Bioorganic Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis, characterization, *in vitro* cytotoxicity and antimicrobial investigation and evaluation of physicochemical properties of novel 4-(2-methylacetamide)benzenesulfonamide derivatives

Mustafa Durgun [a], Hasan Turkmen [b], Gulay Zengin [c,*], Huseyin Zengin [c], Mehmet Koyunsever [a], Ismail Koyuncu [d]

[a] Department of Chemistry, Faculty of Science and Literature, Harran University, Sanliurfa 63190, Turkey
[b] Department of Medical Pharmacology, Faculty of Medicine, Harran University, Sanliurfa 63190, Turkey
[c] Department of Chemistry, Faculty of Science and Literature, Gaziantep University, Gaziantep 27310, Turkey
[d] Department of Biochemistry, Faculty of Medicine, Harran University, Sanliurfa 63190, Turkey

ARTICLE INFO

Article history:
Received 14 June 2016
Revised 14 November 2016
Accepted 17 December 2016
Available online xxxx

Keywords:
Synthesis
Sulfonamide derivatives
Cytotoxicity
Antimicrobial activity
Structure-activity relationships
Photoluminescence

ABSTRACT

In this study, several sulfonamide derivatives, 4-(2-methylacetylamino)benzenesulfonamides were synthesized. Chemical structures of the derivatives were characterized by ^{1}H NMR, ^{13}C NMR, LC–MS–MS, UV–Vis, FTIR, photoluminescence and elemental analysis. Sulfanilamide was reacted with 2-bromopropionyl bromide, in the presence of pyridine, to form bromo-substituted sulfonamide key intermediates, which were subsequently treated with secondary amines to obtain novel sulfonamide derivatives. All the synthesized compounds were evaluated for *in vitro* antimicrobial activities and cytotoxicity. Increases in ring size, and rings bearing a nitrogen heteroatom led to improvements in antimicrobial activities. As the presence of CA IX and CA XII enzymes have been implicated in some cancerous tumors, the studies presented herein focuses on targeting these enzymes. It was found that the synthesized derivatives had *in vitro* anti-cancer properties, where compounds (**3**–**6**) were found to be active against all cancerous cells, and no cytotoxic effects on normal cells were observed.

1. Introduction

It is well documented that the zinc-metalloenzyme carbonic anhydrase (CA, EC 4.2.1.1) catalyzes the interconversion of carbon dioxide and water to bicarbonate, with H^+ ions generated in the reaction process; thus this reaction plays a crucial role in the pH regulation of many tissues, organs and organisms. To date there are six genetically different CA families being widespread in living organisms. In humans, fifteen different CA isoforms have been described so far and are involved in many physiological processes, such as, pH regulation, secretion of electrolytes, biosynthetic processes, tumorigenesis, etc. CA inhibition and activation are well known mechanisms, where CA type inhibitors bind to metal centers, while activators plug the assess to active site cavities [1–7].

The importance of the CA isoform hCA has been exhibited by a wide number of physiological processes, where abnormally high amounts, or activities of this enzyme have often been linked to various ill human conditions. Recently, numerous hCA isozyme types have been targeted for the strategic design and development of improved inhibitors, or activators for biomedical applications [8–12].

Sulfonamides, known for inhibiting the metalloenzyme carbonic anhydrase, have been widely investigated due to their attractive use as drugs in the treatment of diseases, and represent an important class of pharmaceutical compounds with a wide spectrum of biological activities, such as, anticancer, CA inhibitory effects, antibacterial, antimalarial, antitumor, antihypertensive, anti-inflammatory and antiprotozoal activities, among others [13–19]. Further, numerous sulfonamides have been found to act as antitumor agents via CA inhibition, where one such derivative is currently under evaluation in Phase I clinical trials as an anticancer/antimetastatic agent [20–24]. The literature shows two CA isozymes, specifically CA IX and CA XII being prominently connected with, and profoundly expressed, in many tumor states [25,26]. The immense value of sulfonamide drugs was noted upon the report of the first antibacterial drug sulfonylamide [27,28]. The information in the literature supports the fact that sulfonamides were used as the first effective chemotherapeutic agents for the systematic prevention, and cure of human and animal bacterial

* Corresponding author.
E-mail address: gzengin@gantep.edu.tr (G. Zengin).

http://dx.doi.org/10.1016/j.bioorg.2016.12.007
0045-2068/

infections [29,30]. Subsequently, formulation improvements in an effort to achieve greater effectiveness and lower toxicity have led to the synthesis of a multitude of compounds, bearing the sulfanilamide scaffold.

To the best of our knowledge, there are no previous studies in the literature on the sulfonamide derivatives presented herein. In this study, new 4-(2-methylacetamide)benzenesulfonamide derivatives were synthesized and screened for *in vitro* cytotoxicity, and antimicrobial activities for assisting in the development of drug alternatives of potentially low toxicity.

2. Results and discussion

2.1. Reaction syntheses for 4-(2-methylacetylamino) benzenesulfonamide derivatives (3–6)

The aim of this study was to synthesize novel sulfonamide derivatives (**3**–**6**). Sulfanilamide (**1**) was reacted with 2-bromopropionyl bromide, in the presence of pyridine, to give the bromo-substituted amide key intermediate (**2**), which was then treated with secondary amines, such as, pyrrolidine for the synthesis of (**3**), piperazine for (**4**), 1-methylpiperazine for (**5**), and 4-methylpiperidine for (**6**) in dry THF, in separate reactions, to obtain the desired derivatives (**3**–**6**). The synthesis reactions of the sulfonamide derivatives (**3**–**6**) are illustrated in Scheme 1. The synthesized derivatives (**3**–**6**) were obtained as white solid products that were stable at room temperature.

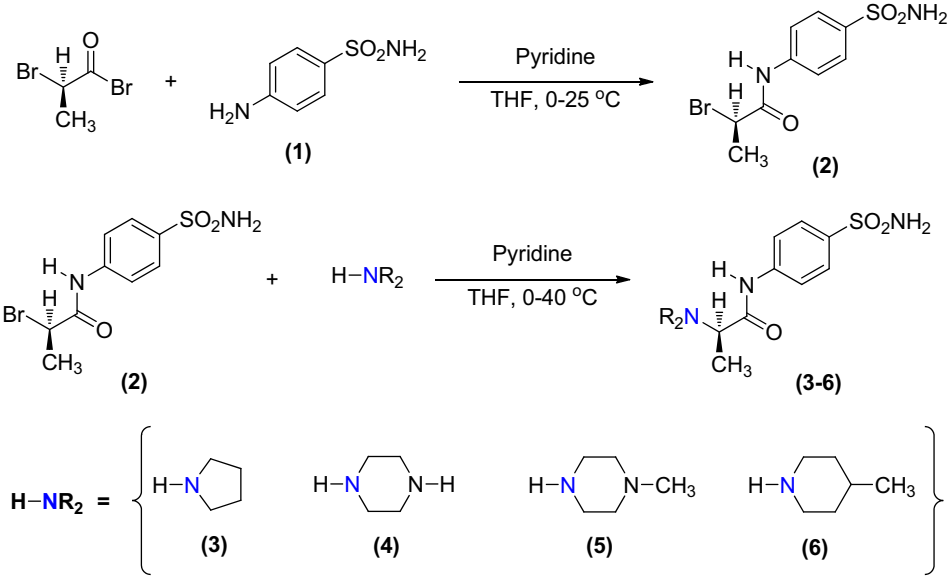
The sulfonamide derivatives were characterized by FT-IR, UV–Vis, ^{1}H and ^{13}C NMR, LC–MS–MS and elemental analysis and data collected are provided in the experimental section, and were in good agreement with calculated values. Characteristic spectral bands and chemical shifts of the synthesized compounds were consistent with those obtained in previous studies for other sulfonamide derivatives [9–11,13,31] and with literature [21].

2.2. Antimicrobial activity

Antimicrobial susceptibility testing may be carried out by a number of methods. In regard to the studies presented herein, where the substrates were sulfonamides, two common methods are the dihydropteroate synthase (DHPS) inhibitory and agar disk diffusion techniques. Sulfonamides are known to be competitive inhibitors of DHPS, the enzyme present in microbial cells [32,33], and the inhibition of DHPS results in damaging effects that lead to eventual microbial cell death. Thus DHPS assays may be carried out for the sulfonamides used in this study, where possible and plausible explanations for observed activities could be due to the ability or inability of these compounds to bind to the active sites. However for our study, the agar disk diffusion technique was used, and growth inhibition measurements were carried out, and the observed antimicrobial activities were discussed as below.

The results of antimicrobial screening are given in Table 1. The synthesized compounds revealed antimicrobial activity with 11–13 mm inhibition zones. No activity was observed against fungi *C. albicans* and *S. cerevisiae*. Synthesized compounds (**3**) and (**6**) revealed antimicrobial properties, and compounds (**4**) and (**5**) were inactive against all the microorganisms. The greatest activities observed were for compound (**3**) and (**6**) against *E. coli* (12 mm, compound **3**), and *P. aeruginosa* and *Y. lipolytica* (13 mm, compound **6**) microorganisms. The antimicrobial activities of parent compound (**1**) were comparable to those of synthesized compound (**3**), exhibiting the highest activity against *B. Subtilis* (12 mm); further the parent compound (**1**) was active against a greater number of microbial lines than compound (**3**).

Pyrrolidine, piperazine and piperidine are nitrogen heterocycles having structural motifs, common to many natural and synthetic bioactive compounds. Nitrogen atoms may provide favorable electrostatic interactions between its protonated form and the anionic region of microbial cell components, and thus lead to the eventual disruption of cell processes. These structural units are frequently used in medicines, and were exploited herein. Variations of substituent on the nitrogen atom heterocycles can have an important effect on selectivity and potency against biological targets. Compound (**3**) has a pyrrolidinyl substituent, while (**4**) and (**5**) both have piperazinyl substituents, and (**6**) has a piperidinyl substituent. Introduction of a second nitrogen atom on a nitrogen heterocycle was expected to enhance antimicrobial activity, but the contrary, where no activity was observed for both two-nitrogen heterocycles, piperazinyl systems (**4**) and (**5**). Nitrogen atom substitution with a methyl group as for compound (**5**), compared to compound (**4**) with no nitrogen substituent, can be expected to reduce the polarity and increase lipophilicity, allowing



Scheme 1. Reaction diagram for the synthesis of 4-(2-methylacetylamino)benzenesulfonamide derivatives (**3**–**6**).