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Carbonic anhydrase inhibitors: Design, synthesis, kinetic, docking and molecular dynamics analysis of novel glycine and phenylalanine sulfonamide derivatives

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ABSTRACT

The inhibition of two human cytosolic carbonic anhydrase isozymes I and II, with some novel glycine and phenylalanine sulfonamide derivatives were investigated. Newly synthesized compounds **G1–4** and **P1–4** showed effective inhibition profiles with K_I values in the range of 14.66–315 μM for hCA I and of 18.31–143.8 μM against hCA II, respectively. In order to investigate the binding mechanisms of these inhibitors, in silico docking studies were applied. Atomistic molecular dynamic simulations were performed for docking poses which utilize to illustrate the inhibition mechanism of used inhibitors into active site of CAII. These sulfonamide containing compounds generally were competitive inhibitors with 4-nitrophenylacetate as substrate. Some investigated compounds here showed effective hCA II inhibitory effects, in the same range as the clinically used sulfonamide, sulfanilamide or mafenide and might be used as leads for generating enzyme inhibitors possibly targeting other CA isoforms which have not been yet assayed for their interactions with such agents.

1. Introduction

Carbonic anhydrase (EC 4.2.1.1, CA) is a family of metalloenzymes that catalyze the rapid conversion of CO_2 to HCO_3^- and H^+, and involved in the biochemical process.[1] 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, ureagenesis, gluconeogenesis, lipogenesis and electrolyte secretion.[1–4] Many 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.[1,4]

Our groups recently investigated the interaction of some mammalian CA isozymes with several types of sulfonamide derivatives, such as sulfanilamide and a series of chromone containing sulfonamides, benzenesulfonamides, for example, and some of their derivatives.[5] Here we extend these earlier investigations to series of sulfonamides, some of which are widely used as prodrug or as drugs. Sulfonamides possess many types of biological activities, and representatives of this class of pharmacological agents are widely used in clinic as antibacterial, hypoglycemic, diuretic, anti-hypertensive and antiviral drugs among others.[1,4–6] Recently, a host of structurally novel sulfonamide derivatives have been reported to show substantial antitumor activity in vitro and/or in vivo.[6–8]

In the present study we have purified CA I and II (hCA I and hCA II) from human erythrocytes and examined the in vitro inhibition effects of above mentioned aminoacid derivatives on these enzymes, using the esterase activity of hCA I and II, with 4-nitrophenyl acetate as substrate. Molecular modeling studies also

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applied for better understanding of molecular mechanisms of used compounds.

2. Results and discussion

2.1. Chemistry

The rationale of investigating sulfonamide derivatives as CA inhibitors (CAIs) is due to the fact that the simple benzenesulfonamide ($PhSO_2NH_2$) has been shown to be competitive inhibitor with both CO_2 and 4-nitrophenyl acetate as substrate for CA isoforms.[1,5a] Sulfonamide type inhibitors bind to CAs, with coordination to the Zn(II) ion from the enzyme active site by substituting the fourth, non-protein ligand, a water molecule or hydroxide ion, such as for example acetazolamide (**AZA**), a clinically used compound since 1954.[8,9]

The X-ray crystal structure has been extensively used for understanding the inhibition mechanism of CAIs. For example, for the adduct of hCA II with sulfamide,[8a,9] it has been observed that the compound binds to CA by anchoring its NH moiety to the zinc ion of the enzyme active site, through a hydrogen bond, as well as through a second hydrogen bond to the NH amide of Thr199, an amino acid conserved in all α-CAs and critically important for the catalytic cycle of these enzymes.[6–9] Only recently, our groups investigated the interactions of some methanesulfonates, chromone containing sulfonamides, benzenesulfonamides, salicylic acid derivatives, some pesticides, some natural product polyphenols and phenolic acids with all mammalian isozymes, CA I–XV,[8–11] evidencing some low micromolar/submicromolar inhibitors as well as the possibility to design isozyme selective CAIs. Indeed, the inhibition profile of various isozymes with this class of agents is very variable, with inhibition constants ranging from the millimolar to the submicromolar range for many simple sulfonamides (Fig. 1).[5a,10]

2.2. CA purification, assay and inhibition with some amino acid derivatives

The purification of the two CA isozymes used here was performed with a simple one step method by a Sepharose-4B-aniline-sulfanilamide affinity column chromatoghrapy.[12] Inhibitory effects of **G1**–**4** and **P1**–**4** compounds on enzyme activities were tested under in vitro conditions; K_I values were calculated from by using the Cheng–Prusoff equation and are given in Table 1.[13]

We report here the first study on the inhibitory effects of amino acid derivatives **G1**–**4** and **P1**–**4** on the esterase activity of hCA I and II:

(i) Against the slow cytosolic isozyme hCA I, **G3** and **P2** behave as good inhibitors, with K_I values in the range of 29.62 and 63.7 μM. Thus, the natural compound derivatives of the groups in hydrophobic benzoic moiety strongly influences hCA I inhibitory activity. It is also interesting to note that the **P3** was much better hCA I inhibitors as compared to the corresponding **G3** and **P2** from which they were prepared. Kinetic investigations indicate that similarly to sulfonamides and inorganic anions,[4,5,8] all the investigated compounds act as competitive 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.[14]

(ii) A better inhibitory activity has been observed with **G3** and **4** investigated here for the inhibition of the rapid cytosolic isozyme hCA II (Table 1). Structure–activity relationship (SAR) is thus quite sharp for this small series of these compounds: the –COOH **G1** are ineffective leads, with carboxylic acid moieties is already a submicromolar hCA II inhibitor. The

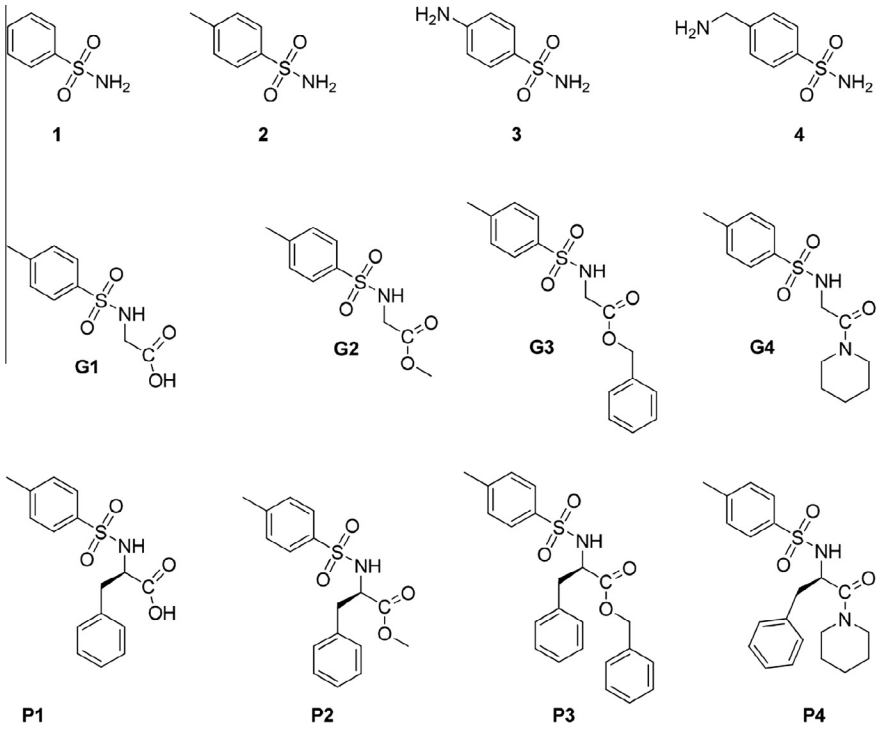

Figure 1. Chemical structures of synthesized and used compounds for this study.

Table 1
hCA I and II inhibition data with studied compounds, by an esterase assay with 4-nitrophenylacetate as substrate[14]

Compound	K_I* (μM)	
	hCA I	hCA II
Benzenesulfonamide	39.96[a]	1.103[a]
p-Toluenesulfonamide	118.47[a]	8.138[a]
Sulfanilamide	42.87[a]	0.628[a]
Mafenide	41.91[a]	0.612[a]
G1	120.1	143.8
G2	105.9	56.40
G3	29.62	18.31
G4	NE	19.32
P1	315	103.9
P2	63.7	97.4
P3	14.66	56.0
P4	100.4	NE

* Mean from at least three determinations. Errors in the range of 3–8% of the reported value (data not shown).
[a] Ref. 5a.

best hCA II inhibitor in this series of derivatives were **G3** and **G4**, which with a K_I of 18.31–19.33 μM. It must be stressed that K_Is measured with the esterase method are always in the micromolar range because hCA I and II are weak esterases.[8,14]

The sulfonamide zinc-binding group is thus superior to the thiol one (from the thioxolone hydrolysis product) for generating CA inhibitors with a varied and sometimes isozyme-selective inhibition profile against the mammalian enzymes. 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. Although there are several studies regarding the interactions of sulfonamide derivatives with CA isoenzymes, it is critically important to explore further classes of potent CAIs in order to detect compounds with a different inhibition profile to find novel applications for these CAIs (Figs. 2 and 3).

2.2.1. Molecular dynamic (MD) simulations

In this section we tried to study the influence of mafenide and **G4** into the active site of CAII on protein structure. For this purpose, three independent MD simulations were set up including of a control system (ligand-free structure) and two complexes. Root-mean-square deviation (RMSD) of backbone atoms of CAII in complexes with mafenide and G4 were calculated based on MD trajectory frames. The evolution of RMSD of each system during 10 ns MD simulations was profiled in Figure 4. Studying through this profile, we have observed the structural stabilities of backbone atoms along simulations. Mafenide-bound CAII is so mobile, but inversely, apo and G4-bound systems exist in a stable

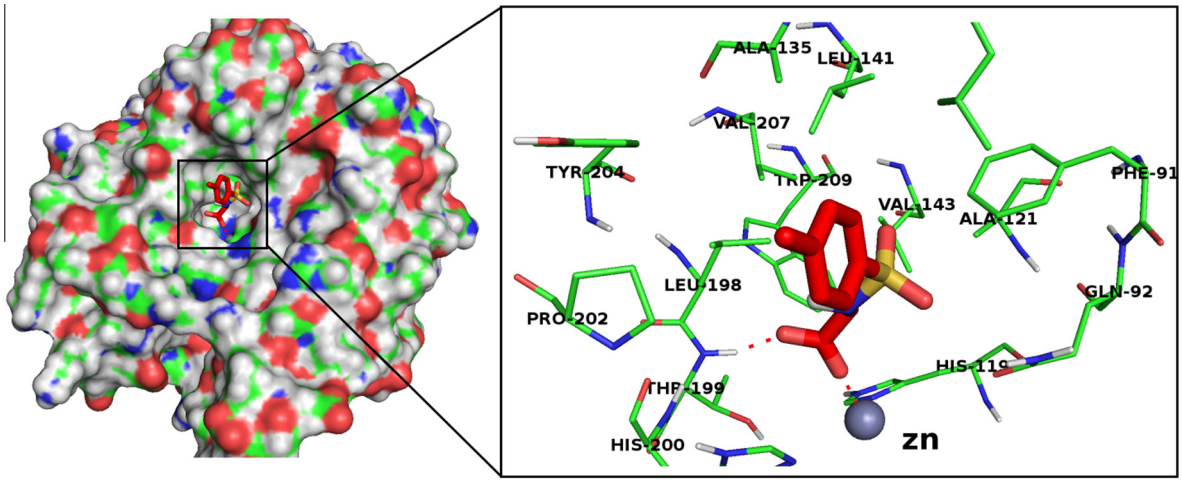

Figure 2. G1 compound was docked into carbonic anhydrase I (CAI).

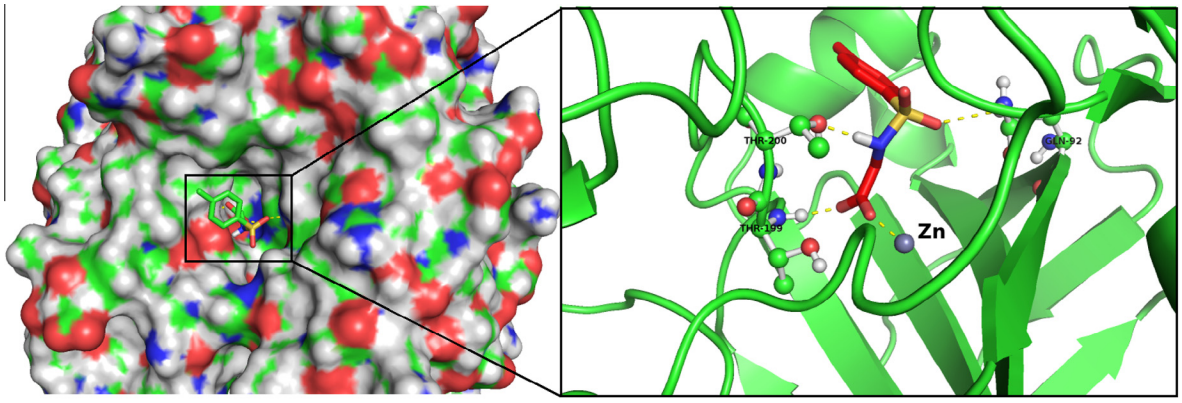

Figure 3. G1 compound was docked into carbonic anhydrase II (CAII).

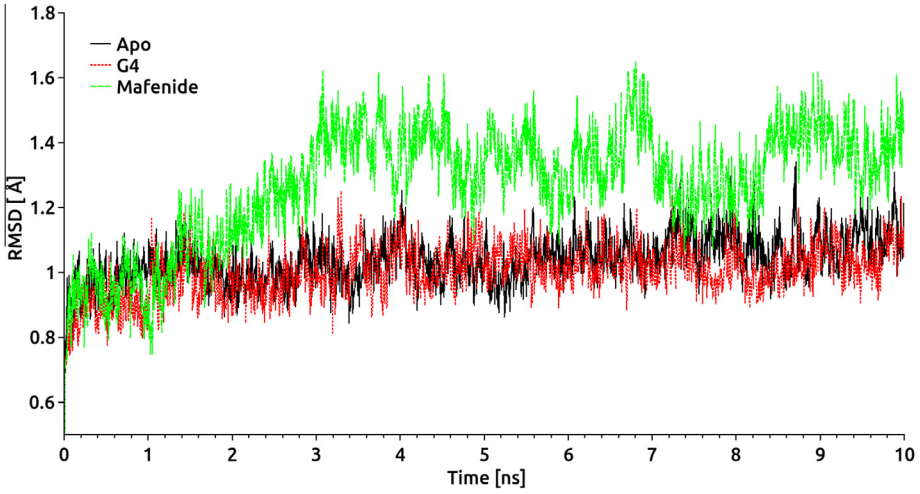

Figure 4. RMSD evolution of backbone atoms of mafenide, G4-bound CAII and apo system during 10 ns MD simulations.

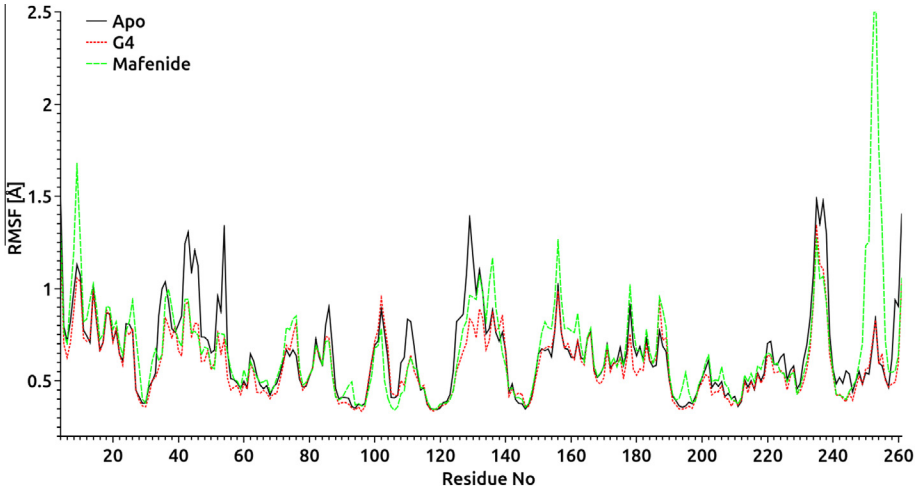

Figure 5. RMSF values for individual backbone atoms of residues along MD simulations.

dynamic. CAII atoms were increasingly influenced upon binding of mafenide, which may be connected to increasing of entropy terms. Increasing of the entropy term in this system is describing the more favorable binding of mafenide. In addition, root-mean-square fluctuations of individual backbone atoms of residues were calculated along the simulation for each system. The results were profiled in Figure 5, the values revealed the stability of each amino acid. Studying through these calculations, we have found that structural stability of mafenide-bound system is low in most of the domains in CAII. And also, in some part of the apo form, atoms have increasingly fluctuated.

3. Conclusions

G1–**4** and **P1**–**4** used in this study affect the activity of CA isozymes due to the presence of the different functional groups (for instance –COOH, $–COOCH_3$, and –COOPh) present in their aromatic scaffold. Our findings here indicate thus another class of possible CAIs of interest, in addition to the well-known sulfonamides/sulfamates/sulfamides, the bearing bulky moieties in their molecules. Indeed, some amino acid derivatives compounds investigated here showed effective hCA I and II inhibitory activity, in the low micromolar range, by the esterase method which usually gives K_I-s an order of magnitude higher as compared to the CO_2 hydrase assay.[14] These findings point out that substituted compounds may be used as leads for generating potent CAIs eventually targeting other isoforms which have not been assayed yet for their interactions with such agents.

4. Experimental

4.1. Chemicals

Benzenesulfonamide, sulfanilamide, *p*-toluenesulfonamide, mafenide, Sepharose 4B, protein assay reagents, 4-nitrophenylacetate were obtained from Sigma–Aldrich Co. Glycine, L-phenylalanine, *p*-toluenesulfonyl chloride were purchased from Sigma Aldrich and used as received. Glycine and L-phenylalanine methyl ester hydrochlorides, glycine and L-phenylalanine benzyl ester hydrochlorides were obtained from Sigma–Aldrich. Glycine and L-phenylalanine piperidinylamides were synthesized from BOC-protected derivatives according to the reported procedure.[15] All other chemicals and solvents were analytical grade and obtained from Merck. Column chromatography was performed using silica gel 60 (43–60 nm, Merck). Thin layer chromatography was performed using silica gel plates (kieselgel 60 F254, 0.2 mm, Merck). Characterization of synthesized compounds was performed using ^{1}H NMR, ^{13}C NMR spectroscopy (Varian 400 MHz).

Elemental analysis data was obtained using a Leco CHNS-932 apparatus (Michigan, USA).

4.2. Syntheses of *N*-tosyl α-amino acid derivatives G(1–4) and P (1–4)

Syntheses of **G1** and **P1**: To a stirred solution of glycine (0.75 g, 10.0 mmol) or L-phenylalanine (1.65 g, 10.0 mmol) in 20 mL of 1 M NaOH solution at 25 °C, *p*-toluenesulfonyl chloride (1.91 g, 10.0 mmol) was added. After overnight stirring at room temperature, the solid residue was filtered off and the aqueous reaction portion was acidified with 1 M HCl. The obtained solid was filtered and purified by column chromatography on silica with hexane and EtOAc (1:2) to obtain compounds G1 and P1 as white solids. (Yields, 81% and 76%, respectively.)

Compound **G1**: Yield 81% as white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.66 (broad singlet, 1H, –COO*H*), 7.93 (t, J = 6.1 Hz, 1H, –N*H*–), 7.67 (d, J = 8.4 Hz, 2H, Ar*H*), 7.37 (d, J = 8.3 Hz, 2H, Ar*H*), 3.54 (d, J = 6.1 Hz, 2H, –CH_2–), 2.37 (s, 3H, –CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 143.8, 136.3, 129.9, 127.3, 40.3, 21.6 ppm. Anal. Calcd [$C_9H_{11}NO_4S$]: C, 47.15; H, 4.84; N, 6.11; S, 13.99. Found: C, 48.36; H, 5.11; N, 5.93; S, 14.27.

Compound **P1**: Yield 76% as white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.70 (broad singlet, 1H, –COO*H*), 8.17 (d, J = 8.8 Hz, 1H, –N*H*–), 7.45 (d, J = 8.2 Hz, 2H, Ar*H*), 7.24–7.10 (m, 7H, Ar*H*), 3.84 (ddd, J_1 = 8.8 Hz, J_2 = 8.8 Hz, J_3 = 5.8 Hz, 1H, –C*H*–), 2.92 (dd, $^2J_1$ = 13.7, $^3J_2$ = 5.7 Hz, 1H, –CH$_a$H$_b$–), 2.70 (dd, $^2J_1$ = 13.7, $^3J_2$ = 8.8 Hz, 1H, –CH$_a$H$_b$–), 2.34 (s, 3H, –CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.7, 143.8, 136.6, 135.0, 129.8, 129.7, 128.7, 127.4, 127.2, 56.5, 39.0, 21.7 ppm. Anal. Calcd [$C_{16}H_{17}NO_4S$]: C, 60.17; H, 5.37; N, 4.39; S, 10.04. Found: C, 59.36; H, 5.11; N, 4.83; S, 9.27.

Syntheses of **G(2–4)** and **P(2–4)**: General experimental procedure is as follows: Glycine or L-phenyl alanine derivative (as HCl salt) (10 mmol) and Na_2CO_3 (2.1 g, 20 mmol) were dissolved in anhydrous dichloromethane (50 mL) and cooled in an ice bath before tosyl chloride (1.9 g, 10 mmol) was added. The resultant reaction mixture was stirred overnight at room temperature. After the reaction, more solvent was added and the residue was washed sequentially with 0.1 M HCl, saturated $NaHCO_3$ and brine. Organic portion was dried with Na_2SO_4 and concentrated in vacuo to give crude product. The product was purified by column chromatography on silica with hexane and EtOAc (1:1).

Compound **G2**: Yield 78% as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, J = 8.3 Hz, 2H, Ar*H*), 7.31 (d, J = 8.3 Hz, 2H, Ar*H*), 5.04 (t, J = 5.1 Hz, 1H, –N*H*–), 3.78 (d, J = 5.1 Hz, 2H, –CH_2–), 3.64 (s, 3H, –OCH_3), 2.42 (s, 3H, –CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 144.0, 136.3, 129.9, 127.4, 52.7, 44.2, 21.7 ppm. Anal. Calcd. [$C_{10}H_{13}NO_4S$]: C, 49.37; H, 5.39; N, 5.76; S, 13.18. Found: C, 50.63; H, 5.09; N, 5.36; S, 12.87.

Compound **P2**: Yield 81% as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, J = 8.3 Hz, 2H, Ar*H*), 7.25–7.20 (m, 5H, Ar*H*), 7.08–7.05 (m, 2H, Ar*H*), 5.03 (d, J = 9.1 Hz, 1H, –N*H*–), 4.21 (dt, J_1 = 9.1 Hz, J_2 = 6.0 Hz, 1H, –C*H*–), 3.49 (s, 3H, –OCH_3), 3.03 (d, J = 6.0 Hz, 2H, -CH_2-), 2.40 (s, 3H, –CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 143.8, 136.8, 135.1, 129.7, 129.5, 128.7, 127.4, 127.3, 56.7, 52.5, 39.5, 21,6 ppm. Anal. Calcd [$C_{17}H_{19}NO_4S$]: C, 61.24; H, 5.74; N, 4.20; S, 9.62. Found: C, 60.67; H, 5.39; N, 4.46; S, 9.17.

Compound **G3**: Yield 86% as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, J = 8.3 Hz, 2H, Ar*H*), 7.37–7.23 (m, 7H, Ar*H*), 5.05 (s, 2H, –OCH_2–), 5.02 (broad singlet, 1H, –N*H*–), 3.82 (d, J = 5.5 Hz, 2H, –NCH_2–), 2.42 (s, 3H, –CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.8, 144.0, 136.3, 134.8, 129.9, 129.8, 128.8, 128.4, 127.4, 67.7, 44.3, 21.7 ppm. Anal. Calcd [$C_{16}H_{17}NO_4S$]: C, 60.17; H, 5.37; N, 4.39; S, 10.04 Found: C, 59.61; H, 5.79; N, 4.73; S, 9.34.

Compound **P3**: Yield 82% as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (d, J = 8.3 Hz, 2H, Ar*H*), 7.34–6.98 (m, 12H, Ar*H*), 5.05 (d, J = 9.3 Hz, 1H, –N*H*–), 4.87 (s, 2H, –OCH_2), 4.25 (dt, J_1 = 9.3 Hz, J_2 = 6.0 Hz, 1H, –C*H*–), 3.04–3.02 (dd, $^3J_1$ = 6.0 Hz, $^4J_2$ = 1.7 Hz, 2H, –CH_2–), 2.39 (s, 3H, –CH_3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 143.7, 136.8, 134.9, 134.8, 129.8, 129.6, 128.7, 128.6, 127.4, 127.3, 67.5, 56.7, 39.6, 21.7 ppm. Anal. Calcd [$C_{23}H_{23}NO_4S$]: C, 67.46; H, 5.66; N, 3.42; S, 7.83. Found: C, 66.74; H, 5.19; N, 3.62; S, 7.44.

Compound **G4**: Yield 78% as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, J = 8.3 Hz, 2H, Ar*H*), 7.30 (d, J = 8.3 Hz, 2H, Ar*H*), 5.73 (broad singlet, 1H, –N*H*–), 3.71 (s, 2H, –CH_2–), 3.47–3.45 (m, 2H, aliphatic ring hydrogens), 3.20–3.17 (m, 2H, aliphatic ring hydrogens), 2.41 (s, 3H, –CH_3), 1.64–1.44 (m, 6H, aliphatic ring hydrogens) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.8, 143.7, 136.2, 129.8, 127.4, 45.5, 43.6, 26.2, 25.4, 24.3, 21.7 ppm. Anal. Calcd [$C_{14}H_{20}N_2O_3S$]: C, 56.73; H, 6.80; N, 9.45; S, 10.82. Found: C, 56.21; H, 6.29; N, 9.69; S, 10.36.

Compound **P4**: Yield 80% as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 8.3 Hz, 2H, Ar*H*), 7.26–7.11 (m, 7H, Ar*H*), 5.77 (d, J = 9.4 Hz, 1H, –N*H*–), 4.33 (ddd, J_1 = 9.4 Hz, J_2 = 8.5 Hz, J_3 = 6.1 Hz, 1H, –C*H*–), 3.20–2.58 (m, 6H, aliphatic hydrogens), 2.38 (s, 3H, –CH_3), 1.42–0.76 (m, 6H, aliphatic hydrogens) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5, 143.5, 137.1, 135.8, 129.8, 129.6, 128.6, 127.5, 127.3, 53.4, 46.3, 43.1, 41.2, 25.5, 25.0, 24.1, 21.6 ppm. Anal. Calcd [$C_{21}H_{26}N_2O_3S$]: C, 65.26; H, 6.78; N, 7.25; S, 8.30. Found: C, 65.91; H, 6.23; N, 7.68; S, 7.87.

4.3. Purification of carbonic anhydrase isozymes from human erythrocytes by affinity chromatography

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.[9a] Firstly, benzoyl chloride was stirred for four hours at room temperature in CH_2Cl_2 cellulose. After the spacer arm cellulose added as a benzyl group and finally diazotized sulfanilamide clamped to the para position of benzyl group as ligand. The hemolysate was applied to the prepared Cellulose-benzyl-sulfanilamide affinity column 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 (hCA I and hCA II) isozymes were eluted with 1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6), respectively. All procedures were performed at 4 °C.[11]

4.4. Esterase activity assay

Carbonic anhydrase activity was assayed by following the change in absorbance at 348 nm of 4-nitrophenylacetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25 °C using a spectrophotometer (CHEBIOS UV–VIS) according to the method described by Verpoorte et al.[13a] The enzymatic reaction, in a total volume of 3.0 mL, 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. A reference measurement was obtained by preparing the same cuvette without enzyme solution. The

inhibitory effects of resveratrol, catechin, silymarin, dobutamine and curcumin were examined. All compounds were tested in triplicate at each concentration used. Different inhibitor concentrations were used. hCA-I enzyme activities were measured for **G1–4** and **P1–4** at cuvette concentrations. Control cuvette activity in the absence of inhibitor was taken as 100%. For each inhibitor an Activity (%)-[Inhibitor] graphs were drawn. K_I values were calculated from by using the Cheng–Prusoff equation.[13]

4.5. Protein determination

Protein during the purification steps was determined spectrophotometrically at 595 nm according to the Bradford method, using bovine serum albumin as the standard.[16]

4.6. 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% Coommassie Brilliant Blue R-250 in 50% methanol and 10% acetic acid, then destained with several changes of the same solvent without the dye.[17]

4.7. Molecular docking simulations

Glide docking protocols[18] were applied for the prediction of positions of used compounds into the active sites of CA I and CA II enzymes. Active site of the enzyme was defined from the co-crystallized ligands from PDB files. Crystal structures of hCA I, II and IX are retrieved from Protein Data Bank server, (PDB codes: 2FW4, 5AML and 3IAI, respectively[19–21]). Throughout the docking simulations both partial flexibility and full flexibility around the active site residues are performed by Glide XP.

4.8. Molecular dynamic simulations

In order to investigate and track the behavior of used inhibitors into the active site of the CAII, atomistic molecular dynamic (MD) simulation was utilized. For this aim three systems, including control (apo form), G4-bound and mafenide-bound structures were constructed based on top-docking poses. CAII was selected as a target for all systems and three classical MD simulations were independently performed using Desmond code.[22] All complexes were solvated by TIP3P water model and then naturalized by adding Na^+ and Cl^- ions. The thickness of water layer was set to 10 Å. Before the MD simulations the systems were minimized with a maximum iteration of 2000 steps. Then, the systems were submitted in 1 ns and 10 ns MD simulations for equilibration and production MD runs for each systems. Temperature and pressure were assigned on 300 K and 1.01325 bar, respectively using Isothermal–isobaric (NPT) ensemble. Nose–hoover chain[23] and Martyna–Tobias–Klein[24] were implemented thermostat and barostat methods, respectively. Cut-off radios of 9 Å and long-range method of smooth Particle Mesh Ewald were used for Coulomb interactions.

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