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Synthesis and structure–activity relationships of novel benzene sulfonamides with potent binding affinity for bovine carbonic anhydrase II

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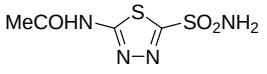
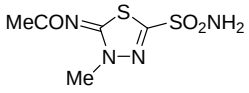
Abstract—This manuscript reports the identification of a novel series of mono- and bis- benzene sulfonamides with potent binding affinity for bovine carbonic anhydrase II (bCAII). These compounds exhibited nanomolar equilibrium dissociation constants with K_i's ranging from 4.7 to 9.3 nM. All compounds were ester derivatives of the weak affinity bCAII inhibitor, 4-carboxybenzenesulfonamide. Structure–activity relationships for this novel series of compounds are discussed.

The carbonic anhydrase (CA) family of Zn(II) metalloenzymes (EC 4.2.1.1) catalyzes the reversible hydration of CO_2 to HCO_3^-. This regulatory reaction underpins many physiological processes associated with pH control, ion transport, and fluid secretion.[1,2] Classically, an aromatic or heteroaromatic sulfonamide moiety ($ArSO_2NH_2$) was the primary recognition element necessary for small molecules to bind the active site of CA.[1,2] Coordination of the nitrogen atom of the ionized sulfonamide anion ($ArSO_2NH^-$) to the active site Zn(II) of CA facilitates this protein–small molecule interaction.[1,2] The inhibition of CAs has been exploited clinically for several decades for the treatment of a variety of conditions including glaucoma, epilepsy, and gastric ulcers.[1,2] More recently, CA inhibition has been implicated as playing an important role in cancer tumor progression.[3,4] Clinically used CA inhibitors include acetazolamide (AAZ), methazolamide (MZA), ethoxazolamide (EZA), dichlorophenamide (DCP), brinzolamide (BRZ) and dorzolamide (DZA). Indisulam (IND) is in Phase II clinical trials as an anticancer agent to treat solid tumors.[5]



AAZ



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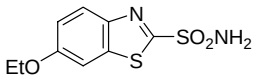

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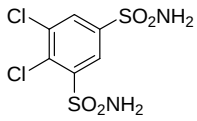

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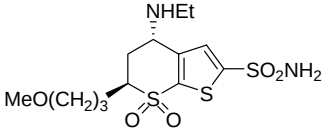
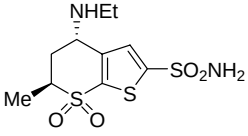
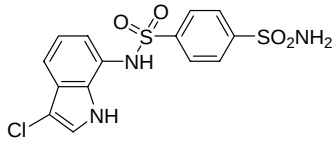


BRZ



DZA



IND

In 1958, Beasley and colleagues reported that 4-carboxybenzenesulfonamide (**1**), containing the well-known primary recognition motif for CA enzyme binding, was minimally more active (4-fold) than the then lead compound sulfanilamide (**2**) for the inhibition of CA

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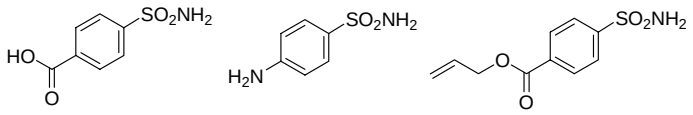
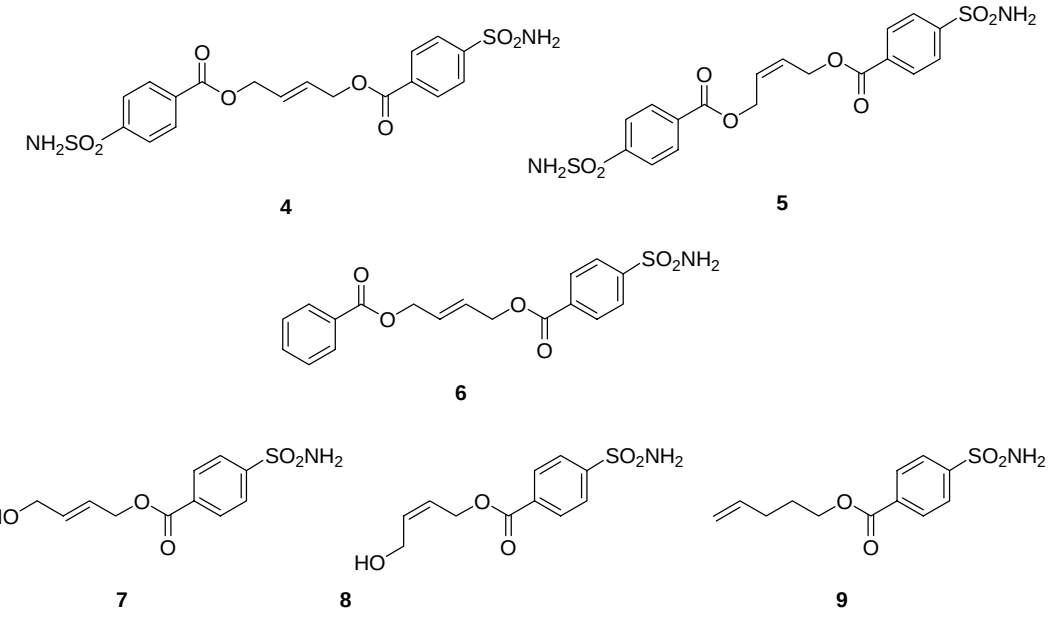


in vitro.[6] A remarkable increase in activity was, however, observed for simple aliphatic esters of **1**. In this study compound **3**, the allyl ester of **1**, possessed 900-fold greater in vitro activity than that of sulfanilamide **2**, while the propyl ester analogue of **1** was 500-fold greater in activity. It is now extensively documented that significant enhancement of CA activity (especially for the most abundant CA isoform CAII) can be achieved through coupling the primary recognition aromatic sulfonamide motif with secondary binding elements, the so-called 'tail' approach.[1,7,8] Even so, since this early study of Beasley no further literature analysis of the CA activity of **3** or CA structure–activity study based on **3** has been reported.

The dual functionality of **3**, possessing both an aromatic sulfonamide and a terminal alkene group, made it an ideal candidate building block for our ongoing investigation into the development of the cross metathesis reaction for dynamic combinatorial chemistry, with bCAII as the protein target.[9] The benzene sulfonamide motif gives reliable bCAII affinity, while the alkene permits the evaluation of cross metathesis chemistry to generate libraries for screening against bCAII.

The symmetrical *trans* bis sulfonamide **4** and *cis* bis-sulfonamide **5** are the two possible homodimer reaction products from cross metathesis of **3**. These homodimers will form the background bCAII binding affinity response in libraries generated from cross metathesis of **3** with other alkene containing building blocks. An accurate determination of the bCAII binding affinity of authentic **4** and **5** was therefore of interest. Early reports of CAII activity of unrelated symmetrical bis-sulfonamides have shown improved CAII activity compared to the corresponding mono-sulfonamide analogues.[6,10] As the bis-sulfonamides of this study were symmetrical, the effect of the second sulfonamide moiety could be determined by synthesis and evaluation of compound **6**, which lacks the second sulfonamide group of **4**. Compounds **7** and **8** were prepared to delineate the effect on bCAII affinity of a small, polar hydroxyl group (in place of the bulky, largely hydrophobic substituents of **4**–**6**) on the allyl functionality. The pentenyl analogue **9** is a two-methylene chain extension of **3**. Compound **9** was prepared to investigate the effect on bCAII affinity of increasing chain length between the aromatic sulfonamide motif and the terminal alkene moiety. This manuscript discusses synthesis and preliminary structure–activity relationships for this family of novel ester-linked benzene sulfonamides.

The synthesis of the lead compound **3** was carried out by the acid catalyzed esterification of **1** with allyl alcohol.[11] Compound **3** afforded crystalline material suitable for X-ray crystallography analysis.[12–14] An ORTEP-3[15] representation of **3** is shown in Figure 1. The molecular structure is essentially planar with the exception of the peripheral NH_2 and CH_2 groups (O4-C8-C9-C10 = −140.4(6)°, N1-S1-C1-C2 = 104.6(2)°). The molecules are linked through strong intermolecular N-H···O hydrogen bonds between the amide and sulfonyl oxygen O1 and the carbonyl oxygen O3, respectively. The synthesis of **4** and **5** proceeded by the 1,3-dicyclohexylcarbodiimide (DCC) mediated esterification of **1** with 0.5 equiv of *trans*-2-butene-1,4-diol and *cis*-2-butene-1,4-diol, respectively.[11] Reaction of **1** in the presence of an excess of each of these diols generated the mono-



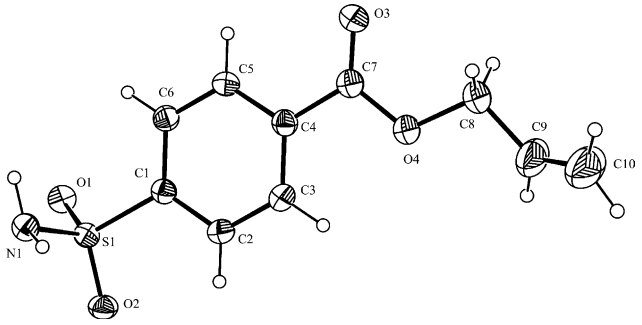
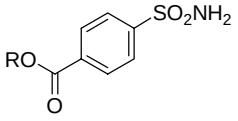


Figure 1. ORTEP-3 plot for **3**.[15]

Table 1. bCAII enzyme binding assay results expressed as K_i in nM



Compound	R	BCAII K_i[a] (R^2)
1	H	151 (0.97)
3	$CH_2CH{=}CH_2$	8.6 (0.97)
4	*trans*-$CH_2CH{=}CHCH_2OCOPhSO_2NH_2$	4.9 (0.97)
5	*cis*-$CH_2CH{=}CHCH_2OCOPhSO_2NH_2$	9.3 (0.98)
6	*trans*-$CH_2CH{=}CHCH_2OCOPh$	4.7 (0.96)
7	*trans*-$CH_2CH{=}CHCH_2OH$	5.3 (0.97)
8	*cis*-$CH_2CH{=}CHCH_2OH$	9.1 (0.95)
9	$CH_2CH_2CH_2CH{=}CH_2$	7.7 (0.96)

[a] bCAII binding data utilizing competitive displacement of DNSA from bCAII, experiments performed in triplicate. K_d of DNSA was 0.3 μM.[16]

esters **7** and **8**, respectively. Esterification of *trans*-2-butene-1,4-diol with 1 equiv of benzoyl chloride followed by 1 equiv of **1** produced **6** in two steps. Compound **9** was synthesized by the DCC mediated esterification of **1** with 1-pentene-5-ol. All compounds were extensively characterized.[11]

Parent compound **1**, lead compound **3**, and the families of novel compounds **4**–**9** were each assayed for bCAII enzyme binding by a fluorescent competitive binding assay, results of which are presented in Table 1.[16] The fluorescence-based assay relies on the competition for the active site of bCAII between the ligand 5-(dimethylamino)-1-naphthalenesulfonamide (DNSA) and the test compounds.[1,7,17] Upon excitation at 290 nm (an absorption minimum for DNSA) fluorescence is detected at 460 nm (from the bCAII–DNSA complex). The equilibrium dissociation constant (K_d) of DNSA was measured as 0.3 μM.[16]

The parent compound **1** was a relatively weak inhibitor of bCAII, with an equilibrium dissociation constant (K_i) of 151 nM. The simple derivatization of the carboxylic acid of **1** to generate esters **4**–**9** yielded compounds with high affinity for bCAII. The allyl ester **3** had a K_i of 8.6 nM, 17-fold > **1**. The bis-sulfonamide *trans* isomer **4** had higher bCAII affinity again (K_i = 4.9 nM, 31-fold > **1**, 1.8-fold > **3**), while the *cis* isomer **5** (K_i = 9.3 nM) had similar affinity to **3**. Compound **6**, which lacks the second sulfonamide moiety of **4**, had identical bCAII affinity to **4** (K_i = 4.7 nM). This result demonstrates that the second sulfonamide moiety is clearly not important for bCAII affinity. Compounds **7** and **8**, with a hydroxyl group in place of the second aromatic sulfonamide moiety of **4** and **5**, exhibited minimal change in affinity when compared to **4** and **5**, respectively (K_i of **7** = 5.3 nM, K_i of **8** = 9.1 nM). The *trans* isomers **4** and **7** were each ~2-fold higher in affinity than their *cis* counterparts **5** and **8**, as well as the unsubstituted **3**. Collectively compounds **4**, **5**, **7**, and **8** demonstrate that (i) the enzyme is very tolerant to functionality on the allyl substituent of **3** (size, polarity, and hydrophobicity) and (ii) the enzyme exhibits a preference for *trans* stereochemistry about the substituted alkene. The pentenyl ester analogue **9**, with two additional methylenes between the ester linkage and terminal alkene when compared to **3**, had a K_i of 7.7 nM, similar to that for **3**. This result also confirms that bCAII exhibits tolerance for the steric nature of the secondary binding elements.

This study has demonstrated that simple allyl ester derivatives of 4-carboxybenzenesulfonamide have produced compounds with high affinity for bCAII. A terminal substituent on the allyl alkene bond revealed both a tolerance from bCAII for the properties of this functionality (steric, hydrophobic, and polar) and also a subtle steric preference for *trans* stereochemistry over *cis* stereochemistry.

Acknowledgments

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11. **Preparation of allyl-4-(aminosulfonyl)benzoate (3)**. To a solution of **1** (2.0 g, 9.94 mmol) in allyl alcohol (80 mL) at 0 °C was added H_2SO_4 (4 drops). The reaction was

stirred at 70 °C for 48 h, then cooled to room temperature and neutralized by addition of solid $NaHCO_3$. The crude reaction mixture was concentrated and the residue was dissolved in DCM (25 mL). The organic phase was washed with H_2O (2×25 mL), dried over $MgSO_4$, filtered, and the solvent removed. The crude material was recrystallized from MeOH to yield compound **3** (690 mg, 29% yield). ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 8.15–8.11 (m, 2H, ArH), 7.95–7.92 (m, 2H, ArH), 7.54 (br s, 2H, NH_2), 6.07–5.98 (m, 1H, =CH), 5.33 (ddt, 2H, J = 16.6, 10.4 Hz, =CH_2), 4.81 (dt, 2H, J = 5.2, 1.6 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 165.1 (CO), 148.8 (=CH), 133.0, 132.9, 130.6, 126.8 (ArCH), 118.9 (=CH_2), 66.3 (CH_2); ESI MS: m/z $[M-H]^-$ 240.3; HRMS (ESI). Calculated for m/z $[M-H]^-C_{10}H_{10}N_1O_4S^-$: 240.0336. Found: 240.0341; Anal. Calcd for $C_{10}H_{11}N_1O_4S$: C, 49.78; H, 4.60; N, 5.81. Found: C, 49.82; H, 4.66; N, 5.67; mp: 106 °C (in agreement with literature[6]).

Preparation of (2*E*)-but-2-en-1,4-diyl-bis[(aminosulfonyl)benzoate] (4) as a representative of substituted allyl ester-benzene sulfonamides 4, 5, 7–9. To a mixture of **1** (0.2 g, 1 mmol) and (2*E*)-butene-1,4-diol (43 mg, 0.5 mmol) in DMF (10 mL) were added DCC (206 mg, 1 mmol) and DMAP (5 mg, 0.041 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under high vacuum to give a clear oil. The crude material was purified by solid-phase extraction on normal-phase silica sorbent (eluted with DCM/methanol 20:1, v/v) to give a white solid. Recrystallization from MeOH yielded **4** (168 mg, 74% yield) as small white needles. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 8.13–8.10 (m, 4H, ArH), 7.94–7.92 (m, 4H, ArH), 7.53 (br s, 4H, NH_2), 5.94–5.92 (m, 2H, =CH), 5.01–5.00 (m, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 165.3 (CO), 148.8 (=CH), 132.9, 130.6, 128.9, 126.8 (ArCH), 61.8 (CH_2); ESI-MS: $m/z$$[M-H]^-$ 453.0; Anal. Calcd for $C_{18}H_{18}N_2O_8S_2$: C, 47.57; H, 3.99; N, 6.16. Found: C, 47.58; H, 4.03; N, 6.06.

(2*Z*)-But-2-en-1,4-diyl-bis[(aminosulfonyl)benzoate] (5). Preparation from (2*Z*)-butene-1,4-diol. (Yield 21%). ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 8.13-8.10 (m, 4H, ArH), 7.94–7.91 (m, 4H, ArH), 7.53 (br s, 4H, NH_2), 5.94–5.92 (m, 2H, =CH), 5.01–5.00 (m, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 165.3 (CO), 148.8 (=CH), 132.9, 130.6, 128.9, 126.8 (ArCH), 61.8 (CH_2); ESI-MS: m/z $[M-H]^-$ 452.9. Anal. Calcd for $C_{18}H_{18}N_2O_8S_2$: C, 47.57; H, 3.99; N, 6.16. Found: C, 47.41; H, 4.02; N, 6.11.

(2*E*)-4-Hydroxybut-2-enyl-4-(aminosulfonyl)benzoate (7). Preparation from (2*E*)-butene-1,4-diol. (Yield 26%). ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 8.11–8.08 (m, 2H, ArH), 7.94–7.91 (m, 2H, ArH), 7.49 (br s, 2H, NH_2), 5.74 (dtt, 1H, J = 11.2, 5.6, 1.2 Hz, =C*H*CH_2OH), 5.61 (dtt, 1H, J = 11.2, 6.4, 1.6 Hz, OCH_2C*H*=), 4.89–4.87 (m, 2H, OCH_2CH=), 4.79 (br s, 1H, OH), 4.09–4.08 (m, 2H, =CHCH_2OH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 165.3 (CO), 148.8 (OCH_2*C*H=CH), 136.1, 133.0, 130.6, 126.7, 124.1 (ArCH, =*C*HCH_2OH), 61.9 (O*C*H_2CH=), 57.9 (=CH*C*H_2OH); ESI-MS: m/z $[M-H]^-$ 269.9. Anal. Calcd for $C_{11}H_{13}NO_5S$: C, 48.70; H, 4.83; N, 5.16. Found: C, 48.67; H, 4.86; N, 4.97.

(2*Z*)-4-Hydroxybut-2-enyl-4-(aminosulfonyl)benzoate (8). Preparation from (2*Z*)-butene-1,4-diol. (Yield 32%). ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 8.12-8.09 (m, 2H, ArH), 7.94–7.91 (m, 2H, ArH), 7.53 (br s, 2H, NH_2), 5.74 (dtt, 1H, J = 11.2, 6.0, 1.6 Hz, =C*H*CH_2OH), 5.61 (dtt, 1H, J = 11.2, 4.8, 1.2 Hz, OCH_2C*H*=), 4.89–4.87 (m, 2H, OCH_2CH=), 4.80 (br s, 1H, OH), 4.09–4.08 (m, 2H, =CHCH_2OH); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 165.3 (CO), 148.8 (OCH_2*C*H=), 136.1, 133.0, 130.6, 126.7, 124.1 (ArCH,=*C*HCH_2OH), 61.9 (O*C*H_2CH=), 57.9 (=CH*C*H_2OH); ESI-MS: m/z $[M-H]^-$ 269.9. Anal. Calcd for $C_{11}H_{13}NO_5S$: C, 48.70; H, 4.83; N, 5.16. Found: C, 48.73; H, 4.85; N, 5.18.

1-Pentenyl-4-(aminosulfonyl)benzoate (9). Preparation from 4-penten-1-ol. (Yield 55%). ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 8.12–8.09 (m, 2H, ArH), 7.94–7.91 (m, 2H, ArH), 7.52 (br s, 2H, NH_2), 5.83 (tt, 1H, J = 17.2, 10.4, 6.4 Hz, =CH), 5.00 (ddt, 1H, J = 17.2, 10.4 Hz, =CH_2), 4.28 (t, 2H, J = 13.2 Hz, OCH_2), 2.15 (m, 2H, $OCH_2CH_2CH_2$), 1.79 (m, 2H, $OCH_2CH_2CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 165.5 (CO), 148.7 (=CH), 138.4, 133.2, 130.6, 126.7 (ArCH), 116.1 (=CH_2), 65.3 (OCH_2), 30.3 ($OCH_2CH_2CH_2$), 27.9 ($OCH_2CH_2CH_2$), ESI-MS: m/z $[M-H]^-$ 268.1. Anal. Calcd for $C_{12}H_{15}NO_4S$: C, 53.52; H, 5.61; N, 5.20. Found: C, 53.53; H, 5.36; N, 5.07.

Preparation of (2*E*)-4-(benzoyloxy)but-2-enyl-4-(aminosulfonyl)benzoate (6). To a solution of (2*E*)-butene-1,4-diol (1 g, 11.3 mmol) in CH_2Cl_2/pyridine (4:1, v/v, 20 mL) was added benzoyl chloride (397 mg, 2.8 mmol) dropwise over 2 h. The solution was stirred at room temperature for 24 h and then concentrated. The residue was redissolved in CH_2Cl_2 (25 mL) and washed with 1 M HCl solution (2×20 mL), saturated $NaHCO_3$ (1×20 mL), and saturated brine (1×20 mL). The organic phase was dried over $MgSO_4$ and evaporated to afford the monobenzoate intermediate (1.7 g, 78%), which was used in the next step without further purification. To a mixture of the mono-benzoate in DMF (10 mL) were added **1** (2.3 g, 11.3 mmol), DCC (2.33 g, 11.3 mmol), and DMAP (50 mg, 0.4 mmol). The solution was stirred at room temperature for 6 h. The reaction mixture was filtered through Celite and the filtrate was concentrated to give a clear oil. Purification by solid-phase extraction on normal-phase silica sorbent (eluted with DCM/methanol 20:1, v/v) to generate **6** as a white solid (198 mg) in 9 % yield over two steps. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 8.12–8.09 (m, 2H, ArH), 7.95–7.991 (m, 4H, ArH), 7.65–7.61 (m, 1H, ArH), 7.44 (br s, 2H, NH_2), 7.51–7.47 (m, 2H, ArH), 5.93–5.91 (m, 2H, =CH), 5.00–4.96 (m, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 165.9 (CO),163.2 (CO), 148.8 (OCH_2*C*H=CH), 148.7 (OCH_2*C*H=CH), 134.1, 133.1, 130.6, 129.8, 129.3, 128.7, 126.7 (ArCH), 61.7 (O*C*H_2CH=), 61.2 (O*C*H_2CH=): ESI-MS: m/z $[M-H]^-$ 374.0. Anal. Calcd for $C_{18}H_{17}NO_6S$: C, 57.59; H, 4.56; N, 3.73. Found: C, 57.93; H, 4.79; N, 3.78.

12. Crystal data for **3** were obtained with a Rigaku AFC7R diffractometer, Mo K_α radiation (λ = 0.71073 Å), graphite monochromator, $C_{10}H_{11}NO_4S$, monoclinic, space group $P2_1/a$, cell dimensions a = 16.180(2), b = 5.162(1), c = 13.495(2) Å, β = 98.14(1), V = 1115.7(3) Å^3, D_{calc} = 1.44 g cm^{-3}, Z = 4, $F(000)$ = 504, and μ = 0.288 mm^{-1}. Data were collected at 295(2) K using $\omega - 2\theta$ scans in the range θ = 2.80–25.0°. A total of 2236 reflections were collected, 1961, were unique (R_{int} = 0.0192). The structure was refined by full-matrix least squares on F^2. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were constrained as riding atoms with C–H = 0.95 Å, N–H = 0.85 Å. U_{iso}(H) values were set to 1.2 U_{eq} of the parent atom. Atom coordinates, bond lengths, angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre: (CCDC 278809)..

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16. Procedure for bCAII enzyme binding assay. Compounds **1**, **3**–**9** were assessed for their ability to inhibit the binding of DNSA to bCAII (CAII from bovine erythrocytes, Sigma–Aldrich, catalog No. C2522, lot number 044K6064). Enzyme assays were carried out in 96-well microtiter plates (Nunc F96) in an assay volume of 200 μL. Each assay contained bCAII (180 nM); DNSA (3 μM, equals 10 times the K_d value), incubation buffer (phosphate buffer, pH 7.2), and test compound (at 15 concentrations, triplicate determinations) in DMSO. The final DMSO concentration in the assay was 1%, this concentration of DMSO did not decrease control binding. The assay was incubated for 4 h at 25 °C. Fluorescence measurements were carried out on a Varian Cary-Eclipse spectrophotometer in fluorescence mode using a multiwell plate reader at 25 °C (excitation wavelength of 290 nM, emission wavelength of 460 nM). Known compounds (AAZ and EZA) were used to characterize this assay procedure. Data were fitted to a sigmoidal dose–response equation using nonlinear regression analysis (GraphPad Prism V4, San Diego, CA, USA). The measurement of the K_d of DNSA was determined by titrating bCAII (180 nM in pH 7.2 phosphate buffer) with DNSA (100–3500 nM) and monitoring the fluorescence as described above. Data were fitted to an equilibrium one-site binding model using nonlinear regression analysis. The K_d of DNSA was calculated as 0.3 μM and is comparable with the literature.[2]
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