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Letter

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Sulfonamides with Potent Inhibitory Action and Selectivity against the α-Carbonic Anhydrase from *Vibrio cholerae*

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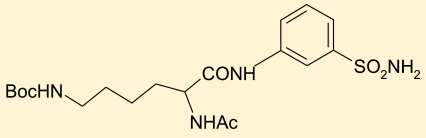
Supporting Information

ABSTRACT: By using *N*-α-acetyl-L-lysine or GABA scaffolds and the conversion of the terminal amino group to the guanidine one, benzenesulfonamides incorporating water solubilizing moieties were synthesized. The new compounds were medium potency inhibitors of the cytosolic carbonic anhydrase (CA, EC 4.2.1.1) isoforms I and II, and highly effective, nanomolar inhibitors of the pathogenic bacterial α-CA from *Vibrio cholerae*. These sulfonamides possess good selectivity for inhibiting the bacterial over the mammalian isoforms and may be used as tools to understand the role of bacterial CAs in pathogenesis.



K_I (VchCA) = 8.8 nM, SI (hCA I/II) > 550

KEYWORDS: *Carbonic anhydrase, enzyme inhibitor, sulfonamide, amino acid, Vibrio cholerae*

Carbonic anhydrases (CAs, EC 4.2.1.1) catalyze a simple but physiologically relevant reaction in all life kingdoms, carbon dioxide hydration to bicarbonate and protons.[1−6] These enzymes are involved in many physiologic processes in organisms all over the phylogenetic tree, including bacteria or other microorganisms.[1,2] In humans, 15 CA isoforms belonging to the α-CA family are known, which constitute drug targets for diuretics, antiglaucoma agents, antiepileptics, antiobesity, and antitumor drugs.[1−5] There are five genetic families encoding CAs in organisms all over the phylogenetic tree, the α-, β-, γ-, δ-, and ζ-CAs, and all of them are metalloenzymes.[1−6]

With the cloning of genomes of many pathogenic organisms, the possibility to explore alternative pathways for inhibiting virulence factors or proteins essential for the life cycle of such pathogens emerged, among which the CAs.[7−12] Bacteria encode CAs belonging to three such families, the α-, β-, and γ-CAs. Some of them were shown to be druggable targets in the last years in several pathogenic bacteria such as *Helicobacter pylori, Brucella suis, Mycobacterium tuberculosis, Salmonella enterica, Streptococcus pneumoniae, Porphyromonas gingivalis, Clostridium perfringens, Legionella pneumophila*, etc., as in many of them, the CAs are essential for their life cycle.[9−12]

Recently we have cloned an α-CA from the human pathogen *Vibrio cholerae*, denominated VchCA.[12] This enzyme showed a significant catalytic activity for the CO_2 hydration reaction and was inhibited by sulfonamides and sulfamates, the most investigated CA inhibitors (CAIs).[1,2,12] As bicarbonate is a virulence factor of this bacterium and ethoxzolamide, a potent CAI was reported to inhibit the *in vivo* virulence of *V. cholerae*,[13] we proposed VchCA as a new target for antibiotic development. In this letter we continue our interest in sulfonamide CAIs as potential antibacterial agents and report the synthesis of benzenesulfonamides incorporating amino acid scaffolds and guanidine moieties. These new compounds were investigated for the inhibition of the physiologically dominant, cytosolic isoforms CA I and II, as well as the bacterial enzyme VchCA.

Results and Discussion. *Chemistry.* Although sulfonamides are highly effective CAIs,[1−3] the main drawbacks of this class of pharmacological agents is the fact that they possess a low water solubility. This is the reason why we considered the attachment of amino acyl moieties as tails to the scaffolds of aromatic or heterocyclic sulfonamides, as a possibility to increase the hydrosolubility.[17] We employed *N*-α-acetyl-L-lysine or GABA scaffolds, as well as the conversion of their terminal amino group to the guanidine one, for synthesizing compounds incorporating 4-aminoethyl/methyl-benzenesulfonamide as well as metanilamide/sulfanilamide as heads for binding to the zinc ion from the enzyme (Schemes 1 and 2).

Starting from *N*-α-acetyl-L-lysine **1**, by protecting the ε-amino group with the Boc moiety and coupling with benzenesulfonamides **2** or **3**, the ε-Boc-α-acetyl-protected-lysyl sulfonamides **4** and **5** were prepared (Scheme 1). After removal of the Boc protecting group in 3 M HCL,

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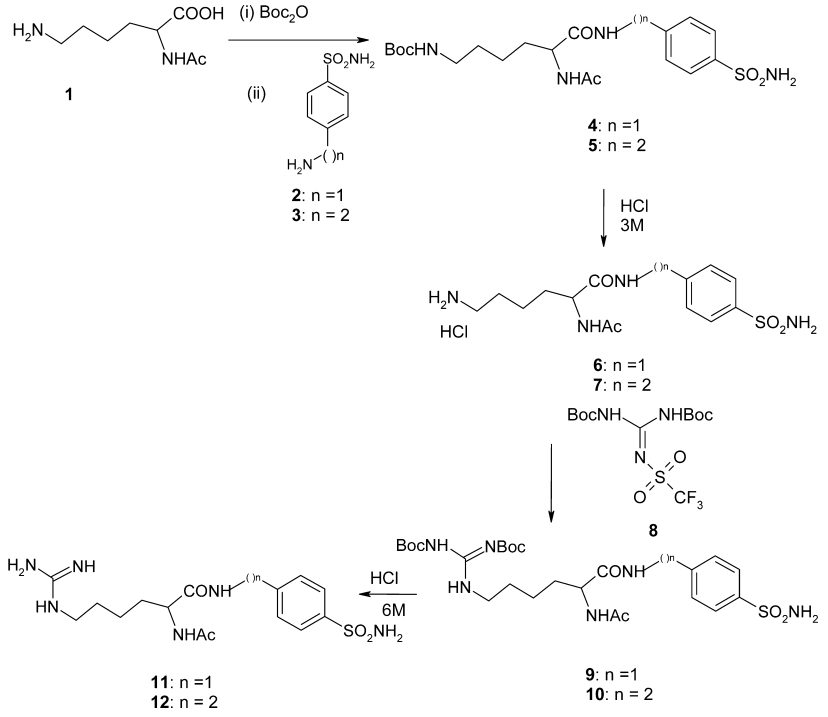
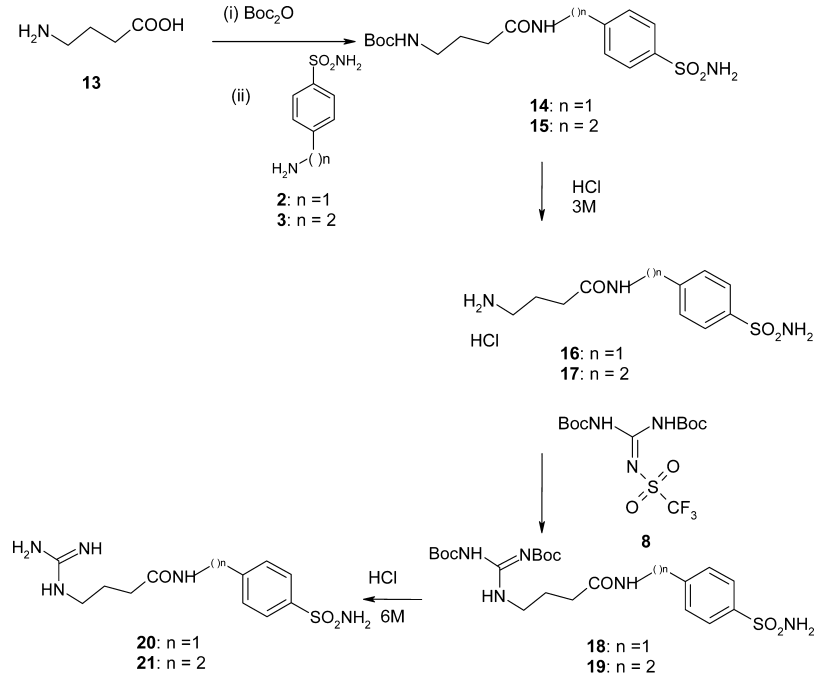
Scheme 1. Synthesis of *N*-α-Acetyl-L-lysine Sulfonamides 4−12



Scheme 2. Synthesis of GABA-Containing Sulfonamides 14−21



hydrochlorides **6** and **7** were treated with *N*,*N*′-di-Boc-*N*″-trifluoromethane-sulfonylguanidine **8** for converting the terminal amino moiety to the di-Boc-protected guanidines **9** and **10**. Deprotection of the guanidine moiety in the presence of acid led to the gunaidini-substituted sulfonamides **11** and **12** (Scheme 1).

By a similar approach, starting from GABA **13** and following similar derivatization reactions, compounds **14**−**21** incorporating guanidine-GABA moieties were obtained (Scheme 2). Furthermore, several metanilamide (**22**, **23**), sulfanilamide (**24**), and 4-carboxy-benzenesulfonamide (**25**) derivatives incorporating lysyl- or GABA moieties were also obtained by the same synthetic procedure (see structures below).

CA Inhibition. Inhibition data with the set of compounds **4**−**25** reported here, against the human (h) CA isoforms hCA I and II and the bacterial enzyme VchCA, are shown in Table 1. The two human isoforms were included in the assay as they may be the main off-targets.[1−3] The following structure−activity relationship (SAR) was observed:

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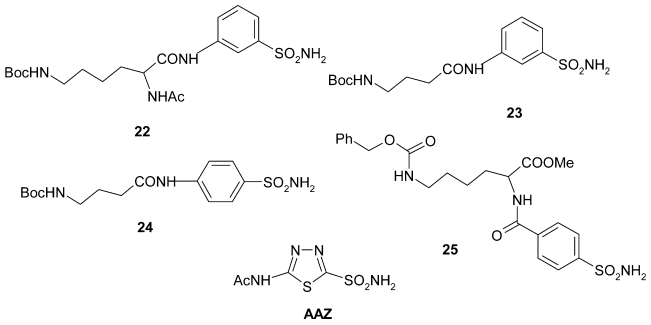

Table 1. Inhibition Data against Human (h) Isoforms hCA I and II (Cytosolic) and Bacterial Enzyme VchCA of Sulfonamides 4−25 and Acetazolamide AAZ (as Standard Inhibitor) by a Stopped-Flow CO_2 Hydrase Assay[14]

	K_I (nM)[a]			selectivity ratios	
compd	hCA I	hCA II	VchCA	VchCA/hCA I	VchCA/hCA II
4	484	71.2	8.9	54.3	8.0
5	430	75.9	76.7	5.6	0.98
6	2330	563	29.1	80.0	19.3
7	626	473	66.5	9.4	7.1
9	2310	384	9.1	253.8	42.2
10	519	452	9.3	55.8	48.6
11	3150	824	8.8	358.0	93.6
12	289	44.0	36.8	7.9	1.20
14	19.8	9.8	6.1	3.2	1.6
15	253	91.9	304.0	0.8	0.30
16	2270	83.5	65.3	33.7	1.3
17	517	197	77.0	6.7	2.5
18	193	83.1	5.8	33.3	14.3
19	267	71.3	95.2	2.8	0.75
20	972	470	6.4	151.9	73.4
21	2120	847	29.1	72.8	29.1
22	6030	5100	8.8	685.2	579.5
23	22.1	5.9	7.6	276.2	73.7
24	151.3	29.4	6.9	21.9	4.26
25	88.9	4.4	6.2	14.34	0.71
AAZ	250	12	6.8	36.7	1.76

[a]Errors in the range of ±10% of the reported value (from 3 different assays).

(i) Against the cytosolic slow isoform hCA I, the new sulfonamides were ineffective or medium potency inhibitors, with inhibition constants ranging between 88.9−6030 nM. Two sulfonamides, **14** and **23**, showed effective inhibitory activity, with K_Is of 19.8−22.1 nM. Both of them incorporate Boc-GABA moieties. For all substitution patterns, 4-aminoethyl-benzenesulfonamides were more effective inhibitors compared to the corresponding 4-aminomethylbenzenesulfonamides.

(ii) The physiologically dominant human isoform hCA II was effectively inhibited by **14** and **22−25**, with K_Is in the range of 4.4−29.4 nM, similar to acetazolamide **AAZ** (K_Is of 12 nM). These compounds incorporate the Boc-GABA moieties, except **25**, which is a derivative of 4-carboxybenzenesulfonamide. Some of the remaining derivatives, such as **4**, **5**, **12**, **15**, **16**, **18**, and **19**, were medium potency hCA II inhibitors (K_Is in the range of 44.0−91.9 nM). The other compounds were ineffective hCA II inhibitors, with K_Is of 384−5100 nM (Table 1).

(iii) The bacterial enzyme VchCA was highly sensitive to inhibition by these sulfonamides, with K_Is in the range of 6.1−77.0 nM. Several low nanomolar VchCA inhibitors were discovered, such as **4**, **9−11**, **14**, **18**, **20**, and **22−25**, with K_Is of 6.1−9.3 nM. The best activity was correlated with the presence of derivatized GABA moieties and 4-amino-methylbenzenesulfonamide headgroup, in most compounds, except **10**, which is a 4-amimoethyl-benzenesulfonamide, and **25**, which has a different scaffold. The guanidine-GABA functionality seems to be a beneficial substitution patterns for obtaining effective VchCA inhibitors. Generally the 4-amino-methyl-benzenesulfonamides were better inhibitors than the corresponding 4-aminoethylbenzenesulfonamides possessing the same substitution pattern. The GABA derivatives were better VchCA inhibitors compared to the *N*-α-acetyl-L-lysines possessing similar sulfonamide and protecting group scaffolds. The least effective VchCA inhibitors were **5−7**, **15−17**, and **21**, which showed K_Is in the range of 29.1−304.0 nM, being medium potency inhibitors.

(iv) Many effective VchCA inhibitors also possessed excellent selectivity ratios for inhibiting the bacterial over the human enzymes. Selectivity ratios of 685.2 against hCA I and 579.5 against hCA II were observed for compound **22**. Other sulfonamides with good selectivity ratios for inhibiting the bacterial over the human enzymes were **9−11**, **14**, and **22−24**. **AAZ** has low selectivity ratios for inhibiting VchCA against hCA I/II, although it acts as an efficient VchCA inhibitor.

Conclusions. New benzenesulfonamides were synthesized by using the tail approach, incorporating amino acid scaffolds, which were further derivatized for achieving increased water solubility, by introducing guanidine groups. Most of them were medium potency or ineffective as inhibitors of hCA I and II, but significantly inhibited the bacterial enzyme VchCA, with efficacy in the low nanomolar range, and selectivity for inhibiting the bacterial over the human enzymes. Some of them may constitute interesting tools for better understanding the physiologic/pathologic roles of α-CAs in the life cycle of bacteria.

MATERIALS AND METHODS

Chemistry. Anhydrous solvents and all reagents were purchased from Aldrich, Merck, or Carlo Erba. All reactions involving air- or moisture-sensitive compounds were performed under nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Details regading the equipment for compound characterization are given in the Supporting Information.

Synthesis of 2-Acetylamino-6-tert-butoxycarbonylamino-hexanoic Acid. A solution of *N*-α-acetyl-lysine **1** (0.3 g, 1.0 equiv) in a 1,4-dioxane/water (50:50) mixture (17.6 mL) was adjusted to pH 11 with 1.0 M NaOH solution and cooled down to −10 °C in a salt ice bath. Di-*tert*-butyl dicarbonate (0.35 g, 1.0 equiv) was then added and the pH maintained at 11 if necessary. The reaction mixture was stirred for 4 h at 0 °C in an ice bath. At the end of the reaction time the solution was acidified to pH 1 using a 3.0 M HCl solution. The solution was then extracted with ethyl acetate. The organic layer was then dried over anhydrous sodium sulfate, filtered, and the solvent removed under reduced pressure to give 0.15 g of pure product as a white solid: 32.5% yield. δ_H (400 MHz, DMSO-d_6): 8.10 (d, 1H, N*H*, J 8), 6.81 (appt, 1H, N*H*, J 6), 4.15 (m, 1H, C*H*), 2.91 (q, 2H, CH_2, J 8), 1.87 (s, 3H, CH_3), 1.74−1.68 (m, 2H, CH_2), 1.57−1.51 (m, 2H, CH_2), 1.41 (s, 9H, $3CH_3$), 1.35−1.25 (m, 2H, CH_2). δ_C (100 MHz, DMSO-d_6): 174.71 (COOH), 170.16 (C=O), 156.9 (C=O), 78.24 (*C*-Boc),

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52.73 (αCH), 31.69 (CH_2), 30.04 (CH_2), 29.18 (CH_3–Boc), 23.72 (CH_3), 23.25 (CH_2).

Synthesis of [5-Acetylamino-5-(4-sulfamoyl-benzylcarbamoyl)-pentyl]-carbamic Acid tert-Butyl Ester ***4***. 2-Acetamido-6-*tert*-butoxycarbonylamino-hexanoic acid (0.11 g, 1.0 equiv) and 4-aminomethylbenzenesulfonamide hydrochloride **2** (0.085g, 1.0 equiv) were added to a round flask with 20 mL of acetonitrile and stirred for few minutes. 1-Hydroxy-7-azabenzotriazole (HOAt) (0.052 g, 1.0 equiv) was then added to the reaction mixture followed by *N*,*N*-diisopropylethylamine (DIPEA) (0.1 g, 2.0 equiv). The solution was cooled to 0 °C in an ice bath for 10 min. *N*-(3-(dimethylamino)-propyl)-*N*′-ethyl-carbodiimide hydrochloride (EDCI, 0.08 g, 1.05 equiv) was then added to the solution turning it yellow. It was then stirred overnight at room temperature. The solvent was removed under vacuum leaving a yellow oil. This oil was dissolved in water and the solution acidified to pH 1 using a 3.0 M HCl solution. The product was then extracted with ethyl acetate (∼30 mL). The organic layer was dried under anhydrous Na_2SO_4 then filtered. The solvent was removed under vacuum to leave 0.08 g of pure product as a white solid.

Compound **4**: 47.6% yield. δ_H (400 MHz, DMSO-d_6): 8.54 (t,1 H, N*H*, J 6), 8.04 (d, 1H, N*H*, J 8), 7.79 (d, 2H, C*H*, J 8), 7.60 (d, 2H, C*H*, J 8), 7.33 (s, 2H, NH_2), 6.80 (appt, 1H, N*H*), 4.36 (t, 2H, CH_2, J 5.8), 4.2 (m, 1H, C*H*), 2.91 (td, 2H, CH_2, J 6.4), 1.89 (s, 3H, CH_3), 1.68–1.63 (m, 2H, CH_2), 1.59–1.50 (m, 2H, CH_2), 1.41 (s, 9H, $3CH_3$), 1.35–1.25 (m, 2H, CH_2). δ_C (100 MHz, DMSO-d_6): 173.17 (C═O), 170.40 (C═O), 156.65 (C═O), 144.71 (C-Ar), 143.61 (C-Ar), 128.40 (CH–Ar), 126.67 (CH–Ar), 78.41(C-Boc), 53.80 (αCH), 42.71 (CH_2), 39.38 (CH_2), 32.65 (CH_2), 30.31 (CH_2), 29.34 (CH_3–Boc), 23.92 (CH_3), 23.60 (CH_2). *m*/*z* (ESI negative), 455.3 $[M - H]^-$.

Compound **5**: 52% yield. δ_H (400 MHz, DMSO-d_6): 8.03 (t,1 H, N*H*, J 5.2), 7.95 (d, 1H, N*H*, J 8), 7.76 (d, 2H, C*H*, J 8), 7.42 (d, 2H, C*H*, J 8), 7.32 (s, 2H, NH_2), 6.83 (appt, 1H, N*H*), 4.16 (m, 1H, C*H*), 3.32 (m, 2H, CH_2), 2.91 (td, 2H, CH_2, J 6.4), 2.81 (m, 2H, CH_2), 1.87 (s, 3H, CH_3), 1.57 (m, 2H, CH_2), 1.40 (s, 9H, $3CH_3$), 1.23 (m, 4H, $2CH_2$). δ_C (100 MHz, DMSO-d_6): 172.70 (C═O), 170.03 (C═O), 156.48 (C═O), 144.57 (C-Ar), 142.96 (C-Ar), 130.07 (CH–Ar), 126.54 (CH–Ar), 78.26 (C-Boc), 53.49 (αCH), 40.55 (CH_2), 39.39 (CH_2), 35.69 (CH_2), 32.70 (CH_2), 30.20 (CH_2), 29.19 (CH_3–Boc), 23.68 (CH_3), 23.43 (CH_2). *m*/*z* (ESI negative), 469.9 $[M - H]^-$.

Synthesis of 5-Acetylamino-5-(4-sulfamoyl-benzylcarbamoyl)-pentyl-ammonium Chloride ***6***. [5-Acetylamino-5-(4-sulfamoyl-benzylcarbamoyl)-pentyl]-carbamic acid *tert*-butyl ester **4** (0.1 g) was suspended in 3.0 M HCl solution (2 mL). and the reaction mixture was stirred overnight at room temperature. The aqueous solution was dried under reduced pressure to give a pale yellow oil. The oil was washed with 2 × 10 mL dichloromethane and triturated with diethyl ether to afford 0.09 g of the desired product as a yellow waxy solid.

Compound **6**: quantitative yield. δ_H (400 MHz, DMSO-d_6): 8.65 (t,1 H, N*H*, J 6, exchange D_2O), 8.18 (d, 1H, N*H*, J 8, exchange D_2O), 8.04 (brs, 3H, NH_3^+, exchange D_2O), 7.79 (d, 2H, C*H*, J 8), 7.44 (d, 2H, C*H*, J 8), 7.37 (s, 2H, NH_2, exchange D_2O), 4.36 (appt, 2H, CH_2), 4.25 (m, 1H, C*H*), 2.77 (appq, 2H, CH_2), 1.91 (s, 3H, CH_3), 1.73–1.67 (m, 2H, CH_2), 1.63–1.55 (m, 2H, CH_2), 1.45–1.25 (m, 2H, CH_2). δ_C (100 MHz, DMSO-d_6): 172.99 (C═O), 170.48 (C═O), 144.61 (C-Ar), 143.48 (C-Ar), 128.31 (CH–Ar), 126.56 (CH–Ar), 53.63 (αCH), 42.61 (CH_2), 39.38 (CH_2), 32.14 (CH_2), 27.47 (CH_2), 23.46 (CH_2), 22.13(CH_3). *m*/*z* (ESI positive), 357.4 $[M - Cl]^+$.

Synthesis of N-Acetylamino-N′*-di(tert-butyl-oxycarbonyl)-guanidino-hexanoic Acid [2-(4-Sulfamoyl-phenyl)-methyl]-amide* ***9***. *N*,*N*′-di-Boc-*N*″-trifluoromethanesulfonylguanidine **8** (0.1 g, 1.0 equiv) was added to a solution of 4-oxo-5-acetylamino-5-(4-sulfamoyl-benzylcarbamoyl)-pentyl-ammonium chloride **6** (0.11 g, 1.1 equiv) and triethylamine (0.028 g, 1.1 equiv) in chloroform, and the mixture was stirred at room temperature until *N*,*N*′-di-Boc-*N*″-trifluoromethanesulfonylguanidine was consumed as evidenced by TLC (10% methanol/dichloromethane). After the reaction was complete, the mixture was diluted with chloroform and washed with 2.0 M sodium bisulfate, saturated sodium bicarbonate, and brine. The organic extract was then dried over sodium sulfate and the solvent removed under reduced pressure to give 0.09 g of pure product as a white solid.

Compound **9**: 58% yield. δ_H (400 MHz, DMSO-d_6): 11.54 (s, 1 H, N*H*, exchange D_2O), 8.56 (t,1 H, N*H*, J 4, exchange D_2O), 8.32 (t,1 H, N*H*, J 4, exchange D_2O), 8.08 (d, 1H, N*H*, J 8, exchange D_2O), 7.79 (d, 2H, C*H*, J 8), 7.44 (d, 2H, C*H*, J 8), 7.33 (s, 2H, NH_2, exchange D_2O), 4.42 (m, 2H, CH_2), 4.24 (m, 1H, C*H*), 3.28 (appq, 2H, CH_2), 1.89 (s, 3H, CH_3), 1.76 (m, 2H, CH_2), 1.51 (s, 9H, $3CH_3$), 1.43(s, 9H, $3CH_3$), 1.21 (m, 4H, $2CH_2$). δ_C (100 MHz, DMSO-d_6): 173.0 (C═O), 170.29 (C═O),), 164.04 (C═N), 156.12 (C═O), 153.09 (C═O), 144.52 (C-Ar), 143.45 (C-Ar), 128.26 (CH–Ar), 126.52(CH–Ar), 83.82 (C-Boc), 79.05(C-Boc), 53.50 (αCH), 42.58 (CH_2), 40.79 (CH_2), 32.41 (CH_2), 28.93 (CH_3–Boc), 28.85 (CH_2), 28.54 (CH_3–Boc), 23.81 (CH_2), 23.42 (CH_3). *m*/*z* (ESI positive), 599.17 $[M - H]^+$.

Synthesis of N-Acetylamino-N′*-guanidino-hexanoic Acid [2-(4-Sulfamoyl-phenyl)-methyl]-amide Hydrochloride* ***11***. A suspension of *N*-acetylamino-*N*′-di-Boc-guanidino-hexanoic acid [2-(4-sulfamoyl-phenyl)-methyl]-amide **9** (0.09 g, 1.0 equiv) and 6.0 M HCl (10 mL) was stirred overnight at room temperature. The solvent was removed under reduced pressure to give a brownish oil. The oil was washed with dichloromethane and triturated with diethyl ether to give 0.04 g of the pure product as a light brown gel.

Compound **11**: 60% yield. δ_H (400 MHz, DMSO-d_6): 8.63 (appt,1 H, N*H*, exchange D_2O), 8.14 (d, 1H, N*H*, J 8, exchange D_2O), 7.79 (d, 2H, C*H*, J 8), 7.75 (appt, 1 H, N*H*, exchange D_2O), 7.44 (d, 2H, C*H*, J 8), 7.35 (s, 2H, NH_2, exchange D_2O), 7.1 (bs, 3 H, NH_3^+, exchange D_2O), 4.36 (m, 2H, CH_2), 4.28 (m, 1H, C*H*), 3.11 (btd, 2H, CH_2), 1.91 (s, 3H, CH_3), 1.77 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 1.26 (m, 4H, $2CH_2$). δ_C (100 MHz, DMSO-d_6): 172.93 (C═O), 170.34 (C═O), 157.8 (C═N), 144.52 (C-Ar), 143.46 (C-Ar), 128.26 (CH–Ar), 126.52(CH–Ar), 53.52 (αCH), 46.40 (CH_2), 42.56 (CH_2), 41.45 (CH_2), 32.30 (CH_2), 29.96 (CH_2), 23.50 (CH_3). *m*/*z* (ESI positive), 399.133 $[M - Cl]^+$.

The synthesis and characterization of the other compounds are shown in the Supporting Information.

CA Inhibition Studies. A stopped-flow assay method has been used to measure the inhibition of the CA catalyzed CO_2 hydration activity,[14] as reported earlier.[15,16]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full characterization of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare the following competing financial interest(s): C.T.S. is an author on many patents claiming carbonic anhydrase inhibitors.

■ ABBREVIATIONS USED

AAZ, acetazolamide; CA, carbonic anhydrase; EDCI, *N*-(3-(dimethylamino)propyl)-*N*′-ethyl-carbodiimide hydrochloride; GABA, γ-aminobutyric acid; HOAt, 1-hydroxy-7-azabenzotriazole; NMR, nuclear magnetic resonance; SAR, structure–activity relationship; TLC, thin layer chromatography; VcCA, *Vibrio cholerae* CA

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