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Inhibition of carbonic anhydrase isozymes I, II and IX with benzenesulfonamides containing an organometallic moiety

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Abstract—A novel series of benzenesulfonamides that contain ferrocenyl or ruthenocenyl moieties were synthesized and investigated for their ability to inhibit the enzymatic activity of physiologically relevant carbonic anhydrase (CA) isozymes: hCA I, II and tumour-associated IX (h = human). This manuscript describes the regioselective synthesis of both the 1,4- and 1,5-disubstituted-1,2,3-triazole benzenesulfonamides from ethynylmetallocene substrates. This is the first report describing the covalent attachment of organometallic moieties to the arylsulfonamide ($ArSO_2NH_2$) CA recognition pharmacophore. At hCA I these metallocene derivatives were either nanomolar or low micromolar inhibitors, while against hCA II and IX inhibition in the range of 9.7–80 nM and 10.3–85 nM, respectively, was observed. The ruthenocenyl derivatives gave superior CA inhibition compared to the ferrocenyl compounds across all three CA isozymes. These compounds constitute a new organometallic class of CA inhibitors with promising biological activity.

Carbonic anhydrases (CAs, EC 4.2.1.1) are abundant zinc metalloenzymes that are present in a diversity of organisms including higher vertebrates, green plants, algae, bacteria and archaea.[1,2] So far 16 isozymes have been characterized in humans (designated hCA) – 12 of these possess catalytic activity for the reversible hydration of carbon dioxide to bicarbonate anion and a proton: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$.[2,3] Different isozymes exhibit variable enzyme kinetics, tissue distribution, expression levels and subcellular locations and these differences underpin a general drug discovery strategy that aims to develop selective inhibitors for a range of specific therapeutic applications.[2,4] Recently, a role for CA inhibition as an anticancer therapy has been identified owing to the overexpression of some CA isozymes (CA IX and CA XII) in cancer cells. CA IX is of particular interest owing to its ectopic expression in carcinomas derived from cervix, uteri, kidney, lung, oesophagus, breast, colon and so on, contrasting with minimal expression in normal tissues. Inhibition of CA IX may constitute a novel approach for the treatment of cancers in which it is overexpressed and this is now an area of research under intensive investigation.[4]

An aromatic or heteroaromatic sulfonamide moiety ($ArSO_2NH_2$) is the classical recognition fragment necessary for small molecules to bind the active site of CA.[1–3] The deprotonated sulfonamide moiety ($ArSO_2NH^-$) coordinates to the CA active site Zn^{2+} and inhibits the enzyme by impeding the normal catalytic cycle. Arylsulfonamides are a reliable CA 'anchor' fragment upon which tethering 'tail' groups is a proven means to optimize physicochemical properties of inhibitors.[3] Clinically used CA inhibitors containing the $ArSO_2NH_2$ motif include acetazolamide (AZA), methazolamide (MZA), ethoxazolamide (EZA), dichlorophenamide (DCP), brinzolamide (BRZ) and dorzolamide (DZA). Metal-based sulfonamides that involve the coordination of a cationic metal to the heteroatoms of heterocyclic sulfonamides such as AZA and MZA have been synthesized and investigated as inhibitors of CA by others.[5,6] These metal complexes are typically 10- to 100-fold more potent than the parent sulfonamides and are among the

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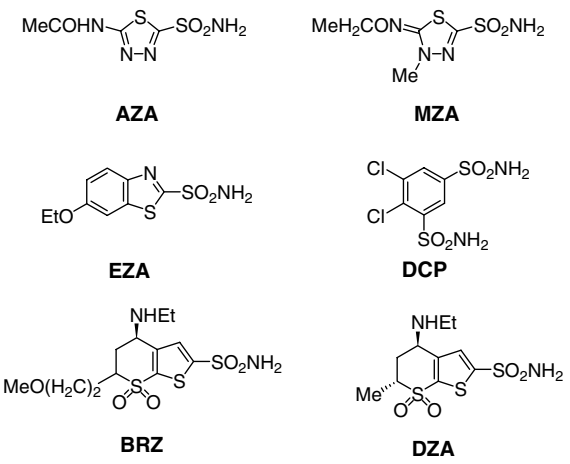
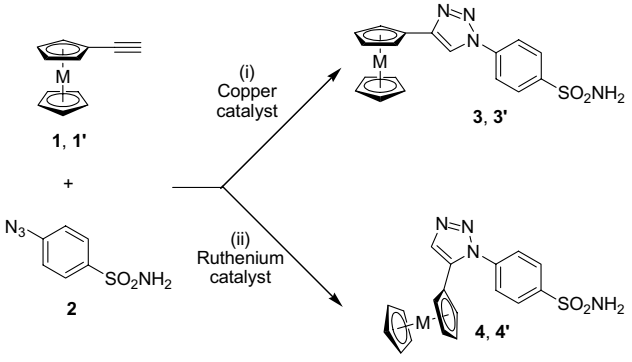
most potent CA inhibitors reported (picomolar inhibition has been observed).[6] Presently, there are no known reports of the covalent attachment of organometallic moieties to the aromatic sulfonamide CA pharmacophore using standard organic synthetic methodology.



Ferrocene and ruthenocene belong to the metallocene family of organometallic compounds, wherein a metal is sandwiched between two cyclopentadienyl (Cp) ligands. The electronic structure of these metallocenes renders them aromatic and they undergo reactions typical of organic aromatic compounds. Attaching an organometallic fragment to a known organic drug is becoming recognized as a strategy to endow the drug with new and improved therapeutic properties.[7,8] The incorporation of the organometallic ferrocenyl fragment into clinically used organic scaffolds has been extensively investigated with the breast cancer drug tamoxifen (to generate ferrocifens) and the antimalarial agent chloroquine (to generate ferroquine).[7–10] The results from these investigations are encouraging and importantly are attributable to the metallocene component of the conjugate. Ferroquine is effective against chloroquine resistant malaria parasites and is in late Phase II clinical trials, while the ferrocifens have antiproliferative effects against both hormone-dependent and non-hormone-dependent breast cancer tumours and are poised to commence clinical trials.[7–11] The ferrocenyl moiety is stable in both air and aqueous environments rendering this fragment compatible with drug lead discovery. In a therapeutic context ruthenocene is less well studied, it differs from ferrocene with regard to the orientation of the Cp rings. In ferrocene there is a small energy barrier separating the staggered and eclipsed rotational orientations of the two Cp rings, whilst ruthenocene exhibits only the lower energy eclipsed conformation.[12] Furthermore, since ruthenium is located below iron in group 8 of the periodic table, the ionization potential of metallocene–organic conjugates should be considerably higher when ferrocene is replaced for ruthenocene.[13] If inhibitor oxidation plays a role in the inhibition of CA, it is expected that the ruthenium derivatives would be more effective inhibitors than their ferrocene counterparts.

The Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction (1,3-DCR; 'click chemistry') as popularized by Sharpless and Meldal[14,15] allows the selective synthesis of the 1,4-disubstituted-1,2,3-triazole regioisomer from azide and acetylene substrates. Without catalyst a mixture of triazoles is obtained by reaction at elevated temperatures, however regioselective synthesis of the other possible product of 1,3-DCR, that is, the 1,5-disubstituted-1,2,3-triazole, has recently become possible using ruthenium complexes such as $[Cp^*RuCl(PPh_3)_2]$ as catalyst.[16,17] Ruthenium catalysts have been less accessible than Cu(I) sources, consequently this reaction is so far much less investigated compared to the Cu(I)-catalyzed variant – significantly there are no medicinal chemistry applications reported (the $[Cp^*RuCl(PPh_3)_2]$ catalyst has only recently become commercially available). We have previously demonstrated the versatility of the 1,3-DCR to generate 1,4-disubstituted-1,2,3-triazole glycoconjugate benzenesulfonamides with varied sugar tail fragments.[18–20] This versatility has encouraged us to further explore our 'click-tailing' strategy to append metallocene tails onto the CA $ArSO_2NH_2$ recognition fragment to generate metallocene-based CA inhibitors. This manuscript describes the regioselective synthesis for both 1,4- and 1,5-disubstituted-1,2,3-triazole benzenesulfonamides from ethynylmetallocene substrates. The target metallocene derivatives **3**, **4**, **3′** and **4′** were synthesized by the 1,3-DCR of ethynylferrocene (**1**, commercially available) or ethynylruthenocene (**1′**, synthesized by literature methods)[21] and 4-azidobenzenesulfonamide (**2**), Scheme 1.[22] The 1,4-disubstituted-1,2,3-triazole regioisomers **3** and **3′** were prepared by the Cu(I)-catalyzed 1,3-DCR while the 1,5-disubstituted-1,2,3-triazole regioisomers **4** and **4′** were prepared by the ruthenium-catalyzed 1,3-DCR using $[Cp^*RuCl(PPh_3)_2]$[23] as catalyst. Synthesis details are described fully in Supplementary data. All compounds were investigated for their ability to inhibit the enzymatic activity of CA isozymes: hCA I, II and IX.

The singlet resonance in the ^1H NMR spectrum attributable to the aryl proton of the triazole moiety is evidence for the formation of the click product. This proton res-



Scheme 1. M = Fe (**1**,**3**,**4**), M = Ru (**1′**,**3′**,**4′**). Reagents and conditions: (i) metallocene (0.05–0.113 mM), azide (1 equiv), sodium ascorbate (0.4 equiv), $CuSO_4{\cdot}5H_2O$ (0.2 equiv), 1:1 *t*-BuOH/H_2O, 40 °C, 24 h, 21–50%; (ii) metallocene (0.04–0.13 mM), azide (1 equiv), $[Cp^*RuCl(PPh_3)_2]$ (6 mol%), toluene, 110 °C, Argon, 58–96%.

onates at δ 9.00 and 8.88 ppm for the 1,4-disubstituted triazoles **3** and **3′**. This is ~1 ppm downfield of the corresponding resonance in the 1,5-disubstituted analogues **4** and **4′** (δ 8.11 and 7.90 ppm, respectively). ^{13}C NMR spectroscopy revealed the resonances expected for the triazole C_{ipso} and CH carbons. For the 1,4-disubstituted triazole **3** these carbons resonate at δ 147.9 and 119.2, respectively, while for the 1,5-disubstituted triazole **4** these carbons resonate at δ 138.0 and 133.2, respectively. These chemical shifts are fully consistent with those reported previously for 1,4- and 1,5-disubstituted 1,2,3-triazoles.[24,25] The regiochemistry assignment of the 1,4-disubstituted triazole was further investigated by 1D NOE experiments carried out on the ferrocene derivative **3**. Selective saturation of the triazole proton resonance (δ 9.00 ppm) resulted in strong NOE enhancements of the arylbenzene protons, $H_{AA'}$, and the ferrocenyl protons, $H_{\alpha\alpha'}$, with a weaker NOE enhancement of ferrocenyl protons, $H_{\beta\beta'}$, Figure 1a. Reversal of the experiment by selective saturation of $H_{\alpha\alpha'}$ on the monosubstituted Cp ring (δ 4.79–4.80 ppm) was then conducted. This resulted in the NOE enhancement of the remaining ferrocenyl protons and the triazole proton, with no NOE enhancement observed for the aryl protons $H_{AA'}$, Figure 1b. These results are fully consistent with the central positioning of the triazole proton – between the ferrocenyl and arylsulfonamide moieties as in the 1,4-regioisomer. Related experiments on the ferrocene 1,5-regioisomer **4** were unable to provide unambiguous confirmation of the regiochemistry assignment owing to near co-incident chemical shifts of the triazole and aryl protons – prohibiting the selective saturation of the triazole proton.

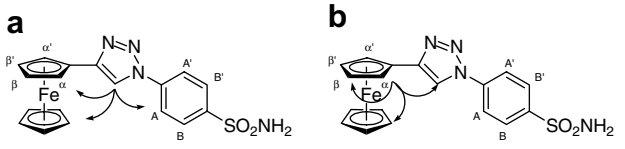


Figure 1. Observed 1D NOE enhancements of the ferrocene derivative **3**. (a) With selective saturation of the triazole proton; (b) with selective saturation of $H_{\alpha\alpha'}$.

Data for the inhibition of isozymes hCA I, II and IX (determined via assaying the CA hydration of CO_2)[26] for the azidosulfonamide **2**, the metallocene-sulfonamides and clinically used CA inhibitors are presented in Table 1. The inhibition constant (K_i) selectivity ratios for hCA IX compared with hCA I and II are also provided.

At isozyme hCA I, the ferrocene sulfonamides **3** and **4** exhibited equal or only slightly improved inhibition, respectively, compared to the parent azide scaffold **2** which had a K_i of 3900 nM. The ruthenocene derivatives **3′** and **4′** showed much greater inhibition, with K_is of 44 and 9 nM, they were 88- and 433-fold more potent than **2**, respectively. The 1,5-substituted triazoles (**4** and **4′**) were more potent than the 1,4-substituted triazoles (**3** and **3′**). For isozyme hCA II the parent compound **2** had a K_i of 47 nM. The ruthenocene derivatives **3′** and **4′** again showed greater inhibition than the ferrocene derivatives, ~5-fold more potent than parent sulfonamide **2** with K_is of 9.7 and 12.3 nM, respectively. The ferrocene derivative **4** had a similar K_i to **2** (K_i = 36 nM), whereas **3** was less potent than **2** (K_i = 80 nM). At isozyme hCA IX the parent azidosulfonamide **2** had a K_i of 105 nM. The ruthenocenyl-based 1,4-regioisomer **3′** was found to be the most potent inhibitor (K_i = 10.3 nM, 10-fold more potent than **2** and more potent than all standard inhibitors). The remaining metallocene derivatives were only slightly more potent than **2** (K_is of 85, 65 and 64 nM). Distinct for this isozyme was that the ruthenocene 1,4- and 1,5-analogues were equipotent (K_is of 64 and 65 nM), whereas for all other regioisomer pairs the 1,5-regioisomer was significantly more potent than the 1,4-regioisomer.

Collectively, the CA inhibition results demonstrate that both the metal (Fe or Ru) and the triazole substitution pattern (1,4- or 1,5-) can markedly influence CA inhibition characteristics. In terms of isozyme selectivity the intention for therapeutic applications of CA is to generate inhibitors that are selective for hCA IX – particularly over the physiologically dominant isozyme hCA II. Of note in our results is that the 1,4-regioisomers **3**

Table 1. Carbonic anhydrase inhibition and selectivity data for metallocene-sulfonamides and standard inhibitors

	K_i (nM)[a]			Selectivity ratios[b]	
	hCA I[c]	hCA II[c]	hCA IX[d]	K_i (hCA I)/K_i (hCA IX)	K_i (hCA II)/K_i (hCA IX)
2	3900	47	105	37.1	0.45
3 (Fe; 1,4-)	3900	80	85	45.9	0.94
3′(Ru; 1,4-)	44	9.7	10.3	4.3	0.94
4 (Fe; 1,5-)	1600	36	65	24.6	0.55
4′(Ru; 1,5-)	9	12.3	64	0.14	0.19
AZA	250	12	25	10.0	0.48
MZA	50	14	27	1.85	0.52
EZA	25	8	34	0.74	0.24
DCP	1200	38	50	24.0	0.76
BRZ	450	3	47	9.6	0.06
DZA	500	9	52	9.6	0.17

[a] Error in the range of ±5–10% of the reported value, from three determinations.
[b] K_i ratios are indicative of isozyme selectivity.
[c] Human (cloned) isozymes, by the CO_2 hydration method.[26–30]
[d] Catalytic domain of human (cloned) isozyme, by the CO_2 hydration method.[26–30]

and **3′** were approximately equipotent at hCA IX and II, with the ratio of K_is approaching unity. Although these compounds are not yet selective, the trend is towards improved hCA IX selectivity, especially when compared to the parent compound **2** and all clinically used sulfonamides (where the K_i(hCA II)/K_i(hCA IX) selectivity ratios range from 0.06 to 0.76). Importantly compounds **3**, **3′** and **4** were also selective towards hCA IX over the other physiologically abundant isozyme hCA I.

In summary, we have explored the synthesis and CA inhibition of novel benzenesulfonamides bearing triazole-tethered metallocene 'tails'. This study is the first to (i) incorporate an organometallic fragment onto the aromatic sulfonamide CA recognition pharmacophore via a covalent bond; and (ii) the first medicinal chemistry application of the regioselective 1,3-DCR between an azide and acetylene substrates using the ruthenium catalyst [$Cp^*RuCl(PPh_3)_2$]. The attachment of the ruthenocenyl fragment to the benzenesulfonamide scaffold gave superior CA inhibition compared to the ferrocenyl fragment across all three CA isozymes investigated (with the exception only of compound **4′** at hCA IX). Furthermore, the orientation of the metallocene tail appears to affect both potency and isozyme selectivity, with the 1,4-triazole regioisomer typically improving hCA IX selectivity over the 1,5-triazole regioisomer. The CA inhibition profiles for the metallocene derivatives demonstrate that these compounds are worthy of further investigation. Future studies will aim to investigate how the differing metal (Fe or Ru) imparts this striking influence on enzyme inhibition. In addition other organometallic and inorganic metal fragments will be investigated.

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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.2007.07.024.

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