EXPERT OPINION

Novel sulfonamide compounds for inhibition of metastatic tumor growth (WO2012021963)

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A series of novel ureido-sulfonamides was prepared by reaction of aminobenzenesulfonamide with alkyl/aryl isocyanate. These compounds are claimed for use as therapeutic agents of metastatic tumors, which are poorly responsive to classical chemotherapies and constitute a conceptually novel approach for cancer treatment.

Keywords: antimetastatic agents, carbonic anhydrase IX, metastatic tumor, ureido-sulfonamide

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1. Introduction

Targeted therapies currently constitute an important concept in anticancer chemotherapy, and are becoming the weapon of choice to fight against the disease by aiming at the key mechanisms of oncogenesis.

The progress recently brought by molecular biology allowed identifying molecular targets specifically activated in the carcinogenesis as the growth and the scattering of tumors. So the rational for a targeted chemotherapy, will be in the development of compounds acting on therapeutic targets and focusing against the tumor or its microenvironment [1,2]. The best knowledge of the physiopathological molecular mechanisms of tumors and quite particularly the tumor hypoxia responsible for an aggravated glycolytic metabolism, recently allowed bringing to the foreground carbonic anhydrases (CAs) as of new potential targets in the treatment of the cancer [3]. Recently this zinc metalloenzyme, which catalyzes the reversible hydration of cell-generated carbon dioxide into protons and bicarbonate ions, has emerged as a potential target in cancer therapy [4,5]. Mammalian cells express different CA isozymes, which differ in their tissue distribution and cellular localization [3]. Membrane-bound CA isozymes IX and XII are expressed at high levels and with a high prevalence in different tumor tissues, whose normal counterparts do not contain this protein. The most studied e.g., the isoform IX, is a membrane glycoprotein [6] overexpressed in several types of cancers (kidneys, colon, etc.) while being present in very few normal tissues. The expression of CA IX is known to increase in a dramatic way in the hypoxic conditions (natural phenotype of the solid tumors recognized as a factor limiting in the success of treatments by the conventional methods of radio and chemotherapy) so constituting a marker of bad prognosis [7].

Furthermore, CA IX plays a very important role in the acidification of the tumor microenvironment contributing on one hand to the phenomenon of metastasis, and on the other hand to the decrease in the absorption of basic anticancer drugs, so modulating the answer of the tumor cells [8]. Sulfonamide based compounds are effective in inhibiting tumor cell growth *in vitro* and *in vivo*. Inhibition of CA IX (and CA XII) explains the antitumor activity of these inhibitors. The *in vivo* proof-of-concept study that sulfonamide CA IX inhibitors may indeed show

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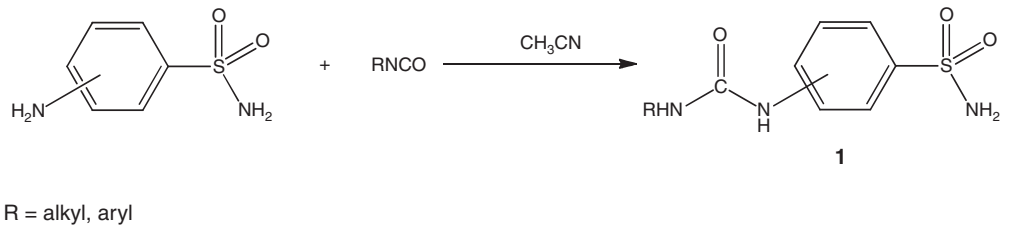

Figure 1. Synthesis of ureido-subsituted benzenesulfonamides.

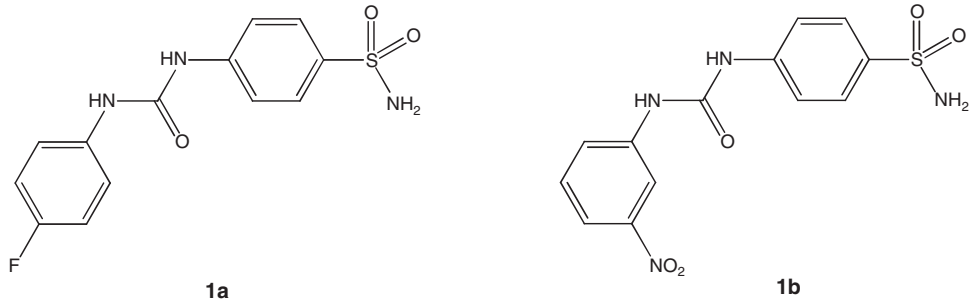

Figure 2. Sulfonamides tested *in vivo*.

antitumor activity has been only very recently published by Neri's group [9]. Thus, sulfonamide based compounds appear very effective in inhibiting specifically the tumor associated isoform CA IX.

In the patent discussed here, the applicants disclose novel ureido-sulfonamides that preferentially inhibit the activity of CA IX. Two of the claimed compounds strongly inhibit the formation of metastases by the highly aggressive 4T1 mammary tumor cells and human breast carcinoma.

2. Chemistry

The typical procedure used for the preparation of these ureido-sulfonamides (**1**) is described in **Figure 1**. The compounds claimed in the patent were prepared by reaction of amino-substituted aromatic sulfonamides with aryl/alkyl isocyanates [10]. All compounds reported in the patent (48 derivatives) were fully characterized by physicochemical and spectroscopic methods which confirmed their structures.

It should be noted that the chemical diversity in this series of compounds was achieved by varying the position of the amino group in the aromatic sulfonamide and/or the nature of the isocyanate. In **Figure 2** the compounds tested *in vivo* are shown.

3. Biological activity

The claim describes the different biological assays that were performed on this series of compounds.

3.1 Carbonic anhydrase inhibition assays

The 48 compounds claimed in this application have been tested against four carbonic anhydrase isoforms (CA I, CA II, CA IX and CA XII) by the CO_2 hydration method. The cytosolic isoforms CA I was inhibited by the ureido-sulfonamides with a wide range of potency, with inhibition in the range 9.0 – 5530 nm. The second offtarget isoform, the cytosolic CA II was inhibited with K_i values in the range of 0.93 – 9640 nm. The tumor-associated CA IX was generally better inhibited by these compounds compared to CA I and II with inhibition in the range 0.5 – 575 nm. In the case of CA XII, the sulfonamides exhibited very good potency with K_i values in the range of 4.2 – 67.3 nm. It should be noted that several compounds showed excellent selectivity ratios for inhibiting the tumor-associated over the offtarget isoforms.

3.2 Inhibition of metastasis *in vitro*

Also described in this application is the inhibition of metastatic MDA-MB-231 (breast carcinoma) cells grown in hypoxia. The ureido-sulfonamides inhibited migration and spreading of tumor cells under oxygen-decreased conditions as found in solid tumors.

3.3 Inhibition of metastases *in vivo*

Mouse 4T1 mammary tumor model has been used in the present patent to test the antimetastatic activity of two of the ureido-sulfonamides claimed. Mouse were injected with

4T1 cells intravenously and the ability of the cells to form lung metastases using bioluminescent imaging techniques were tested. The data showed that compounds **1a** and **1b** are effective in attenuating formation of metastases [11].

4. Expert opinion

The patent claims a series of sulfonamides prepared by reaction of aminobenzenesulfonamide with aryl/alkyl isocyanates. Some of them displayed high specificity at nanomolar levels for the tumor associated CA IX/XII isoforms. Two of them significantly inhibited the formation of metastases by the highly aggressive 4T1 mammary tumor and human breast carcinoma cells. Also the applicants demonstrated that metastatic tumors overexpress CA IX and these cells induce CA IX during hypoxia. Thus the patent validates that the inhibition of this isozyme is involved in the antimetastatic and antitumor activity of the compounds reported. Importantly, administration of the inhibitors did not result in significant weight loss in any animals, indicating that the two compounds **1a** and **1b** were not generally toxic. Further *in vivo* investigations are required to analyze the effects in the treatment of other metastatic tumors.

Although only two compounds have been tested *in vivo*, this patent is of high importance as it constitutes a proof of concept tool for demonstrating that sulfonamide based CA IX inhibitors are very interesting candidates for the development of conceptually novel anticancer and antimetastatic drugs.

Declaration of interest

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