrick, G. M. *SHELXS97 and SHELXL97*; University of Göttingen: Germany, 1997.