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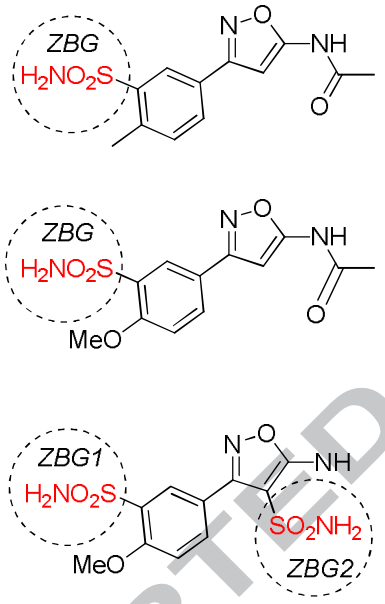

	K_i, nM		
*h*CA I	*h*CA II	*h*CA IV	*h*CA VII
>10,000	390.4	>10,000	84.5
	'detrimental methoxy'		
>10,000	>10,000	>10,000	>10,000
	alternative binding mode?		
>10,000	>10,000	9.3	>10,000

Human carbonic anhydrase inhibitory profile of mono- and bis-sulfonamides synthesized via a direct sulfochlorination of 3-and 4-(hetero)arylisoxazol-5-amine scaffolds

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ABSTRACT

Three distinct series of isoxazole-based primary mono- and bis-sulfonamides have been synthesized via direct sulfochlorination, each of them delivering nanomolar inhibitors of human carbonic anhydrase. Certain pronounced SAR trends have been established and rationalized by in silico docking. These findings expand the structure-activity knowledge base for heterocycle-containing sulfonamide carbonic anhydrase inhibitors and further validate the power of direct electrophilic sulfochlorination as a means of introducing the pharmacophoric primary sulfonamide group into structurally diverse aromatic precursors.

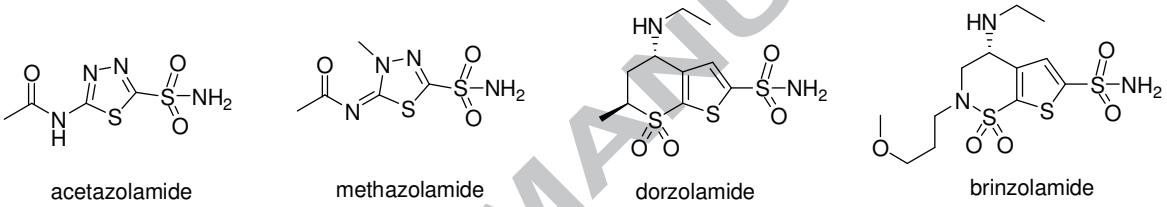
Keywords: carbonic anhydrase inhibitors, isoform selectivity, zinc binding group, primary sulfonamide, aromatic sulfochlorination, alternative binding mode.

1. Introduction

Primary aromatic and heteroaromatic sulfonamides represent a robust platform for the discovery of novel, isoform-selective inhibitors of human carbonic anhydrase (*h*CA) as they ensure specific binding of the enzyme's active site zinc ion.[1] Once anchored to the prosthetic metal ion, the periphery groups in the primary sulfonamide template can be either serendipitously screened or judiciously optimized to improve ligand's affinity and ability to selectively inhibit any of the 16

known *h*CA isoforms.[2] The availability of true isoform-selective inhibitors is essential from the standpoint of 'cleaner' drug discovery strategies unencumbered by the need to tackle off-target effects.[3] Additionally, isoform-selective *h*CA inhibitors are of value as chemical biology probes which can greatly aid in further deciphering the implications of the normal and aberrant functioning of particular isoforms for the onset and treatment of disease.[4] The majority of the clinically used carbonic anhydrase inhibitors (CAIs), exemplified by primary sulfonamides acetazolamide (**1**), methazolamide (**2**), dorzolamide (**3**) and brinzolamide (**4**) (Figure 1), are non-selective ligands for several *h*CA isoforms.[5] At the same time, the concept of isoform-selective *h*CA targeting is substantiated by the ample pre-clinical data on numerous advanced leads[6] and by the recently completed Phase I clinical study involving selective *h*CA IX inhibitor SLC-0111.[7]

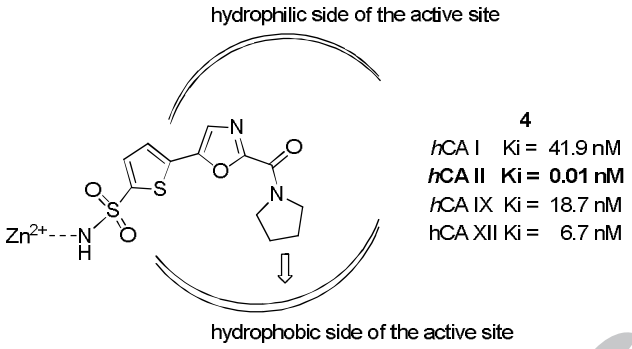
Figure 1. Examples clinically used primary sulfonamide drugs – pan-CA inhibitors.



Direct sulfochlorination of aromatic substrates, followed by a reaction of the respective sulfonyl chloride with ammonia, offers a convenient and straightforward way of introducing the primary sulfonamide zinc-binding group (ZBG) into a wealth of atomatic precursors.[8] While often chemoselective, electrophilic aromatic sulfochlorination can also take several alternative courses and thus generate more chemical diversity in the initial, SAR-probing set of compounds. However, this transformation has been relatively seldom reported in the literature as a direct link between the chemical space of aromatic precursors and that of CA inhibitory sulfonamides (compared to the more frequently reported synthesis of CAIs using reagents already containing the SO_2NH_2 functionality).

Recently, we reported the synthesis and biological profiling of aromatic, 1,3-oxazol-5-yl-substituted primary sulfonamides obtained via chemoselective sulfochlorination of the aromatic and heteroaromatic sybstrates.[9] The compounds were designed to systematically explore opportunities to build additional contacts with the protein environment of the active site where the presence of two distinct parts – hydrophobic and hydrophilic – had been confirmed earlier from crystallography studies.[10] As the result, selective picomolar inhibitors (exemplified by **4**) of *h*CA II were identified and their high potency was rationalized by the efficient interaction of the carboxamide periphery of the molecule with the target's hydrophobic half (Figure 2).

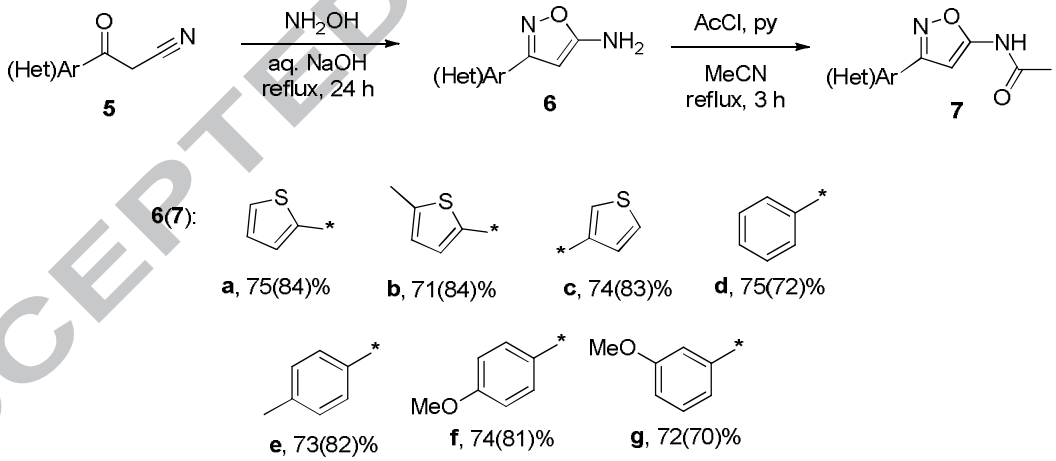
Figure 2. Selective picomolar inhibitor of *h*CA II **4** and its positioning in the enzyme's active site.



This success prompted us to consider applying the same strategy to other azolyl-substituted aromatic substrates – in particular, those that offer an opportunity of introducing two primary sulfonamides ZBGs in the same molecule. Herein, we disclose a series of primary mono- and bis-sulfonamides based on 3- an 4-(hetero)arylisoxazol-5-amine scaffolds and the results of their profiling as inhibitors of several *h*CA isoforms.

2. Results and discussion

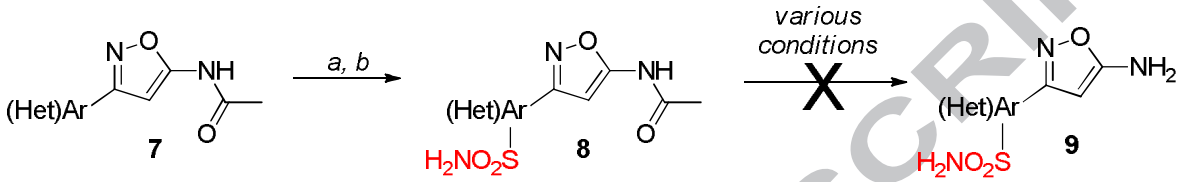
Scheme 1. Synthesis of 5-acetylaminoisoxazole substrates **7** for subsequent sulfochlorination.



A set of isoxazol-5-amines **6a-g** was prepared from readily available 2-cyanoketones **5** on reaction with hydroxylamine – and the amino group in **6** was protected with an acetyl group, to avoid unwanted reactions in the chlorosulfonylation step, to give **7a-g**. The yields of **6** and **7** were good to excellent in all instances (Scheme 1). Direct chlorosulfonylation of **7** proceeded smoothly at 60 ºC in excess chlorosulfonic acid containing thionyl chloride (10:1) and afforded respective primary sulfamides **8a-f**, as a single regioisomer, after the treatment of the respective intermediate sulfonyl chlorides with 25% aqueous ammonia in acetone (Scheme 2). While

compounds **8a-f** contain an acetylamino group, which makes them somewhat similar to acetazolamide and methazolamide, we were curious to see if respective de-acetylated compounds **9** could be obtained. Unfortunately, compounds **8** proved to be totally resistant to de-acetylation, under basic or acidic conditions, whereupon destruction of the isoxazole nucleus was observed on attempts to apply forcing temperature.

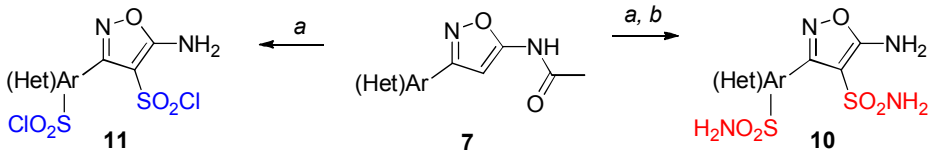
Scheme 2. Synthesis of primary mono-sulfonamides **8**.



Reagents and conditions: (a) HSO_3Cl (10 equiv.), $SOCl_2$ (1.0 equiv.), 60 ºC, 2 h; (b) 25% NH_4OH (5 equiv.), acetone, 50 ºC, 1h.

Interestingly, doubling the quantity of the sulfochlorination reagents and increasing the reaction temperature and time not only allowed achieving double sulfochlorination of substrates **7** but also led to a spontaneous loss of the acetyl group to produce a set of bis-sulfamides **10a-g**. This lability of the acetyl function *en route* to **10** is in sharp contrast to that in compounds **8** (*vide supra*). However, it is perhaps an unsurprising outcome as sulfochlorination of position 4 in isoxazole essentially turns the compounds into vinylogous *N*-acetylsilfamides which would be easy to de-acetylate. The intermediate sulfonyl chlorides **11** are also rather intriguing as they contain two different SO_2Cl moieties in combination with an unprotected amino group (Scheme 3). As they can be of interest as novel linkers for the design of dendritic structures, we also isolated and characterized them as individual compounds.

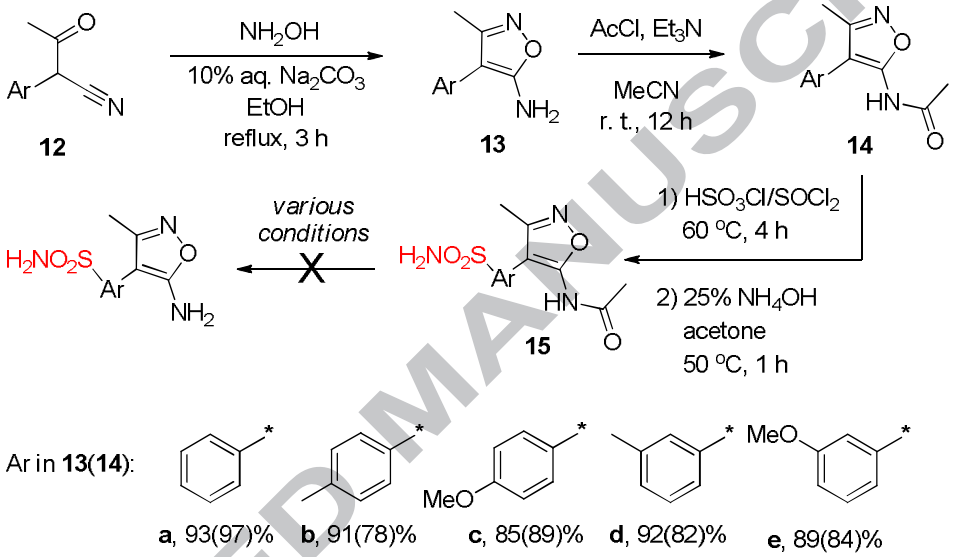
Scheme 3. Synthesis of primary bis-sulfonamides **10** and intermediate bis-sulfonylchlorides **11**.



Reagents and conditions: (a) HSO_3Cl (20 equiv.), $SOCl_2$ (2.0 equiv.), 80-110 ºC, 12-48 h; (b) 25% NH_4OH (10 equiv.), acetone, 50 ºC, 1h.

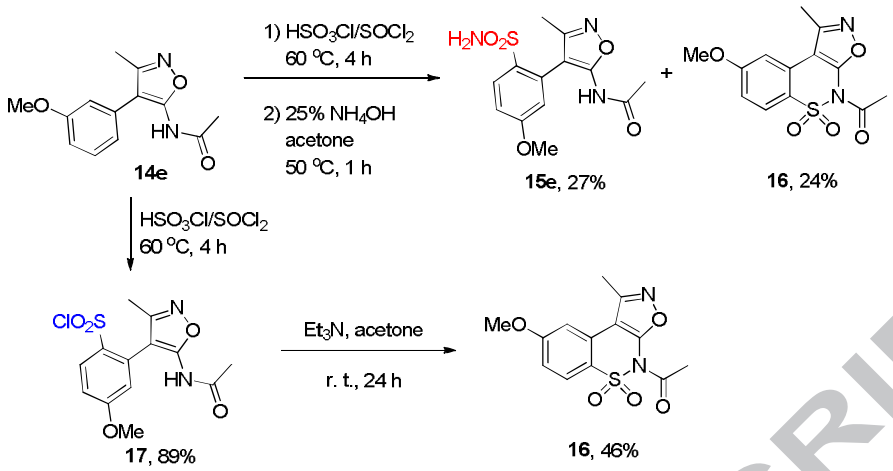
In addition to mono- (**8a-f**) and bis-sulfonamides (**10a-g**) we were interested to explore sulfonamides of different topology where the pharmacophoric ZBG would be positioned, also via direct sulfochlorination, within 4-aryl substituent of respective 5-acetylamino isoxazole substrates. The latter was obtained analogously to **7** from 2-aryl-2-cyanoacetones **12** and sulfochlorinated under the same set of conditions. While sulfochlorination (followed by aqueous ammonia treatment) proved smooth and reasonably yielding, the respective 5-acetylamino compounds **15a-e** were also resistant to all our efforts to remove the acetyl group (Scheme 4).

Scheme 4. Synthesis of primary sulfonamides **15**.



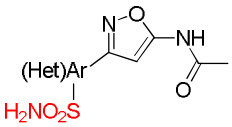
The relatively low yield of compound **15e** was due to the fact that it was accompanied by an intriguing cyclic by-product **16** formed in roughly the same amount. Its formation can be justified by the deprotonation (in basic aqueous ammonia medium) of the nearby acetylamino group and its intramolecular interaction with the sulfonyl chloride moiety prior to its conversion to primary sulfamide. This view was supported by **16** being the sole product (isolated in 46% yield) of treatment of **17** with trimethylamine (Scheme 5).

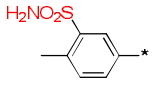
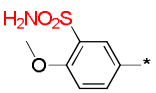
Scheme 5. Formation of tricyclic sultam **16.**

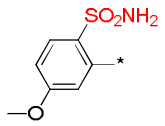
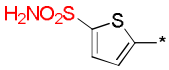
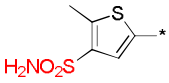
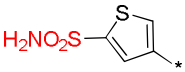


The inhibitory profile of compounds **8a-f** against *h*CA I, II, IV and VII isoforms was assessed in a stopped-flow kinetics assay as reported earlier.[9] Examination of the data presented in Table 1 reveals a strong dependence of the inhibition activity on the topology of the ZBG relative to the periphery of the molecule. In particular, compound **8a** (having a roughly 120º relationship between the sulfonamide and the isoxazole moiety) displays a remarkable (~100-fold) selectivity toward *h*CA II while compound **8b** is completely inactive. Small variations in the substitution pattern in the thiophene-linked compounds **8d-f** appear to have a strong effect on the inhibition profile. A sharp change in geometry drastically increases (**8f**) the overall potency profile of relatively weak pan-inhibitor **8d**. Compound 8e displayed a pronounced (>100-fold) selectivity toward *h*CA II while not inhibiting the other three isoforms. Particularly intriguing if the detrimental effect of methoxy group in **8b** on the *h*CA II and VII potency of its close, methyl-substituted analog **8a**.

Table 1. CA inhibitory profile of sulfonamides **8a-f**.

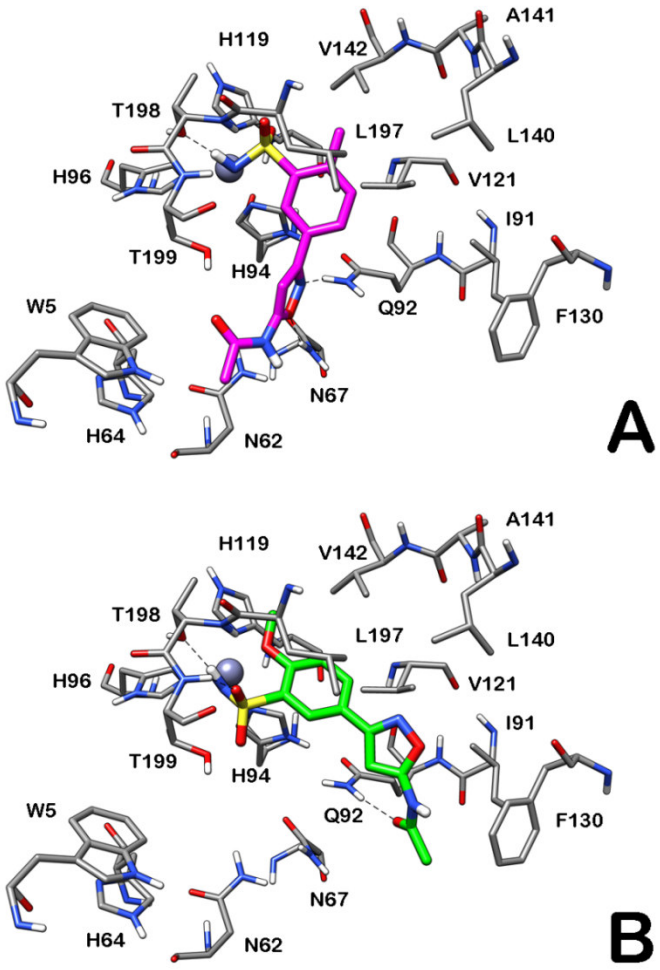


Compound	(Het)ArSO$_2$NH$_2$	Yield (%)	K$_i$, nM			
			*h*CA I	*h*CA II	*h*CAIV	*h*CAVII
8a		61[a]	>10,000	390.4	>10,000	84.5
8b		79	>10,000	>10,000	>10,000	>10,000

8c		65	>10,000	>10,000	>10,000	>10,000
8d		56	615.8	272.4	6148.0	281.6
8e		72	>10,000	89.9	>10,000	>10,000
8f		63	13.6	2.4	94.1	6.7
Acetazolamide			250	12	74	2.5

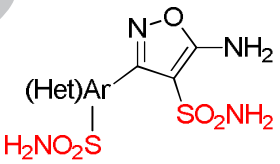
[a] Sulfochlorination reaction time – 8 h.

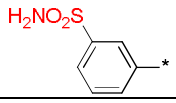
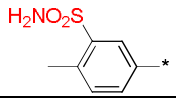
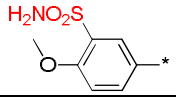
Figure 3. Docking of compounds **8a** (A) and **8b** (B) into *h*CA II.

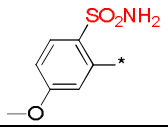
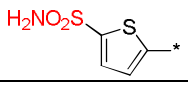
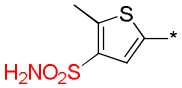
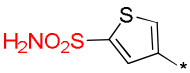


In order to understand the observed difference in *h*CA II potency between compounds **8a** and **8b**, we docked both of them into the crystal structure of *h*CA II[12] (PDB[13] code 2AW1) using GOLD software (Figure 3).[14] As expected, the sulfonamide portion of the molecule acts as a zinc binding group, also showing two additional H-bond interactions with the protein backbone and the hydroxyl group of T198. The 4-methylphenyl ring shows lipophilic interactions with V121, V142 and L197, the isoxazole ring forms two H-bonds with the side chains of Q92 and N67 whereas the acetamide portion does not show any important interactions and is directed towards the solvent-exposed entrance of the binding site cavity. Compound **8b** is not able to display the same binding mode of **8a** because of the steric clash effects between the methyl group of the methoxy substituent and the sidechain of V142. For this reason, as shown in Figure 3B compound **8b** displays an unusual distorted trigonal bipyramidal coordination of the zinc ion with both the nitrogen of the sulfonamide and oxygen of the methoxy group interacting with the zinc ion. Therefore, the impossibility for compound **8b** to display the common sulfonamide coordination of the zinc ion could explain the sharp inhibition activity decrease observed in **8b** relative to **8a**. Moreover, this finding does not contradict similar 'detrimental methoxy' SAR trend observed earlier for *o*-methoxy (vs. *o*-methyl) substituted arenesulfonamides as *h*CA II inhibitors.[9, 15]

Table 2. CA inhibitory profile of bis-sulfonamides **10a-g**.



Compound	(Het)ArSO$_2$NH$_2$	Yield **10** (%)	Yield **11** (%)	K$_i$, nM			
				*h*CA I	*h*CA II	*h*CAIV	*h*CAVII
10(11)a[a]		31	40	3,649	376.3	1,752	553.5
10(11)b[b]		54	77	3,661	6.0	8.9	3.8
10(11)c[c]		61	82	>10,000	>10,000	9.3	>10,000

10(11)d[c]		37	49	>10,000	>10,000	>10,000	>10,000
10(11)e[d]		49	57	802.5	20.3	530.5	8.5
10(11)f[d]		48	59	792.3	67.6	533.4	78.1
10(11)g[d]		41	53	66.3	24.6	184.5	10,1
Acetazolamide				250	12	74	2.5

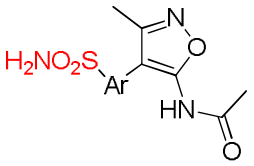
Conditions for the bis-sulfochlorination step:
[a] 110 ºC over 48 h.
[b] 90 ºC over 24 h.
[c] 80 ºC over 24 h.
[d] 90 ºC over 12 h.

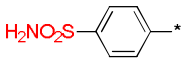
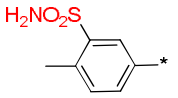
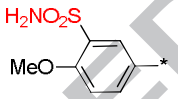
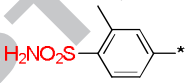
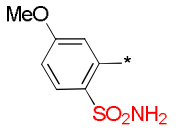
Some SAR trends observed for bis-sulfonamide CAIs **10a-g** (Table 2) appear to parallel those of mono-sulfonamides **8a-f**. The 'detrimental methoxy' trend seems to be maintained for *h*CA I, II and VII inhibition (*cf.* **10b** and **10c**). Likewise, *o*-isoxazolyl-substituted sulfonamide **10d** was similarly inactive. These instances suggest that the second, isoxazole-bound primary sulfonamide group probably does not act as a ZBG and is rather an element of the isoxazole periphery. However, certain inhibitory properties that were not observed for mono-sulfonamides appear to be restored in bis-sulfonamides, thus strongly suggesting the existence of an alternative zinc anchoring mode for the bis-sulfonamide compounds. The most notable instances are: (i) the restoration of *h*CA VII potency of compound **10b** (in comparison to its *N*-acetyl mono-sulfonamide counterpart **8a**); (ii) the low nanomolar potency of compound **10c** toward *h*CA IV (compared to the inactivity of **8b** toward this isoform) and the pronounced selectivity (>1,000-fold) vs. the other three isoforms; (iii) the restoration of inhibitory potency (in particular, against *h*CA I, VI and VII) for compound **10f** in comparison to its mono-sulfonamide congener **8e**. These observations suggest that introduction of a second primary sulfonamide ZBG[16] into isoxazole nucleus can carry strong advantages for selective targeting of certain CA isoforms, in particular *h*CA IV and VII.

Analysis of the structure-activity relationships for the isoxazol-4-yl arenesulfonamides **15a-e** (Table 3) also reveals several notable trends. Firstly, the 'detrimental methoxy' phenomenon (manifesting itself in compound **15c**) seems to persist in this series of CAIs, in particular, when it comes to ablating the nanomolar inhibitory activity of **15b** against *h*CA II, IV and VII. The lack

of inhibitory activity for the *o*-isoxazolyl benzene sulfonamide **15e** *vs.* all four isoforms parallels that of compounds **8c** and **10d** (*vide supra*). The effect of the methyl group in **15d** on *h*CA VII activity in comparison to the unsubstituted phenyl-linked compound **15a** is quite pronounced. The latter compound is rather exemplary of the strong *h*CA VII inhibition (in the low nanomolar range) observed for selected compounds in all three series (*cf.* **8f**, **10b**, **10e**).

Table 3. CA inhibitory profile of sulfonamides **15**.



Compound	$ArSO_2NH_2$	Yield (%)	K_i, nM			
			*h*CA I	*h*CA II	*h*CAIV	*h*CAVII
15a		56	96.1	5.4	207.2	4.5
15b		52	7,488	14.9	7.5	9.2
15c		61	>10,000	>10,000	>10,000	>10,000
15d		47	872.9	197.1	>10,000	707.1
15e		27	>10,000	>10,000	>10,000	>10,000
Acetazolamide			250	12	74	2.5

3. Conclusion

In summary, we have described synthesis and inhibitory activity against four isoforms of human carbonic anhydrase of three distinct series of primary mono- and bis-sulfonamides. Nanomolar levels of inhibition were achieved in all three series. Certain SAR trends have been established, in particular, the ablation of *h*CA inhibitory potency by replacing an *o*-methyl substituent with *o*-

methoxy (the 'detrimental methoxy phenomenon') as well as a possibility of an alternative binding mode for bis-sulfonamides and the suitability of the latter for selective targeting of *h*CA IV and VII isoforms.

4. Experimental section

4.1. General experimental

All reactions were carried out in oven-dried glassware in atmosphere of nitrogen. Melting points were measured with a Buchi B-520 melting point apparatus and are uncorrected. Thin-layer chromatography was carried out on Silufol UV-254 silica gel plates using an appropriate mixture of ethyl acetate and hexane. Compounds were visualized with short-wavelength UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker MSL-300 spectrometers in DMSO-d_6 using TMS as an internal standard. Elemental analyses were obtained at Research Institute for Chemical Crop Protection (Moscow, Russia) using Carlo ErbaStrumentazione 1106 analyzer. Mass spectra were recorded using Shimadzu LCMS-2020 system with electron impact (EI) ionization. All and reagents and solvents were obtained from commercial sources and used without purification.

4.2. Synthetic organic chemistry

4.2.1. General procedure for the preparation of 5-aminoisoxazoles 6a-g.

Ketonitrile **5** (100 mmol) was added to 15% aqueous NaOH solution (100 mL) followed by hydroxylamine (200 mmol). The resulting mixture was heated at reflux for 14 h, cooled down to r. t., the resulting precipitate was isolated by filtration and crystallized from isopropyl alcohol.

4.2.1.1. 3-(Thiophen-2-yl)-1,2-oxazol-5-amine (6a)

Yield 12,450 mg (75%). White solid, m.p. 96-98 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.60 (dd, $J_{3\text{-}4}$=5.0 Hz, $J_{3\text{-}5}$=1.0 Hz, 1H, 3-$H_{thiophene}$), 7.51 (dd, $J_{4\text{-}5}$=3.6 Hz, $J_{3\text{-}5}$=1.0 Hz, 1H, 5-$H_{thiophene}$), 7.13 (dd, $J_{3\text{-}4}$=5.0 Hz, $J_{4\text{-}5}$=3.6 Hz, 1H, 4-$H_{thiophene}$), 6.81 (s, 2H, NH_2), 5.36 (s, 1H, $H_{isoxazole}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 171.3, 158.4, 131.9, 128.1, 128.0, 127.8, 75.6. LC MS (ESI): *m/z* $[M+H]^+$ 167. Anal. calcd for $C_7H_6N_2OS$ (166.20): C, 50.59; H, 3.64; N, 16.86; found: C, 50.51; H, 3.64; N, 16.83.

4.2.1.2. 3-(5-Methylthiophen-2-yl)-1,2-oxazol-5-amine (6b)

Yield 12,780 mg (71%). White solid, m.p. 134-136 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.29 (d, *J*=3.30 Hz, 1H, $H_{thiophene}$), 6.80 (d, *J*=3.30 Hz, 1H, $H_{thiophene}$), 5.29 (s, 2H,

NH_2), 2.45 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 171.1, 158.5, 141.4, 129.6, 128.0, 126.4, 75.3, 15.4. LC MS (ESI): *m/z* $[M+H]^+$ 181. Anal. calcd for $C_8H_8N_2OS$ (180.23): C, 53.31; H, 4.47; N, 15.54; found: C, 53.28; H, 4.48; N, 15.50.

4.2.1.3. 3-(Thiophen-3-yl)-1,2-oxazol-5-amine (6c)

Yield 12,284 mg (74%). White solid, m.p. 177-178 °C (i-PrOH). ^1H NMR (300 MHz, DMSO-d_6) δ ppm 7.94 (dd, $J_{2\text{-}5}$=3.0 Hz, $J_{2\text{-}4}$=1.3 Hz, 1H, 2-$H_{thiophene}$), 7.61 (dd, $J_{4\text{-}5}$=5.0 Hz, $J_{2\text{-}5}$=3.0 Hz, 1H, 4-$H_{thiophene}$), 7.41 (dd, $J_{4\text{-}5}$=5.0 Hz, $J_{2\text{-}4}$=1.3 Hz, 1H, 4-$H_{thiophene}$), 6.73 (s, 2H, NH_2), 5.34 (s, 1H, $H_{isoxazole}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 171.1, 159.2, 131.8, 127.6, 126.0, 125.1, 75.9. LC MS (ESI): *m/z* $[M+H]^+$ 167. Anal. calcd for $C_7H_6N_2OS$ (166.20): C, 50.59; H, 3.64; N, 16.86; found: C, 50.56; H, 3.64; N, 16.90.

4.2.1.4. 3-Phenyl-1,2-oxazol-5-amine (6d)

Yield 12,000 mg (75%). White solid, m.p. 109-112 °C (i-PrOH). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.72 (m, 2H, H_{Ar}), 7.44 (m, 3H, H_{Ar}), 6.76 (s, 2H, NH_2), 5.39 (s, 1H, $H_{isoxazole}$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 171.5, 163.0, 130.5, 129.9, 129.2, 126.7, 75.7. LC MS (ESI): *m/z* $[M+H]^+$ 161. Anal. calcd for $C_9H_8N_2O$ (160.18): C, 67.49; H, 5.03; N, 17.49; found: C, 67.44; H, 5.03; N, 17.50.

4.2.1.5. 3-(4-Methylphenyl)-1,2-oxazol-5-amine (6e)

Yield 12,702 mg (73%). White solid, m.p. 155-156 °C (i-PrOH). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.60 (d, *J*=8.0 Hz, 2H, H_{Ar}), 7.24 (d, *J*=8.0 Hz, 2H, H_{Ar}), 6.73 (s, 2H, NH_2), 5.35 (s, 1H, $H_{isoxazole}$), 2.33 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 171.3, 162.9, 139.5, 129.8, 127.7, 126.6, 75.5, 21.3. LC MS (ESI): *m/z* $[M+H]^+$ 175. Anal. calcd for $C_{10}H_{10}N_2O$ (174.20): C, 68.95; H, 5.79; N, 16.08; found: C, 68.91; H, 5.79; N, 16.06.

4.2.1.6. 3-(4-Methoxyphenyl)-1,2-oxazol-5-amine (6f)

Yield 14,060 mg (74%). White solid, m.p. 132-134 °C (i-PrOH). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 7.65 (m, 2H, H_{Ar}), 6.98 (m, 2H, H_{Ar}), 6.68 (s, 2H, NH_2), 5.32 (s, 1H, $H_{isoxazol}$), 3.79 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 171.2, 162.6, 160.6, 128.1, 122.8, 114.6, 75.4, 55.6. LC MS (ESI): *m/z* $[M+H]^+$ 191. Anal. calcd for $C_{10}H_{10}N_2O_2$ (190.20): C, 63.15; H, 5.30; N, 14.73; found: C, 63.10; H, 5.30; N, 14.73.

4.2.1.7. 3-(3-Methoxyphenyl)-1,2-oxazol-5-amine (6g)

Yield 13,680 mg (72%). White solid, m.p. 83-85 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.39 (m, 3H, H_{Ar}), 7.07 (m, 1H, H_{Ar}), 6.68 (s, 2H, NH_2), 5.34 (s, 1H, $H_{isoxazol}$), 3.80 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 171.3, 162.8, 159.9, 130.3, 119.2, 116.5, 115.4, 111.5, 75.3, 55.6. LC MS (ESI): *m/z* $[M+H]^+$ 191. Anal. calcd for $C_{10}H_{10}N_2O_2$ (190.20): C, 63.15; H, 5.30; N, 14.73; found: C, 63.07; H, 5.30; N, 14.76.

4.2.2. General procedure for the preparation of 5-acetylaminoisoxazoles 7a-g.

A solution of 5-aminoisoxazole **6** (50 mmol) and pyridine (100 mmol) in acetonitrile (800 mL) was cooled to 0 ºC and treated with acetyl chloride (50 mmol). The resulting mixture was heated at reflux for 3 h, cooled to r. t., concentrated in vacuo and the residue was treated with water (800 mL). The resulting precipitate was isolated by filtration and crystallized from ethanol.

4.2.2.1. *N*-[3-(Thiophen-2-yl)-1,2-oxazol-5-yl]acetamide (7a)

Yield 8,778 mg (84%). White solid, m.p. 132-134 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.62 (br.s., 1H, AcNH), 7.73 (dd, $J_{4\text{-}5}$=3.6 Hz, $J_{3\text{-}5}$=1.0 Hz, 1H, 5-$H_{thiophene}$), 7.71 (dd, $J_{3\text{-}4}$=5.0 Hz, $J_{3\text{-}5}$=1.0 Hz, 1H, 3-$H_{thiophene}$), 7.19 (dd, $J_{3\text{-}4}$=5.0 Hz, $J_{4\text{-}5}$=3.6 Hz, 1H, 4-$H_{thiophene}$), 6.67 (s, 1H, $H_{isoxazole}$), 2.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.4, 162.5, 158.7, 132.5, 128.4, 128.2, 127.9, 86.6, 15.5. LC MS (ESI): *m/z* $[M+H]^+$ 209. Anal. calcd for $C_9H_8N_2O_2S$ (208.24): C, 51.91; H, 3.87; N, 13.45; found: C, 51.89; H, 3.88; N, 13.44.

4.2.2.2. *N*-[3-(5-Methylthiophen-2-yl)-1,2-oxazol-5-yl]acetamide (7b)

Yield 9,324 mg (84%). White solid, m.p. 193-195 °C (EtOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.66 (s, 1H, AcNH), 7.50 (d, *J*=3.3 Hz, 1H, $H_{thiophene}$), 6.86 (d, *J*=3.3 Hz, 1H, $H_{thiophene}$), 6.61 (s, 1H, $H_{isoxazole}$), 2.47 (s, 3H, Th-CH_3), 2.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.4, 162.3, 158.6, 142.5, 129.4, 128.2, 126.8, 85.8, 23.5, 15.5. LC MS (ESI): *m/z* $[M+H]^+$ 223. Anal. calcd for $C_{10}H_{10}N_2O_2S$ (222.27): C, 54.04; H, 4.54; N, 12.60; found: C, 53.99; H, 4.54; N, 12.63.

4.2.2.3. *N*-[3-(Thiophen-3-yl)-1,2-oxazol-5-yl]acetamide (7c)

Yield 8,632 mg (83%). White solid, m.p. 197-199 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.65 (br s, 1H), 8.19 (dd, $J_{2\text{-}5}$=2.8 Hz, $J_{2\text{-}4}$=1.0 Hz, 1H, 2-$H_{thiophene}$), 7.68 (dd, $J_{4\text{-}5}$=5.0, $J_{2\text{-}5}$=2.8 Hz, 1H, 5-$H_{thiophene}$), 7.52 (dd, $J_{4\text{-}5}$=5.0 Hz, $J_{2\text{-}4}$=1.0 Hz, 1H, 4-$H_{thiophene}$), 6.66 (s, 1H, $H_{isoxazole}$), 2.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.4, 162.0, 159.4, 131.9, 127.2, 125.5, 125.3, 86.5, 21.5. LC MS (ESI): *m/z* $[M+H]^+$ 209. Anal. calcd for $C_9H_8N_2O_2S$ (208.24): C, 51.91; H, 3.87; N, 13.45; found: C, 51.82; H, 3.87; N, 13.40.

4.2.2.4. *N*-(3-Phenyl-1,2-oxazol-5-yl)acetamide (7d)

Yield 7,272 mg (72%). White solid, m.p. 152-155 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.68 (br s, 1H, AcNH), 7.84 (m, 2H, H_{Ar}), 7.49 (m, 3H, H_{Ar}), 6.69 (s, 1H, $H_{isoxazole}$), 2.13 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.4, 162.9, 162.7, 130.6, 129.5, 129.3, 126.9, 86.1, 23.6. LC MS (ESI): *m/z* $[M+H]^+$ 203. Anal. calcd for $C_{11}H_{10}N_2O_2$ (202.21): C, 65.34; H, 4.98; N, 13.85; found: C, 65.25; H, 4.99; N, 13.80.

4.2.2.5. *N*-[3-(4-Methylphenyl)-1,2-oxazol-5-yl]acetamide (7e)

Yield 8,856 mg (82%). White solid, m.p. 197-199 °C (EtOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.64 (br s, 1H, AcNH), 7.72 (d, *J*=7.9 Hz, 2H, H_{Ar}), 7.29 (d, *J*=7.9 Hz, 2H, H_{Ar}), 6.65 (s, 1H, $H_{isoxazole}$), 2.35 (s, 3H, $ArCH_3$), 2.13 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.4, 162.9, 162.5, 140.3, 126.8, 126.5, 86.0, 23.5, 21.4. LC MS (ESI): *m/z* $[M+H]^+$ 217. Anal. calcd for $C_{12}H_{12}N_2O_2$ (216.24): C, 66.65; H, 5.59; N, 12.96; found: C, 66.61; H, 5.60; N, 13.00.

4.2.2.6. *N*-[3-(4-Methoxyphenyl)-1,2-oxazol-5-yl]acetamide (7f)

Yield 9,396 mg (81%). White solid, m.p. 166-168 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.62 (br s, 1H, AcNH), 7.77 (m, 2H, H_{Ar}), 7.03 (m, 2H, H_{Ar}), 6.62 (s, 1H, $H_{isoxazole}$), 3.80 (s, 3H, OCH_3), 2.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.5, 162.6, 162.3, 159.7, 129.8, 122.1, 114.7, 86.5, 55.7, 20.5. LC MS (ESI): *m/z* $[M+H]^+$ 233. Anal. calcd for $C_{12}H_{12}N_2O_3$ (232.24): C, 62.06; H, 5.21; N, 12.06; found: C, 61.98; H, 5.21; N, 12.11.

4.2.2.7. *N*-[3-(3-Methoxyphenyl)-1,2-oxazol-5-yl]acetamide (7g)

Yield 8,120 mg (70%). White solid, m.p. 153-156 °C (EtOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.68 (br s, 1H), 7.40 (m, 3H, H_{Ar}), 7.05 (m, 1H, H_{Ar}), 6.71 (s, 1H, $H_{isoxazol}$), 3.82 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.4, 162.9, 162.6, 160.1, 130.6, 119.3, 116.6, 111.8, 86.3, 55.7, 23.5. LC MS (ESI): *m/z* $[M+H]^+$ 233. Anal. calcd for $C_{12}H_{12}N_2O_3$ (232.24): C, 62.06; H, 5.21; N, 12.06; found: C, 62.00; H, 5.21; N, 12.00.

4.2.3. *N*-[3-(4-methyl-3-sulfamoylphenyl)-1,2-oxazol-5-yl]acetamide (8a).

Compound **7d** (10 mmol) was added portionwise to a stirred and cooled mixture of chlorosulfonic acid (100 mmol) and thionyl chloride (10 mmol). The resulting mixture was heated at 60 °C over 8 h, cooled to r. t. and poured over crushed ice. The resulting precipitate was isolated by filtration and dissolved in dichloromethane (100 mL). The solution was washed with 5% aqueous K_2CO_3 (50 mL), dried over anhydrous $CaCl_2$, filtered and concentrated *in*

vacuo. The residue was briefly fractionated on silica gel using 25% ethyl acetate in hexanes and the fractions containing the intermediate sulfonyl chloride were pooled and concentrated *in vacuo*. The residue was dissolved in acetone (40 mL) and the solution was treated with 25% aqueous ammonia (25 mmol). The resulting mixture was heated at 50 °C over 1 h, cooled and the volatiles were removed *in vacuo*. The residue was treated with ice-cold water (40 mL) and the mixture was stirred until a thick precipitate formed. The latter was filtered off and crystallized from isopropyl alcohol to provide the title compound.

Yield 1,805 mg (61%). White solid, m.p. 237-239 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.70 (s, 1H, AcNH), 8.30 (d, $J_{2\text{-}6}$=1.3 Hz, 1H), 7.94 (dd, $J_{5\text{-}6}$=7.9 Hz, $J_{2\text{-}6}$=1.3 Hz, 1H, 6-H_{Ar}), 7.50 (m, 3H, 5-H_{Ar}, NH_2), 6.72 (s, 1H, $H_{isoxazol}$), 2.64 (s, 3H, Ar-CH_3), 2.14 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.5, 162.9, 162.0, 143.4, 138.4, 133.5, 129.9, 127.2, 125.2, 86.0, 23.6, 20.2. LC MS (ESI): *m/z* $[M+H]^+$ 296. Anal. calcd for $C_{12}H_{13}N_3O_4S$ (295.32): C, 48.81; H, 4.44; N, 14.23; found: C, 48.79; H, 4.44; N, 14.21.

4.2.4. General procedure for the synthesis of sulfonamides 8b-f.

The procedure was identical to that for the preparation of compound **8a** but the heating in the first step continued for 2 h.

4.2.4.1. *N*-[3-(4-Methoxy-3-sulfamoylphenyl)-1,2-oxazol-5-yl]acetamide (8b)

Yield 2,457 mg (79%). White solid, m.p. 278-280 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.66 (s, 1H, AcNH), 8.17 (d, $J_{2\text{-}6}$=1.6 Hz, 1H, 2-H_{Ar}), 8.04 (dd, $J_{5\text{-}6}$=8.6 Hz, $J_{2\text{-}6}$=1.6 Hz, 1H, 6-H_{Ar}), 7.32 (d, $J_{5\text{-}6}$=8.6 Hz, 1H, 5-H_{Ar}), 7.19 (s, 2H, SO_2NH_2), 6.67 (s, 1H, $H_{isoxazol}$), 3.97 (s, 3H, OCH_3), 2.14 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.5, 162.4, 158.2, 135.9, 129.5, 126.9, 120.1, 112.8, 85.6, 56.1, 23.6. LC MS (ESI): *m/z* $[M+H]^+$ 312. Anal. calcd for $C_{12}H_{13}N_3O_5S$ (311.32): C, 46.30; H, 4.21; N, 13.50; found: C, 46.24; H, 4.21; N, 13.55.

4.2.4.2. *N*-[3-(5-Methoxy-2-sulfamoylphenyl)-1,2-oxazol-5-yl]acetamide (8c)

Yield 2,022 mg (65%). White solid, m.p. 207-209 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 11.71 (s, 1H, AcNH), 7.98 (d, $J_{3\text{-}4}$=9.0 Hz, 1H, 3-H_{Ar}), 7.21 (dd, $J_{3\text{-}4}$=9.0 Hz, $J_{4\text{-}6}$=2.3 Hz, 1H, 4-H_{Ar}), (s, 2H, NH_2), 7.09 (d, $J_{4\text{-}6}$=2.3 Hz, 1H, 6-H_{Ar}), 6.57 (s, 1H, $H_{isoxazol}$), 3.87 (s, 3H, OCH_3), 2.13 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 168.4, 167.4, 163.1, 161.7, 135.1, 130.4, 129.5, 117.4, 115.2, 90.3, 66.8, 23.5. LC MS (ESI): *m/z* $[M+H]^+$ 312. Anal. calcd for $C_{12}H_{13}N_3O_5S$ (311.32): C, 46.30; H, 4.21; N, 13.50; found: C, 46.18; H, 4.21; N, 13.44.

4.2.4.3. *N*-[3-(5-Sulfamoylthiophen-2-yl)-1,2-oxazol-5-yl]acetamide (8d)

Yield 1,607 mg (56%). Brown solid, m.p. 201-203 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 13.40 (br s, 2H, SO_2NH_2), 11.67 (s, 1H, AcNH), 7.48 (d, *J*=3.63 Hz, 1H, $H_{thiophene}$), 7.12 (d, *J*=3.63 Hz, 1H, $H_{thiophene}$), 6.62 (s, 1H, $H_{isoxazole}$), 2.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.5, 162.5, 158.5, 153.6, 130.3, 128.3, 126.5, 86.1, 23.5. LC MS (ESI): *m/z* $[M+H]^+$ 288. Anal. calcd for $C_9H_9N_3O_4S_2$ (287.32): C, 37.62; H, 3.16; N, 14.63; found: C, 37.60; H, 3.16; N, 14.60.

4.2.4.4. *N*-[3-(5-Methyl-4-sulfamoylthiophen-2-yl)-1,2-oxazol-5-yl]acetamide (8e)

Yield 2,167 mg (72%). Brown solid, m.p. 161-165 °C (i-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.75 (br s, 2H, SO_2NH_2), 11.68 (br s, 1H, AcNH), 7.47 (s, 1H, $H_{thiophene}$), 6.54 (s, 1H, $H_{isoxazole}$), 2.55 (s, 3H, Th-CH_3), 2.11 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.5, 162.5, 158.1, 145.8, 139.5, 129.6, 124.6, 85.7, 23.5, 14.5. LC MS (ESI): *m/z* $[M+H]^+$ 302. Anal. calcd for $C_{10}H_{11}N_3O_4S_2$ (301.35): C, 39.86; H, 3.68; N, 13.94; found: C, 39.82; H, 3.68; N, 13.98.

4.2.4.5. *N*-[3-(5-Sulfamoylthiophen-3-yl)-1,2-oxazol-5-yl]acetamide (8f)

Yield 1,808 mg (63%). White solid, m.p. 226-228 °C (*i*-PrOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.70 (br s, 1H, AcNH), 8.45 (s, 1H, $H_{thiophene}$), 7.91 (s, 1H, $H_{thiophene}$), 7.79 (s, 2H, SO_2NH_2), 6.72 (s, 1H, $H_{isoxazole}$), 2.13 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 167.5, 162.7, 158.5, 147.7, 130.7, 130.2, 127.9, 86.4, 23.6. LC MS (ESI): *m/z* $[M+H]^+$ 288. Anal. calcd for $C_9H_9N_3O_4S_2$ (287.32): C, 37.62; H, 3.16; N, 14.63; found: C, 37.59; H, 3.16; N, 14.61.

4.2.5. 5-Amino-3-(3-sulfamoylphenyl)-1,2-oxazole-4-sulfonamide (10a).

Compound **7d** (10 mmol) was added portionwise to a stirred and cooled mixture of chlorosulfonic acid (200 mmol) and thionyl chloride (20 mmol). The resulting mixture was heated at 60 °C over 1 h, then at 110 ºC for 48 h, cooled to r. t. and poured over crushed ice. The mixture was extracted with dichloromethane (150 mL). The solution was washed with 5% aqueous K_2CO_3 (50 mL), dried over anhydrous $CaCl_2$, filtered and concentrated *in vacuo*. The residue was dissolved in acetone (40 mL) and the solution was treated with 25% aqueous ammonia (50 mmol). The resulting mixture was heated at 50 °C over 1 h, cooled and the volatiles were removed *in vacuo*. The residue was treated with ice-cold water (40 mL) and the mixture was extracted with ethyl acetate (50 mL). The extract was dried over anhydrous $CaCl_2$,

filtered and concentrated *in vacuo*. Chromatography on silica gel using 75% ethyl acetate in hexanes as eluent afforded the title compound.

Yield 986 mg (31%). White solid, m.p. 153-155 °C (AcOEt). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.11 (s, 1H, 2-H_{Ar}), 8.02 (d, *J*=7.9 Hz, 1H, H_{Ar}), 7.95 (d, *J*=7.9 Hz, 1H, H_{Ar}), 7.67 (t, *J*=7.9 Hz, 1H, 4-H_{Ar}), 7.65 (s, 2H, NH_2), 7.47 (s, 2H, NH_2), 7.28 (s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.6, 159.4, 144.7, 132.9, 129.6, 129.4, 127.4, 126.0, 94.5. LC MS (ESI): *m/z* $[M+H]^+$ 319. Anal. calcd for $C_9H_{10}N_4O_5S_2$ (318.33): C, 33.96; H, 3.17; N, 17.60; found: C, 33.93; H, 3.17; N, 17.63.

4.2.6. 5-Amino-3-(4-methyl-3-sulfamoylphenyl)-1,2-oxazole-4-sulfonamide (10b).

Compound **7d** (10 mmol) was added portionwise to a stirred and cooled mixture of chlorosulfonic acid (200 mmol) and thionyl chloride (20 mmol). The resulting mixture was heated at 60 °C over 1 h, then at 90 °C for 24 h, cooled to r. t. and poured over crushed ice. The mixture was extracted with dichloromethane (150 mL). The solution was washed with 5% aqueous K_2CO_3 (50 mL), dried over anhydrous $CaCl_2$, filtered and concentrated *in vacuo*. The residue was briefly fractionated on silica gel using 25% ethyl acetate in hexanes and the fractions containing the intermediate sulfonyl chloride were pooled and concentrated *in vacuo*. The residue was dissolved in acetone (40 mL) and the solution was treated with 25% aqueous ammonia (50 mmol). The resulting mixture was heated at 50 °C over 1 h, cooled and the volatiles were removed *in vacuo*. The residue was treated with ice-cold water (40 mL) and the mixture was and the mixture was extracted with ethyl acetate (50 mL). The extract was dried over anhydrous $CaCl_2$, filtered and concentrated *in vacuo*. Chromatography on silica gel using 75% ethyl acetate in hexanes as eluent afforded the title compound.

Yield 1,793 mg (54%). Beige solid, m.p. 200-202 °C (AcOEt). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.14 (d, $J_{2\text{-}6}$=1.5 Hz, 1H, 2-H_{Ar}), 7.90 (dd, $J_{5\text{-}6}$=7.8 Hz, $J_{2\text{-}6}$=1.5 Hz, 1H, 6-H_{Ar}), 7.59 (s, 2H, NH_2), 7.49 (m, 3H, NH_2, 5-H_{Ar}), 7.24 (s, 2H, NH_2), 2.64 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.6, 159.4, 142. 7, 138.2, 132.7, 132.7, 127.4, 126.6, 94.4, 20.2. LC MS (ESI): *m/z* $[M+H]^+$ 333. Anal. calcd for $C_{10}H_{12}N_4O_5S_2$ (332.36): C, 36.14; H, 3.64; N, 16.86; found: C, 36.14; H, 3.64; N, 16.85.

4.2.7. General procedure for the synthesis of sulfonamides 10c-d.

The procedure is identical to that for the synthesis of compound **10b** but the heating in the first step continued for 24 h at 80 °C.

4.2.7.1. 5-Amino-3-(4-methoxy-3-sulfamoylphenyl)-1,2-oxazole-4-sulfonamide (10c)

Yield 2,123 mg (61%). Beige solid, m.p. 211-213 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.03 (m, 2H, 2-H_{Ar}, 6-H_{Ar}), 7.56 (s, 2H, NH_2), 7.30 (d, $J_{5\text{-}6}$=8.6 Hz, 1H, 5-H_{Ar}), 7.16 (s, 2H, NH_2), 7.21 (s, 2H, NH_2), 3.96 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.6, 159.2, 157.6, 134.9, 131.6, 128.3, 120.2, 113.0, 94.2, 56.8. LC MS (ESI): *m/z* $[M+H]^+$ 349. Anal. calcd for $C_{10}H_{12}N_4O_6S_2$ (348.36): C, 34.48; H, 3.47; N, 16.08; found: C, 34.42; H, 3.47; N, 16.10.

4.2.7.2. *N*-[3-(5-Methoxy-2-sulfamoylphenyl)-4-sulfamoyl-1,2-oxazol-5-yl]acetamide (10d)

Yield 1,288 mg (37%). Beige solid, m.p. 125-129 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.92 (d, $J_{3\text{-}4}$=8.9 Hz, 1H, 3-H_{Ar}), 7.57 (s, 2H, NH_2), 7.21 (dd, $J_{3\text{-}4}$=8.9 Hz, $J_{4\text{-}6}$=2.8 Hz, 1H, 4-H_{Ar}), 7.07 (d, $J_{4\text{-}6}$=2.8 Hz, 1H, 6-H_{Ar}), 6.94 (s, 2H, NH_2), 6.89 (s, 2H, NH_2), 3.85 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 168.5, 161.3, 159.5, 135.2, 130.1, 128.1, 118.1, 115.4, 95.86, 56.3. LC MS (ESI): *m/z* $[M+H]^+$ 349. Anal. calcd for $C_{10}H_{12}N_4O_6S_2$ (348.36): C, 34.48; H, 3.47; N, 16.08; found: C, 34.45; H, 3.47; N, 16.11.

4.2.8. General procedure for the synthesis of sulfonamides 10e-g.

The procedure is identical to that for the synthesis of compound **10b** but the heating in the first step continued for 12 h at 90º C.

4.2.8.1. 5-Amino-3-(5-sulfamoylthiophen-2-yl)-1,2-oxazole-4-sulfonamide (10e)

Yield 1,588 mg (49%). Yellow solid, m.p. 137-139 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.35 (d, *J*=3.63 Hz, 1H, $H_{thiophene}$), 7.27 (br s, 2H, NH_2), 7.17 (d, *J*=3.63 Hz, 1H, $H_{thiophene}$), 7.10 ((br s, 2H, NH_2), 6.93 ((br s, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 172.6, 159.3, 155.3, 131.6, 127.8, 126.1, 94.7. LC MS (ESI): *m/z* $[M+H]^+$ 325. Anal. calcd for $C_7H_8N_4O_5S_3$ (324.36): C, 25.92; H, 2.49; N, 17.27; found: C, 25.89; H, 2.49; N, 17.29.

4.2.8.2. 5-Amino-3-(5-methyl-4-sulfamoylthiophen-2-yl)-1,2-oxazole-4-sulfonamide (10f)

Yield 1,622 mg (48%). Beige solid, m.p. 229-231 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.99 (s, 1H, $H_{thiophene}$), 7.63 (s, 2H, NH_2), 7.34 (s, 2H, NH_2), 7.40 (s, 2H, NH_2), 2.65 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.9, 153.4, 144.5, 140.4, 130.7, 124.6, 93.1, 14.4. LC MS (ESI): *m/z* $[M+H]^+$ 339. Anal. calcd for $C_8H_{10}N_4O_5S_3$ (338.39): C, 28.40; H, 2.98; N, 16.56; found: C, 28.38; H, 2.98; N, 16.58.

4.2.8.3. 5-Amino-3-(5-sulfamoylthiophen-3-yl)-1,2-oxazole-4-sulfonamide (10g)

Yield 1,328 mg (41%). Beige solid, m.p. 215-217 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 8.42 (d, *J*=1.3 Hz, 1H, $H_{thiophene}$), 7.88 (d, *J*=1.3 Hz, 1H, $H_{thiophene}$), 7.77 (s, 2H, NH_2), 7.59 (s, 2H, NH_2), 7.35 (s, 2H, NH_2) ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.7, 154.5, 146.5, 132.6, 129.7, 128.0, 93.5. LC MS (ESI): *m/z* $[M+H]^+$ 325. Anal. calcd for $C_7H_8N_4O_5S_3$ (324.36): C, 25.92; H, 2.49; N, 17.27; found: C, 25.91; H, 2.49; N, 17.27.

4.2.9. 5-Amino-3-[3-(chlorosulfonyl)phenyl]-1,2-oxazole-4-sulfonyl chloride (11a).

Compound **7d** (10 mmol) was added portionwise to a stirred and cooled mixture of chlorosulfonic acid (200 mmol) and thionyl chloride (20 mmol). The resulting mixture was heated at 60 °C over 1 h, then at 110 ºC for 48 h, cooled to r. t. and poured over crushed ice. The mixture was extracted with dichloromethane (150 mL). The solution was washed with 5% aqueous K_2CO_3 (75 mL), dried over anhydrous $CaCl_2$, filtered and concentrated *in vacuo*. The residue was briefly fractionated on silica gel using 25% ethyl acetate in hexanes and the fractions containing the bis-sulfonyl chloride were pooled and concentrated *in vacuo.* The residue was crystallized from diethyl ether to provide the title compound.

Yield 1,428 mg (40%). Beige solid, m.p. 121-124 °C (diethyl ether). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.51 (s, 1H, 2-H_{Ar}), 8.23 (d, *J*=8.0 Hz, 1H, H_{Ar}), 8.23 (d, *J*=8.0 Hz, 1H, H_{Ar}) 7.81 (t, *J*=8.0 Hz, 1H, 5-H_{Ar}), 6.28 (br s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 169.1, 157.6, 144.9, 135.6, 130.2, 129.1, 127.9, 127.8, 99.0. Anal. calcd for $C_9H_6Cl_2N_2O_5S_2$ (357.19): C, 30.26; H, 1.69; N, 7.84; found: C, 30.36; H, 1.73; N, 7.79.

5-Amino-3-[3-(chlorosulfonyl)-4-methylphenyl]-1,2-oxazole-4-sulfonyl chloride (11b).

The procedure is analogous to that for the preparation of compound **10a** but the heating in the first step continued at 90 ºC at 24 h.

Yield 2,857 mg (77%). Beige solid, m.p. 136-138 °C (diethyl ether). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.54 (s, 1H, 2-H_{Ar}), 8.07 (d, *J*=7.9 Hz, 1H, H_{Ar}), 7.60 (d, *J*=7.9 Hz, 1H, H_{Ar}), 6.29 (br s, 2H, NH_2), 2.89 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 169.0, 157.7, 143.4, 141.1, 135.3, 133.8, 129.5, 124.9, 99.0, 20.4. Anal. calcd for $C_{10}H_8Cl_2N_2O_5S_2$ (371.22): C, 32.35; H, 2.17; N, 7.55; found: C, 32.42; H, 2.12; N, 7.59.

4.2.11. General procedure for the synthesis of bis-sulfochlorides 11c-d.

The procedure is analogous to that for the preparation of compound **10a** but the heating in the first step continued at 80 ºC at 24 h.

4.2.11.1. 5-Amino-3-[3-(chlorosulfonyl)-4-methoxyphenyl]-1,2-oxazole-4-sulfonyl chloride (11c)

Yield 3,173 mg (82%). Beige solid, m.p. 148-151 °C (diethyl ether). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.46 (d, $J_{2\text{-}6}$=2.3 Hz, 1H, 2-H_{Ar}), 8.18 (dd, $J_{5\text{-}6}$=8.6 Hz, $J_{2\text{-}6}$=2.3 Hz, 12 H), 7.29 (d, $J_{5\text{-}6}$=8.6 Hz, 1H, 5-H_{Ar}), 6.27 (br s, 2H, NH_2), 4.17 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 169.1, 159.0, 157.5, 137.7, 132.2, 130.8, 118.2, 113.5, 98.9, 57.0. Anal. calcd for $C_{10}H_8Cl_2N_2O_6S_2$ (387.22): C, 31.02; H, 2.08; N, 7.23; found: C, C, 30.97; H, 2.16; N, 7.13.

4.2.11.2. 5-Amino-3-[2-(chlorosulfonyl)-5-methoxyphenyl]-1,2-oxazole-4-sulfonyl chloride (11d)

Yield 1.896 mg (49%). Beige solid, m.p. 124-126 °C (diethyl ether). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.19 (d, $J_{3\text{-}4}$=8.6 Hz, 1H, 3-H_{Ar}), 7.14 (m, 2H, 4-H_{Ar}, 6-H_{Ar}), 6.27 (br s, 2H, NH_2), 3.94 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 169.1, 161.5, 160.4, 134.8, 132.1, 131.3, 118.2, 115.3, 99.9, 56.2. Anal. calcd for $C_{10}H_8Cl_2N_2O_6S_2$ (387.22): C, 31.02; H, 2.08; N, 7.23; found: C, C, 31.07; H, 2.10; N, 7.27.

4.2.12. General procedure for the synthesis of bis-sulfochlorides 11e-g.

The procedure is analogous to that for the preparation of compound **10a** but the heating in the first step continued at 90 ºC at 12 h.

4.2.12.1. 5-Amino-3-[5-(chlorosulfonyl)thiophen-2-yl]-1,2-oxazole-4-sulfonyl chloride (11e)

Yield 2,069 mg (57%). Beige solid, m.p. 116-118 °C (diethyl ether). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.99 (d, J=4.3 Hz, 1H, $H_{thiophene}$), 7.92 (d, J=4.3 Hz, 1H, $H_{thiophene}$), 6.40 (br s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 169.2, 152.2, 146.4, 135.5, 134.6, 131.0, 98.2. Anal. calcd for $C_7H_4Cl_2N_2O_5S_3$ (363.22): C, 23.15; H, 1.11; N, 7.71; found: C, 23.08; H, 1.17; N, 7.66.

5-Amino-3-[4-(chlorosulfonyl)-5-methylthiophen-2-yl]-1,2-oxazole-4-sulfonyl chloride (11f)

Yield 2,224 mg (59%). Beige solid, m.p. 113-116 °C (diethyl ether). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.23 (s, 1H, $H_{thiophene}$), 6.37 (br s, 2H, NH_2), 2.88 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 169.1, 152.1, 152.0, 139.1, 130.4, 123.6, 97.9, 14.8. Anal. calcd for $C_8H_6Cl_2N_2O_5S_3$ (377.24): C, 25.47; H, 1.60; N, 7.43; found: C, 25.42; H, 1.55; N, 7.47.

5-Amino-3-[5-(chlorosulfonyl)thiophen-3-yl]-1,2-oxazole-4-sulfonyl chloride (11g)

Yield 1,924 mg (53%). Beige solid, m.p. 97-99 °C (diethyl ether). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.63 (d, *J*=1.6 Hz, 1H, $H_{thiophene}$), 8.37 (d, *J*=1.6 Hz, 1H, $H_{thiophene}$), 6.32 (br s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 169.0, 152.8, 145.2, 137.0, 134.2, 126.6, 98.3. Anal. calcd for $C_7H_4Cl_2N_2O_5S_3$ (363.22): C, 23.15; H, 1.11; N, 7.71; found: C, 23.13; H, 1.09; N, 7.75.

4.2.13. General procedure for preparation of 3-methyl-4-aryl-1,2-oxazol-5-amines 13a-e.

To a solution of ketonitrile **12** (100 mmol) in ethanol (250 mL) hydroxylamine (100 mL) and 10% aqueous Na_2CO_3 (110 mL) were added. The mixture was stirred at r. t. until the full dissolution of the solids and then heated at reflux for 3 h. It was then cooled to r. t. and the volatiles were removed *in vacuo*. Water (10 mL) was added and the mixture was extracted with ethyl acetate (250 mL). The extract was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to provide the title compounds.

4.2.13.1. 3-Methyl-4-phenyl-1,2-oxazol-5-amine (13a)

Yield 16,182 mg (93%). Yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.35 (m, 4H, H_{Ar}), 7.22 (m, 1H, H_{Ar}), 6.70 (s, 2H, NH_2), 2.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 166.6, 158.9, 131.8, 129.1, 128.3, 126.0, 92.3, 11.6. LC MS (ESI): *m/z* $[M+H]^+$ 175. Anal. calcd for $C_{10}H_{10}N_2O$ (174.20): C, 68.95; H, 5.79; N, 16.08; found: C, 68.95; H, 5.79; N, 16.07.

4.2.13.2. 3-Methyl-4-(4-methylphenyl)-1,2-oxazol-5-amine (13b)

Yield 17,484 mg (91%). Yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.20 (m, 4H, H_{Ar}), 6.60 (s, 2H, NH_2), 2.31 (s, 3H, CH_3), 2.10 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 166.5, 158.9, 135.2, 129.7, 128.8, 128.3, 92.3, 21.2, 11.6. LC MS (ESI): *m/z* $[M+H]^+$ 189. Anal. calcd for $C_{11}H_{12}N_2O$ (188.23): C, 70.19; H, 6.43; N, 14.88; found: C, 69.99; H, 6.43; N, 14.82.

4.2.13.3. 4-(4-Methoxyphenyl)-3-methyl-1,2-oxazol-5-amine (13c)

Yield 17,425 mg (85%). Yellow solid, m.p.76 - 78 °C (AcOEt). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.22 (m, 2H, H_{Ar}), 6.95 (m, 2H, H_{Ar}), 6.53 (s, 2H), 3.75 (m, 3H), 2.06 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 166.4, 159.0, 157.8, 129.7, 123.9, 114.6, 92.1, 55.5, 11.5. LC MS (ESI): *m/z* $[M+H]^+$ 205. Anal. calcd for $C_{11}H_{12}N_2O_2$ (204.23): C, 64.69; H, 5.92; N, 13.72; found: C, 64.66; H, 5.93; N, 13.70.

4.2.13.4. 3-Methyl-4-(3-methylphenyl)-1,2-oxazol-5-amine (13d)

Yield 17,296 mg (92%).Yellow solid, m.p. 93 - 95 °C (AcOEt). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.27 (t, *J*=7.60 Hz, 2H, 5-H_{Ar}), 7.11 (m, 2H, H_{Ar}), 7.03 (d, *J*=7.60 Hz, 1H, H_{Ar}), 6.65 (s, 2H, NH_2), 2.32 (s, 3H, CH_3), 2.11 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 166.6, 158.9, 138.2, 131.7, 128.9, 126.7, 125.3, 92.3, 21.5, 11.6. LC MS (ESI): *m/z* $[M+H]^+$ 189. Anal. calcd for $C_{11}H_{12}N_2O$ (188.23): C, 70.19; H, 6.43; N, 14.88; found: C, 70.17; H, 6.43; N, 14.89.

4.2.13.5. 4-(3-Methoxyphenyl)-3-methyl-1,2-oxazol-5-amine (13e)

Yield 18,156 mg (89%). Yellow solid, m.p. 94 - 96 °C (AcOEt). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.29 (t, $J_{5\text{-}6}$=7.6 Hz, $J_{4\text{-}5}$=8.3 Hz, 1H, 5-H_{Ar}), 6.89 (d, $J_{5\text{-}6}$=7.6 Hz, 1H, 6-H_{Ar}), 6.85 (d, $J_{2\text{-}4}$=2.31 Hz, 1H, 2-H_{Ar}), 6.79 (dd, $J_{4\text{-}5}$=8.3 Hz, $J_{2\text{-}4}$=2.31 Hz, 1H, 4-H_{Ar}), 6.70 (s, 2H, NH_2), 3.79 (m, 3H, CH_3), 2.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 166.7, 159.9, 158.9, 133.2, 130.1, 120.5, 113.6, 111.8, 92.2, 55.4, 11.7. LC MS (ESI): *m/z* $[M+H]^+$ 205. Anal. calcd for $C_{11}H_{12}N_2O_2$ (204.23): C, 64.69; H, 5.92; N, 13.72; found: C, 64.65; H, 5.92; N, 13.75.

4.2.14. General procedure for the synthesis of *N*-(3-methyl-4-aryl-1,2-oxazol-5-yl)acetamides 14a-e.

To a solution of amine **13** (20 mmol) and trimethylamine (4.7 mL, 30 mmol) in acetonitrile (25 mL) acetyl chloride (2 mL, 30 mmol) was added dropwise. The mixture was stirred at r. t. for 12 h and then water (50 mL) was added. The mixture was extracted with ethyl acetate (50 mL), the extract was washed with water (50 mL), dried over anhydrous sodium sulfate, filtered and concentrated. The residue was crystallized from isopropyl alcohol to provide the title compound.

4.2.14.1. *N*-(3-Methyl-4-phenyl-1,2-oxazol-5-yl)acetamide (14a)

Yield 4,210 mg (97%). Yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.46 (br s, 1H, AcNH), 7.39 (m, 5H, H_{Ar}), 2.26 (s, 3H, CH_3), 2.01 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.7, 160.1, 157.7, 129.5, 129.2, 128.8, 128.0, 110.4, 23.1, 11.8. LC MS (ESI): *m/z* $[M+H]^+$ 217. Anal. calcd for $C_{12}H_{12}N_2O_2$ (216.24): C, 66.65; H, 5.59; N, 12.96; found: C, 66.62; H, 5.60; N, 12.97.

4.2.14.2. *N*-[3-Methyl-4-(4-methylphenyl)-1,2-oxazol-5-yl]acetamide (14b)

Yield 3,588 mg (78%). White solid, m.p. 115 - 118 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.42 (br s, 1H, AcNH), 7.26 (m, 4H, H_{Ar}), 2.33 (s, 3H, Ar-CH_3), 2.25 (s, 3H, CH_3), 2.00 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.7, 160.2, 157.5, 137.4, 129.7,

128.7, 126.5, 110.6, 23.1, 21.2, 11.7. LC MS (ESI): *m/z* $[M+H]^+$ 231. Anal. calcd for $C_{13}H_{14}N_2O_2$ (230.27): C, 67.81; H, 6.13; N, 12.17; found: C, 67.80; H, 6.13; N, 12.19.

4.2.14.3. *N*-[4-(4-Methoxyphenyl)-3-methyl-1,2-oxazol-5-yl]acetamide (14c)

Yield 4,379 mg (89%). Yellow solid, m.p. 101 - 103 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.38 (br s, 1H, AcNH), 7.31 (d, *J*=8.8 Hz, 2H, H_{Ar}), 7.01 (d, *J*=8.8 Hz, 2H, H_{Ar}), 3.78 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3), 2.00 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.7, 160.2, 159.2, 157.2, 130.1, 121.5, 114.6, 110.3, 55.5, 23.0, 11.7. LC MS (ESI): *m/z* $[M+H]^+$ 247. Anal. calcd for $C_{13}H_{14}N_2O_3$ (246.27): C, 63.40; H, 5.73; N, 11.38; found: C, 63.38; H, 5.73; N, 11.40.

4.2.14.4. *N*-[3-Methyl-4-(3-methylphenyl)-1,2-oxazol-5-yl]acetamide (14d)

Yield 3,772 mg (82%). Yellow solid, m.p. 85 - 87 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.43 (br s, 1H, AcNH), 7.33 (m, 1H, 5-H_{Ar}), 7.18 (m, 3H, H_{Ar}), 2.34 (s, 3H, Ar-CH_3), 2.26 (s, 3H, CH_3), 2.01 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.7, 160.1, 157.6, 138.4, 129.4, 129.1, 128.7, 125.9, 110.5, 23.07, 21.5, 11.7. LC MS (ESI): *m/z* $[M+H]^+$ 231. Anal. calcd for $C_{13}H_{14}N_2O_2$ (230.27): C, 67.81; H, 6.13; N, 12.17; found: C, 67.77; H, 6.13; N, 12.16.

4.2.14.5. *N*-[4-(3-Methoxyphenyl)-3-methyl-1,2-oxazol-5-yl]acetamide (14e)

Yield 4,133 mg (84%). White solid, m.p. 122 - 124 °C (*i*-PrOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.46 (br s, 1H, AcNH), 7.35 (m, 1H, 5-H_{Ar}), 6.95 (m, 3H, H_{Ar}), 3.77 (s, 3H, OCH_3), 2.27 (s, 3H, CH_3), 2.01 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.7, 160.1, 159.8, 157.7, 130.7, 130.2, 121.1, 114.4, 113.7, 110.4, 55.6, 23.1, 11.8. LC MS (ESI): *m/z* $[M+H]^+$ 247. Anal. calcd for $C_{13}H_{14}N_2O_3$ (246.27): C, 63.40; H, 5.73; N, 11.38; found: C, 63.32; H, 5.74; N, 11.41.

4.2.15. General procedure for the preparation of sulfonamides 15a-d.

Compound **10** (10 mmol) was added portionwise to a stirred and cooled mixture of chlorosulfonic acid (100 mmol) and thionyl chloride (10 mmol). The resulting mixture was heated at 60 °C over 4 h, cooled to r. t. and poured over crushed ice. The resulting mixture was extracted with ethyl acetate precipitate (75 mL). The extract was washed with 5% aqueous K_2CO_3 (75 mL), dried over anhydrous $CaCl_2$, filtered and concentrated *in vacuo*. The residue was briefly fractionated on silica gel using 25% ethyl acetate in hexanes and the fractions

containing the intermediate sulfonyl chloride were pooled and concentrated *in vacuo*. The residue was dissolved in acetone (40 mL) and the solution was treated with 25% aqueous ammonia (25 mmol). The resulting mixture was heated at 50 °C over 1 h, cooled and the volatiles were removed *in vacuo*. The residue was treated with ice-cold water (40 mL) and the mixture was stirred until a thick precipitate formed. The latter was filtered off and crystallized from acetone to provide the title compound.

4.2.15.1. *N*-[3-Methyl-4-(4-sulfamoylphenyl)-1,2-oxazol-5-yl]acetamide (15a)

Yield 1,652 mg (56%). White solid, m.p. 212-214 °C (acetone). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.68 (br s, 1H, AcNH), 7.86 (d, J=8.3 Hz, 2H, H_{Ar}), 7.56 (d, J=8.3 Hz, 2H, H_{Ar}), 7.41 (s, 2H, SO_2NH_2), 2.28 (s, 3H, CH_3), 2.02 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 160.1, 158.3, 143.3, 133.3, 129.2, 126.4, 108.4, 23.2, 11.7. LC MS (ESI): *m/z* $[M+H]^+$ 296. Anal. calcd for $C_{12}H_{13}N_3O_4S$ (295.32): C, 48.81; H, 4.44; N, 14.23; found: C, 48.78; H, 4.44; N, 14.21.

4.2.15.2. *N*-[3-Methyl-4-(4-methyl-3-sulfamoylphenyl)-1,2-oxazol-5-yl]acetamide (15b)

Yield 1,607 mg (52%). White solid, m.p. 173-175 °C (acetone). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.62 (br s, 1H, AcNH), 7.84 (s, 1H, 2-H_{Ar}), 7.47 (m, 4H, 5-H_{Ar}, 6-H_{Ar}, SO_2NH_2), 2.63 (s, 3H, Ar-CH_3), 2.28 (s, 3H, CH_3), 2.06 (m, 3H, $COCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.3, 160.0, 158.1, 142.9, 135.5, 133.1 131.8, 127.6, 127.1, 108.7, 23.1, 20.0, 11.7. LC MS (ESI): *m/z* $[M+H]^+$ 310. Anal. calcd for $C_{13}H_{15}N_3O_4S$ (309.35): C, 50.47; H, 4.89; N, 13.58; found: C, 50.44; H, 4.89; N, 13.56.

4.2.15.3. *N*-[4-(4-Methoxy-3-sulfamoylphenyl)-3-methyl-1,2-oxazol-5-yl]acetamide (15c)

Yield 1,987 mg (61%). White solid, m.p. 176-178 °C (acetone). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.50 (br s, 1H, AcNH), 7.70 (d, $J_{2\text{-}6}$=2.0 Hz, 1H, 2-H_{Ar}), 7.56 (dd, $J_{2\text{-}6}$=8.6 Hz, $J_{5\text{-}6}$=2.31 Hz, 1H, 6-H_{Ar}), 7.28 (d, $J_{5\text{-}6}$=8.6 Hz, 1H, 5-H_{Ar}), 7.10 (s, 2H, SO_2NH_2), 3.94 (s, 3H, OCH_3), 2.25 (s, 3H, CH_3), 2.00 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.5, 160.1, 157.8, 155.8, 134.0, 131.9, 127.8, 121.2, 113.5, 109.0, 56.7, 23.1, 11.7. LC MS (ESI): *m/z* $[M+H]^+$ 326. Anal. calcd for $C_{13}H_{15}N_3O_5S$ (325.35): C, 47.99; H, 4.65; N, 12.92; found: C, 47.94; H, 4.65; N, 12.92.

4.2.15.4. *N*-[3-Methyl-4-(3-methyl-4-sulfamoylphenyl)-1,2-oxazol-5-yl]acetamide (15d)

Yield 1,452 mg (47%). Gray solid, m.p. 215-217 °C (acetone). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.59 (br s, 1H, AcNH), 7.88 (d, $J_{5\text{-}6}$=7.93 Hz, 1H, 5-H_{Ar}), 7.33 (m, 4H, 2-H_{Ar}, 6-H_{Ar},

SO_2NH_2), 2.62 (s, 3H, Ar-CH_3), 2.28 (s, 3H, CH_3), 2.02 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 169.2, 160.1, 158.3, 141.5, 136.7, 133.3, 132.3, 127.9, 126.3, 108.4, 23.2, 20.3, 11.7. LC MS (ESI): *m/z* $[M+H]^+$ 310. Anal. calcd for $C_{13}H_{15}N_3O_4S$ (309.35): C, 50.47; H, 4.89; N, 13.58; found: C, 50.45; H, 4.89; N, 13.59.

4.2.16. *N*-[4-(5-Methoxy-2-sulfamoylphenyl)-3-methyl-1,2-oxazol-5-yl]acetamide 15e and 1-(8-methoxy-1-methyl-5,5-dioxido-4H-[1,2]oxazolo[5,4-c][1,2]benzothiazin-4-yl)ethanone 16.

Compound **14e** (10 mmol) was added portionwise to a stirred and cooled mixture of chlorosulfonic acid (100 mmol) and thionyl chloride (10 mmol). The resulting mixture was heated at 60 °C over 4 h, cooled to r. t. and poured over crushed ice. The resulting mixture was extracted with ethyl acetate (100 mL). The extract was washed with 5% aqueous K_2CO_3 (75 mL), dried over anhydrous $CaCl_2$, filtered and concentrated *in vacuo*. The residue was briefly fractionated on silica gel using 25% ethyl acetate in hexanes and the fractions containing the intermediate sulfonyl chloride were pooled and concentrated *in vacuo*. The residue was dissolved in acetone (40 mL) and the solution was treated with 25% aqueous ammonia (25 mmol). The resulting mixture was heated at 50 °C over 1 h, cooled and the volatiles were removed *in vacuo*. The residue was treated with ice-cold water (40 mL) and the mixture was stirred until a thick precipitate formed. The latter was filtered off and chromatographed on silica gel using 85% ethyl acetate un hexanes as eluent to provide **15e** and **16**.

4.2.16.1. N-[4-(5-Methoxy-2-sulfamoylphenyl)-3-methyl-1,2-oxazol-5-yl]acetamide (15e)

Yield 880 mg (27%). White solid, m.p. 162-164 °C (AcOEt). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 10.37 (br s, 1H, AcNH), 7.90 (d, $J_{3\text{-}4}$=8.8 Hz, 1H, 3-H_{Ar}), 7.12 (dd, $J_{3\text{-}4}$=8.8 Hz, $J_{4\text{-}6}$=2.0 Hz, 1H, 4-H_{Ar}), 6.98 (br s, 2H, SO_2NH_2), 6.83 (d, $J_{4\text{-}6}$=2.0 Hz, 1H, 6-H_{Ar}), 3.83 (s, 3H, OCH_3), 1.97 (s, 3H, CH_3), 1.92 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 168.8, 161.4, 161.3, 157.7, 136.2, 129.9, 129.5, 119.5, 113.7, 106.7, 56.1, 23.1, 11.3. LC MS (ESI): *m/z* $[M+H]^+$ 326. Anal. calcd for $C_{13}H_{15}N_3O_5S$ (325.35): C, 47.99; H, 4.65; N, 12.92; found: C, 47.96; H, 4.65; N, 12.95.

4.2.16.2. 1-(8-Methoxy-1-methyl-5,5-dioxido-4*H*-[1,2]oxazolo[5,4-*c*][1,2]benzothiazin-4-yl)ethanone (16)

Yield 739 mg (24%). White solid, m.p. 155-157 °C (AcOEt). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.58 (d, $J_{6\text{-}7}$=8.6 Hz, 1H, 6-H_{Ar}), 6.90 (d, $J_{7\text{-}9}$=2.0 Hz, 1H, 9-H_{Ar}), 6.78 (dd, $J_{6\text{-}7}$=8.6, $J_{7\text{-}9}$=2.0 Hz, 1H, 7-H_{Ar}), 3.83 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3), 1.91 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 172.4, 171.0, 160.7, 155.8, 131.6, 125.6, 121.6, 110.5, 104.3, 87.6, 55.6,

21.5, 13.3. LC MS (ESI): *m/z* $[M+H]^+$ 309. Anal. calcd for $C_{13}H_{12}N_2O_5S$ (308.31): C, 47.99; H, 3.72; N, 8.61; found: C, 47.98; H, 3.72; N, 8.64.

4.2.17. 2-[5-(Acetylamino)-3-methyl-1,2-oxazol-4-yl]-4-methoxybenzenesulfonyl chloride (17).

Compound **14e** (10 mmol) was added portionwise to a stirred and cooled mixture of chlorosulfonic acid (100 mmol) and thionyl chloride (10 mmol). The resulting mixture was heated at 60 °C over 4 h, cooled to r. t. and poured over crushed ice. The resulting mixture was extracted with ethyl acetate (100 mL). The extract was washed with 5% aqueous K_2CO_3 (75 mL), dried over anhydrous $CaCl_2$, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel using 25% ethyl acetate in hexanes to provide the title compound.

Yield 3,068 mg (89%). Brown solid, m.p. 155-157 °C (acetone). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.14 (d, $J_{5\text{-}6}$=9.0 Hz, 1H, 6-H_{Ar}), 7.95 (br s, 1H, AcNH), 7.08 (dd, $J_{5\text{-}6}$=9.0 Hz, $J_{3\text{-}5}$=2.3 Hz, 1H, 5-H_{Ar}), 6.94 (d, $J_{3\text{-}5}$=2.3 Hz, 1H, 3-H_{Ar}), 3.95 (s, 3H, OCH_3), 2.13 (s, 3H, CH_3), 2.12 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 164.4, 160.8, 157.6, 135.9, 131.7, 120.0, 113.900, 102.9, 56.1, 23.1, 11.1. Anal. calcd for $C_{13}H_{13}ClN_2O_5S$ (344.78): C, 45.29; H, 3.80; N, 8.13; found: C, 45.25; H, 3.80; N, 8.14.

4.2.18. 1-(8-Methoxy-1-methyl-5,5-dioxido-4H-[1,2]oxazolo[5,4-c][1,2]benzothiazin-4-yl)ethanone (16).

Compound **17** (5 mmol) was dissolved in acetone (20 mL) and trimethylamine (7 mmol) was added. The mixture was stirred at r. t. for 24 h and concentrated *in vacuo*. Water (25 mL) was added and the precipitate formed was filtered off, washed with water and air-dried to provide the title compound in 46% yield, with analytical data identical to those presented above (4.2.16.2).

4.3 Carbonic anhydrase inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity.[17] Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Tris (pH 8.3) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10-100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each

inhibitor at least six traces of the initial 5-10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.005 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng-Prusoff equation, as reported earlier, and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house.[18-21]

4.4 In Silico docking

The crystal structure of *h*CA II (pdb code 2AW1[12]) was taken from the Protein Data Bank.[13] After adding hydrogen atoms and removing the complexed ligand, the protein was minimized using Amber 11 software and parm03 force field at 300 K. The protein was placed in a rectangular parallelepiped water box, an explicit solvent model for water, TIP3P, was used and the complex was solvated with a 20 Å water cap. Sodium ions were added as counter ions to neutralize the system. Two steps of minimization were then carried out; in the first stage, we kept the protein fixed with a position restraint of 500 kcal/mol•Å^2 and we solely minimized the positions of the water molecules. In the second stage, we minimized the entire system through 5000 steps of steepest descent followed by conjugate gradient until a convergence of 0.05 kcal/Å•mol. The region of interest used by the docking program GOLD version 5.1 was defined in order to contain the residues within 15 Å from the original position of the ligand in the X-ray structure. Metal coordination in GOLD is modeled as 'pseudohydrogen bonding' in which metals can be considered to bind to H-bond acceptors and the metal will compete with H-bond donors for interaction. The 'allow early termination' option was deactivated, while the possibility for the ligand to flip ring corners was activated. The remaining GOLD default parameters were used, and the ligands were submitted to 30 genetic algorithm runs. The docking analysis was carried out using the ChemScore fitness function imposing the formation of an H bond between the ligands and the hydroxy group of T198.[14, 23] Cluster analysis was performed on the results using an rmsd tolerance of 2.0 Å and the best docked conformation was taken into account.

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Supplementary data

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

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