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Novel sulfonamide incorporating piperazine, aminoalcohol and 1,3,5-triazine structural motifs with carbonic anhydrase I, II and IX inhibitory action

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ABSTRACT

A new series of *s*-triazine derivatives incorporating sulfanilamide, homosulfanilamide, 4-aminoethyl-benzenesulfonamide and piperazine or aminoalcohol structural motifs is reported. Molecular docking was exploited to select compounds from virtual combinatorial library for synthesis and subsequent biological evaluation. The compounds were prepared by using step by step nucleophilic substitution of chlorine atoms from cyanuric chloride (2,4,6-trichloro-1,3,5-triazine). The compounds were tested as inhibitors of physiologically relevant carbonic anhydrase (CA, EC 4.2.1.1) isoforms. Specifically, against the cytosolic hCA I, II and tumor-associated hCA IX. These compounds show appreciable inhibition. hCA I was inhibited with K_Is in the range of 8.5–2679.1 nM, hCA II with K_Is in the range of 4.8–380.5 nM and hCA IX with K_Is in the range of 0.4–307.7 nM. As other similar derivatives, some of the compounds showed good or excellent selectivity ratios for inhibiting hCA IX over hCA II, of 3.5–18.5. 4-[({4-Chloro-6-[(4-hydroxyphenyl)amino]-1,3,5-triazin-2-yl}amino)methyl] benzene sulfonamide demonstrated subnanomolar affinity for hCA IX (0.4 nM) and selectivity (18.50) over the cytosolic isoforms. This series of compounds may be of interest for the development of new, unconventional anticancer drugs targeting hypoxia-induced CA isoforms such as CA IX.

1. Introduction

A few years ago, the isozyme IX of human carbonic anhydrase (hCA IX) was identified as a promising target for the cancer treatment. The concentration of hCA IX increases as a response to the tumor hypoxia [1,2]. This is caused by the transcription factor HIF-1 (hypoxia inducible factor 1) which activates genes responsible for energy production, anaerobic glycolysis, neoangiogenesis and pH regulation, with upregulation of proteins involved in cell apoptosis or survival [3–6]. In general, tumor hypoxia is caused by oxygen deficiency in tumor tissue. It leads to the changes in tumor microenvironment and its metabolism [1,2,7,8]. One of the consequences of these changes is a different, acidic extracellular tumor pH (6.7–7.0) compared to the extracellular pH of normal tissue (7.4) [9,10]. Tumor acidification processes release H^+ ions into the extracellular environment by CO_2 hydration when bicarbonate and protons are formed [11,12]. These acidification processes are catalyzed by hCA IX. Details of connection between hCA IX, hypoxia and tumor growth were described and discussed in many articles and books, for example in ref. [13,14,15].

It was also proved, that hypoxia and acidic extracellular environment play a crucial role not only in tumor growth, but also in spreading of metastasis and tumor resistance against chemotherapy or radiotherapy [1,2,12,16,17].

Due to all above described reasons the hCA IX is interesting target for development of new drugs.

From the literature, various sulfonamides are known as inhibitors of hCAs [18]. Furthermore, it was discovered, that sulfonamides bound to *s*-triazine skeleton have an increased efficiency

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and usually also specificity for isozyme hCA IX over hCA I or hCA II [3,18].

In this paper, we present a new set of *s*-triazine derivatives containing sulfanilamide, homosulfanilamide or 4-aminoethylsulfonamide and other (aminoalcohol or *N*-monosubstituted piperazine) structural motifs.

2. Results and discussion

2.1. Virtual screening

Final compounds were prepared by step by step nucleophile substitution of chlorine atoms in cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) (see Figs. 1 and 2). The virtual combinatorial library was docked into hCA IX active site and scored to predict best hCA IX inhibitors from the set. Best of them were docked also into hCA II active site to predict isozyme selectivity and their ADMET properties were predicted. From previous studies [3,11,18,19] could be concluded simple SAR rule that trisubstituted *s*-triazines with bulky substituents should have decreased inhibition activity. Virtual screening proposed such compounds as good and selective hCA IX inhibitors. There is no disubstituted *s*-triazine among the best scored sulfonamides. Best scored compounds violate all Lipinski's and Webber's rules causing very low predicted oral absorption but good water solubility despite their high molar mass and low penetration through blood–brain barrier. There is also possibility of cardiotoxicity because of predicted higher affinity to HERG. All physicochemical and ADMET properties predicted by QikProp are in Supporting information.

From 20 structures with the best docking score, were selected 11 compounds, which were synthesized. These compounds and their intermediates were tested as inhibitors of physiologically relevant carbonic anhydrase isoforms (hCA I, hCA II and hCA IX).

2.2. Chemistry

Final compounds were prepared by step by step nucleophile substitution of chlorine atoms in cyanuric chloride (2,4,6-tri chloro-1,3,5-triazine). Previously reported protocol [3] was used for the preparation of key monosubstituted *s*-triazine intermediates (**1**, **2**, **3**). Disubstituted or trisubstituted *s*-triazines were prepared by the reaction of corresponding mono-/ disubstituted intermediate with nucleophile (another sulfonamide, aminoalcohol or monosubstituted piperazine) in a molar ratio 1:1. Anhydrous potassium carbonate (1 M equivalent) was used as a base. Substitution of the second or the third chlorine atom was controlled by the temperature mode (the second chlorine was replaced at 35 °C and the third at 100 °C) (see Scheme 1).

2.3. Enzymes inhibition

Synthesized compounds were tested for their inhibition activity against physiologically relevant hCA I and hCA II and against tumor-associated hCA IX. For the evaluation methodology based on stopped flow assay was employed [3]; see Section 4.8. Obtained results are shown in Table 1. As standards, clinically used sulfonamides were used - **AAZ** (Acetazolamide), **BRZ** (Brinzolamide), **DCP** (Dichlorophenamide), **DZA** (Dorzolamide), **EZA** (Ethoxzolamide), **MZA** (Methazolamide) and **IND** (Indisulam; cancled in clinical trials) [3]. (See Table 2.)

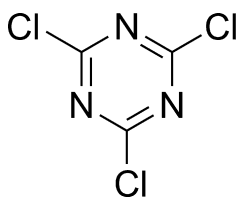

Fig. 1. Cyanuric chloride used as scaffold for virtual combinatorial chemistry.

From the data in Table 1, we can conclude following relationships between structure and activity of the compounds:

(i) All tested compounds act as weak inhibitors of cytosolic isozyme hCA I with K_Is in the range of 8.5 – 2679.1 nM. Two inhibitors of hCA I with the lowest activity, compounds **7** and **22** (K_Is 2372.5 and 2679.1 nM) contain 4-methoxycarbonyl- or 4-(methoxycarbonylethyl)piperazin-1-yl and sulfonamide structural motifs, which are probably too bulky to be bound to the active site of the enzyme. All other compounds have much better inhibitory activity (K_Is in the range of 62.3–929.6 nM) and thus they are better inhibitors than standards **DCP** (dichlorophenamide) and **DZA** (dorzolamide). Two of the tested compounds, specifically **14** (disubstituted *s*-triazine containing homosulfanilamide and aminophenol motif) and **17** (*s*-triazine derivative containing sulfanilamide and two aminoethylsulfonamide motifs), showed much better inhibitory activity than all compared with K_Is in the range of 8.5–16.7 nM (Table 1).

(ii) Against hCA II, one of the physiologically most relevant carbonic anhydrases, synthesized compounds showed significantly better inhibitory activity than against hCA I with K_Is in the range of 4.8–380.5 nM. Just as for hCA I, the worst inhibition data for hCA II were obtained for compounds **7** and **22** as well as for **12**, which is structurally very similar to **7** (K_Is in the range of 140.0–380.5 nM). The reason is probably the same as in the previous case, the effective bonding to enzyme is affected by bulkiness of substituents (as for the most of tested compounds). Some of the tested compounds showed higher inhibitory activity than standard **DCP** with K_Is in the range of 16.8–28.7 nM. These compounds (**6**, **23**, **26**) contained sulfonamides and/or linear alcohol in their structures. The best inhibition activities against hCA II were obtained for molecules **14**, **17**, **18**, **25** and **27** (K_Is in the range of 4.8–7.8 nM) containing combination of three sulfonamides or sulfonamides with 2,3-dihydroxy-propyl-1-aminyl substituent or phenol in the structure. All of the mentioned compounds have higher inhibition activity than all standard drugs except of **BRZ** (Brinzolamide); see Table 1. From obtained inhibitory data it is not easy to determine clear relationship between structure and biological activity, but still some remarks can be done. In the case of disubstituted *s*-triazine containing sulfonamide and piperazine in the structure, the following can be observed: if the piperazine is present in the structure (**7**, **9**, **11**) then with increasing number of CH_2 groups in piperazine motif, also the activity against hCA II is increasing. On the other hand, when the homosulfanilamide is present in the structure (**8**, **10**, **12**), the best inhibitory activity was observed for molecules with one CH_2 group in a piperazine moiety and the worst one for 4-(methoxycarbonylethyl) piperazin-1-yl derivative. It should also be mentioned, that for the activity of **15** and **16** (contain sulfanilamide and 4-acetylphenyl as substituents) the position of acetyl group in the structure is important. Derivative with acetyl group in meta position (**15**) is more efficient against hCAII than para derivative. On the other hand, for the *para*-substituted derivative higher inhibitory activity against hCA I and hCA IX was observed.