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Fluorescent sulfonamide carbonic anhydrase inhibitors incorporating 1,2,3-triazole moieties: Kinetic and X-ray crystallographic studies

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ABSTRACT

Fluorescent sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs) were essential for demonstrating the role played by the tumor-associated isoform CA IX in acidification of tumors, cancer progression towards metastasis and for the development of imaging and therapeutic strategies for the management of hypoxic tumors which overexpress CA IX. However, the presently available such compounds are poorly water soluble which limits their use. Here we report new fluorescent sulfonamides **7**, **8** and **10** with increased water solubility. The new derivatives showed poor hCA I inhibitory properties, but were effective inhibitors against the hCA II (K_Is of 366–127 nM), CA IX (K_Is of 8.1–36.9 nM), CA XII (K_Is of 4.1–20.5 nM) and CA XIV (K_Is of 12.8–53.6 nM). A high resolution X-ray crystal structure of one of these compounds bound to hCA II revealed the factors associated with the good inhibitory properties. Furthermore, this compound showed a three-fold increase of water solubility compared to a similar derivative devoid of the triazole moiety, making it an interesting candidate for ex vivo/in vivo studies.

1. Introduction

Fluorescent sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitors (CAIs)[1–3] such as compounds **A** and **B** reported earlier by our group,[4] were essential for demonstrating the role played by the tumor-associated isoform CA IX in acidification of tumors,[5] cancer progression towards metastasis,[6] and for the development of imaging[7] and therapeutic strategies[8] for the management of hypoxic tumors which overexpress high amounts of CA IX.[9] Indeed, starting from such proof-of-concept experiments,[5,7,8] many interesting, CA IX-selective inhibitors were developed,[10,11] with one such compound, **SLC-0111** currently in advanced Phase I clinical trials for the treatment of hypoxic, metastatic solid tumors (Fig. 1).[12]

However one of the main drawbacks associated with the previously fluorescent sulfonamide CAIs, that is, compounds **A** and **B**, is related to their relatively low water solubility at pH values in the range of 6.5–7.4.[4] This limits their extensive use in many biological situations in which working with higher concentrations of inhibitor is required. As a consequence, there is urgent need to develop fluorescent sulfonamides with increased water solubility, which in principle can be achieved in several ways: either by changing the linker between the benzenesulfonamide head and the fluorescent tail present in **A** and **B**, or by changing the nature of the fluorescent moiety to incorporate hydrophilic moieties, not present in the fluorescein fragment. Herein we report new compounds **7**, **8** and **10** obtained using both synthetic strategies mentioned above, their inhibition potencies and selectivity profiles against the physiologically relevant human (h) CA isoforms such as hCA I, II, IX, XII and XIV, as well an X-ray crystallographic structure at 1.3 Å resolution of one selected compound in adduct with the physiologically dominant isoform hCA II.

2. Results and discussion

2.1. Drug design and chemistry

The first synthetic approach used here consisted of replacing the alkyl spacers between the benzenesulfonamide head and the thioureido-fluorescent moiety (methylene or ethylene for **A** and **B**, respectively) by a 1,2,3-triazolyl spacer, which due to the presence of the heterocyclic ring system rich in heteroatoms should increase water solubility. Since the click chemistry methodology[13] has been successfully applied for obtaining large series of sulfonamide, sulfocoumarin or coumarin CAIs,[14–16] also in this case was the preferred choice.

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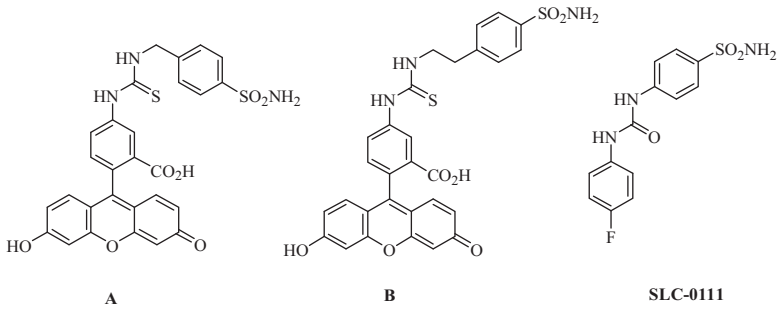
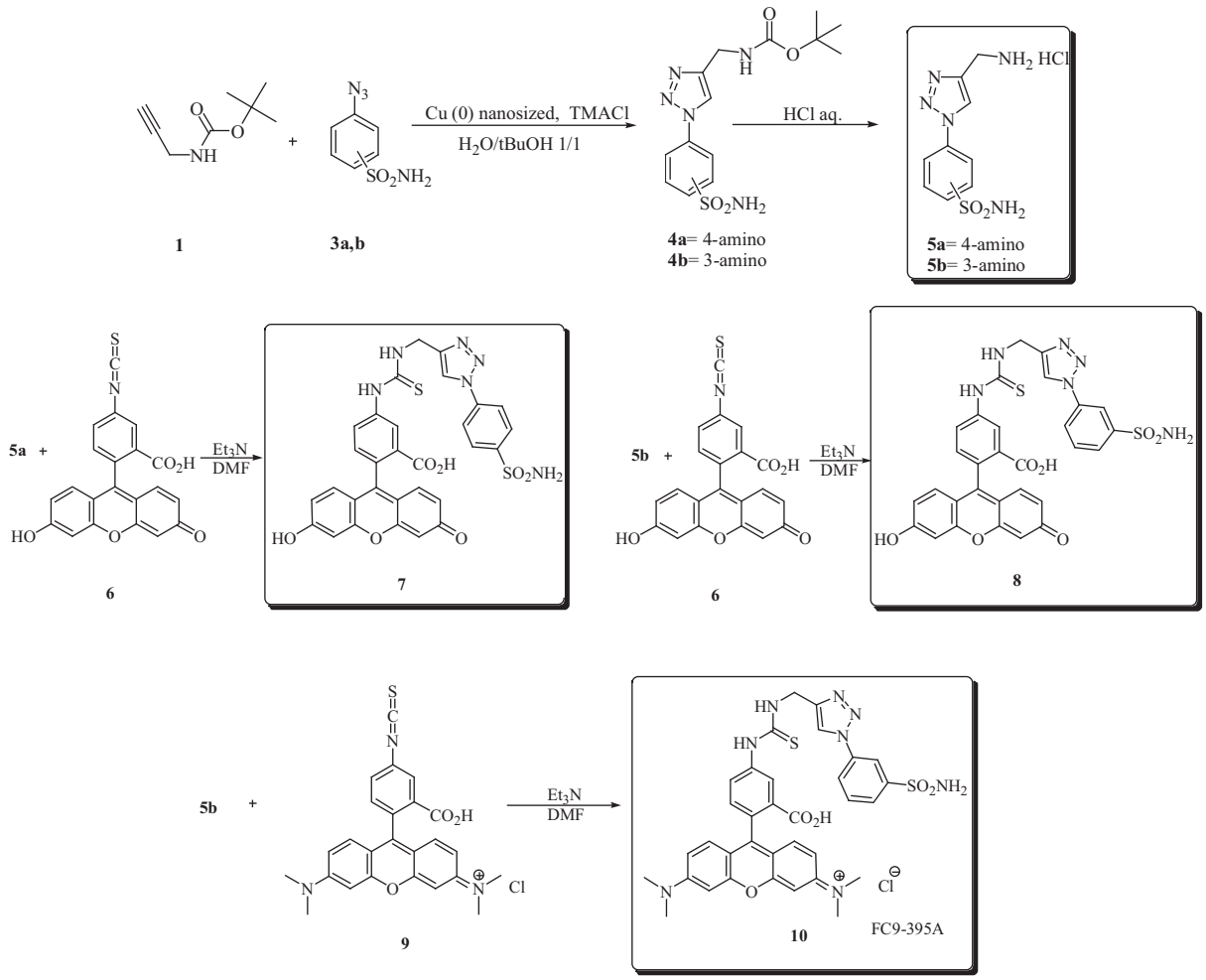


Figure 1. Structures of fluorescent sulfonamides **A**, **B** and the advanced Phase I **SLC-0111**.



Scheme 1. Synthesis of fluorescent-tagged compounds **7**, **8** and **10**.

The 1,2,3-triazolyl-benzenesulfonamide key intermediates **5a** and **5b** were prepared by routine procedures as outlined in Scheme 1. The propargylamine *Boc*-protected **1** was reacted in the presence of copper(0) nanosized catalysts with freshly prepared azidobenzenesulfonamides **3a** and **3b** to afford the desired intermediates **5a** and **5b** upon deprotection of the amino moiety with aqueous 12 M hydrochloric acid. These amines were then coupled with fluorescein isothiocyanate (FITC) leading to the thioureas **7** and **8**, which are analogs of the leads **A** and **B**, but incorporate the water-solubilizing triazole moieties in their molecule (Scheme 1).

The alternative procedure was to replace the fluorescein moiety present in **A** and **B** by a fluorescent tag which promotes a better water solubility itself. Indeed, the replacement of the 6-hydroxy-xanthen-3-one moiety from fluorescein by the (6-dimethylamino-xanthen-3-ylidene)-dimethyl-ammonium present in **10**, is supposed to increase the water solubility as well as the membrane impermeability of compound **10** compared to the structurally-related derivative **8** (Scheme 1). The synthesis of **10** was achieved again by using the amino sulfonamide **5b** and the fluorescent isothiocyanate **9** (see Section 4 for details).

We have compared the phosphate buffer (pH 7.4) solubility of **A**, one of the most investigated fluorescent CAI for a variety of in vitro and in vivo studies,[4–9] with compound **7** designed here. **A** reported a solubility of 1.2 mg/mL whereas **7** had a more than 3-fold increased solubility of 3.8 mg/mL in the same conditions (25 °C, pH 7.4 phosphate buffer).