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Carbonic anhydrase inhibitory properties of novel benzylsulfamides using molecular modeling and experimental studies

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ABSTRACT

In this study, a series of sulfamoyl carbamates and sulfamide derivatives were synthesized. Six commercially available benzyl amines and BnOH were reacted with chlorosulfonyl isocyanate (CSI) to give sulfamoyl carbamates. Pd–C catalyzed hydrogenolysis reactions of carbamates afforded sulfamides. The inhibition effects of novel benzylsulfamides on the carbonic anhydrase I, and II isoenzymes (CA I, and CA II) purified from fresh human blood red cells were determined by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography. In vitro studies were shown that all of novel synthesized benzylsulfamide analogs inhibited, concentration dependently, both hCA isoenzyme activities. The novel benzylsulfamide compounds investigated here exhibited nanomolar inhibition constants against the two isoenzymes. K_i values were in the range of 28.48 ± 0.01–837.09 ± 0.19 nM and 112.01 ± 0.01–268.01 ± 0.22 nM for hCAI and hCA II isoenzymes, respectively. Molecular modeling approaches were also applied for studied compounds.

1. Introduction

Sulfamides are beneficial organic compounds in medicinal chemistry [1]. They show a wide biological activity spectrum. For example, an injectable antibiotic doripenem (**1**) [2], anti-hyperprolactinemic agent quinagolide (**2**) [3] and anticonvulsant **3** (JNJ-26990990) [4] are small molecule drugs containing sulfamide moiety. In addition, anti-trypanosomal [5], anticonvulsant [6], smooth muscle relaxant [7], carbonic anhydrase inhibitory [8] activities of sulfamide **4** has been reported (Fig 1).

The sulfonamide group (–SO_2NH–) is present in many organic compounds that are known as potent inhibitors of the carbonic anhydrases (CA) [9,10]. They bind as anions to the Zn^{2+} ion in the enzyme active site with high affinities for CA isozymes [11]. Zn^{2+} is an essential metal and necessary for more than 300 enzymes functions. The discovery of presence of Zn^{2+} as the catalytic center of CA enzymes was found in 1939 [12]. This exploration was followed by Zn^{2+} characterization in carboxypeptidase in 1950 [13,14]. Then, a study related to analysis on coordination spheres around Zn^{2+} in existing protein crystal structures was published [15]. In this analysis, authors defined the spacer rule for native Zn^{2+} containing proteins. Recently, the general principles for the coordination of Zn^{2+} in proteins have been demonstrated in a number of scientific reports [16,17]. In most cases, Zn^{2+} is arranged by a combination of His, Glu or Asp, and Cys residues. More rarely, Zn^{2+} ion interacts with phenolic group of Tyr residue [18], and the carboxamide oxygen of Glu or Asp residues [19].

The carbonic anhydrase (CA, E.C. 4.2.1.1) is a superfamily of metalloenzymes family. CA catalyzes the interconversion of CO_2 and H_2O to bicarbonate (HCO_3^-) and protons (H^+) using a metal hydroxide nucleophilic mechanism [20]. The sixteen human CA isozymes differ in their subcellular localization and distribution, catalytic activity. They are included in regulation of important physiological and pathological processes such as acid-base balance, respiration, CO_2 and ion transport, gluconeogenesis, bone resorption, lipogenesis, ureagenesis, and body fluid generation [21]. So far, this enzyme has been purified from a large scale of tissues, involving human erythrocytes [22].

Many chemical substances and drugs changed activities of enzyme and affect some metabolic processes. Chemicals usually activate or inhibit activities of several enzymes in vivo and effect

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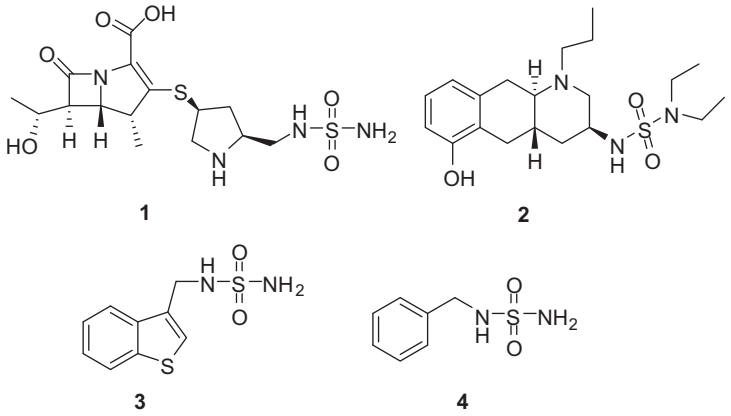


Fig. 1. Sulfamide drugs **1–3**, and biologically active sulfamide **4**.

metabolic pathways [23]. It is well known that some chemicals inhibit CA isoenzymes in different tissues [20,24,25]. So far, inhibition profiles of different chemicals, anions, cations, metal ions, phenolics, sulfamides and sulfonamides have been investigated against many CA isoenzymes. The human CA isoenzymes belong to the α-class. So far, 16 CA isozymes have been found and identified. CA plays an important role in water and ion transport and pH regulation in the kidney, eye, central nervous system, inner ear, and other systems [20–26]. Especially, CA isoenzyme inhibitors are utilized for different purposes particularly for the remedy of glaucoma, epilepsy, diuretics, antitumor agents and diagnostic tools. Hence, finding of novel CA inhibitors targeting various isoenzymes has considerably gained attention nowadays [27]. Recently it was reported that phenols, aryl or alkyl carboxylic acids, sulfonamides, sulfamoylcarbamates and sulfamides may act as CA inhibitors [28].

In our ongoing projects, we have already addressed the synthesis and CA inhibition properties of sulfamides incorporating dopamine [29], indane [20], and tetralin [24] scaffolds. Results concluded from these investigations showed that the synthetic sulfamides are good candidates for CA inhibitors. In the present study, we aimed to synthesize more simple sulfamides, and investigate their CA I, and CA II isoenzymes inhibition properties. In this context, five novel and a known sulfamides were synthesized from methoxylated benzyl amines and they were evaluated for their human hCA I, and hCA II inhibitory properties. In addition, molecular modeling approaches were used to investigate drug–receptor interactions as well as predictions of pharmacokinetic profiles of these compounds.

2. Results and discussion

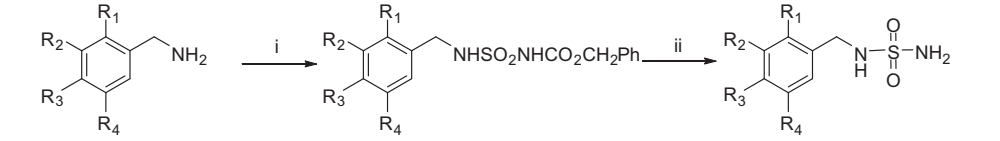
2.1. Chemistry

The synthesis of sulfamide carbamates and sulfamides were started with commercially available benzylamines **5–10**. The reactions of amines in the presence of alcohols with chlorosulfonyl isocyanate (CSI) are giving sulfamoylcarbamates [20,24,29]. By the similar approach, the reactions of benzylamines **5–10** and benzylalcohol with CSI in the presence of Et_3N at 0–25 °C for 4 h yielded novel sulfamoyl carbamates **11–16** in good yields. Pd–C catalyzed hydrogenolysis reaction of carbamates is one of the most convenient procedures for the synthesis of amine related compounds [30–32]. Hence, hydrogenolysis reactions of carbamates with H_2 in the presence of Pd–C catalysis afforded a known sulfamide **18** [33], new benzyl sulfamides **17** and **19–22** with yields 68–77% respectively (Scheme 1). The structures of synthesized compounds **11–22** were characterized by ^{1}H-, ^{13}C-NMR, IR and elemental analysis techniques.

2.2. Biochemistry

Carbonic anhydrase inhibitors (CAIs) are important class of chemical or pharmaceutical agents that suppress and prevent the CA activity. The clinical usage of CAIs has been determined as antiepileptics, antiglaucoma agents, diuretics, in the management of gastric and duodenal ulcers, mountain sickness, osteoporosis, and neurological disorders [9]. Both isoenzymes (hCA I, and hCA II) inhibition profiles were extensively studied. Recently, our groups examined the interaction of both isoenzymes with melatonin [34], morphine [35], caffeic acid phenethyl ester (CAPE) [36], a series of antioxidant phenols [37], a series of phenolic acids [38], a series of natural product polyphenols and phenolic acids [39], some bromophenol derivatives [40], naturally occurring bromophenols and their synthetic derivatives [23,41], dopaminergic compounds [42], a series of sulfamides [20,24,29] and sulfonamides [22,25].

The main goal of this study was to investigate the effect of novel sulfamide carbamates **11–16** and sulfamides **17–22** at hCA I, and hCA II isoenzymes. The inhibition data related to novel sulfamide carbamates **11–16** and sulfamides **17–22** are given in Table 1. As it can be seen from the data presented in Table 1, these novel sulfamide carbamates **11–16** and sulfamides **17–22** showed effective inhibitory activity against both tested isoforms. It is well known that sulfamides have high affinity for all CA isozymes, which leads to a lack of specificity. Two sulfamide NH and NH_2 groups are slightly acidic and provide hydrogen bond donors. Considering the data in Table 1, the following results were obtained. For cytosolic hCA I isoenzyme, novel benzylsulfamides were inhibited with inhibition constants in the low nanomolar level. All of the newly synthesized sulfamide carbamates **11–16** and sulfamides **17–22** (**11–22**), which demonstrated IC_{50} values range of 212.58–577.02 nM. Also, K_i values of these novel benzylamine derivatives are around 28.48 ± 0.01–837.09 ± 0.19 nM (Table 1). Especially, Compound **13**, possessing methoxy group (-OMe) with *para*-position, was the best hCA I inhibitor (K_i: 28.48 ± 0.01 nM). On the other hand, CA II isoenzyme has very important physiological effects in cytosol and increases intraocular pressure, in the anterior uvea of the eye, leading to visual dysfunction. Conversely, the best hCA II inhibitor in benzylsulfamide series was compound **22.** This compound showed the highest inhibition activity on physiologically dominant CA II with K_i values of 112.01 ± 0.01 nM. Many studies performed on sulfamides revealed that inhibition of CA II is brought about by their ability to mimic the tetrahedral transition state when binding to catalytic Zn^{2+} settled at the CA active site [43]. K_i values of novel sulfamide carbamates **11–16** and sulfamides **17–22** were much more effective when compared to clinically used drug aceta-



5 R_1=OMe, R_2=R_3=R_4=H
6 R_2=OMe, R_1=R_3=R_4=H
7 R_3=OMe, R_1=R_3=R_4=H
8 R_1=R_3=OMe, R_2=R_4 =H
9 R_2=R_4=OMe, R_1=R_3=H
10 R_2=R_3=R_4=OMe, R_1=H

11 R_1=OMe, R_2=R_3=R_4=H 77%
12 R_2=OMe, R_1=R_3=R_4=H 83%
13 R_3=OMe, R_1=R_3=R_4=H 81%
14 R_1=R_3=OMe, R_2=R_4 =H 78%
15 R_2=R_4=OMe, R_1=R_3=H 81%
16 R_2=R_3=R_4=OMe, R_1=H 83%

17 R_1=OMe, R_2=R_3=R_4=H 76%
18 R_2=OMe, R_1=R_3=R_4=H 72%
19 R_3=OMe, R_1=R_3=R_4=H 73%
20 R_1=R_3=OMe, R_2=R_4 =H 77%
21 R_2=R_4=OMe, R_1=R_3=H 75%
22 R_2=R_3=R_4=OMe, R_1=H 68%

Scheme 1. Synthesis of sulfamides (i) CSI/NEt_3/BnOH, CH_2Cl_2 0–25 °C, 4 h; (ii) H_2/Pd–C, MeOH, 25 °C, 4 h.

Table 1
Human carbonic anhydrase isoenzymes (hCA I and hCA II) inhibition data for some novel benzylsulfamides **11–22**. Glide/XP and IFD docking scores (in kcal/mol) have been also shown.

Compounds	IC_{50} (nM)		K_I (nM)		Glide/XP		IFD	
	CA I	CA II	CA I	CA II	CA I	CA II	CA I	CA II
11	212.58	167.68	361.63 ± 0.15	197.17 ± 0.02	−5.53	−3.99	−8.68	−7.42
12	373.79	170.14	169.62 ± 0.05	268.01 ± 0.22	−5.41	−4.07	−9.44	−8.24
13	332.37	167.03	28.48 ± 0.01	130.79 ± 0.06	−5.83	−4.93	−8.66	−8.59
14	272.19	156.65	837.09 ± 0.19	237.05 ± 0.02	−5.58	−5.29	−8.62	−7.53
15	301.44	186.79	203.4 ± 0.02	117.35 ± 0.02	−5.37	−5.44	−8.32	−8.09
16	254.03	160.19	285.46 ± 0.13	198.15 ± 0.07	−5.73	−4.00	−8.34	−7.46
17	577.02	199.08	196.71 ± 0.07	224.48 ± 0.07	−4.66	−4.04	−7.32	−6.26
18	371.98	220.77	458.87 ± 0.06	237.04 ± 0.09	−4.68	−4.04	−7.27	−8.25
19	383.93	334.14	214.57 ± 0.05	276.45 ± 0.07	−3.65	−5.08	−6.24	−5.50
20	495.35	225.73	398.67 ± 0.10	167.96 ± 0.04	−4.50	−3.24	−6.49	−6.00
21	282.05	220.07	253.81 ± 0.04	187.95 ± 0.02	−5.20	−3.46	−6.55	−5.75
22	312.58	186.09	335.29 ± 0.13	112.01 ± 0.01	−3.55	−4.64	−6.08	−5.40
AZA	315.52	123.53	184.34 ± 0.28	61.12 ± 0.018	–	–	−3.10*	−4.10*

* From Ref. [40].

zolamide [20] (K_i values are 184.34 ± 0.28 nM and 61.12 ± 0.018 nM for hCA I and hCAII, respectively).

2.3. In silico studies

In order to enrich the experimental studies and predict the binding energies between ligands and protein, an *in silico* calculation was performed [44–48]. Rigid and flexible receptor docking simulations were carried out on twelve ligands against CA I and CA II isoenzymes. Glide/XP and Induced Fit Docking (IFD) docking scores are shown at Table 2. Predicted docking scores using IFD method are so reasonable and suggest a high correlation with experimental results. 3D and 2D ligand interaction diagrams have been shown at Figs. 2 and 3, respectively. Fig. 4 shows the pKa values, which were predicted for each compound.

In addition, pharmacokinetic profiles of compounds are investigated, theoretically. These properties includes molecular weight, octanol/water partition coefficient, apparent Caco-2 cell permeability in nm/s, hERG channels blocking activities, percentage of human oral absorption and Lipinski's rule of five (Table 2).

3. Conclusions

In summary starting from commercially available benzyl amines **5–10**, novel sulfamide carbamates **11–16** and sulfamides **17–22** were synthesized. The synthesized compounds were then evaluated for their hCA I, and hCA II isoenzymes inhibitory properties. Novel sulfamide carbamates **11–16** and sulfamides **17–22** were found to be sufficiently active. The most active inhibitors in the both series was found as sulfamide carbamate **11** which had K_i value of 28.48 ± 0.01 nM for hCA I, and sulfamide **22** with K_i value of 112.01 ± 0.01 nM for hCA II.

4. Experimental

All chemicals and solvents are commercially available and they were used without purification or after distillation and treatment with drying agents. Melting points are uncorrected and they were determined on a capillary melting apparatus (BUCHI 530). IR spectra were obtained from solutions in 0.1 mm cells with a Perkin-Elmer spectrophotometer. The ^1H and ^{13}C-NMR spectra were recorded on a 400 (100)-MHz Varian and 400 (100)-MHz Bruker spectrometer; δ in ppm, Me_4Si as the internal standard. Elemental analyses were performed on a Leco CHNS-932 apparatus. All column chromatography was performed on silica gel (60-mesh, Merck). PLC is preparative thick-layer chromatography: 1 mm of silica gel 60 PF (Merck) on glass plates.

4.1. Synthesis

Compounds **5–10** were purchased from commercially available sources (Sigma–Aldrich, Acros Organics).

4.1.1. General procedure for the synthesis of sulfamoylcarbamates

Benzylalcohol (1.2 mmol) was added to a solution of CSI (1.2 mmol) in CH_2Cl_2 dropwise at 0 °C. A solution of amine (1 mmol) and NEt_3 (1.4 mmol) in CH_2Cl_2 was added to the solution of CSI-Benzylalcohol. The mixture was stirred at 0 °C for 1 h. then at 25 °C for 3 h. The reaction mixture was cooled to 0 °C and it was poured into a solution of 0.1 N HCl (100 mL). Organic phase was separated. After H_2O phase was extracted with CH_2Cl_2 (2 × 30 mL), combined organic layers were dried over Na_2SO_4. The solvent was evaporated and column chromatography of the residue on silica gel (30 g) with EtOAc-hexane (1:4) afforded desired product.

Table 2
ADME properties of synthesized ligands.

Compound	MW[a]	QplogP o/w[b]	QPPCaco[c]	QploghERG[d]	Percent human oral absorption[e]	Rule of five[f]
11	350.389	4.013	363.314	−5.626	96.268	✓
12	350.389	4.217	317.694	−6.610	96.418	✓
13	350.389	4.223	320.593	−6.637	96.525	✓
14	380.415	4.380	336.075	−6.598	100.000	✓
15	380.415	4.298	320.218	−6.480	96.956	✓
16	410.441	4.443	319.250	−6.477	100.000	✓
17	216.254	0.897	300.616	−4.423	76.549	✓
18	216.254	0.830	294.196	−4.123	75.987	✓
19	216.254	0.844	289.033	−4.221	75.931	✓
20	246.281	1.002	387.247	−3.979	79.134	✓
21	246.281	0.940	294.162	−4.047	76.631	✓
22	276.307	1.022	289.720	−4.117	76.994	✓

[a] Molecular weight (reasonable value <500).
[b] Predicted octanol/water partition coefficient (reasonable value from −2.0 to 6.5).
[c] Predicted apparent Caco-2 cell permeability in nm/s (reasonable value >25).
[d] Predicted value of hERG channels blocking activities (reasonable value <−7).
[e] Percentage of human oral absorption (<25% is weak and >80% is strong).
[f] Lipinski's rule of five.

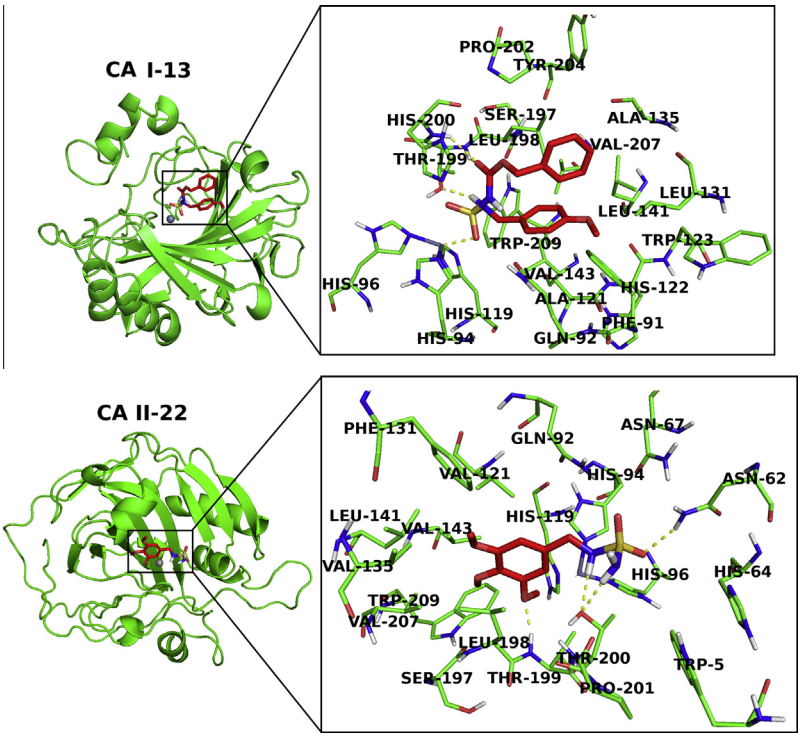


Fig. 2. Top docking poses of compounds **13** and **22** at CA-I and CA-II enzymes, respectively.

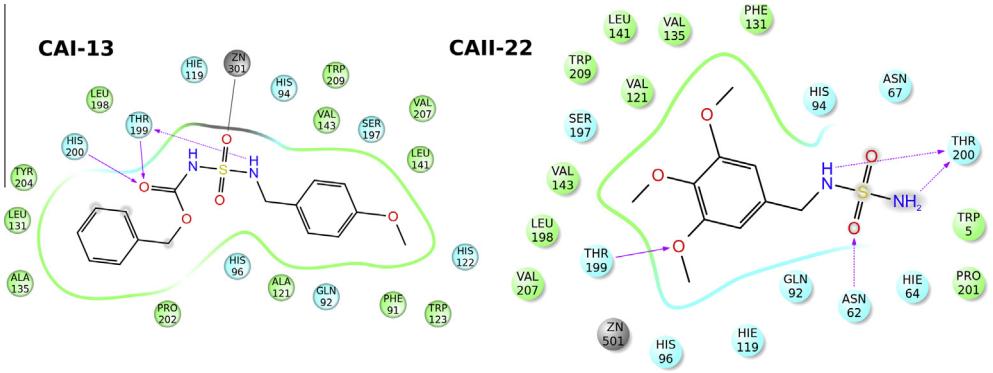


Fig. 3. 2D ligand interaction diagrams of compounds **13** and **22** at CA-I and CA-II enzymes, respectively.

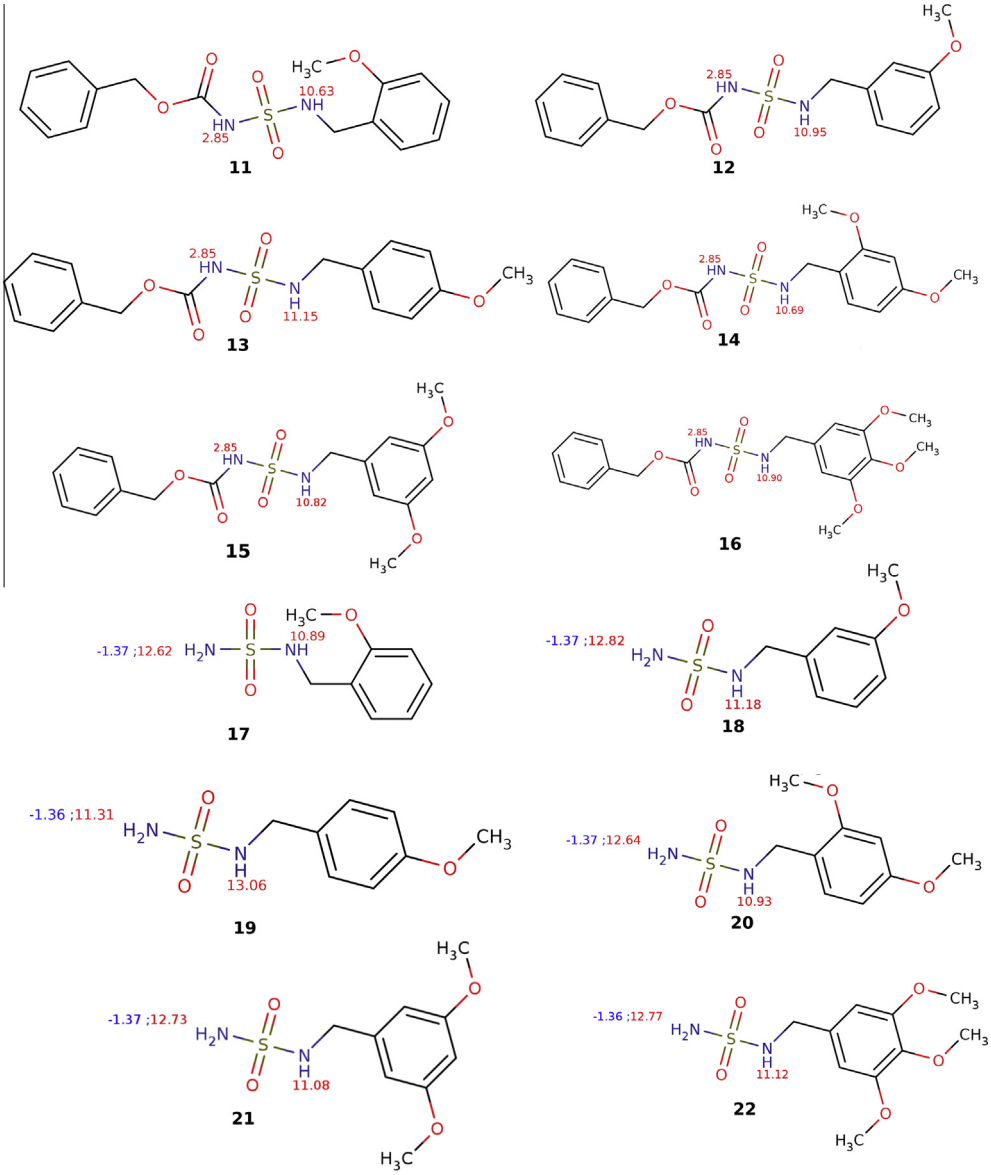


Fig. 4. Predicted pKa values for selected compounds (**11–22**).

*4.1.2. Benzyl N-(2-methoxybenzyl)sulfamoylcarbamate (**11**)*

General procedure described at 4.1.1 was applied to amine **5** to give **11.** White solid, yield 77%. Mp: 134–136 °C. ^{1}H-NMR (400 MHz, Acetone-d6) δ 9.90 (bs, 1H, NH), 7.41–7.33 (m, 6H, Ph–H and Ar–H), 7.29 (td, 1H, Ar–H, *J* = 8.1 and 1.7 Hz), 6.98 (d, 1H, Ar–H, *J* = 8.1 Hz), 6.90 (td, 1H, Ar–H, *J* = 7.5 and *J* = 1.0 Hz), 6.71 (t, 1H, NH, *J* = 6.2 Hz), 5.00 (s, 2H, OCH_2), 4.27 (d, 2H, CH_2N, *J* = 6.2 Hz), 3.85 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, Acetone-d6): 157.7 (CO), 151.7 (C), 136.1 (C of Ph), 129.6 (CH), 129.3 (CH), 128.7 (2CH of Ph), 128.4 (CH of Ph), 128.3 (2CH of Ph), 124.9 (C), 120.5 (CH), 110.6 (CH), 67.3 (OCH_2), 55.1 (OCH_3), 43.4 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3280, 3194, 2943, 2839, 1712, 1604, 1589, 1495, 1475, 1456, 1440, 1426, 1377, 1353, 1293, 1249, 1164, 1156, 1116,1051, 1040, 1030. Anal. Calcd for ($C_{16}H_{18}N_2O_5S$): C, 54.85; H, 5.18; N, 7.99; S, 9.15; Found C, 54.81; H, 5.20; N, 8.01; S, 9.13.

4.1.3. Benzyl N-(3-methoxybenzyl)sulfamoylcarbamate (12)

General procedure described at 4.1.1 was applied to amine **6** to give **12.** White solid, yield 83%. Mp: 106–108 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.26 (bs, 1H, NH), 8.37 (t, 1H, NH, *J* = 6.2 Hz), 7.39–7.32 (m, 5H, Ph–H), 7.20 (t, 1H, Ar–H, *J* = 7.9 Hz), 6.88–6.84 (m, 2H, Ar–H), 6.80 (dd, 1H, Ar–H, *J* = 2.3 and *J* = 8.2 Hz), 5.03 (s, 2H, OCH_2), 4.08 (d, 2H, CH_2N, *J* = 6.2 Hz), 3.71 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): 159.8 (CO), 152.1 (C), 140.0 (C), 136.4 (C of Ph), 129.9 (CH), 129.1 (2CH of Ph), 128.7 (2CH of Ph), 120.3 (CH), 113.7 (CH), 113.3 (CH), 67.3 (OCH_2), 55.6 (OCH_3), 46.9 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3473, 3272, 3064, 2936, 1729, 1598, 1546, 1490, 1465, 1454, 1346, 1263, 1232, 1153, 1093, 1049. Anal. Calcd for ($C_{16}H_{18}N_2O_5S$): C, 54.85; H, 5.18; N, 7.99; S, 9.15; Found C, 54.88; H, 5.21; N, 7.95; S, 9.12.

*4.1.4. Benzyl N-(4-methoxybenzyl)sulfamoylcarbamate (**13**)*

General procedure described at 4.1.1 was applied to amine **7** to give **13.** White solid, yield 81%. Mp: 141–142 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38–7.31 (m, 6H, Ph–H and NH), 7.18 (d, 2H, Ar–H, *J* = 8.7 Hz), 6.83 (d, 2H, Ar–H, *J* = 8.7 Hz), 5.42 (t, 1H, NH, *J* = 6.1 Hz), 5.10 (s, 2H, OCH_2), 4.16 (d, 2H, CH_2N, *J* = 6.1 Hz), 3.77 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): 159.6 (CO), 151.1 (C), 134.5 (C of Ph), 129.6 (2CH), 128.9 (CH of Ph), 128.8 (2CH of Ph), 128.5 (2CH of Ph), 127.3 (C), 114.2 (2CH), 68.6 (OCH_2), 55.3

(OCH_3), 47.6 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3419, 3332, 3273, 3197, 3034, 2934, 1689, 1608, 1445, 1403, 1344, 1151, 1121, 1084, 1071. Anal. Calcd for ($C_{16}H_{18}N_2O_5S$): C, 54.85; H, 5.18; N, 7.99; S, 9.15; Found C, 54.81; H, 5.20; N, 7.97; S, 9.17.

*4.1.5. Benzyl N-(2,4-dimethoxybenzyl)sulfamoylcarbamate (**14**)*

General procedure described at 4.1.1 was applied to amine **8** to give **14.** White solid, yield 78%. Mp: 96–98 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.21 (bs, 1H, NH), 7.87 (t, 1H, NH, *J* = 6.2 Hz), 7.37–7.32 (m, 5H, Ph–H), 7.15 (d, 1H, Ar–H, *J* = 8.4 Hz), 6.50 (d, 1H, Ar–H, *J* = 2.3 Hz), 6.35 (dd, 1H, Ar–H, *J* = 2.3 and 8.4 Hz), 5.04 (s, 2H, OCH_2), 4.01 (d, 2H, CH_2N, *J* = 6.2 Hz), 3.73 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): 160.6 (C), 158.2 (CO), 152.2 (C), 136.5 (C of Ph), 129.8 (CH), 129.1 (2CH of Ph), 128.9 (CH of Ph), 128.7 (2CH of Ph), 118.0 (C), 104.9 (CH), 98.7 (CH), 67.3 (OCH_2), 56.1 (OCH_3), 55.9 (OCH_3), 41.6 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3478, 3287, 3019, 2935, 2851, 2350, 1735, 1614, 1590, 1549, 1508, 1453, 1347, 1290, 1265, 1210, 1158, 1132, 1036. Anal. Calcd for ($C_{17}H_{20}N_2O_6S$): C, 53.67; H, 5.30; N, 7.36; S, 8.43; Found C, 53.70; H, 5.32; N, 7.35; S, 8.40.

*4.1.6. Benzyl N-(3,5-dimethoxybenzyl)sulfamoylcarbamate (**15**)*

General procedure described at 4.1.1 was applied to amine **9** to give **15.** White solid, yield 81%. Mp: 125–127 °C. ^{1}H-NMR (400 MHz, Acetone-d6) δ 9.93 (bs, 1H, NH), 7.43–7.32 (m, 5H, Ph–H), 7.17 (t, 1H, NH, *J* = 6.3 Hz), 6.58 (d, 2H, Ar–H, *J* = 2.3 Hz), 6.39 (t, 1H, Ar–H, *J* = 2.3 Hz), 5.08 (s, 2H, OCH_2), 4.23 (d, 2H, CH_2N, *J* = 6.3 Hz), 3.76 (s, 6H, $2OCH_3$). ^{13}C-NMR (100 MHz, Acetone-d6): 161.2 (2C), 151.7 (CO), 139.8 (C), 136.1 (C of Ph), 128.7 (2CH of Ph), 128.4 (CH of Ph), 128.3 (2CH of Ph), 106.0 (2CH), 99.5 (CH), 67.4 (OCH_2), 54.9 ($2OCH_3$), 47.5 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3277, 3002, 2940, 2841, 1725, 1598, 1458, 1432, 1355, 1297, 1225, 1205, 1157, 1064. Anal. Calcd for ($C_{17}H_{20}N_2O_6S$): C, 53.67; H, 5.30; N, 7.36; S, 8.43; Found C, 53.69; H, 5.34; N, 7.33; S, 8.41.

*4.1.7. Benzyl N-(3,4,5-trimethoxybenzyl)sulfamoylcarbamate (**16**)*

General procedure described at 4.1.1 was applied to amine **10** to give **16.** White solid, yield 83%. Mp: 131–133 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.54 (bs, 1H, NH), 7.36–7.30 (m, 5H, Ph–H), 6.52 (s, 2H, Ar–H), 5.57 (t, 1H, NH, *J* = 6.3 Hz), 5.11 (s, 2H, OCH_2), 4.17 (d, 2H, CH_2N, *J* = 6.3 Hz), 3.83 (s, 6H, $2OCH_3$), 3.82 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$): 153.7 (2C), 151.4 (CO), 134.6 (C), 131.2 (C of Ph), 129.1 (CH of Ph), 129.0 (2CH of Ph), 128.8 (C), 128.7 (2CH of Ph), 105.1 (2CH), 68.9 (OCH_2), 61.1 (OCH_3), 56.4 ($2OCH_3$), 48.4 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3257, 2939, 2834, 1737, 1594, 1546, 1508, 1463, 1420, 1346, 1328, 1235, 1156, 1127, 1003. Anal. Calcd for ($C_{18}H_{22}N_2O_7S$): C, 52.67; H, 5.40; N, 6.83; S, 7.81; Found C, 52.64; H, 5.42; N, 6.84; S, 7.83.

4.1.8. General procedure for the synthesis of sulfamides (Hydrogenolysis of sulfamylcarbamates)

Sulfamoylcarbamate (2.63 mmol) in MeOH (50 mL) and Pd–C (50 mg) were placed into a 100-mL flask. A balloon filled with H_2 gas (3 L) was fitted to the flask. Flushing with H_2 provided deoxygenation. The mixture was hydrogenated at rt for 4 h. Removal of the catalyst by filtration and crystallization of the residue with EtOAc-hexane furnished sulfamides.

*4.1.9. N-(2-methoxybenzyl)sulfamide (**17**)*

General Procedure described at 4.1.8 was applied to sulfamoylcarbamate **11** to give **17**. White solid, yield 76%. Mp: 59–61 °C. ^{1}H-NMR (400 MHz, Acetone-d6) δ 7.40–7.37 (m, 1H, Ar–H), 7.26 (td, 1H, Ar–H, *J* = 8.1 and 1.7 Hz), 6.96 (d, 1H, Ar–H, *J* = 8.1 Hz), 6.91 (td, 1H, Ar–H, *J* = 7.5 and 1.1 Hz), 5.91 (bs, 2H, NH_2), 5.85 (t, 1H, NH, *J* = 6.2 Hz), 4.25 (d, 2H, CH_2N, *J* = 6.2 Hz), 3.84 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, Acetone-d6): 157.5 (C), 129.1 (CH), 128.8 (CH), 126.3 (C), 120.5 (CH), 110.5 (CH), 55.1 (OCH_3), 42.6 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3512, 3355, 3283, 2942, 2839, 1603, 1495, 1464, 1440, 1330, 1291, 1247, 1158, 1120, 1050, 1027. Anal. Calcd for ($C_8H_{12}N_2O_3S$): C, 44.43; H, 5.59; N, 12.95; S, 14.83; Found C, 44.45; H, 5.61; N, 12.98; S, 14.80.

*4.1.10. N-(3-methoxybenzyl)sulfamide (**18**)*

General Procedure described at 4.1.8 was applied to sulfamoylcarbamate **12** to give **18**. White solid, yield 72%. Mp: 47–49 °C (lit. [33] Mp: 45–46 °C) ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.20 (t, 1H, Ar–H, *J* = 8.1 Hz), 7.01 (t, 1H, NH, *J* = 6.6 Hz), 6.91–6.88 (m, 2H, Ar–H), 6.78 (dd, 1H, Ar–H, *J* = 8.1 and 2.2 Hz), 6.60 (bs, 2H, NH_2), 4.02 (d, 2H, CH_2N, *J* = 6.6 Hz), 3.72 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): 158.9 (C), 141.0 (C), 129.9 (CH), 120.5 (CH), 113.8 (CH), 113.1 (CH), 55.7 (OCH_3), 46.7 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3274, 2941, 2838, 1602, 1587, 1491, 1456, 1437, 1326, 1264, 1156, 1045. Anal. Calcd for ($C_8H_{12}N_2O_3S$): C, 44.43; H, 5.59; N, 12.95; S, 14.83; Found C, 44.40; H, 5.57; N, 12.93; S, 14.85.

*4.1.11. N-(4-methoxybenzyl)sulfamide (**19**)*

General Procedure described at 4.1.8 was applied to sulfamoylcarbamate **13** to give **19**. White solid, yield 73%. Mp: 112–114 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.23 (d, 2H, Ar–H, *J* = 8.8 Hz), 6.91 (t, 1H, NH, *J* = 6.2 Hz), 6.86 (d, 2H, Ar–H, *J* = 8.8 Hz), 6.56 (bs, 1H, NH_2), 3.97 (d, 2H, CH_2N, *J* = 6.2 Hz), 3.71 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): 159.0 (C), 131.2 (C), 129.7 (2CH), 114.2 (2CH), 55.7 (OCH_3), 46.3 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3327, 3272, 3114, 1513, 1432, 1403, 1334, 1253, 1151, 1030. Anal. Calcd for ($C_8H_{12}N_2O_3S$): C, 44.43; H, 5.59; N, 12.95; S, 14.83; Found C, 44.46; H, 5.60; N, 12.93; S, 14.81.

*4.1.12. N-(2,4-dimethoxybenzyl)sulfamide (**20**)*

General Procedure described at 4.1.8 was applied to sulfamoylcarbamate **14** to give **20**. White solid, yield 77%. Mp: 88–90 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.22 (d, 1H, Ar–H, *J* = 8.4 Hz), 6.62 (t, 1H, NH, *J* = 6.5 Hz), 6.53 (bs, 1H, NH_2), 6.50 (d, 1H, Ar–H, *J* = 2.3 Hz), 6.47 (dd, 1H, Ar–H, *J* = 8.4 and 2.3 Hz), 3.96 (d, 2H, CH_2N, *J* = 6.5 Hz), 3.75 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): 160.3 (C), 158.1 (C), 129.7 (CH), 119.0 (C), 104.9 (CH), 98.7 (CH), 56.0 (OCH_3), 55.9 (OCH_3), 41.3 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3273, 3109, 3002, 2939, 2834, 1614, 1590, 1508, 1459, 1420, 1328, 1290, 1208, 1157, 1131, 1034. Anal. Calcd for ($C_9H_{14}N_2O_4S$): C, 43.89; H, 5.73; N, 11.37; S, 13.02; Found C, 43.91; H, 5.75; N, 11.36; S, 13.00.

*4.1.13. N-(3,5-dimethoxybenzyl)sulfamide (**21**)*

General Procedure described at 4.1.8 was applied to sulfamoylcarbamate **15** to give **21**. White solid, yield 75%. Mp: 108–110 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.99 (t, 1H, NH, *J* = 6.6 Hz), 6.59 (bs, 1H, NH_2), 6.50 (d, 2H, Ar–H, *J* = 2.2 Hz), 6.34 (t, 1H, Ar–H, *J* = 2.2 Hz), 3.98 (d, 2H, CH_2N, *J* = 6.6 Hz), 3.70 (s, 6H, $2OCH_3$). ^{13}C-NMR (100 MHz, DMSO-d_6): 161.0 (2C), 141.7 (C), 106.2 (2CH), 99.4 (CH), 55.8 ($2OCH_3$), 46.8 (CH_2N)). IR (CH_2Cl_2, cm^{-1}): 3350, 3327, 3266, 3002, 2935, 2834, 1737, 1598, 1464, 1429, 1404, 1356, 1317, 1206, 1130, 1049. Anal. Calcd for ($C_9H_{14}N_2O_4S$): C, 43.89; H, 5.73; N, 11.37; S, 13.02; Found C, 43.90; H, 5.74; N, 11.35; S, 13.01.

*4.1.14. N-(3,4,5-trimethoxybenzyl)sulfamide (**22**)*

General Procedure described at 4.1.8 was applied to sulfamoylcarbamate **16** to give **22**. White solid, yield 68%. Mp: 149–151 °C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 6.99 (t, 1H, NH, *J* = 6.5 Hz), 6.66 (s, 2H, Ar–H), 6.63 (bs, 2H, NH_2), 4.01 (d, 2H, CH_2N, *J* = 6.5 Hz), 3.77 (s, 6H, $2OCH_3$), 3.63 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6): 153.3 (2C), 136.9 (C), 134.8 (C), 105.5 (2CH), 60.7 (OCH_3), 56.4 ($2OCH_3$),

46.9 (CH_2N). IR (CH_2Cl_2, cm^{-1}): 3245, 3098, 3019, 2924, 2831, 1734, 1630, 1595, 1546, 1507, 1462, 1422, 1342, 1264, 1216, 1149, 1125. Anal. Calcd for ($C_{10}H_{16}N_2O_5S$): C, 43.47; H, 5.84; N, 10.14; S, 11.60; Found C, 43.45; H, 5.5.82; N, 10.13; S, 11.62.

4.2. Biochemistry

4.2.1. CA isoenzymes purification

CA I, and CA II isoenzymes were purified from fresh human blood erythrocytes by Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography [49]. In this purification method, CA isoenzymes were purified via a simple one-step described previously [34,49].

4.2.2. Esterase activity assay

Carbonic anhydrase activity was determined according to the method of Verpoorte et al. [50]. In this spectrophotometer detection, the change in absorbance at 348 nM of 4-nitrophenylacetate (NPA) to 4-nitrophenylate ion over a period of 3 min at 25 °C was recorded. This CA activity determination was described in detail by our previous studies [37–40].

4.2.3. Protein determination

Quantity of protein was determined spectrophotometrically at 595 nm during the purification steps according to the Bradford method [51] as explained previously [52]. Bovine serum albumin was used as standard [52].

4.2.4. SDS–polyacrylamide gel electrophoresis

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out after purification procedure of the CA isoenzymes. It was performed in acrylamide (10% and 3%) for the running and the stacking gel, containing SDS (0.1%) [47] using a Minigel system (Mini-PROTEAN Tetra System). This protein visualization method clearly described in previous studies [53]. Briefly, Gel was fixed, stained with Coomassie Brilliant Blues R-250, than destained using standard methods to detect protein bands belonging to purified both CA isoenzymes [54].

4.2.5. Inhibition assays

The effect of novel benzylsulfamides was examined using by the hydratase activity and recorded in triplicate analysis at the each used concentration [25]. For this purpose, different concentrations of novel benzylsulfamides were determined in preliminary assays. CA isoenzyme activities were measured in the presence of different quantity of novel benzylsulfamides. The control sample activity in the absence of a novel benzylsulfamides was taken as 100%. For each novel benzylsulfamides, an Activity (%)-[Benzylsulfamides] was drawn using conventional polynominal regression software (Microsoft Office 2000, Excel). The half maximal inhibitory concentration (IC_{50}) of each novel benzylsulfamides was calculated from graphs [55]. IC_{50} values are measure of the effectiveness of novel benzylsulfamides in inhibiting both CA isoenzymes. For determination of K_i values, three different inhibitor concentrations were used. K_i values reflect the binding affinity of the inhibitor to both CA isoenzymes. In this way, The IC_{50} value is converted to an absolute inhibition constant K_i value. In these experiments, PNA was used as substrate at five different concentrations. Then, Lineweaver–Burk curves were drawn [56].

5. In silico studies

5.1. Input structures preparation

Crystal structures of CA I, and II are retrieved from Protein Data Bank server, (PDB: 2NMX and 3M04, respectively) [57]. All water molecules are removed form crystal structures. Zn^{2+} ion was conserved in the both systems. All missing hydrogen atoms are added by employing Protein Preparation Wizard of Maestro [58] program. Amino acids are ionized at the physiological pH (pH = 7.4) using PROPKA program [59]. Finally both repaired proteins are relaxed utilizing of restrained minimization with a cutoff of 0.3 Å.

5.2. Ligands preparation

All docked ligand are prepared in marvinsketch [60] program as follows: (i) Drawing of 2D chemical structures; (ii) Converting 2D chemical structures to 3D structures, (iii) applying the molecular mechanics (MM) optimization, (iv) checking protonation states at the pH 7.4.

5.3. Docking protocol

All rigid receptor-docking simulations were preformed using Glide/XP [61] program of Schroedinger molecular modeling package. The co-crystallized ligand in the active sites of proteins is used as centered for docking box in gridding step. No constraints are used for docking simulations.

5.4. Induced fit docking

In addition, flexible receptor docking is applied utilizing IFD [62] method in order to predict the binding energies of protein/ligand complexes with more sophisticated approach compared to rigid docking. This protocol is carried out in three steps; (i) all ligands are docked against the rigid proteins using Glide/SP method, (ii) all amino acids within 5 Å of the ligand are refined using OPLS-2001 [63] force field, (iii) finally all prior docked ligands were re-docked into refined proteins using Glide/XP method.

5.5. ADME studies

The absorption, distribution, metabolism and excretion (ADME) of compounds are examined employing QikProp program [64].

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2014.07.009.

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