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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.



## 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

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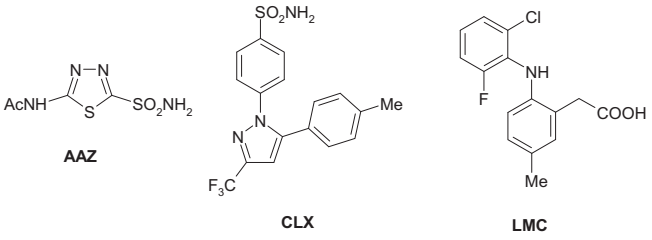
regulation processes but also in protecting tissue from oxidative stress.[12] Other recent studies reported an enhanced expression of some CA isoforms (among which CA II) in states connected to chronic pain, such as thrombus-induced ischemic pain,[13a] or chronic musculoskeletal pain in humans.[13b] 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]



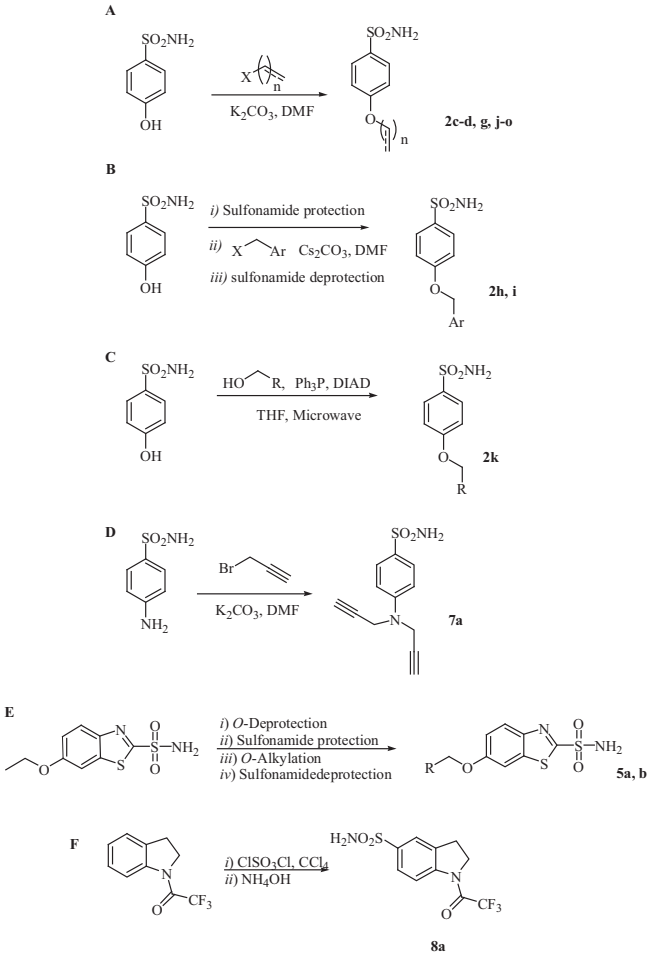

Considering all these interesting and recent reports connecting CA and its inhibition to pain, we report here the drug design and in vitro/in vivo investigations of a class of sulfonamides possessing interesting pain modulating properties in an animal model of neuropathic pain. As far as we know, this 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).

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 Section 4 for details).




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