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Novel sulfonamides bearing pyrrole and pyrrolopyrimidine moieties as carbonic anhydrase inhibitors: Synthesis, cytotoxic activity and molecular modeling

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ABSTRACT

Novel pyrrole and pyrrolopyrimidine scaffold-based sulfonamides were designed and synthesized. The carbonic anhydrase (CA) inhibition ability of all derivatives was assessed against the human (h) cytosolic isoforms hCA I and II and the transmembrane, tumor-associated isoforms hCA IX and XII. Some of these sulfonamides were 6–8 fold more potent than the reference drug acetazolamide (AZA, K_i = 5.7 nM)) against hCA XII showing subnanomolar activity. The *in vitro* cytotoxicity of these derivatives was evaluated against MCF-7, where some derivatives were more cytotoxic than doxorubicin (IC_{50} = 8.02 μM) displaying IC_{50} values between 6.46 and 7.56 μM. Docking of these sulfonamides with CA XII was performed and their binding modes were comparable with that of AZA.

1. Introduction

Cancer is a disease of striking impact on the people's health in the world today. It is the second leading cause of death in the world after cardiovascular diseases and it is projected to be the primary cause of death within the coming years [1,2]. The discovery of novel small molecules with potential usefulness as potent, selective and less toxic anticancer agents is still a major challenge to medicinal chemistry researchers [3]. Despite the important advances achieved over recent decades in the research and development of various cancerostatic drugs, current antitumor chemotherapy still suffers from two major limitations, the first being the lack of selectivity of conventional chemotherapeutic agents for cancer tissues, causing unwanted side effects. The second is the acquisition of multiple-drug resistance by cancer cells, rendering them unresponsive to conventional chemotherapeutic agents. Unwanted side effects of antitumor drugs could be overcome with agents capable of discriminating tumor cells from normal proliferative cells and the resistance is minimized using combined modality approach with different complementary mechanisms of action [4]. Pyrrolo(2,3-*d*)pyrimidines are purine analogs that display remarkable biological activities, such as ant-inflammatory [5], anticancer [6–10], antimicrobials [11,12], and antiviral [13–15]. Also, natural products and synthetic drugs displaying a wide range of biological activities were found to contain a pyrrole moiety as their key skeleton [16,17], subsequently substituted pyrroles have become one of the major targets in synthetic chemistry. On the other hand, sulfonamides and their different derivatives are extensively used in medicine due to their bioactivity as antibacterial agents and diuretics [11,12,18]. In addition, sulfonamides have been reported for potent antitumor activity *in vitro* and *in vivo* against numerous types of cancers [19,20]. A number of mechanisms of action were

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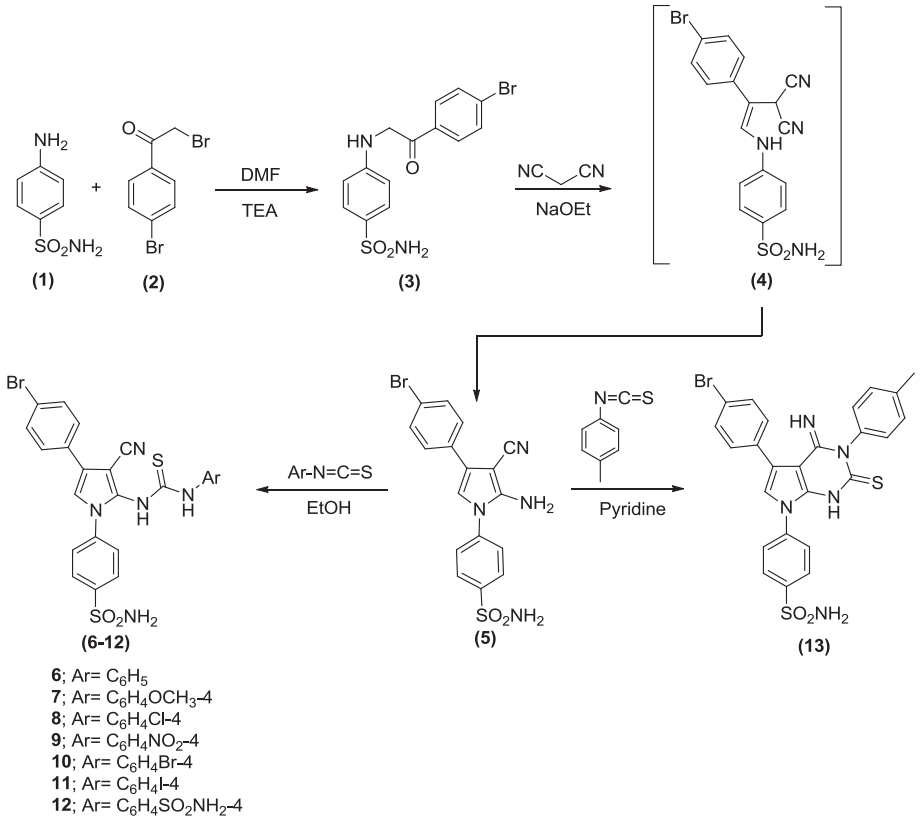

Scheme 1. Synthesis of sulfonamide derivatives **5**–**13**.

reported to be associated with the anticancer ability of sulfonamides, the most prominent being their power to act as carbonic anhydrase inhibitors (CAIs) [21]. Carbonic anhydrases (CA, EC 4.2.1.1) are a group of metalloenzymes that catalyze the hydration of carbon dioxide (cellular waste product) to bicarbonate and a proton [22–27]. This family of enzymes comprises five main classes, *viz*. α, β, γ, δ and ζ. Among the 16 known CA isoforms belonging to class α, the human cytosolic isoforms hCA I and hCA II are ubiquitous in the body and are targets for anticonvulsant, diuretic and anti-glucoma drugs. In addition, the transmembrane isoforms hCA IX and hCA XII, possessing an extracellular active site, were found to be associated with some types of cancers being induced by tumor hypoxia. This being the case, these two isoforms justifiably represent good candidates for antitumor drug design [23,28,29]. In lights of the previous facts and based on our previous work in the field of design and synthesis of sulfonamide based molecules as cytotoxic agents [30–33], we herein report the results achieved from the design and synthesis of novel pyrrole and pyrrolo(2,3-*d*) pyrimidine derivatives carrying biologically active sulfonamide moiety. All the newly synthesized compounds were subjected to cytotoxic screening against the breast cancer cell line MCF-7. Assessment of the *in vitro* enzyme-inhibitory capacity of these novel sulfonamides against hCA I, II, IX and XII was performed. The observed activity of these derivatives was compared with the well-known CAI acetazolamide (AZA). Finally, molecular docking of all compounds in the active site of CA XII was carried out in an attempt to speculate the possible binding mode of these molecules in the active site of the target enzyme.

2. Results and discussion

2.1. Chemistry

The synthesis of the sulfonamide derivatives in this study is depicted in Schemes 1–3. The synthesis of derivatives **5**–**13** is displayed in Scheme 1. Reaction of sulfanilamide **1** with 2-bromo-1-(4-bromophenyl)ethanone **2** yielded the corresponding 4-(2-(4-bromophenyl)-2-oxoethylamino)benzenesulfonamide **3**, which upon reaction with malononitrile in absolute ethanol in presence of sodium ethoxide afforded the pyrrole derivative **5** *via* the formation of intermediate **4**, followed by intramolecular cyclization. The structure of compound **3** was verified by elemental analysis and spectral data. The IR spectrum of compound **3** showed the presence of the characteristic bands for the NH, NH_2, C=O and SO_2 groups. Also, the ^{1}H NMR spectrum indicated the presence of a singlet at 4.7 ppm, which could be assigned to the CH_2 group. The IR spectrum of compound **5** exhibited bands for the NH_2, C≡N and SO_2 functions. In addition, the ^{1}H NMR spectrum of compound **5** revealed two D_2O exchangeable signals, one at 6.1 ppm assigned to the NH_2 group and another at 7.9 ppm for the SO_2NH_2 group.

The reaction of compound **5** with isothiocyanates under different conditions was studied. Thus, reaction of compound **5** with various aryl isothiocyanates in refluxing absolute ethanol gave the corresponding thiourea derivatives **6**–**12**. On the other hand, treatment of **5** with 1-isothiocyanato-4-methylbenzene in pyridine furnished the corresponding pyrrolopyrimidine derivative **13**. The structures of compounds **6**–**13** were established through the IR spectral data. While IR spectra of sulfonamides **6**–**13** showed the presence of a characteristic C=S band in the range of 1230–1271 cm^{-1}, only compounds **6**–**12** showed the presence of a