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New Dihydrothiazole Benzensulfonamides: Looking for Selectivity toward Carbonic Anhydrase Isoforms I, II, IX, and XII

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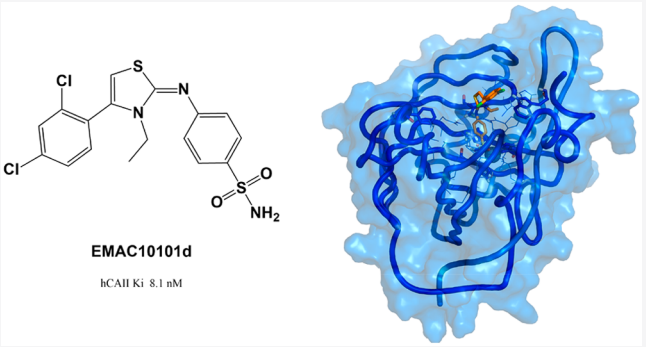
ABSTRACT: In the present study we investigated the structure−activity relationships of a new series of 4-[(3-ethyl-4-aryl-2,3-dihydro-1,3-thiazol-2-ylidene)amino]benzene-1-sulfonamides (**EMAC10101a−m**). All synthesized compounds, with the exception of compound **EMAC10101k**, preferentially inhibit off-target hCA II isoform. Within the series, compound **EMAC10101d**, bearing a 2,4-dichorophenyl substituent in position 4 of the dihydrothiazole ring, was the most potent and selective toward hCA II with an inhibitory activity in the low nanomolar range.



KEYWORDS: *Dual enzyme inhibitors, carbonic anhydrase, anticancer agents, docking, benzensulfonamides*

Human carbonic anhydrases (hCAs) belongs to a family of ubiquitous metalloenzymes that catalyze an essential yet simple physiological reaction, the reversible hydration of carbon dioxide to bicarbonate and protons ($CO_2 + H_2O \leftrightarrows HCO_3^- + H^+$). Sixteen different α-CA isozymes or CA-related proteins (CARPs) were identified and described so far differing in cellular localization and tissue distribution.[1,2] Thus, cytosolic forms (hCA I−III, VII), membrane-bound (hCA IV, IX, XII, and XIV), mitochondrial form (hCA V), and secreted (hCA VI) isozymes can be distinguished.[1] According to their cellular and tissue localization, hCAs are involved in crucial physiological processes such as respiration and transport of CO_2/bicarbonate between metabolizing cell/tissues and lungs, pH and CO_2 homeostasis, bone resorption, calcification, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (such as gluconeogenesis, lipogenesis, and urea-genesis), and tumor genesis and progression.[1,3−8] Not surprisingly the physiological and pathological role of hCA has been extensively studied and the role of potential inhibitors and/or activators investigated,[9,10] in particular the implication of hCAs IX and XII in different types of cancers,[11−13] the overexpression of hCAs II and XII in glaucoma,[14−16] as well as the role of CAs inhibitors (CAIs) as diuretics, antiepileptic, in the management of altitude sickness, antiobesity, diagnostic tools, painkillers, and anti-infective drugs.[17−20] However, the design of isozyme specific inhibitors still remains an issue, due to the high similarity between the catalytic sites of this family of enzymes.[2,3,8,21−25] Indeed, several mechanisms of inhibition have been reported and new classes of CAIs have been therefore investigated, besides the classical zinc binders.

Nevertheless, most of CAIs are generally characterized by a zinc-binding group (ZBG), which could be a sulfonamide or a bioisoster. The ZBG is often linked to a scaffold capable of interacting with one or both hydrophobic and hydrophilic halves of the active site and by a tail that may interact with the most variable sites of the enzyme such as the entrance of the cavity, thus enhancing the isoform selectivity profile of CA inhibitors.[25] Carboxylates, hydroxamates, dithiocarbamates, and isosters are included in this class.[26−28] However, the most important and largely represented ZBG is the sulfonamide group. Accordingly, the majority of the clinically used CAIs belong to the sulfonamide's family. They comprise acetazolamide (**1**), ethoxzolamide (**2**), sulthiame (**3**), dorzolamide (**4**) (Figure 1), and more. On the basis of the above and pursuing our research of sulfonamide based hCA inhibitors,[29−33] we have synthesized a series of 4-[(3-ethyl-4-aryl-2,3-dihydro-1,3-thiazol-2-ylidene)amino]benzene-1-sulfonamides (**EMAC10101a−m**) and evaluated their activity against the hCA I, II, IX, and XII isozymes. Compounds

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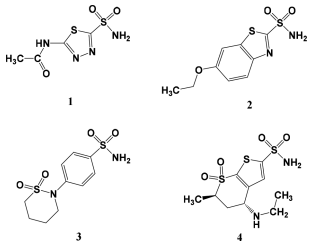
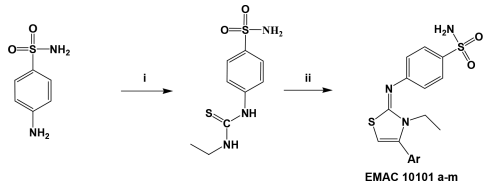


Figure 1. Examples of sulfonamides in clinical use.

Scheme 1. Synthetic Pathway to Compounds EMAC10101a−m[a]

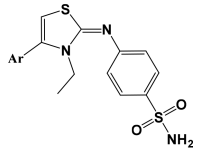


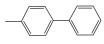
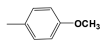
[a]Reagents and conditions: (i) ethyl isothiocyanate, 2-propanol, reflux; (ii), α-halogen arylketone, rt/80°C.

EMAC10101a−m were synthesized by slightly modifying a previously reported procedure (Supporting Information)[34] (Scheme 1).

All the obtained compounds were characterized by means of analytical and spectroscopic methods (Figures S1−S39 and Tables S1−S3) and then submitted to biological evaluation to assess their activity against hCA I, II, IX, and XII (Table 1). When tested on hCA I, none of the new derivatives exhibited activity in the nanomolar range. Only compounds **EMAC10101b** and **EMAC10101c**, bearing a 3 nitro- and a 2,4-difluorophenyl substituent, respectively, were active in the low micromolar range. Conversely, most of the EMAC compounds were active toward the hCAII isozyme in the nanomolar range. Only compounds **EMAC10101a** and **EMAC10101h** exhibited activity in the low micromolar range. Consequently, the introduction of a chlorine atom or of a nitro group in position 4 of the phenyl substituent was detrimental for the inhibition activity. When a second halogen is introduced in position 2 of the phenyl ring, the activity is restored. Thus, compounds **EMAC10101c** and **EMAC10101d** were the most potent hCA II inhibitors exhibiting K_i values of 9.2 and 8.1, respectively. The other EMAC derivatives, with

Table 1. Inhibition Data toward hCA I, II, IX, XII of Compounds EMAC10101a−m[a]



Compound	Ar	Ki (nM)			
		hCA I	hCA II	hCA IX	hCA XII
EMAC10101a		4472.4	724.8	108.8	1390.0
EMAC10101b		750.5	55.3	214.1	448.2
EMAC10101c		698.0	9.2	143.8	367.6
EMAC10101d		9627.4	8.1	224.6	154.9
EMAC10101e		9067.2	65.5	3391.0	1379.4
EMAC10101f		9703.4	90.3	4305.7	1751.4
EMAC10101g		4958.5	65.5	4333.3	1820.6
EMAC10101h		8720.1	285.3	4320.2	1795.6
EMAC10101i		2868.6	75.1	3930.0	1689.2
EMAC10101j		1604.2	60.8	357.7	157.2
EMAC10101k		8155.2	44.0	205.3	60.9
EMAC10101l		9625.0	55.1	297.1	1118.8
EMAC10101m		8622.7	60.9	247.9	175.1
AAZ		250	12.1	25.8	5.7

[a]Values are the mean from three different assays, by a stopped flow technique (errors were in the range of ±5−10% of the reported values).

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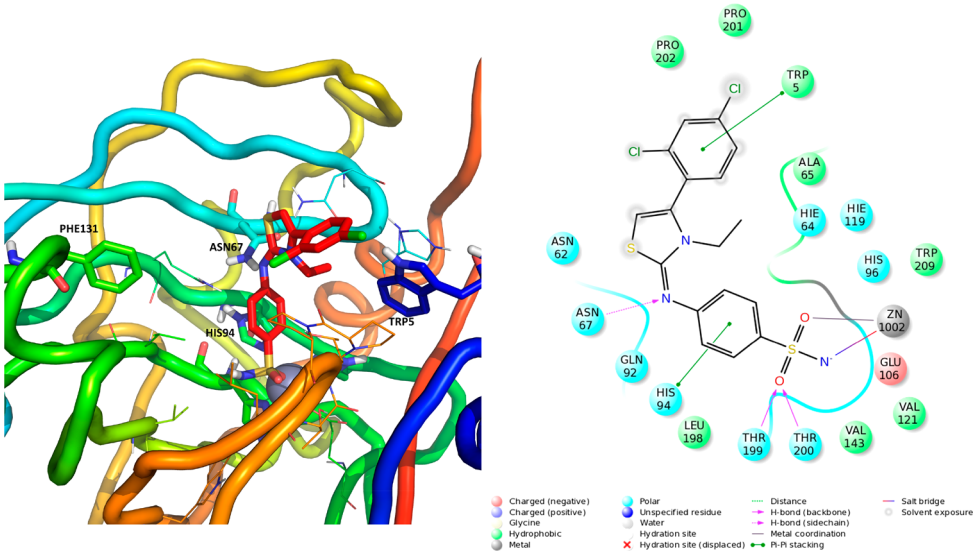

Figure 2. 3D representation of the putative binding mode obtained by docking experiment of **EMAC10101d** into hCA II and relative 2D representation of the complex stabilizing interactions with the binding site residues.

the exception of the above-mentioned **EMAC10101a** and **EMAC10101h**, exhibited K_i values on hCA II ranging from 44.0 to 90.3. Regarding hCA IX, only compound **EMAC10101a** exhibited some selectivity with a K_i value of 108.8 nM. Moreover, its K_i values toward the other hCA isozymes are 4472.4, 724.8, and 1390.0 toward hCA I, II, and XII, respectively.

Docking experiments of compound **EMAC10101d** into hCA II (PDB code 3k34),[35] showed that the benzensulfonamide moiety is able to accommodate close to the catalytic site and chelate the Zn ion. Although the 4-aryl-2,3-dihydro-1,3-thiazol-2-ylidene moiety is bulkier with respect to the known unselective hCA IIi (acetazolamide (**1**), dorzolamide (**4**), Figure S40a), the tail is not able to affect the selectivity toward the membrane isoforms IX and XII. Furthermore, the substitutions in position 3 of thiazolidine moiety with bulky groups was shown to be detrimental for the inhibitory activity K_i, C_5H_6 > Et > Me,[29−31] because of the lack of space in the binding pocket. Hence, to achieve isozyme selectivity, the optimization of these series of compounds should be directed toward the modification of the tail in order to point to more external residues that are bulkier in hCA II than in the isoforms IX and XII and in particular toward the residue Phe131 which is mutated in valine in hCA IX and alanine in XII, Figure S40b.

Overall the complex **EMAC10101d** is stabilized by a hydrogen bond with ASN67, which in hCA XII is substituted by a lysine, and $\pi-\pi$ interactions with HIS94 and TRP5 and by several hydrophobic interactions with surrounding residues. Finally, the sulfonamide moiety is anchored to the Zn^{2+} and residues THR199 and THR200 (Figure 2).

Small changes in the Ar ring produced activity cliffs, showing that the pocket is highly sensitive to the substituent and molecule interactions. As an example, in **EMAC10101a** the loss of a Cl caused a drop of the activity due to the loss of a hydrogen bond and contacts with surrounding residues (Figure S41, Table S4).

In conclusion our data highlighted the relevance of the dihydrothiazole benzensulfonamide scaffold for the inhibition of hCA isoforms. Moreover, indication for future isozyme selectivity optimization was achieved, thus paving the way for the design and synthesis of new derivatives with enhanced properties.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.9b00644.

Experimental procedures and characterization of compounds (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

hCA, human carbonic anhydrase; ZBG, zinc-binding group

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