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Inhibition of human mitochondrial carbonic anhydrases VA and VB with *para*-(4-phenyltriazole-1-yl)-benzenesulfonamide derivatives

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ABSTRACT

A library of 10 novel benzenesulfonamides containing triazole-tethered phenyl 'tail' moieties were synthesized by a Cu(I) catalyzed 1,3-dipolar cycloaddition reaction (DCR) (i.e., click chemistry) between 4-azido benzenesulfonamide and a panel of variously substituted phenyl acetylenes. These compounds were very effective inhibitors (low nanomolar) of the human mitochondrial carbonic anhydrase isozymes VA and VB. Mitochondrial carbonic anhydrases are potential targets for anti-obesity therapies, acting to reduce lipogenesis through a novel mechanism of action. The inhibitors reported here should prove valuable as lead compounds to further investigate the potential of CA inhibition for this novel therapeutic application.

An observed side effect of the widely used antiepileptic drugs Zonisamide (**ZNS**) and Topiramate (**TPM**) is a significant loss of body weight when administered to obese patients. These compounds contain a sulfonamide ($-SO_2NH_2$ in **ZNS**) or sulfamate ($-OSO_2NH_2$ in **TPM**) moiety, (Fig. 1).[1] These functional groups are both well characterized zinc binding functions (ZBFs) that have prominent representation amongst known carbonic anhydrase (CA) inhibitors, wherein a ZBF is a critical component of the CA inhibition pharmacophore model. These facts have directed Supuran and co-workers to commence an investigation of the possibility of a connection between CA inhibition by these compounds and weight loss, in particular inhibition of cytosolic CA II and mitochondrial CA VA and VB. These studies have shown that both **ZNS** and **TPM** are good inhibitors of CA II, VA and VB.[2] The X-ray crystal structures of both these drugs complexed with the human CA II isozyme have also been reported.[2] These 3-dimensional enzyme-inhibitor structures reveal the molecular interactions that readily account for the good inhibition observed for these compounds at CA II.

The carbonic anhydrases (CA, EC 4.2.1.1) are a family of zinc(II) metalloenzymes that catalyze the reversible hydration of carbon dioxide (CO_2) to give bicarbonate (HCO_3^-) and a proton (H^+).

In humans 15 different CA isozymes and CA related proteins (CARP) have been identified and characterized. These isozymes exhibit variable kinetic parameters, subcellular localization, expression levels and tissue/organ localization.[1a,3] Several hCA isozymes are cytosolic (I–III, VII and XIII), four are membrane bound or transmembrane proteins (IV, IX, XII and XIV), one is secreted into the saliva and milk (VI) and two are mitochondrial (VA and VB). The activity of hCAs modulates cellular processes associated with respiration and transport of $CO_2HCO_3^-$, provision of HCO_3^- as a substrate for biosynthetic pathways, pH regulation, electrolyte and fluid secretion, as well as bone resorption and calcification.[1a,3] At least 25 clinically used drugs have significant hCA inhibition activity, and the modulation of specific hCAs by inhibition is an avenue for the treatment of a wide range of acquired and inherited diseases.[1a]

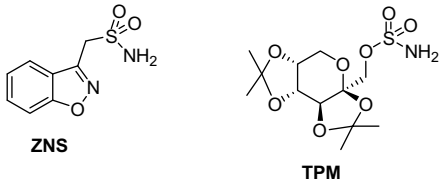

Figure 1. Zonisamide (**ZNS**) and Topiramate (**TPM**).

Mitochondria are located in the cytoplasm of human cells wherein they play significant roles in a number of cellular functions. The dysfunction of mitochondria contributes to a range of human diseases including obesity.[4] The discovery and characterization of mitochondrial hCA isozymes VA and VB[5] led to the hypothesis that the manipulation of mitochondrial CA activity

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may provide an avenue for the development of anti-obesity therapeutics with a novel mechanism of action.[1a] CA isozymes are critical to fatty-acid biosynthesis. The mitochondrial CA isozymes provide the necessary mitochondrial supply of HCO_3^- substrate (the mitochondrial membrane is impermeable to HCO_3^-) for biosynthetic processes dependant upon the mitochondrial enzyme pyruvate carboxylase. Eventually this pathway leads to the formation of citrate from pyruvate. Citrate is translocated from mitochondria to the cytoplasm, then, through a biosynthetic pathway again requiring HCO_3^- substrate (provided by cytosolic CA II), leads to de novo lipogenesis.[1a] Isozymes hCA VA and VB have different tissue distribution profiles, with isozyme VA found predominantly in the liver, whilst isozyme VB has a much wider tissue distribution. The physiological impact of the tissue distribution profile differences is as yet unknown. As well, the pharmacological explanation for the weight loss observed with **ZNS** and **TPM** is still under investigation. The results so far available from a number of studies underpin a working hypothesis that inhibition of mitochondrial CA isozymes, possibly in conjunction with that of the ubiquitous cytosolic isozyme CA II, may represent targets for novel anti-obesity therapies, acting to reduce lipogenesis through inhibition of these CAs.[1c,6]

The Cu(I) catalyzed ligation of a terminal acetylene to an organic azide to form regiospecifically a 1,4-disubstituted-1,2,3-triazole, that is, 'click chemistry', has become a valued synthetic tool in medicinal chemistry.[7] Rather than being used as a passive linker between two respective fragments of structural space, the 1,2,3-triazole moiety can actively contribute to an enzyme-inhibitor pharmacophore model. The triazole has moderate dipole character, hydrogen bonding capability, rigidity and stability in the in vivo environment.[8] We have recently demonstrated the versatility of the click chemistry methodology as an expedient method of generating both glycoconjugate and metallocene-based hCA inhibitors.[9,10] These compounds contain a benzenesulfonamide moiety linked via a 1,2,3-triazole to either a sugar or a metallocene 'tail' moiety, and have so far been evaluated for their inhibition in vitro of hCA I, II and IX; in some cases also for transmembrane CAs XII and XIV (Fig. 2).[9d]

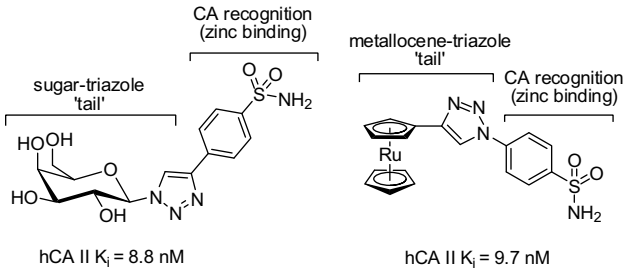
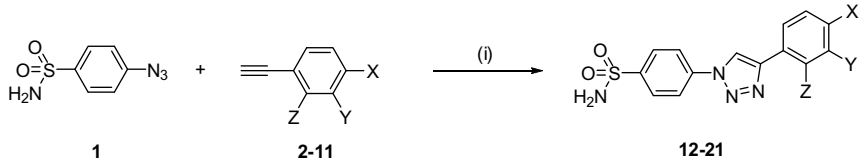


Figure 2. Glycoconjugate and metallocene-based hCA inhibitors.[9,10]

The impressive potency and selectivity of many of these inhibitors has prompted us to investigate this efficient chemistry to target small molecule CA inhibitors towards the mitochondrial CA isozymes. The compounds described here have been designed with a lipophilic tail moiety. The lipophilicity should enhance the membrane permeability of these new compounds, an important physicochemical property owing to the intracellular location of the target CA isozymes.

A library of 10 benzenesulfonamides (**12–21**) containing triazole-tethered, phenyl tail groups were synthesized by Cu(I) catalyzed 1,3-DCR of the azido benzenesulfonamide fragment **1** with a panel of variously substituted phenyl acetylenes (**2**–**11**) (Scheme 1).[11] The members of this library represent a novel class of compounds with no reported examples of 1,4-disubstituted-1,2,3-triazoles wherein the triazole *N*-1 substituent is a benzenesulfonamide and the triazole *C*-4 substituent derived from a phenyl acetylene fragment. The variously substituted phenyl acetylene panel encompassed commercially available compounds as well as 4-ethynyl benzenesulfonamide (Scheme 1).

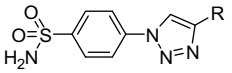
The inhibition data of the parent 4-azido benzenesulfonamide **1**; new sulfonamides **12**–**21**; and clinically used compounds **ZNS** and **TPM** against the human cytosolic isozymes CA I, CA II, and the mitochondrial isozymes CA VA and CA VB are presented in Table 1. Inhibition constants have been determined using the CA cat-

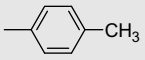
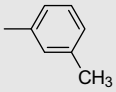
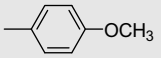
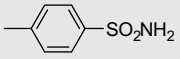
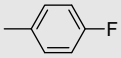
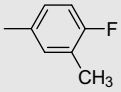
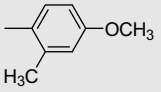


	X	Y	Z
2,12	H	H	H
3,13	CH_3	H	H
4,14	H	CH_3	H
5,15	H	H	CH_3
6,16	OCH3	H	H
7,17	SO_2NH_2	H	H
8,18	F	H	H
9, 19	F	CH_3	H
10, 20	OCH_3	H	CH_3
11, 21	H	H	CF_3

Scheme 1. Synthesis of benzenesulfonamides with triazole-tethered, variously substituted phenyl tail groups. Reagents and conditions: (i) azide **1** (0.2–0.5 M), arylacetylene **2**–**11** (1 equiv), $CuSO_4{\cdot}5H_2O$ (0.1–0.2 equiv), sodium ascorbate (0.2–0.4 equiv), 1:1 *t*-BuOH/H_2O, 45 °C, 1-h overnight, 55–88%.[11]

Table 1
Inhibition data for **1**, new sulfonamides **12**–**21** and standard inhibitors **ZNS** and **TPM** against human isozymes hCA I, II, VA and VB (h = human) [12]



Compound	R	K_i (nM)[a]			
		CA I[b]	CA II[b]	CA VA[c]	CA VB[c]
ZNS	–	56	35	20	6033
TPM	–	250	10	63	30
1	–	3900	47.0	56.0	55.8
12		5100	7.9	17.8	10.6
13		2100	18.6	12.8	10.6
14		4000	33.8	19.6	54.2
15		3000	7.7	9.3	11.4
16		2200	8.4	10.6	51.8
17		100	8.1	15.1	11.9
18		4600	40.3	14.2	11.2
19		6500	10.7	19.1	52.3
20		5800	11.7	16.9	10.5
21		5500	8.3	17.1	12.9

[a] Errors in the range of ±5–10% of the reported value, from three determinations.
[b] Human (cloned) isozymes, by the CO_2 hydration method.
[c] Human recombinant full length isozyme, by the CO_2 hydration method.[12]

alyzed CO_2 hydration assay, which measures the absorbance change of a pH indicator and so is directly responsive to the endogenous CA catalyzed reaction.

ZNS inhibits isozymes I, II and VA with nanomolar K_i values, whilst it inhibits more weakly isozyme VB (K_i = 6033 nM). **TPM** inhibits all isozymes efficiently, with a K_i of 10 nM for isozyme II, 63 nM for isozyme VA and 30 nM for isozyme VB.

The parent azido benzenesulfonamide fragment (**1**) had similar good efficacy against hCA II, VA and VB (K_is of 47.0, 56.0 and 55.8 nM, respectively). This azido compound was also a much weaker inhibitor of hCA I (K_i = 3900 nM). The inhibition profile for **1** compared to **ZNS** and **TPM** demonstrates that the classical CA pharmacophore (i.e., an Ar–SO_2NH_2) is effective at targeting the CA isozymes of interest in development of potential anti-obesity therapies.

At the physiologically dominant hCA I the variously substituted phenyl moieties had minimal influence on enzyme inhibition characteristics. Nine of the ten derivatives had micromolar K_is in a compact grouping with a value similar to that of the azido parent **1**: K_is ranged from 2200 to 6500 nM, the K_i of **1** was 3900 nM. The only outlier in this library was the bis-sulfonamide **17**, with two ZBFs and an inhibition constant an order of magnitude better than for all other compounds (K_i of 100 nM). This compound can bind to the hCA active site zinc cation in two possible orientations, the orientation of the triazole moiety being reversed in the two binding modes. Thus, in the 1,4-diaryl-1,2,3-triazole, the triazole orientation appears to have a significant impact on hCA I binding that is worthy of further investigation, and we intend to follow this up in a future report.

At isozyme hCA II the parent azido scaffold **1** had a K_i of 47 nM. Seven of the phenyl triazoles (compounds **12**, **15**–**17**, **19**, **20**, **21**) were potent low nanomolar inhibitors of hCA II (K_is ranged from 7.7 to 11.7 nM). Compound **18** (*para*-fluoro derivative) had a K_i value similar to that of the azido parent **1** (K_is 40.3 nM), whilst those for compounds **13** and **14** were in between (K_is 18.6 and 33.8 nM, respectively). All triazole compounds were selective for hCA II over hCA I, typically three orders of magnitude (except the bis-sulfonamide **17**, which is two orders of magnitude selective). These results demonstrate, as did our earlier work, that the 'tail' approach for hCA inhibitors can readily discriminate selectivity between the physiologically dominant hCA isozymes I and II.[9]

At the mitochondrial CA isozyme VA the parent azido fragment **1** had a K_i of 56.0 nM. All aryl triazole inhibitors were stronger inhibitors of hCA VA than **ZNS**, **TPM** or **1**, with tightly clustered low nanomolar K_is that ranged from 9.3 to 19.6 nM. The new compounds are typically three orders of magnitude more potent inhibitors than that at cytosolic hCA I, and of similar potency for inhibition of hCA II, with the exception of compounds **14** (*meta*-methyl derivative) and **18** (*para*-fluoro derivative) which were better inhibitors of hCA VA than hCA II. At hCA VB the parent azido scaffold **1** had a K_i of 55.8 nM, equipotent with that at hCA VA. At this isozyme the aryl triazoles had two distinct inhibition profiles—strongest inhibitors were compounds **12**, **13**, **15**, **17**, **18**, **20**, **21** (K_i range of 10.5–12.9 nM) and intermediate potency inhibitors were compounds **14**, **16**, **19** (K_i range 51.8–54.2 nM). All compounds were much better inhibitors than **ZNS** (K_i = 6033 nM), whilst the strongest inhibitors were ~3-fold better inhibitors than **TPM**. As hCA VB has a wider tissue distribution this finding may prove valuable when discriminating the mitochondrial hCA isozymes is desirable.

Typically the chemical nature of the substituent on the aryl tail moiety had less impact on the CA inhibition constants than did the position of the substituent (*ortho*, *meta* or *para*). Compounds **12**–**15** allow the influence of a methyl group substitution to be assessed in the *ortho*, *meta* and *para* positions of the phenyl tail. Compound **12** (where R = phenyl, that is, no methyl group substitution) has similar K_is to the *para*- (**13**) and *ortho*- (**15**) methyl substituted phenyl groups whilst the *meta* analogue (**14**) is weaker at hCA II, VA and VB. Similarly, the other *meta* modified derivative (**19**) was also in the weaker hCA VB inhibitor group described above. These K_i profiles indicate that *meta* substitution is in general detrimental to hCA inhibition whilst *ortho* and *para* substitution patterns do not effect inhibition constants greatly at either hCA II, VA or VB. There is one exception to this general SAR: the *para*-methoxy compound (**16**) had a weaker K_i at hCA VB than other *para* substituted compounds.

In conclusion, an inhibition study of the human cytosolic isozymes CA I and II, and the mitochondrial isozymes CA and VB with a novel library of benzenesulfonamides generated by click chemistry is presented. These compounds were low to mid nanomolar inhibitors of hCA isozymes II, VA and VB, and weaker micromolar inhibitors of hCA I. The inhibition profiles for these novel derivatives should prove valuable lead work in the discovery of isozyme

selective CA inhibitors targeting the mitochondrial hCA isozymes VA and VB with potential use as anti-obesity agents.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.010.

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11. General procedure for synthesis of phenyl triazole benzenesulfonamides **(12–21)**. A mixture of azide (**1**, 1.0 equiv) and acetylene (**2**–**11**, 1.0 equiv) was suspended in *tert*-butyl alcohol and distilled water (1:1, 0.2–0.5 M final concentration). A solution of sodium ascorbate (0.2 equiv) in water, followed by a solution of $CuSO_4{\cdot}5H_2O$ (0.1 equiv) in water was successively added. The bright yellow heterogeneous mixture was stirred vigorously at 40 °C until TLC indicated reaction completion. The mixture was evaporated under reduced pressure and the resulting residue was purified by flash chromatography. Full synthesis details and spectroscopic data for **12–21** can be found in the Supplementary Material.
12. The CO_2 hydration assay procedure is described in the Supplementary Material.