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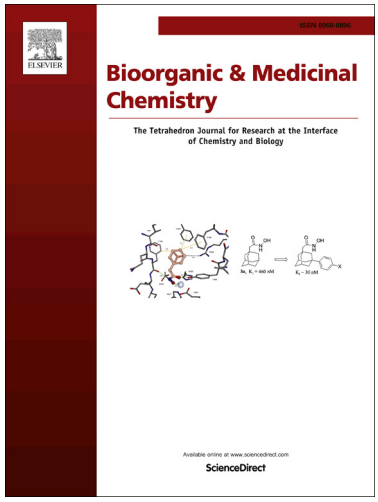

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A class of sulfonamide carbonic anhydrase inhibitors with neuropathic pain modulating effects

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Abstract. A series of benzene sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitors which incorporate lipophilic 4-alkoxy- and 4-aryloxy moieties, together with several derivatives of ethoxzolamide and sulfanilamide are reported. These derivatives were investigated as inhibitors of the metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) of which multiple isoforms are known, and some appear to be involved in pain. These sulfonamides showed modest inhibition against the cytosolic isoform CA I, but were generally effective, low nanomolar CA II, VII, IX and XII inhibitors. X-ray crystallographic data for the adduct of several such sulfonamides with CA II allowed us to rationalize the good inhibition data. In a mice model of neuropathic pain induced by oxaliplatin, one of the strong CA II/VII inhibitors reported here induced a long lasting pain relieving effect, a fact never observed earlier. This is the first report of rationally designed sulfonamide CA inhibitors with pain effective modulating effects.

Key words: sulfonamide, metalloenzyme, carbonic anhydrase, pain, analgesic, X-ray crystallography.

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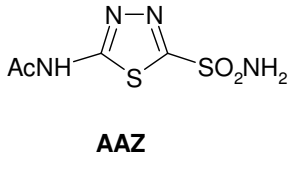
1. Introduction

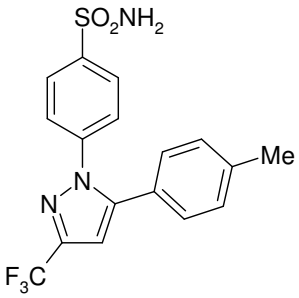
The metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) catalyzes the interconversion between carbon dioxide and bicarbonate, when H^+ ions are also generated in the hydration reaction, being thus one of the main players of pH regulation in many tissues, organs and organisms.[1,2] Indeed, six genetically different CA families are known to date, which are widespread in organisms all over the tree of life.[3,4] In humans, 15 different α-CA isoforms were described so far, which are involved in many physiologic processes connected to pH regulation, secretion of electrolytes, biosynthetic processes, tumorigenesis, etc.[1,2] Interference with the activity of these enzymes, some of which are extremely catalytically active for the physiologic reaction,[1,2] leads to pharmacologic effects which were exploited for obtaining diuretics,[5] antiglaucoma agents,[6] antiepileptics,[7] antiobesity drugs,[8] agents effective for treating high-altitude sickness,[9] and ultimately anticancer/antimetastatic drugs targeting hypoxic tumors.[10] So many types of pharmacological effects are due to the fact that different isoforms among the 15 human (h) hCAs are involved in diverse physiologic processes, and are targeted by such drugs,[1,2] which may be sometimes a rather challenging task considering the fact that many of these isoforms are rather similar from the structural view point and even subcellular localization.[1b,c]

There seem to be some connections between CA activity and pain, although this field has been less investigated for the moment. Kaila and Price's groups[11a] showed that CA inhibition with the clinically used sulfonamide acetazolamide (**AAZ**) augments $GABA_A$ receptor-mediated analgesia via a spinal mechanism of action. These authors explain their finding as being due to a reduced HCO_3^--dependent depolarization via $GABA_A$ receptors when the function of the neuron-specific potassium-chloride (KCl) cotransporter KCC2 is compromised. CA inhibition with acetazolamide thus mitigates the negative effects of loss of KCC2 function after nerve injury resulting in an enhancement of analgesic effects for several $GABA_A$ allosteric modulators, being proposed that this effect might be useful for designing agents effective in the management of neuropathic pain. The same group showed earlier that acetazolamide and midazolam act synergistically in inhibiting neuropathic pain,[11b] and the CA isoform thought to be involved in this process was proposed to be the cytosolic, brain-specific CA VII.[11c] Although not exclusively found in this organ, CA VII is in fact widely expressed in various brain tissues, and its physiologic function is still elusive, being for example recently showed that it is involved not only in pH regulation processes but also in protecting tissue from oxidative stress.[12] Other recent studies reported an enhanced expression of some CA isoforms (among which CA II) in states connected to

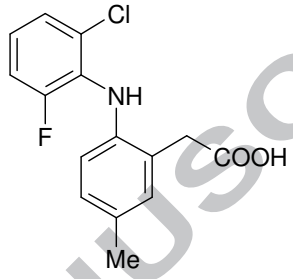
chronic pain, such as thrombus-induced ischemic pain,[13a] or chronic musculoskeletal pain in humans.[13b] Furthermore, acetazolamide **AAZ** [14a] and celecoxib **CLX**,[14b,c] two sulfonamides with known potent CA inhibitory properties,[15] were shown to lead to analgesic effects in various animal models of pain, such as chemical-stimulated pain, peripherally induced inflammatory pain, etc. The mechanisms by which these drugs exert their analgesic effect seem to be correlated with CA inhibition since the COX-2 inhibitor lumiracoxib **LMC**, which unlike **CLX** is not a CA inhibitor (CAI), does not possess analgesic effects in the investigated animal models.[14b]



AAZ



CLX



LMC

Considering all these interesting and recent reports connecting CA and its inhibition to pain, we report here the drug design and in vitro/in vivo investigations of a class of sulfonamides possessing interesting pain modulating properties in an animal model of neuropathic pain. As far as we know, this is the first structure-based drug design study of sulfonamide CAIs with pain-modulating effects.

2. Results and Discussion

2.1. Chemistry and Drug Design. Apart heterocyclic derivatives such as acetazolamide **AAZ**, the benzene sulfonamides (of which **CLX** is a clinically used representative)[15] constitute a much investigated class of CAIs,[16] with many such compounds reported so far being derivatives of 3- or 4-amino-substituted benzenesulfonamides. A much less investigated class of CAIs is that derived from 4-hydroxybenzene sulfonamide **1**.[17] In fact apart an earlier study of Vernier et al.,[17a] who reported ethers of **1**, recently we investigated the possibility to obtain 4-sulfamoylphenyl-ω-aminoalkyl ethers incorporating 2-6 carbon atoms aliphatic chains, which proved to possess a good water solubility and excellent in vivo antiglaucoma activity in an animal model of the disease.[17b] As the chemistry of ethers of sulfonamide **1**, which incorporate simple aliphatic and aromatic R moieties in the RO-C_6H_4-SO_2NH_2 scaffold was poorly investigated to date, we report here an extensive such study. In this paper, our main goal was to obtain compounds with an increased lipophilicity in order to target brain-associated CA isoforms such as CA II and VII, unlike the

previous studies in which we were interested in obtaining water soluble derivatives for topical antiglaucoma activity. Thus, we decided to incorporate small aliphatic, saturated and unsaturated R moieties, such as Et, *n*-Pr, *n*-Bu, allyl or propargyl in the molecules of ethers derivated from sulfonamide **1**, which have been obtained as outlined in Scheme 1. Three different approaches were used for obtaining the series of ethers **2a-2o** reported here: (i) Williamson ether synthesis directly from sulfonamide **1** and alkyl/alkenyl/alkynyl/benzyl halides (route A in Scheme 1); (ii) protection of the sulfamoyl moiety of **1** (as *N,N*-dimethylformimidamide) followed by reaction of the phenolic OH functionality with the corresponding substituted-benzyl halide and deprotection at the sulfamoyl moiety (route B in Scheme 1); and (iii) Mitsunobu reaction of **1** and the corresponding benzyl alcohol (route C in Scheme 1). Two ethers derived from ethoxzolamide **3**, which was de-alkylated to the corresponding ethoxzolamide phenol **4**, were also obtained after protecting the sulfamoyl moiety as described above, followed by *O*-alkylation and removal of the sulfonamide protecting group (compounds **5a** and **5b**, route E, Scheme 1). We also prepared the 4-(bis-N,N-propargyl) derivative of sulfanilamide **6**, compound **7a**, by reacting sulfanilamide at the aromatic amino group with propargyl bromide (route D, Scheme 1). In order to increase the lipophilicity of some of our derivatives, the compounds incorporating alkyne moieties, such as **2e** and **7a**, were converted to the corresponding hexacarbonyl dicobalt(0) complexes **2f** and **7b**, respectively, as it has been reported[18] that such derivatives containing diverse alkynes as (bio)ligands showed interesting potential for application as antitumor agents, hormonally active drugs or diagnostic agents.[18] Finally, sulfonamide **8** incorporating a poorly investigated scaffold up until now, the indoline-5-sulfonamide one, was also prepared and derivatized with the trifluoroacetyl moiety in order to increase its lipophilicity (route F, Scheme 1).

Scheme 1 here

All compounds **2a-2o, 5a, 5b, 7a, 7b** and **8** reported here were completely characterized by spectroscopic and chromatographic methods which confirmed their structures (see Experimental protocols for details).

Table 1 here

2.2. Carbonic anhydrase inhibition. Inhibition data against five physiologically relevant CA isoforms, i.e., h (human) CA I, II, VII, IX and XII; are shown in Table 1. We included these isoforms in the study due to the fact that CA I and II are highly abundant cytosolic isoforms which may be considered either drug targets (for antiglaucoma effects, diuretics, etc) or as offtargets (when isoforms such as CA VA/B or CA IX/XII are targeted).[1,2] hCA VII, as mentioned in the Introduction, seems to be one of the isoforms involved in pain modulation and was included for this purpose, whereas CA IX/XII are antitumor targets, these isoforms being present in high

concentrations in hypoxic, solid tumors.[2] The following structure-activity relationship (SAR) can be drawn from the data of Table 1:

(i) Except for the indoline-5-sulfonamide derivative **8a** and the benzyloxy-derivative of ethoxzolamide **5a**, which were effective inhibitors of hCA I (K_Is in the range of 34.3 – 48.1 nM), the remaining sulfonamides investigated here were ineffective as inhibitors of the slow hCA I isoform, with inhibition constants ranging between 143 and 6400 nM (Table 1).

(ii) The physiologically dominant hCA II was generally highly inhibited by the sulfonamides reported here, except for the sulfanilamide propargyl derivative **7a** and its dicobalt hexacarbonyl complex **7b**, which were rather ineffective CAIs (K_Is of 162-1785 nM, Table 1). The best hCA II inhibitors were the ethers derived from sulfanilamide **2c, 2k** and **2l**, incorporating *n*-Bu, 4-nitrobenzyl and phenethyl moieties, and the ethoxzolamide derivative **5b** incorporating benzyl instead of ethyl moiety (K_Is in the range of 1.4 – 7.8 nM). All these compounds are more effective than acetazolamide AAZ (K_I of 12 nM). The remaining derivatives showed effective hCA II inhibitory properties, with a flat SAR and K_Is in the range of 12.0 – 52.1 nM, rather similar with that of celecoxib **CLX** (K_I of 21 nM, Table 1).

(iii) A very interesting inhibition profile was registered against hCA VII. Except for three benzene sulfonamide ethers (**2m-2o**) and one ethoxzolamide derivative **5a**, all of which incorporate rather bulky R moieties (1-phenyl-propargyl, diphenylethyl- and *N*-morpholyl-ethyl) and which were medium potency hCA VII inhibitors (K_Is of 70.7 – 97.2 nM), all other sulfonamides reported here were highly effective hCA VII inhibitors. The SAR is in fact quite flat, with a variation of the K_Is of only between 0.9 and 10.1 nM. Thus, all compact aliphatic, aromatic and even organometallic moieties were well tolerated in the molecules of these compounds, leading to low nanomolar hCA VII inhibitors (Table 1). Interestingly, the sulfanilamide derivatives **7a** and **7b** showed a very similar activity with the corresponding ethers **2e** and **2f**.

(iv) The transmembrane, tumor-associated isoform hCA IX was effectively inhibited by all sulfonamides investigated here, with inhibition constants in the range of 0.86 – 34.1 nM (Table 1). The most effective hCA IX inhibitors were **2i** (incorporating the benzene sulfonamide ether and pentafluorobenzyl moieties) and **5a** (incorporating the ethoxzolamide and benzyl scaffolds), with subnanomolar potency. Many derivatives with K_Is < 10 nM were detected, such as **2a, 2e-2g, 2j-2l, 7a, 7b** and **8a**. Thus all scaffolds investigated here may lead to interesting inhibitors targeting CA IX, with the nature of the R moiety from the ether being the crucial factor influencing biological activity.

(v) The second transmembrane isoform investigated here, hCA XII; was also effectively inhibited by most compounds reported in the paper. Thus, only **2m** and **2n** (incorporating the bulky moieties

1-phenyl- propargyl, diphenylethyl) were less effective hCA XII inhibitors (K_Is of 33.8 – 50.4 nM) with the remaining sulfonamides showing inhibition constants in the range of 0.72 – 13.6 nM (Table 1). The most effective substitution patterns for hCA XII inhibition were propargyl and the corresponding dicobalt hexacarbonyl complex in ethers **2e** and **2f**, pentafluorobenzyl (**2i**), 4-nitrobenzyl (**2k**), phenethyl (**2l**), etc. The remaining scaffolds (ethoxzolamide in **5a, 5b**; bis-propargyl-sulfanilamide in **7a, 7b**, or indoline-5-sulfonamide in **8a**) also led to quite effective hCA XII inhibitors.

Figs. 1-3 and Table 2 here

2.3. X-ray crystallography. In order to understand the high efficacy of this class of benzenesulfonamides incorporating ether moieties for inhibition of various CA isoforms, we report the high resolution X-ray crystallographic structure for the adduct of hCA II with sulfonamides **2a** (Figure 1a), **2b** (Figure 1b), and **2d** (Figure 1c),, all acting as highly efficient hCA II, VII, IX and XII inhibitors (Table 2 and Fig. 1a).

Each inhibitor-soaked crystals crystallized in the monoclinic space group, $P2_1$ (Table 2). For the CA II-**2a** complex, data was collected to a resolution of ~1.6 Å with unit cell dimensions: a = 42.3, b = 41.2, c = 71.9 Å and β = 104.3°. The final refined model had a R_{crys} and R_{free} value of 15.5% and 19.2%, respectively. The CA II-**2b** complex data was collected to a resolution of ~1.1 Å, with the following unit cell dimensions: a = 42.3, b = 41.1, c = 71.7 Å and β = 104.3°. The final refined model had a R_{crys} and R_{free} value of 20.1% and 21.7%, respectively. The CA II-**2d** complex data was collected to a resolution of ~1.1 Å, with the following unit cell dimensions: a = 42.3, b = 41.2, c = 71.8 Å and β = 104.3°. The final refined model had a R_{crys} and R_{free} value of 15.2% and 16.2%,

The initial F_o–F_c electron density maps observed in *Coot* for each complex showed the inhibitors bound in the active site of the enzyme (Fig. 2). The tetrahedral conformation about the zinc(II) ion in the inhibitor bound state usually seen with sulfonamides was observed in each enzyme-inhibitor complex. Like in most sulfonamides inhibitors the nitrogen atom of the sulfonamide co-ordinated with the zinc in the active site at a distance of approximately 2.0 Å.

In the absence of an inhibitor the Zn(II) ion is tetrahedrally coordinated by His94, His96 and His119 (CA II numbering) and a water molecule or hydroxyl ion (PDB ID: 3KS3). This Zn(II) ion lies at the base of a deep conical cavity where the zinc bound solvent also forms hydrogen bond interactions with the hydroxyl group of Thr199 which in turn interacts with the carboxylate group of Glu106. These interactions form part of the conserved hydrogen bond network in the active site

of CA II, which allows the enzyme to increase the nucleophilcity of the zinc-bound solvent as well as to proper orient the CO_2 substrate in the hydrophobic pocket bordering the active site entrance.

In each protein-inhibitor complex, the conserved zinc-bound water molecule is displaced by the inhibitor's sulfonamide group. The NH of the same moiety also participated in a strong hydrogen bond with the OH group of Thr199, again as in all sulfonamide complexes investigated so far. [20,21] The aryl ring of **2a** and the alkoxy moiety in *para* to the sulfonamide were stabilized by van der Waals interactions with hydrophobic amino acid residues from the enzyme active site, such as Val121, Val135, Val143, Phe131, Leu198 and Pro202. There were no polar interactions between the inhibitor and the enzyme, apart the Zn(II) coordination and the anchoring to Thr199 mentioned above, which probably explains the rather similar inhibitory properties of **2a** against 4 out of 5 CA isoforms (Table 1). However, in this work we were interested in a pain-modulating CA inhibitor as the brain has high amounts of CA II, CA VII and CA XII and we do not know which of these isoforms are targeted with respect to this phenomena involving the neuropathic pain.

Sulfonamides **2b** and **2d** were also co-crystallized with CA II, as they also contain a short hydrophobic tail, similar to **2a**. Derivatives **2b** and **2d** differ from **2a** only by the presence of a $-CH_2$ group and a double bond in the tail of the inhibitor, respectively. Based on the crystallographic structures both drugs bind in the CA II active site in a similar manner to **2a** (Fig 1b and 2c). All three inhibitors formed stable interactions with the same residues mentioned above for **2a**. All three inhibitors interacted with hydrophobic amino acid residues that are generally conserved among the catalytically active CA isoforms (Fig. 3). Even the residues that are not conserved were also hydrophobic in nature and this explains the modest change in binding affinity for the three inhibitors among various CAs. In addition the high degree of amino acid conservation observed in the active site of CA II, VII, IX and XII[1] may provide some insight into the similar inhibition profile among these isoforms.

Fig. 4 here

2.4. Pain modulating effects of CA inhibitors. We investigated the potent sulfonamide CAI **2a** in comparison to acetazolamide (AAZ), as possible pain relievers in a mice model of neuropathic pain induced by oxaliplatin (Fig. 4). This third-generation platinum derivative has become a first-line chemotherapy in metastatic colorectal cancer and a valid option as adjuvant therapy in several types of cancer.[22] The limiting side effect is a painful neuropathy that persists between cycles [23] and is correlated with characteristic alterations of the nervous system.[24] Neuropathy development results in therapy dose reduction or discontinuation and negatively influences quality of life on cancer survivors.[25]

In mice repeatedly treated with oxaliplatin, a single administration of **2a** reverted the lowering of pain threshold to cold stimuli (cold plate test, Fig. 2). On day 14 of oxaliplatin treatment, when neuropathy was well established,[24,26] **2a** relieved pain dose-dependently (10 – 50 mg kg^{-1} p.o.), the higher dose was able to restore pain threshold up to the value of control group. Significant pain relief was induced between 15 and 45 min after treatment (Figure 2). In the same model **AAZ** was also effective but with less potency and efficacy compared to **2a**. The minimum effective dose of **AAZ** was 3 fold higher (30 mg kg^{-1} p.o.) in comparison to **2a**. AAZ dosed at 100 mg kg^{-1} did not completely revert pain, and effectiveness was limited to 15 min (Figure 2).

The 2014 clinical practical guideline from the American Society of Clinical Oncology states that there are no agents recommended for the prevention of chemotherapy-induced neuropathic pain.[25] The efficacy of CAIs against oxaliplatin-induced pain improves the knowledge about the physiopathology of neuropathy and offers a new pharmacological target for this purpose.

3. Conclusions

A series of benzene sulfonamide ethers were obtained from 4-hydroxybenzenesulfonamide or the phenol derivative of ethoxzolamide. They incorporate lipophilic moieties, such as alkyloxy, alkenyloxy, alkynyloxy, substituted-benzyloxy, etc. Two dicobalt(0) hexacarbonyl complexes of the obtained alkynes were also prepared. The new derivatives showed modest inhibition against the cytosolic isoform CA I, but were generally effective, low nanomolar CA II, VII, IX and XII inhibitors. X-ray crystallographic data for the adduct of one such sulfonamide with CA II allowed us to rationalize the good inhibition data. In a mice model of neuropathic pain induced by oxaliplatin, one of the strong CA II/VII inhibitors reported here induced a long lasting pain relieving effect. This is the first report of rationally designed sulfonamide CA inhibitors with pain modulating effects.

4. Experimental protocols

4.1. Chemistry. Anhydrous solvents and all reagents were purchased from Sigma-Aldrich, Alfa Aesar and TCI. All reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using dried glassware and syringes techniques to transfer solutions. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR) spectra were recorded using a Bruker Advance III 400 MHz spectrometer in DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s,

singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet; dd, double of doublets. The assignment of exchangeable protons (O*H* and N*H*) was confirmed by the addition of D_2O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates. Flash chromatography purifications were performed on Merck Silica gel 60 (230-400 mesh ASTM) as the stationary phase and ethylacetate/*n*-hexane were used as eluents. Melting points (mp) were carried out in open capillary tubes and are uncorrected.

4.1.1. General procedure for the synthesis of *O-(N)*-alkyl/benzyl-benzenesulfonamides 2a-o, 7a, and *O*-alkyl benzo[*d*]thiazole-2-sulfonamides 5a,b.

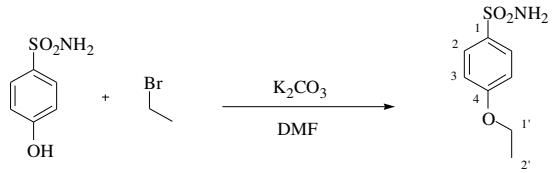
a) **General procedure for *(O)-N* nucleophilic alkylations.**

4-Hydroxybenzenesulfonamide **1** (1.0 eq) (**scheme A**), *N,N*-dimethyl-*N'*-(phenylsulfonyl)formimidamide (1.0 eq) (**scheme B**) or sulfanilamide (1.0 eq) (**scheme D**), were treated with the appropriate base in dry DMF (10ml) and the suspension was stirred at r.t. for 20 min under a nitrogen atmosphere. Then the corresponding alkyl/benzyl halide was added and the reaction was stirred at r.t. until starting material was consumed (TLC monitoring). The reaction mixture was quenched with slush and the precipitate formed was collected by filtration, dried under *vacuo* and purified by silica gel column chromatography eluting with ethyl acetate/*n*-hexane to afford the desired product.

b) General procedure for *(O)*-alkylations via Mitsunobu coupling.

A solution of 4-hydroxybenzenesulfonamide (1.0g, 1.0 eq), alcohol (1.0 eq) and triphenylphospine (1.0 eq) in dry THF (20 ml) was sonicated at 0°C for 5 min (**scheme C**). Then DIAD (1.1 eq) was added drop-wise and the orange solution sonicated at the same temperature for 20 min, quenched with H_2O (20 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic layers were washed with H_2O (3 x 20 ml), dried over Na_2SO_4, filtered and concentrated in *vacuo* to give a sticky residue that was purified by silica gel column chromatography eluting with 50 % ethyl acetate/n-hexane 50 % v/v to and the white solid obtained was recrystallized from IPA.

Synthesis of 4-ethoxybenzenesulfonamide (**2a).**

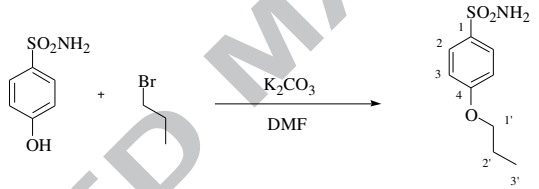


1 **2a**

The synthesis was carried out according to the general procedure in **scheme 1, route A** to afford the desired product as a white solid.

4-Ethoxybenzenesulfonamide **2a**: 60 % yield; silica gel TLC R_f 0.60 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 145 °C (lit.[27] 149° C); δ_H (400 MHz, DMSO-d_6) 1.38 (3H, t, *J* 7.2, 2'-H_3), 4.13 (2H, q, *J* 7.2, 1'-H_2), 7.11 (2H, d, *J* 8.8, 2 x 3-H), 7.23 (2H, s, exchange with D_2O, SO_2NH_2), 7.76 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 161.8, 136.9, 128.6, 115.3, 64.5, 15.4; *m/z* (ESI positive) 202.05 $[M+H]^+$.

Synthesis of 4-*n*-propoxybenzenesulfonamide (**2b).**

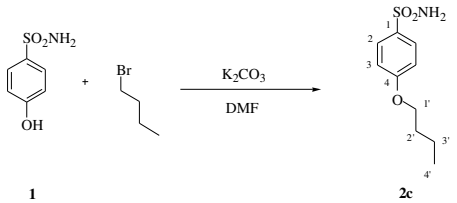


1 **2b**

The synthesis was carried out according to the general procedure in **scheme 1, route A** to afford the desired product as a white solid.

4-*n*-Propoxybenzenesulfonamide **2b**: 57 % yield; silica gel TLC R_f 0.50 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 120 °C; δ_H (400 MHz, DMSO-d_6) 1.02 (3H, t, *J* 7.6, 3'-H_3), 1.78 (2H, m, 2'-H_2), 4.04 (2H, t, *J* 7.6, 1'-H_2), 7.10 (2H, d, *J* 8.8, 2 x 3-H), 7.24 (2H, s, exchange with D_2O, SO_2NH_2), 7.76 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 162.0, 136.9, 128.6, 115.3, 70.3, 22.8, 11.2; *m/z* (ESI positive) 216.06 $[M+H]^+$.

Synthesis of 4-*n*-butoxybenzenesulfonamide (**2c).**

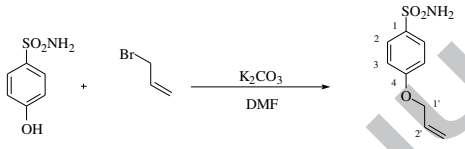


1 **2c**

The synthesis was carried out according to the general procedure in **scheme 1, route A** to afford the desired product as a white solid.

4-*n*-Butoxybenzenesulfonamide **2c**: 67 % yield; silica gel TLC R_f 0.42 (Ethyl acetate/*n*-hexane 30 % v/v); m.p. 112 °C (lit.[28] 108 °C); δ_H (400 MHz, DMSO-d_6) 0.97 (3H, t, *J* 7.2, 4'-H_3), 1.48 (2H, m), 1.76 (2H, m), 4.08 (2H, t, *J* 7.2, 1'-H_2), 7.12 (2H, d, *J* 8.8, 2 x 3-H), 7.24 (2H, s, exchange with D_2O, SO_2NH_2), 7.77 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 162.0, 136.9, 128.6, 115.3, 68.9, 31.5, 19.6, 14.6; *m/z* (ESI positive) 230.09 $[M+H]^+$.

Synthesis of 4-(allyloxy)benzenesulfonamide (**2d).**

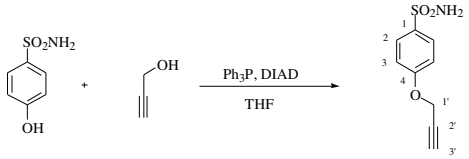


1 **2d**

The synthesis was carried out according to the general procedure in **scheme 1, route A** to afford the desired product as a white solid.

4-(Allyloxy)benzenesulfonamide **2d**: 70 % yield; silica gel TLC R_f 0.47 (Ethyl acetate/*n*-hexane 50 % v/v); m.p. 128 °C (lit.[29] 132-133 °C); δ_H (400 MHz, DMSO-d_6) 4.68 (2H, d, *J* 5.2, 1'-H_2), 5.42 (2H, m, 3'-H_2), 7.12 (2H, d, *J* 8.8, 2 x 3-H), 7.24 (2H, s, exchange with D_2O, SO_2NH_2), 7.78 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 161.5, 137.2, 134.1, 128.6, 118.9, 115.6, 69.4; *m/z* (ESI positive) 214.05 $[M+H]^+$.

Synthesis of 4-(prop-2'-ynyloxy)benzenesulfonamide (**2e).** [30]



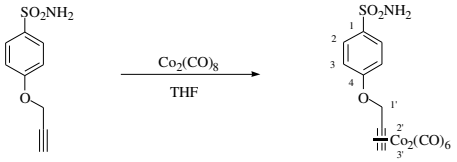
1 **2e**

The synthesis was carried out according to the general procedure in **scheme C** to afford the desired product as a white solid.

4-(Prop-2'-ynyloxy)benzenesulfonamide **2e**: 53 % yield; silica gel TLC R_f 0.31 (Ethyl acetate/*n*-hexane 50 % v/v); m.p. 96 °C; δ_H (400 MHz, DMSO-d_6) 3.66 (1H, t, J 1.9, 3'-H), 4.93 (2H, d, J 1.9,

1'-H_2), 7.17 (2H, d, *J* 8.8, 2 x 3-H), 7.27 (2H, s, exchange with D_2O, SO_2NH_2), 7.79 (2H, d, *J* 8.8, Ar-H), 7.43 (8H, m, 2 x 2-H), 7.76 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 160.5, 137.9, 128.7, 116.0, 79.8, 79.7, 56.8; *m/z* (ESI positive) 212.03 $[M+H]^+$.

Synthesis of 4-(prop-2'-ynyloxy)benzenesulfonamidehexacarbonyldicobalt **(2f).**[31]

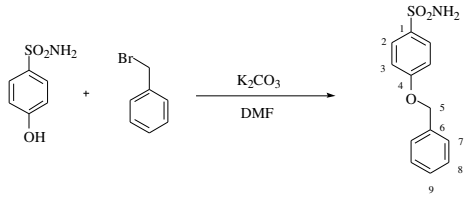


2e **2f**

4-(Prop-2'-ynyloxy)benzenesulfonamide **2e** (0.1g, 1.0 eq) was dissolved in THF (10ml) and then cobalt carbonyl (1.05 eq) was added. The black solution was stirred at r.t. for 40 min. Then SiO_2 (0.3g) was added and solvent removed under *vacuo* to give a black solid that was purified by silica gel column chromatography eluting with 50 % ethyl acetate in *n*-hexane to give the title compound as a red solid.

4-(Prop-2'-ynyloxy)benzenesulfonamidehexacarbonyldicobalt **2f**: yield 82 % yield; silica gel TLC R_f 0.56 (Ethyl acetate/*n*-hexane 50 % v/v); m.p. 174 °C; δ_H (400 MHz, DMSO-d_6) 5.44 (2H, s, 1'-H_2), 6.86 (1H, s, 3'-H), 7.17 (2H, d, *J* 8.8, 2 x 3-H), 7.28 (2H, s, exchange with D_2O, SO_2NH_2), 7.81 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 200.9, 161.3, 137.7, 128.8, 115.7, 90.1, 74.0, 69.3; *m/z* (ESI positive) 512.90 $[M+H]^+$.

Synthesis of 4-(benzyloxy)benzenesulfonamide **(2g).**



1 **2g**

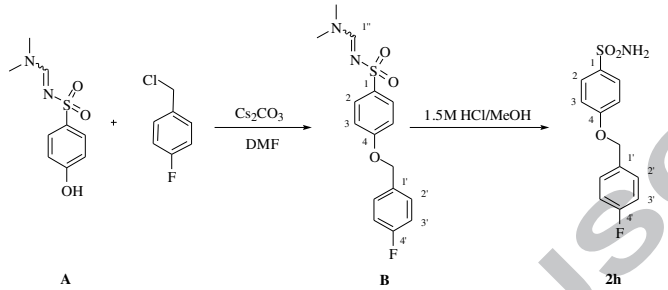
The synthesis was carried out according to the general procedure in **scheme A** to afford the desired product as a white solid.

4-(Benzyloxy)benzenesulfonamide **2g**: 83 % yield; silica gel TLC R_f 0.38 (Ethyl acetate/*n*-hexane 50 % v/v); δ_H (400 MHz, DMSO-d_6) 5.23 (2H, s, 5-H_2), 7.19 (2H, d, *J* 8.8, 2 x 3-H), 7.24 (2H, s, exchange with D_2O, SO_2NH_2), 7.38-7.50 (5H, m, 2 x 7-H, 2 x 8-H, 9H), 7.79 (2H, d, *J* 8.8, 2 x 2-

H); δ_C (100 MHz, DMSO-d_6) 161.6, 137.4, 137.3, 129.4, 128.9, 128.6, 128.6, 70.4; *m/z* (ESI positive) 264.04 $[M+H]^+$.

Experimental data are in agreement with reported data. [32]

Synthesis of 4-(4'-fluorobenzyloxy)benzenesulfonamide **(2h).**



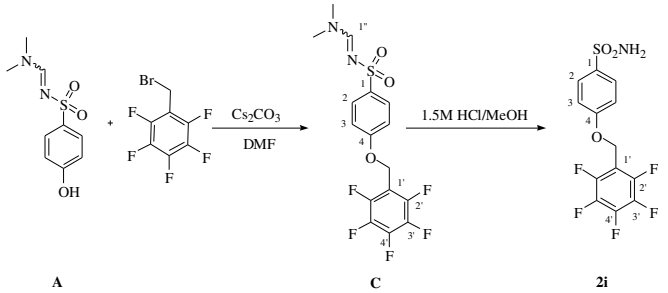
A **B** **2h**

The synthesis was carried out according to the general procedure in **scheme B** to afford the desired product **B** as a white solid that was dissolved in a 1.5M HCl/MeOH solution (5 ml) and the reaction was stirred at 60 °C in a sealed tube for 4 h, concentrated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with ethyl acetate/*n*-hexane 50 % *v/v* affording **2h** as a white solid.

N'-(4-(4'-Fluorobenzyloxy)phenylsulfonyl)-*N,N*-dimethylformimidamide **B**: yield 55 % yield; silica gel TLC R_f 0.24 (Ethyl acetate/*n*-hexane 70 % *v/v*); m.p. 150 °C; δ_H (400 MHz, DMSO-d_6) 2.92 (3H, s, N-CH_3), 3.17 (3H, s, N-CH_3), 4.81 (2H, s, CH_2), 7.04-7.29 (4H, m, Ar-H), 7.50-7.80 (4H, m, Ar-H), 8.22 (1H, s, 1''-H); *m/z* (ESI positive) 337.10 $[M+H]^+$.

4-(4'-Fluorobenzyloxy)benzenesulfonamide **2h**: 42 % yield; silica gel TLC R_f 0.31 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 182 °C; δ_H (400 MHz, DMSO-d_6) 5.21 (2H, s, CH_2), 7.25 (6H, m, Ar-H, SO_2NH_2, exchange with D_2O), 7.55 (2H, m, Ar-H), 7.78 (2H, d, *J* 8.8, Ar-H); δ_F (376 MHz, DMSO-d_6) –114.16; *m/z* (ESI positive) 282.05 $[M+H]^+$.

Synthesis of 4-(perfluorobenzyloxy)benzenesulfonamide **(2i).**



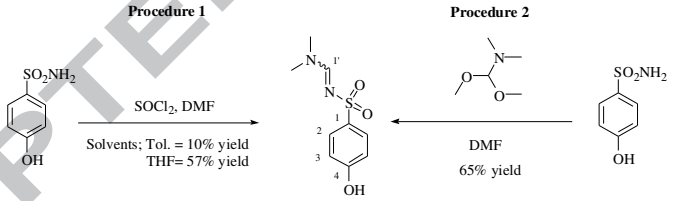
A **C** **2i**

The synthesis was carried out according to the general procedure in **scheme B** to afford the desired product **C** as a white solid that was dissolved in a 1.5M HCl/MeOH solution (5 ml) and the reaction was stirred at 60 °C in a sealed tube for 4 h, concentrated under *vacuo* to give a residue that was purified by silica gel column chromatography eluting with ethyl acetate/*n*-hexane 50 % *v/v* affording **2i** as a pale yellow solid.

N,N-Dimethyl-*N'*-(4-(perfluorobenzyloxy)phenylsulfonyl)formimidamide **C**: yield 69 % yield; silica gel TLC R_f 0.22 (Ethyl acetate/*n*-hexane 70 % v/v); m.p. 191 °C; δ_H (400 MHz, DMSO-d_6) 2.93 (3H, s, N-CH_3), 3.17 (3H, s, N-CH_3), 5.33 (2H, s, CH_2), 7.20 (2H, d, *J* 8.8, Ar-H), 7.56 (2H, d, *J* 8.8, Ar-H), 8.24 (1H, s, 1"-H); *m/z* (ESI positive) 409.06 $[M+H]^+$.

4-(Perfluorobenzyloxy)benzenesulfonamide **2i**: yield 35 % yield; silica gel TLC R_f 0.75 (Ethyl acetate/*n*-hexane 70 % v/v); m.p. 163 °C; δ_H (400 MHz, DMSO-d_6) 5.34 (2H, s, CH_2), 7.25 (2H, d, *J* 8.8, Ar-H), 7.31 (2H, s, SO_2NH_2, exchange with D_2O), 7.82 (2H, d, *J* 8.8, Ar-H); δ_F (376 MHz, DMSO-d_6) –142.8 (2F, d, $^3J_{F\text{-}F}$ 22.0, 2 x F-2'), -152.9 (1F, t, $^3J_{F\text{-}F}$ 21.0, F-4'), -164.21 (2F, m, 2 x F-3'); *m/z* (ESI positive) 354.01 $[M+H]^+$.

Synthesis of *N'*-(4-hydroxyphenylsulfonyl)-*N,N*-dimethylformimidamide **(A).**



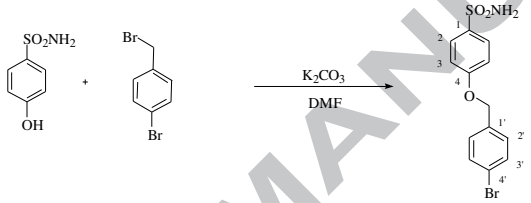
Procedure 1:[30] Thionyl chloride (3.43 g, 5.0 eq) was added to a suspension of 4-hydroxybenzenesulfonamide (1.0g, 1.0eq) in toluene or THF (15 ml), followed by addition of DMF (2.95g, 7.0 eq). The yellow suspension was stirred at 70°C O.N., quenched with slush, extracted with ethyl acetate (3 x 20 ml) and the combined organic layers were washed with brine (3 x 20 ml), H_2O (3 x 20 ml), dried over Na_2SO_4, filtered and concentrated in *vacuo* to give a residue that was purified by silica gel column chromatography eluting with ethyl acetate/*n*-hexane 70 % *v/v* to afford the desired product as a white solid.

Procedure 2:[33] Dimethoxy-*N,N*-dimethylmethanamine (4.54 g, 6.6 eq) was added to a solution of 4-hydroxybenzenesulfonamide (1.0 g, 1.0 eq) in DMF (0.5ml). The yellow solution was stirred at 40 °C 5h, quenched with slush, extracted with ethyl acetate (3 x 20 ml) and the combined organic

layers were washed with H_2O (7 x 15 ml), dried over Na_2SO_4, filtered and concentrated in *vacuo* to give a residue that was purified by silica gel column chromatography eluting with ethyl acetate/*n*-hexane 70 % *v/v* to afford the desired product as a white solid.

N'-(4-Hydroxyphenylsulfonyl)-*N*,*N*-dimethylformimidamide **A**: silica gel TLC R_f 0.10 (Ethyl acetate/*n*-hexane 70 % *v/v*); m.p. 178 °C; δ_H (400 MHz, DMSO-d_6) 2.92 (3H, s, N-CH_3), 3.16 (3H, s, N-CH_3), 7.12 (2H, d, *J* 6.8, Ar-H), 7.61 (2H, d, *J* 6.8, Ar-H), 8.18 (1H, s, 1'-H), 10.29 (1H, brs, exchange with D_2O, O*H*); δ_C (100 MHz, DMSO-d_6) 161.3, 160.3, 134.2, 129.0, 116.2, 41.7, 35.9; *m/z* (ESI positive) 229.10 $[M+H]^+$.

Synthesis of 4-(5'-bromobenzyloxy)benzenesulfonamide **(2j).**

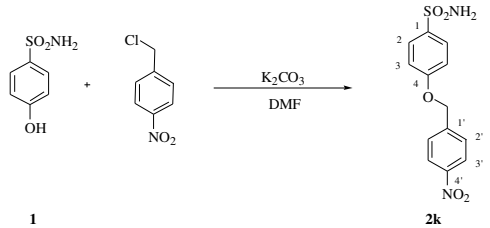


1 **2j**

The synthesis was carried out according to the general procedure in **scheme A** to afford the desired product as a yellow solid.

4-(5'-bromobenzyloxy)benzenesulfonamide **2j**: 63 % yield; silica gel TLC R_f 0.40 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 178 °C; δ_H (400 MHz, DMSO-d_6) 5.22 (2H, s, CH_2), 7.19 (2H, d, *J* 8.0, Ar-H), 7.25 (2H, s, exchange with D_2O, SO_2NH_2), 7.46 (2H, d, *J* 8.0, Ar-H), 7.64 (2H, d, *J* 8.0, Ar-H), 7.78 (2H, d, *J* 8.0, Ar-H); δ_C (100 MHz, DMSO-d_6) 161.4, 137.4, 136.9, 132.4, 130.8, 128.6, 122.1, 115.8, 69.6; *m/z* (ESI positive) 341.97 $[M+H]^+$.

Synthesis of 4-(4'-nitrobenzyloxy)benzenesulfonamide **(2k).**

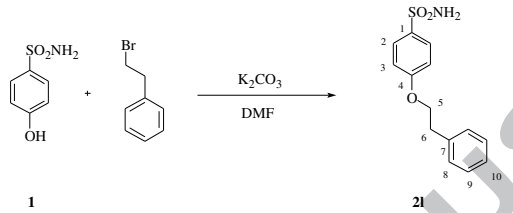


1 **2k**

The synthesis was carried out according to the general procedure in **scheme A** to afford the desired product as a yellow solid.

4-(4'-Nitrobenzyloxy)benzenesulfonamide **2k**: 78 % yield; silica gel TLC R_f 0.35 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 189 °C; δ_H (400 MHz, DMSO-d_6) 4.54 (2H, s, CH_2), 7.22 (2H, d, *J* 8.8, Ar-H), 7.27 (2H, s, exchange with D_2O, SO_2NH_2), 7.77 (2H, d, *J* 8.8, Ar-H), 7.78 (2H, d, *J* 8.8, Ar-H), 8.32 (2H, d, *J* 8.8, Ar-H); δ_C (100 MHz, DMSO-d_6) 161.2, 148.0, 145.3, 137.7, 129.2, 128.7, 124.6, 115.9, 69.2; *m/z* (ESI positive) 309.05 $[M+H]^+$.

Synthesis of 4-phenethoxybenzenesulfonamide **(2l).**

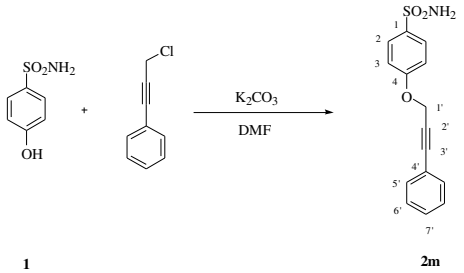


1 **2l**

The synthesis was carried out according to the general procedure in **scheme A** to afford the desired product as a yellow solid.

4-Phenethoxybenzenesulfonamide **2l**: 62 % yield; silica gel TLC R_f 0.41 (Ethyl acetate/n-hexane 50 % *v/v*); m.p. 112 °C; δ_H (400 MHz, DMSO-d_6) 3.09 (2H, t, *J* 6.8, 6-H_2), 4.31 (2H, t, *J* 6.8, 5-H_2), 7.12 (2H, d, *J* 8.8, 2 x 3-H), 7.23 (2H, s, exchange with D_2O, SO_2NH_2), 7.25-7.37 (5H, m, 2 x 8-H, 2 x 9-H, 10-H), 7.56 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 161.7, 139.0, 137.1. 129.9, 129.3, 128.6, 127.3, 115.4. 69.5, 35.6; *m/z* (ESI positive) 278.08 $[M+H]^+$.

Synthesis of 4-(3'-phenylprop-2'-ynyloxy)benzenesulfonamide **(2m).**



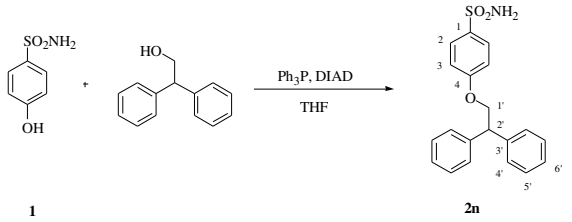
1 **2m**

The synthesis was carried out according to the general procedure in **scheme A** to afford the desired product as a yellow solid.

4-(3'-Phenylprop-2'-ynyloxy)benzenesulfonamide **2m**: 55 % yield; silica gel TLC R_f 0.36 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 138 °C; δ_H (400 MHz, DMSO-d_6) 5.19 (2H, s, 1'-H_2), 7.23 (2H, d, *J* 8.8, 2 x 3-H), 7.25 (2H, s, exchange with D_2O, SO_2NH_2), 7.45 (5H, m, 2 x 5'-H, 2 x 6'-H, 7-H),

7.83 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 160.5, 137.8, 132.4, 130.1, 129.7, 128.6, 122.3, 115.9, 87.7, 85.2, 57.3; *m/z* (ESI positive) 287.07 $[M+H]^+$.

Synthesis of 4-(2',2'-diphenylethoxy)benzenesulfonamide **(2n).**

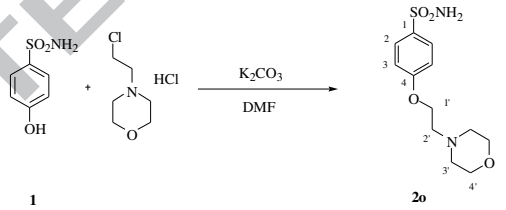


1 **2n**

The synthesis was carried out according to the general procedure in **scheme C** to afford the desired product as a yellow solid.

4-(2',2'-Diphenylethoxy)benzenesulfonamide **2n**: 53 % yield; silica gel TLC R_f 0.44 (Ethyl acetate/*n*-hexane 50 % *v/v*); m.p. 176 °C; δ_H (400 MHz, DMSO-d_6) 4.56 (1H, t, *J* 7.2, 2'-H), 4.68 (2H, d, *J* 7.2, 1'-H_2), 7.12 (2H, d, *J* 8.8, Ar-H), 7.23 (2H, s, exchange with D_2O, SO_2NH_2), 7.26 (2H, d, *J* 8.8, Ar-H), 7.43 (8H, m, Ar-H), 7.76 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 161.7, 142.7, 137.2, 129.4, 129.0, 128.5, 127.5, 115.6, 71.2, 50.6; *m/z* (ESI positive) 354.11 $[M+H]^+$.

Synthesis of 4-(2'-morpholinoethoxy)benzenesulfonamide **(2o).**



1 **2o**

The synthesis was carried out according to the general procedure in **scheme A** using 2.2 eq. of K_2CO_3 to afford the desired product as a white solid.

4-(2'-Morpholinoethoxy)benzenesulfonamide **2o**: 62 % yield; silica gel TLC R_f 0.12 (Ethyl acetate); m.p. 158 °C; δ_H (400 MHz, DMSO-d_6) 2.49 (4H, m, 2 x 3'-H_2), 2.74 (2H, t, *J* 5.6, 2'-H_2), 3.61 (4H, m, 2 x 4'-H_2), 4.20 (2H, t, *J* 5.6, 1'-H_2), 7.12 (2H, d, *J* 6.8, 2 x 3-H), 7.23 (2H, s, exchange with D_2O, SO_2NH_2), 7.76 (2H, d, *J* 6.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 161.8, 137.1, 128.6, 115.4, 67.1, 66.7, 57.7, 54.5; *m/z* (ESI positive) 286.10 $[M+H]^+$.

Synthesis of 6-hydroxybenzo[*d*]thiazole-2-sulfonamide **(4).**[34]

3 4

Ethoxzolamide (2.0g, 1.0 eq) was suspended in DCM (40 ml) and the $AlCl_3$ (3.61g, 3.5 eq) was added portion-wise at 0°C. The reaction mixture was stirred vigorously at r.t. O.N. cooled down to 0° C and quenched with 3.0 M aqueus hydrochloric acid (100 ml). The solid formed was collected by filtration, washed with H_2O (150 ml), dried under *vacuo* and used as it is

6-Hydroxybenzo[*d*]thiazole-2-sulfonamide **4**: 87 % yield; δ_H (400 MHz, DMSO-d_6) 7.14 (1H, dd, *J* 8.8 2.4, 5-H), 7.53 (1H, d, *J* 2.4, 7-H), 7.99 (1H, d, *J* 8.8, 4-H), 8.23 (2H, s, exchange with D_2O, SO_2NH_2), 10.28 (1H, s, exchange with D_2O, O*H*); δ_C (100 MHz, DMSO-d_6) 166.1 (C-2), 158.1 (C-6), 146.1 (C-4a), 138.5 C-7a), 125.9 (C-4), 118.5 (C-5), 107.9 (C-7); *m/z* (ESI positive) 230.98 $[M+H]^+$.

Spectroscopic data are in agreement with reported data.[34]

Synthesis of *N*'-(6-hydroxybenzo[*d*]thiazol-2-ylsulfonyl)-*N,N*-dimethylformimidamide **(4a).**[34]

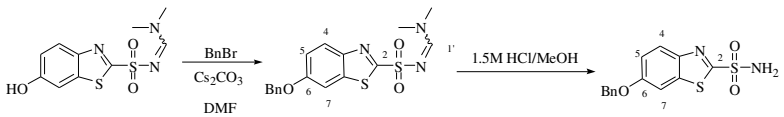
4 4a

The synthesis was carried out according to the general procedure **2** for the intermediate **A** to afford the desired product as a white solid.

N'-(6-Hydroxybenzo[*d*]thiazol-2-ylsulfonyl)-*N,N*-dimethylformimidamide **4a**: 55 % yield; silica gel TLC R_f 0.36 (Ethyl acetate); δ_H (400 MHz, DMSO-d_6) 3.12 (3H, s, N-CH_3), 3.18 (3H, s, N-CH_3), 7.12 (1H, dd, *J* 8.8 2.4, 5-H), 7.50 (1H, d, *J* 2.4, 7-H), 7.97 (1H, d, *J* 8.8, 4-H), 8.40 (1H, s, 1'-H), 10.35 (1H, brs, exchange with D_2O, O*H*); δ_C (100 MHz, DMSO-d_6) 165.5, 161.8, 158.1, 146.4, 138.5, 126.0, 118.4, 107.7, 42.2, 36.4; *m/z* (ESI positive) 286.02 $[M+H]^+$.

Spectroscopic data are in agreement with reported data.[34]

Synthesis of 6-(benzyloxy)benzo[*d*]thiazole-2-sulfonamide **(5a).**



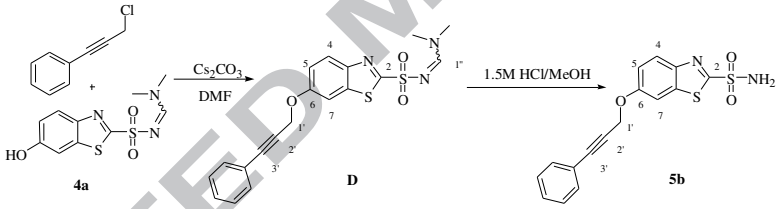
4a C 5a

The synthesis was carried out according to the general procedure in **scheme E** to afford the desired product as a white solid.

N'-(6-(Benzyloxy)benzo[*d*]thiazol-2-ylsulfonyl)-*N,N*-dimethylformimidamide **C**: 64 % yield; silica gel TLC R_f 0.20 (Ethyl acetate); δ_H (400 MHz, DMSO-d_6) 3.03 (3H, s, N-CH_3), 3.28 (3H, s, N-CH_3), 5.25 (2H, s, CH_2), 7.30-7.50 (6H, m, Ar-H, 5-H), 7.91 (1H, d, *J* 2.4, 7-H), 8.08 (1H, d, *J* 8.8, 4-H), 8.41 (1H, s, 1'-H); *m/z* (ESI positive) 375.07 $[M+H]^+$.

6-(Benzyloxy)benzo[*d*]thiazole-2-sulfonamide **5a**: 40 % yield; silica gel TLC R_f 0.35 (Ethyl acetate/*n*-hexane 50 % v/v); δ_H (400 MHz, DMSO-d_6) 5.11 (2H, s, CH_2), .6.97 (1H, dd, *J* 8.8 2.4, 5-H), 7.05 (1H, d, *J* 8.8, 4-H), 7.32-7.50 (8H, m, Ar-H, 7-H, SO_2NH_2 exchange with D_2O); δ_C (100 MHz, DMSO-d_6) 171.8, 155.8, 138.3, 131.4, 130.0, 129.5, 129.3, 125.8, 115.8, 113.8, 110.4, 71.4; *m/z* (ESI positive) 320.03 $[M+H]^+$.

Synthesis of 6-(3'-phenylprop-2'-ynyloxy)benzo[*d*]thiazole-2-sulfonamide **(5b).**



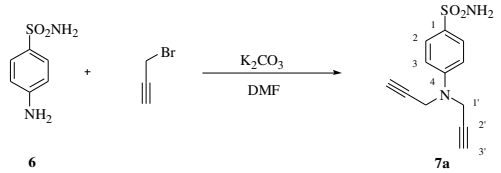
The synthesis was carried out according to the general procedure in **scheme E** to afford the desired product as a white solid.

N,N-Dimethyl-*N'*-(6-(3'-phenylprop-2'-ynyloxy)benzo[*d*]thiazol-2-ylsulfonyl)formimidamide **D**: 24 % yield; silica gel TLC R_f 0.32 (Ethyl acetate); δ_H (400 MHz, DMSO-d_6) 3.03 (3H, s, N-CH_3), 3.27 (3H, s, N-CH_3), 5.21 (2H, s, CH_2), 7.37-7.50 (6H, m, Ar-H, 5-H), 7.94 (1H, d, J 2.4, 7-H), 8.11 (1H, d, *J* 8.8, 4-H), 8.41 (1H, s, 1"-H); δ_C (100 MHz, DMSO-d_6) 167.3, 161.9, 157.6, 147.8, 138.3, 132.5, 130.1, 129,7, 126.0, 122.3, 118.7, 107.4, 87.7, 85.3, 57.8, 42.2, 36.5; *m/z* (ESI positive) 400.07 $[M+H]^+$.

6-(3'-Phenylprop-2'-ynyloxy)benzo[*d*]thiazole-2-sulfonamide **5b**: 12 % yield; silica gel TLC R_f 0.28 (Ethyl acetate/*n*-hexane 10 % v/v); δ_H (400 MHz, DMSO-d_6) 5.17 (2H, s, 1'-H_2), 7.28 (1H, dd, *J* 8.8 2.4, 5-H), 7.40-7.51 (7H, m, Ar-H, SO_2NH_2 exchange with D_2O), 7.86 (1H, d, *J* 2.4, 7-H),

7.94 (1H, d, *J* 8.8, 4-H); δ_C (100 MHz, DMSO-d_6) 156.6, 151.0, 146.1, 137.9, 132.5, 130.1, 129.7, 124.0, 122.4, 117.4, 107.7, 87.7, 85.5, 57.7; *m/z* (ESI positive) 345.03 $[M+H]^+$.

Synthesis of 4-(diprop-2'-ynylamino)benzenesulfonamide **(7a).**

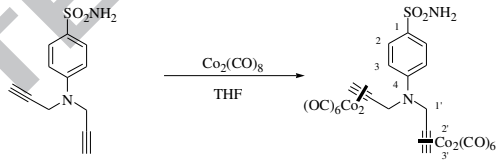


6 **7a**

The synthesis was carried out according to the general procedure in **scheme D** using propargylbromide (2.0 eq) and potassium carbonate (2.2 eq) to afford the desired product as a yellow solid.

4-(Diprop-2'-ynylamino)benzenesulfonamide **7a**: 78 % yield; silica gel TLC R_f 0.56 (Ethyl acetate/*n*-hexane 30 % v/v); m.p. 124 °C; δ_H (400 MHz, DMSO-d_6) 3.26 (2H, t, J 2.4, 2 x 3'-H), 4.03 (4H, d, J 1.9, 1'-H_2), 6.12 (2H, s, exchange with D_2O, SO_2NH_2), 6.63 (2H, d, *J* 8.8, 2 x 3-H), 7.46 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 154.3, 130.5, 122.8, 113.5, 77.8, 77.4, 36.9; *m/z* (ESI positive) 249.06 $[M+H]^+$.

Synthesis of 4-(diprop-2'-ynylamino)benzenesulfonamidehexacarbonyldicobalt **(7b).**

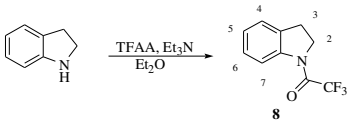


7a **7b**

The synthesis was carried out according to the previous procedure for **2f** using cobalt carbonyl (2.10 eq) to afford the desired product as a reddish solid.

4-(Diprop-2'-ynylamino)benzenesulfonamidedihexacarbonyldicobalt **7b**: 79 % yield; silica gel TLC R_f 0.16 (Ethyl acetate/*n*-hexane 20 % v/v); m.p. >300 °C; δ_H (400 MHz, DMSO-d_6) 4.75 (4H, s, 2 x 1'-H_2), 6.16 (2H, s, 2 x 3'-H), 6.54 (2H, s, exchange with D_2O, SO_2NH_2), 6.69 (2H, d, *J* 8.8, 2 x 3-H), 7.52 (2H, d, *J* 8.8, 2 x 2-H); δ_C (100 MHz, DMSO-d_6) 200.3, 154.3, 130.1, 126.0, 113.6, 89.3, 75.5, 49.1; *m/z* (ESI positive) 850.80 $[M+H]^+$.

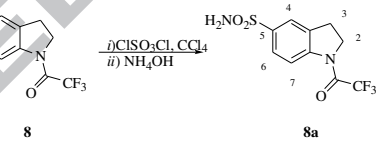
Synthesis of 2,2,2-trifluoro-1-(indolin-1-yl)ethanone **(8)**.[34, 35]



TFAA (7.0g, 2.0eq) was added drop-wise to a solution of indoline (2.0g, 1.0 eq), Et_3N (5.09g, 3.0eq) in Et_2O (60 ml) at 0°C. The solution was stirred at r.t. until starting material was consumed (TLC monitoring), quenched with H_2O (50 ml) and the organic layer was washed with brine (3x 20 ml), dried over Na_2SO_4, filtered and concentrated in vacuo to give a residue that was purified by silica gel column chromatography eluting with ethyl acetate/n-hexane 10 % v/v to afford the title compound as a yellow solid.

2,2,2-Trifluoro-1-(indolin-1-yl)ethanone **8**: 94 % yield; silica gel TLC R_f 0.29 (Ethyl acetate/*n*-hexane 10 % v/v); δ_H (400 MHz, DMSO-d_6) 3.29 (2H, t, *J* 8.0, 3-H_2), 4.31 (2H, t, *J* 8.0, 2-H_2), 7.21 (1H, t, *J* 7.2, Ar-H), 7.25 (1H, t, *J* 7.2, Ar-H), 7.41 (1H, d, *J* 7.2, 4-H), 8.1 (1H, d, *J* 7.2, 7-H); δ_F (376 MHz, DMSO-d_6) –71.49; *m/z* (ESI positive) 216.06 $[M+H]^+$.
Spectroscopic data are in agreement with reported data.[34]

Synthesis of 1-(2,2,2-trifluoroacetyl)indoline-5-sulfonamide **(8a).**[35]



A 0.1M solution of 2,2,2-trifluoro-1-(indolin-1-yl)ethanone **8** (0.5g, 1.0eq) in CCl_4 was added drop-wise to chlorosulfonic acid (0.81g, 3.0 eq) at 0°C. The brown solution was stirred at r.t. until starting material was consumed (TLC monitoring), then added to slush and the mixture was extracted with ethyl acetate (3 x 20 ml). The combined organic layers were washed with H_2O (3 x 15ml), brine (2 x 20 ml), dried over Na_2SO_4, filtered and concentrated in *vacuo* to give a residue that was treated with a concentrated ammonium hydroxide aqueous solution (2.0 eq) at 0°C. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The reaction was quenched with H_2O (30 ml), extracted with ethyl acetate (3 x 20 ml) and the combined organic layers were washed with H_2O (3 x 15ml), dried over Na_2SO_4, filtered and concentrated in *vacuo* to give a residue that was purified by silica gel column chromatography eluting with ethyl acetate/*n*-hexane 50 % *v/v* to afford the title compound as a pale yellow solid.

1-(2,2,2-Trifluoroacetyl)indoline-5-sulfonamide **8a**: 0.6 % yield; silica gel TLC R_f 0.25 (Ethyl acetate/*n*-hexane 50 % v/v); δ_H (400 MHz, DMSO-d_6) 3.32 (2H, t, J 8.0, 3-H_2), 4.39 (2H, t, J 8.0, 2-H_2), 7.39 (2H, s, SO_2NH_2 exchange with D_2O), 7.80 (2H, m, 4-H, 6-H), 8.19 (1H, d, J 7.2, 7-H); δ_F (376 MHz, DMSO-d_6) –71.69; *m/z* (ESI positive) 295.03 $[M+H]^+$.
Spectroscopic data are in agreement with reported data.[35]

4.2. CA inhibition assay

An Applied Photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity[19]. 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 Hepes (pH 7.4) 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 0.01 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,[21] and represent the mean from at least three different determinations. All CA isoforms were recombinant proteins obtained in-house as reported earlier.[8,9,12]

4.3. X-ray Crystallography

Co-crystallization

In this study, 4-ethoxybenzenesulfonamide (**2a**), 4-propoxybenzenesulfonamide (**2b**) and 4-(allyloxy)benzenesulfonamide (**2d**) were co-crystallized with CA II. A 600 mM concentration of compound **2a** was prepared and 2 µl of this solution was added to a 500 µl 1.6 M Sodium citrate, 50 mM Tris-HCL pH 7.8 reservoir solution. 1 µl of this reservoir solution was added to 5 µl of CA II at a final concentration of 10 mg/ml so that the final drug concentration was at 0.24 mM. Hanging-drops were set up and crystals were seen within 5 days. This was repeated for compound **2b** and **2d.**

Diffraction Data and Collection

Drug soaked CA II crystals were immersed in 20% (w/v) glycerol (~0.6 M) cryoprotectant for approximately 5 seconds and immediately flash cooled at 100 K for data collection. Diffraction data for CA II-**2a**, CA II-**2b** and CA II-**2d** complexes were collected at the F1 station at Cornell High Energy Synchrotron Source (CHESS F1; $\lambda = 0.9177$ A°) on an ADSC Q-270 detector. Images were collected every 1° with an exposure time of 5 minutes at a detector distance of 100 mm. The crystal data were integrated, merged and scaled using *HKL2000.*[36]

Structure Determination

Phasing of each complex was carried out in the PHENIX[37] suite of programs using the Auto Molecular Replacement procedure to obtain the initial phases using a previously solved CA II structure with water molecules removed (PDB ID: 3KS3[38]). The graphics program COOT[39] was used to view the electron density map, and each structure was adjusted based on the calculated electron density. Topology files of the inhibitors were generated using the PRODRG[40] server, and these files were used to model the drug into the density generated; refinement was then continued using PHENIX.REFINE until the R_{crys} and R_{free} were minimized. The geometric restraints of the final model were analyzed using PROCHECK.[41] The data diffraction and final model refinement statistics are summarized in Table 2.

4.4. Biological data

Animals. CD-1 albino mice (23-25 g) were used in the experiments. The animals were fed with a standard laboratory diet and tap water ad libitum, and kept at 23 ± 1 °C with a 12 h light/dark cycle, light on at 7 a.m. Animal handling was carried out according to the European Community guidelines for animal care (DL 116/92, application of the European Communities Council Directive 86/609/EEC). The ethical policy of the University of Florence conforms with the Guide for the Care and Use of Laboratory Animals of the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the experiments described herein was obtained from the Italian Ministry of Health (N°54/2014-B) and from the Animal Subjects Review Board of the University of Florence. Experiments involving animals have been reported according to ARRIVE guidelines.[42]

Oxaliplatin model and pharmacological treatments

Oxaliplatin neuropathy was induced in mice administering 2.4 mg kg^{-1} oxaliplatin, intraperitoneally (i.p.) for 5 consecutive days every week for 2 weeks.[26] Oxaliplatin was dissolved in 5% glucose

solution. Control animals received an equivalent volume of 5% glucose i.p. (vehicle). Behavioral tests were performed on day 14. **2a** (1 - 50 mg kg^{-1}) and **AAZ** (10 - 100 mg kg^{-1}) were suspended in the vehicle (1% carboxymethylcellulose) and per os (p.o.) acutely administered on day 14.

Cold plate test

The animals were placed in a stainless box (12 cm x 20 cm x 10 cm) with a cold plate as floor. The temperature of the cold plate was kept constant at 4°C ± 1°C. Pain-related behaviors (i.e. lifting and licking of the hind paw) were observed and the time (s) of the first sign was recorded. The cut-off time of the latency of paw lifting or licking was set at 60 s.[43]

Statistical analysis

Behavioral measurements were performed on 12 mice for each treatment carried out in 2 different experimental sets. Results were expressed as means ± s.e.m. and the analysis of variance was performed by one way ANOVA. A Bonferroni's significant difference procedure was used as post-hoc comparison. *P* values of less than 0.05 or 0.01 were considered significant. Data were analyzed using the "Origin 9" software (OriginLab, Northampton, USA).

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Table 1: hCA I, II, VII, IX and XII inhibition data of the newly synthesized sulfonamides **2-8a** by a stopped flow CO_2 hydrase assay.[19] Acetazolamide (**AAZ**) and celecoxib (**CLX**) inhibition data are included for comparison reasons.

No	R	K_I (nM) *				
		hCA I	hCA II	hCA VII	hCA IX	hCA XII
2a	Et	246	23.1	9.5	8.1	13.6
2b	*n*-Pr	780	12.8	10.1	13.2	7.9
2c	*n*-Bu	797	7.3	9.8	10.1	8.5
2d	CH_2=CH-CH_2	785	12.0	9.9	13.5	6.8
2e	CH≡C-CH_2	2340	28.7	9.6	8.2	2.3
2f	(CH≡CH-CH_2)$Co_2(CO)_6$	1345	40.9	8.2	5.4	0.90
2g	$PhCH_2$	762	20.3	8.3	17.9	10.5
2h	4-$FC_6H_4CH_2$	315	23.8	9.7	7.4	8.0
2i	$C_6F_5CH_2$	231	26.0	8.9	0.86	0.75
2j	4-$BrC_6H_4CH_2$	246	27.8	8.9	3.5	6.4
2k	4-$O_2NC_6H_4CH_2$	143	1.4	0.9	1.3	0.72
2l	$PhCH_2CH_2$	207	7.8	9.4	2.4	1.2
2m	PhC≡C-CH_2	3450	41.6	97.2	21.6	33.8
2n	Ph_2CHCH_2	5675	22.1	70.7	34.1	50.4
2o	N-morpholyl-CH_2CH_2	453	31.7	86.4	12.6	10.7
5a	Ph	48.1	7.6	7.7	0.89	2.5
5b	PhC≡C-CH_2	2418	52.1	73.8	10.3	6.2
7a	bis-CH≡C-CH_2	6400	1785	8.6	9.0	4.9
7b	bis(CH≡CH-CH_2)$Co_2(CO)_6$	876	162	8.9	6.2	7.5
8a	-	34.3	9.2	8.3	6.9	4.1
AAZ	-	250	12	2.5	25	5.7
CLX	-	50000	21	2170	16	18

* Mean from 3 different assays, errors in the range of +/- 10 % of the reported values.

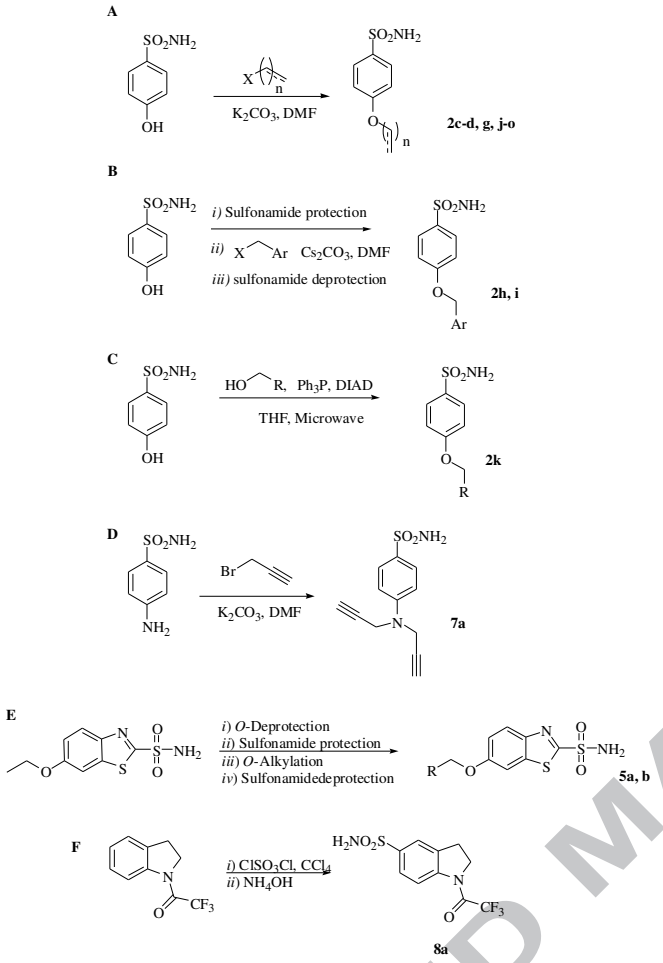
Table 2: X-ray crystallographic data set and refinement statistics for hCA II in complex with **2a, 2b** and **2d.**

Data and Refinement Statistics	**2a**	**2b**	**2d**
PDB ID	4RUZ	4RUY	4RUX
Space Group	$P2_1$		
Unit-cell parameters *a,b,c* (Å) β (°)	42.3, 41.2, 71.9 104.3	42.3, 41.1, 71.7 104.3	42.3, 41.2, 71.8 104.3
Resolution (Å)	20-1.63 (1.69-1.63)	20-1.14 1.18-1.14	20-1.14 (1.18-1.14)
Total No. of reflections	99152	300668	301355
No. unique reflections	29579	85660	84118
Redundancy	3.4	3.5	3.6
Completeness	(97.0) 96.1	(94.8) 95.0	(92.9) 94.4
R_{sym} [a]	0.046	0.060	0.057
R_{cryst}/R_{free} [b]	0.155/0.192	0.201/0.217	0.152/0.162
Rmsd for bond lengths (Å), angles (°)	0.007,1.10	0.005,1.13	0.005,1.18
Average B-Factors (Å^2) main,side,drug	18.3, 23.4, 20.9	9.70, 13.8, 11.8	9.57,14.1, 11.9
No. of protein atoms main,side	1045,1082	1047,1084	1043,1061
No. water molecules	279	203	247
Ramachandran Statistics (%) most favored, additional, generously allowed	88.4,11.1,0.5	88.4, 11.1, 0.5	88.9, 10.6, 0.5

[a] $R_{sym} = (\sum|I - \langle I\rangle|/\sum \langle I\rangle)$

[b] $R_{cryst} = (\sum|F_o - F_c|/\sum |F_o|)$. R_{free} is calculated in the same way as R_{cryst} except it is for data omitted from refinement (5% of reflections for all data sets).

[c] Values in parentheses represent highest resolution bin.



Scheme 1: Synthesis of sulfonamides **2-8a** investigated in this paper.

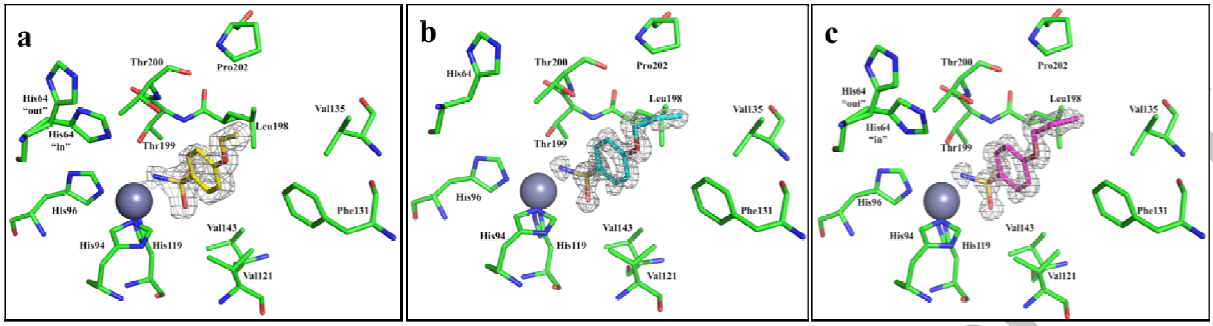


Fig 1: Crystal structures of **2a** (PDB ID: 4RUZ) (a) **2b** (PDB ID: 4RUY) (b) and **2d** (PDB ID: 4RUX) (c) complexed with CA II. The Zn(II) ion (grey sphere), its three His ligands (His94, 96 and 119) and amino acid residues involved in the binding are shown. The proton-shuttling residue His64 was present with both conformations (in and out) for the complexes CA II-**2a** and CA II-**2d**. The electron density is represented by a 1.4 σ-weighted $2F_o$-F_c Fourier map (grey mesh).

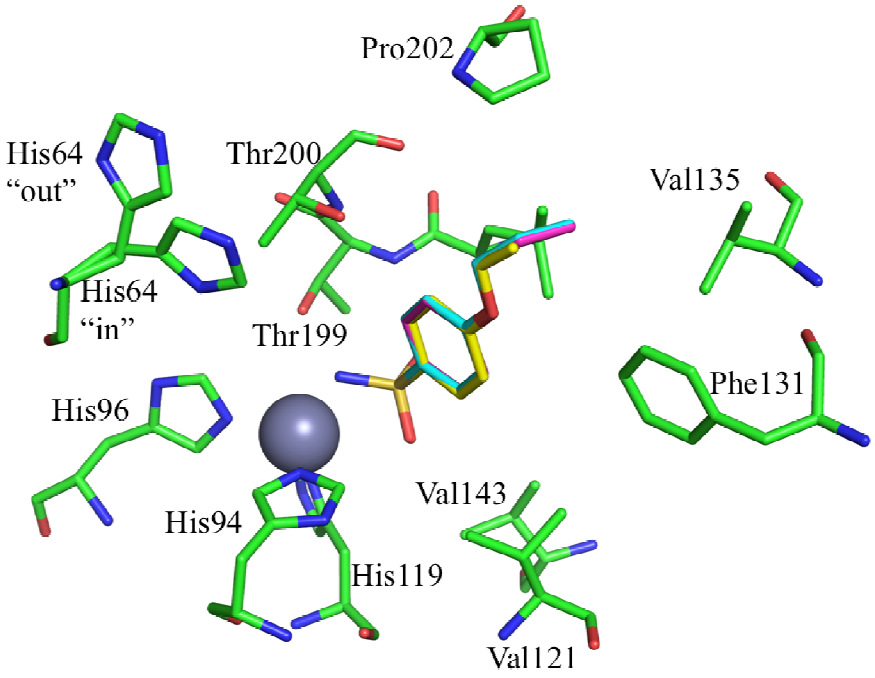


Figure 2: An overlay of **2a** (PDB ID: 4RUZ, yellow), **2b** (PDB ID: 4RUY, sky blue) and **2d** (PDB ID: 4RUX, magenta) complexed with CA II

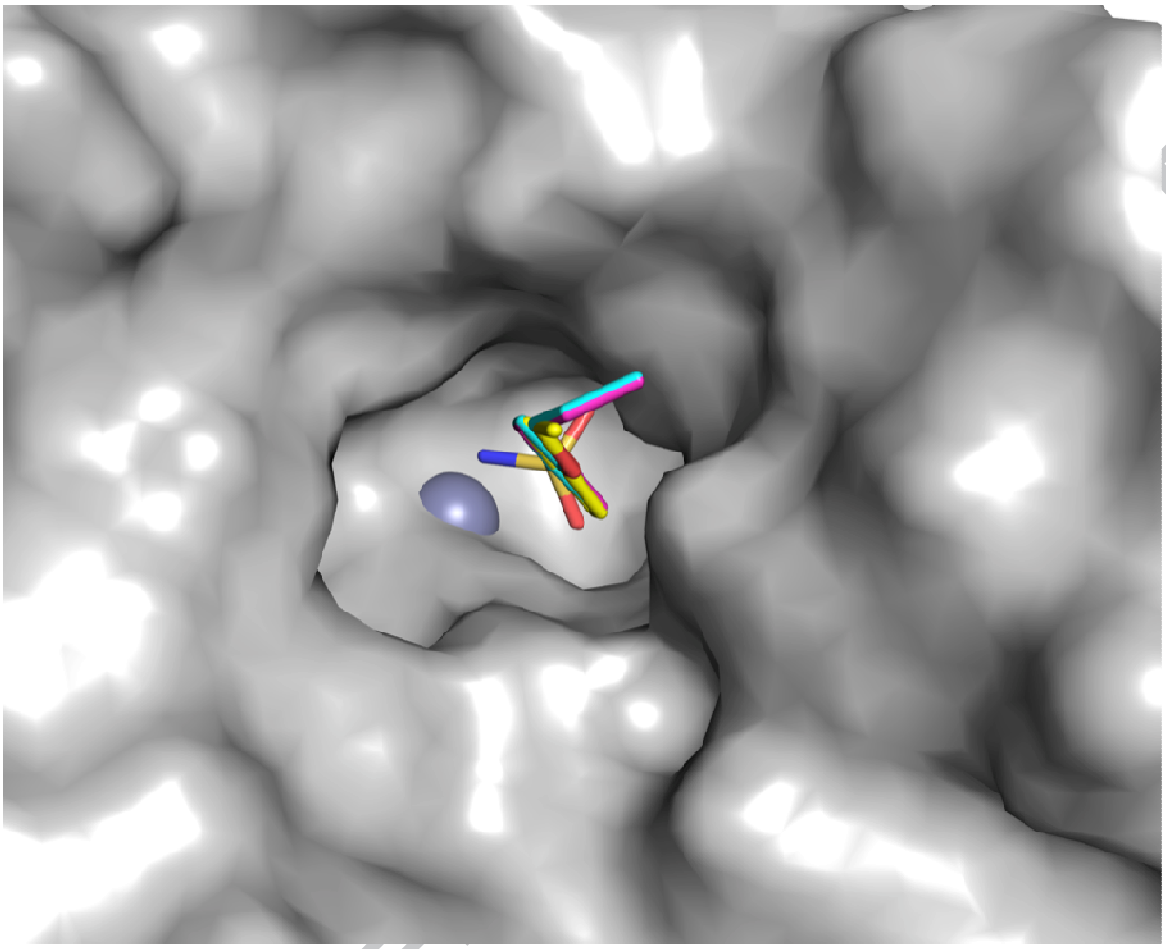


Figure 3: Surface rendition showing an overlay of **2a** (PDB ID: 4RUZ), **2b** (PDB ID: 4RUY) and **2d** (PDB ID: 4RUX) complexed with CA II. The compounds bind in the middle of the active site cavity, are almost superimposable with each other and do not participate in polar interactions with amino acid residues from the CA II active site.

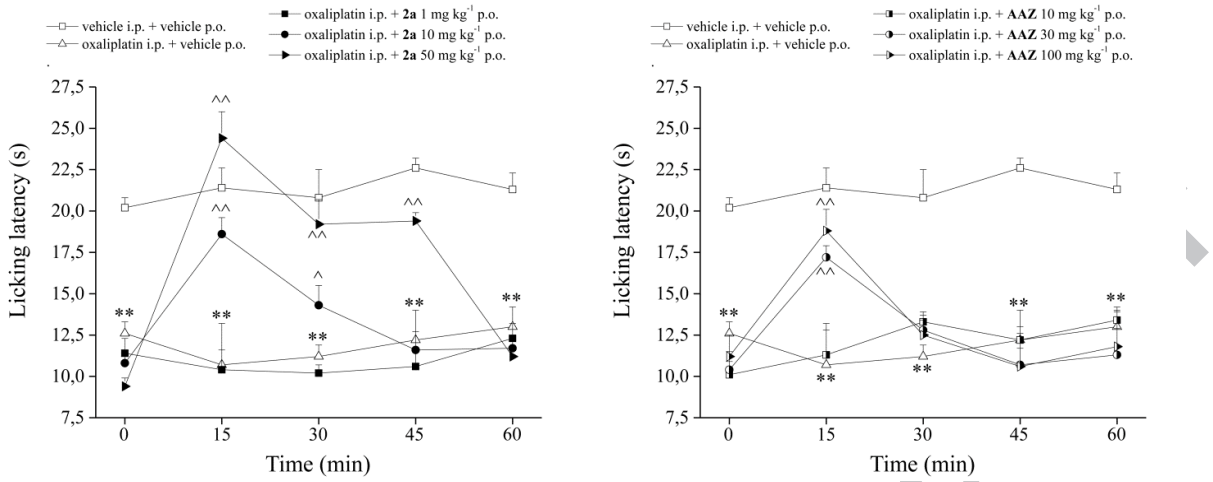


Figure 4: Effect of **2a** on oxaliplatin-induced neuropathic pain in comparison to **AAZ**. On day 14 of oxaliplatin administration (2.4 mg kg^{-1} i.p. administered daily), the response to a thermal stimulus was evaluated by the Cold plate test measuring the latency to pain-related behavior (lifting or licking of the paw). The pain reliever effects of **2a** and **AAZ** were evaluated after a single p.o. administration (time 0) over time. **P<0.01 in comparison to vehicle + vehicle; ^P<0.05 and ^^P<0.01 in comparison to oxaliplatin + vehicle. Each value represents the mean of 12 mice performed in 2 different experimental set.

A class of sulfonamide carbonic anhydrase inhibitors with neuropathic pain modulating effects

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