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Tuning the Dual Inhibition of Carbonic Anhydrase and Cyclooxygenase by Dihydrothiazole Benzensulfonamides

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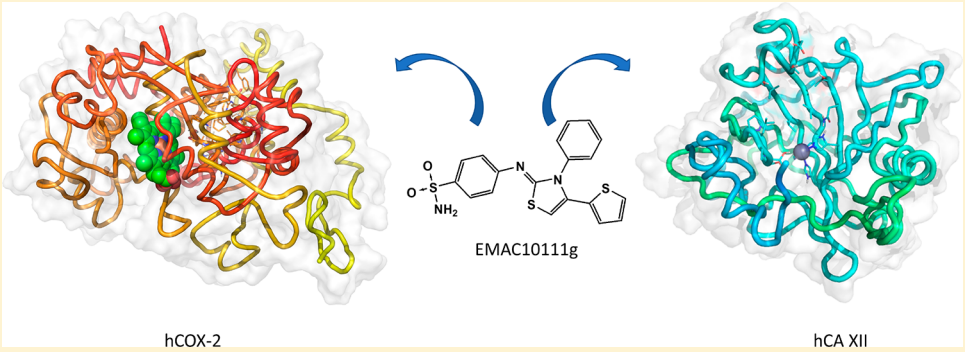
Supporting Information



ABSTRACT: A novel series of of 4-[(3-phenyl-4-aryl-2,3-dihydro-1,3-thiazol-2-ylidene)amino]benzene-1-sulfonamides (**EMAC10111a−g**) was synthesized and assayed toward both human carbonic anhydrase isozymes I, II, IX, and XII and cyclooxygenase isoforms. The majority of these derivatives preferentially inhibit hCA isoforms II and XII and hCOX-2 isozyme, indicating that 2,3,4-trisubstituted 2,3-dihydrothiazoles are a promising scaffold for the inhibition of hCA isozymes and of hCOX-2 enzyme. The nature of the substituent at the dihydrothiazole ring position 4 influenced the activity and selectivity toward both enzyme families. **EMAC10111g** resulted as the best performing compound toward both enzyme families and exhibited preferential activity toward hCA XII and hCOX-2 isozymes.

KEYWORDS: *Dual inhibitors, hCA XII, hCA II, hCOX-2, tumor, molecular docking*

The potential of human carbonic anhydrases (hCAs) and human cyclooxygenase (hCOX) dual inhibitors for the treatment of cancer is an attractive yet challenging goal in the field of medicinal chemistry. CAs are a class of well-studied metalloenzymes that are widely distributed in all living organisms.[1−4] These enzymes are encoded by seven different gene families, αCA, βCA, γCA, δCA, ζCA, ηCA, and θCA.[5−8] Sixteen αCA isozymes have been identified in humans so far, each differing for cellular localization and tissue distribution.[9] 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.[9] The role of hCAs in the regulation of hypoxic-tumors pH has been extensively reported,[4,10−19] and isoforms II, IX, and XII are validated targets for cancer therapy.[20−22] On the other hand, the relevant role of hCOX 1 and 2 in different tumors has been

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outlined.[23−26] Celecoxib, a selective hCOX-2 inhibitor, has been approved by the FDA for adjuvant treatment of patients with familial adenomatous polyposis.[27] Furthermore, the role of Aspirin as an adjuvant agent in the prevention and treatment of several solid tumors has been reported.[28−30] Furthermore, a COX-dependent evasion of immunity has been observed in tumors.[31] Therefore, hCOX inhibitors could be considered as adjuvant agents for the therapy of different cancers. On the basis of the above, the identification of dual inhibitors of hCOX and hCA might lead to highly efficient anticancer agents, capable of simultaneously interacting with two different tumor metabolic pathways. Such compounds are intrinsically advantageous, as they are less prone to induce drug resistance and drug−drug interactions and, not last, they might increase drug-compliance. A key step for the design of dual inhibitors is represented by the identification of the common pharmacophoric features between hCAs inhibitors (hCAIs) and hCOX inhibitors (hCOXIs), particularly hCOX-2Is.

In this respect, it could be evident that the sulfonamide moiety, although with different specific roles,[32−34] is a common chemical feature of several inhibitors of hCAs and hCOX-2 (Figure 1).

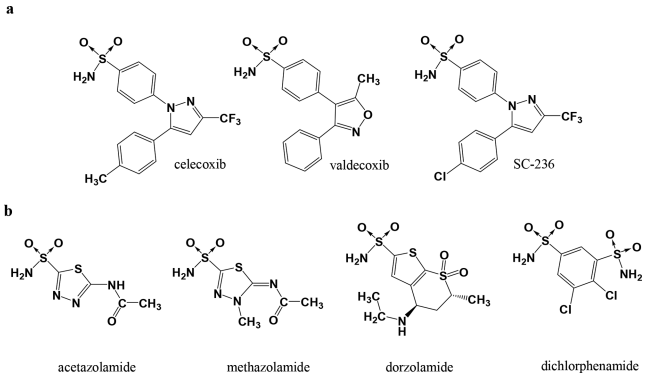
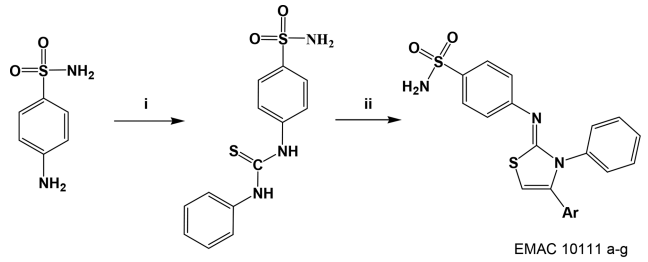


Figure 1. Structurally representative sulfonamide-based (a) COX-2 selective and (b) hCA inhibitors.

The sulfonamide moiety is widely represented within the hCAIs.[33,35,36] Moreover it is a synthetically accessible and versatile scaffold that can be appropriately decorated to achieve isozyme selectivity.[21,35,37−41] In the case of hCOX-2Is a benzene-sulfonamide or a benzene-sulfonyl methyl moiety is present in most of the active compounds in order to occupy a hydrophilic pocket that is made accessible mainly by the substitution of the hCOX-1 Ile523 residue by a smaller valine in hCOX-2. The substitution of the residues Ile434 and His513 of COX-1 with valine and arginine in hCOX-2, respectively, further contributes to enlarge the hydrophilic pocket and to differentiate the two isozyme inhibitors sensitivity.[32] Prompted by these considerations and pursuing our studies on hCAIs,[35,42−44] we have designed and synthesized a small library of 4-[(3-phenyl-4-aryl-2,3-dihydro-1,3-thiazol-2-ylidene)amino]benzene-1-sulfonamides (**EMAC10111a−g**) and evaluated their activity against the hCA I, II, IX, and XII isozymes as well as their inhibition activity toward hCOX 1 and 2 isoforms. These compounds represent a new example of hybrid structures with CA inhibitory activity.[45,46]The synthetic pathway toward compounds **EMAC10111a−g** consists of the reaction of equimolar amounts of 4-amidobenzensulfonamide with phenyl-isothiocyanate in refluxing 2-propanol (Scheme 1). The obtained 1-phenyl-3-(4-sulphamoylphenyl)thiourea was further reacted with the appropriate α-haloketone to give the desired compounds in good yields.

Scheme 1. Synthetic Pathway to Compounds EMAC10111a−g[a]



[a]Reagents and conditions: (i) phenyl-isothiocyanate, 2-propanol, reflux; (ii), α-haloketone, RT/50 °C, 1−2 h.

Compounds **EMAC10111a−g** were characterized by means of analytical and spectroscopic methods (Figures S2−22 and Table S1−2) and then submitted for biological evaluation toward hCA isoforms I, II, IX, and XII and hCOX isozymes 1 and 2 (Table 1). Acetazolamide (AAZ) was chosen as reference compound for hCA activity while indomethacin, diclofenac, FR122047, nimesulide, and DuP 697 were selected as reference compounds for hCOX activity.

With respect to the hCA inhibition, the majority of EMAC derivatives exhibited a preferential activity toward the isoforms hCA II and hCA XII. Interestingly compound **EMAC10111b**, bearing a 2,4-dichlorophenyl moiety in the position 4 of the dihydrothiazole ring, exhibited the highest activity toward hCA II with a K_i value equal to 0.053 μM. All the other compounds, with the exception of **EMAC10111f**, resulted as almost equipotent in the inhibition of hCA II with K_i values ranging from 0.28 to 0.86 μM.

Compounds **EMAC10111a**, **b**, and **d** were the most potent for the inhibition of hCA I isoform. **EMAC10111a** was the most active toward hCA IX, but, on the other hand, it was demonstrated to be one of the less selective derivatives toward a specific hCA isoform, within the studied compounds. With respect to the hCA XII isoform, compound **EMAC10111g**, bearing a thiophene substituent in the position 4 of the dihydrothiazole ring, was identified as the most potent and selective inhibitor, with a K_i value of 0.06 μM and with a selective index (K_i hCA II/K_i hCA XII) higher than 10 fold.

When tested toward the two isoforms of hCOX, none of the new compounds exhibited activity on the hCOX-1 isozyme up to the concentration of 25 μM. Unfortunately, at higher concentrations the compounds precipitated from the test solution. On the contrary, all compounds, except for **EMAC10111d** and **EMAC10111f**, were active toward the COX-2 at concentrations comparable with those of the reference inhibitors indomethacin, diclofenac, and nimesulide.

Among the new derivatives, **EMAC10111g** resulted as the most potent COX-2 inhibitor, with an IC_{50} equal to 12.61 μM. Interestingly, **EMAC10111g** was the most selective inhibitor of hCA XII indicating that the presence of the thiophene group in the position 4 of the dihydrothiazole could be optimal for the interaction with both COX-2 and hCA XII. To achieve a better understanding on the recognition of **EMAC10111g** by both targets and to obtain useful information to further develop

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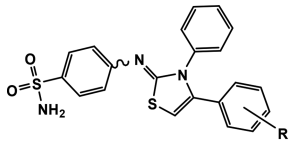
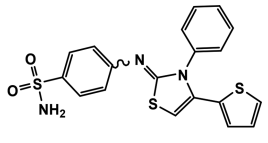
Table 1. Inhibition Data toward hCA I, II, IX, XII, and hCOX 1 and 2 Isozymes of Compounds EMAC10111a−g





		K_i(μM)				IC_{50} (μM)	
Compound EMAC	R	hCA I	hCA II	hCA IX	hCA XII	hCOX-1	hCOX-2
10111a	4-Cl	0.49	0.28	1.25	0.65	*	16.21
10111b	2,4-Cl	0.75	0.053	2.78	0.34	*	18.32
10111c	4-Br	3.46	0.32	2.44	0.80	*	19.73
10111d	4-CH_3	0.96	0.26	2.26	0.78	*	*
10111le	4-OCH_3	3.37	0.28	2.25	0.84	*	21.78
10111f	H	8.02	3.10	3.54	0.30	*	*
10111g	//	9.07	0.86	3.43	0.06	*	12.61
Reference compounds							
AAZ	/	0.25	0.01	0.02	0.006	/	/
Indomethacin	/	/	/	/	/	12.16	35.20
Diclofenac	/	/	/	/	/	18.23	23.62
FR122047	/	/	/	/	/	0.09	**
Nimesulide	/	/	/	/	/	***	23.14
DuP 697	/	/	/	/	/	22.61	0.12

*Inactive at 25 μM (highest concentration tested). At higher concentrations, the compounds precipitate. **Inactive at 100 μM (highest concentration tested). At higher concentrations, the compound precipitates. ***Inactive at 500 μM (highest concentration tested). At higher concentrations, the compound precipitates.

such compounds as tumor hCAs and COX-2 dual inhibitors, molecular modeling studies were performed. Due to the presence of the double bond between the amino benzene sulfonamide and the dihydrothiazole moieties, merging known theoretical approaches,[47,48] the population of "*E*" and "*Z*" isomers was preliminarily investigated. Conformational search (Supporting Information) was carried out on both **EMAC10111g** isomers. The internal energy of each generated conformer was considered in a Boltzmann analysis computed at 300 K. Interestingly theoretical results indicated the "*Z*" isomer population of about 100%, basically discarding the presence of "*E*" isomer. As a consequence, further molecular modeling was carried out taking into account the (*Z*)-**EMAC10111g** configuration only.

Docking protocols were first validated with self- and cross-docking experiments. In particular, the validation highlighted the ability of all settings protocols to reproduce, with acceptable RMSD, the experimental binding mode of most ligands.

Furthermore, all methods clearly confirmed that highly selective hCOX-2Is cannot be docked inside hCOX-1.

Therefore, not surprisingly, we observed that (*Z*)-**EMAC10111g** was not able to recognize the hCOX-1 active site, thus corroborating biological results. On the contrary, the putative binding mode depicted in Figure 2 shows that the compound can be accommodated into the hCOX-2 pocket. The theoretical complex was stabilized by hydrophobic interactions with several residues such as Val89, Pro86, Leu123, Tyr115, Ala527, Val116, Tyr355, Leu531, Leu83, and Pro84. Furthermore, the sulfonamide moiety was involved in a hydrogen bond with Leu82. Finally, the aromatic moieties interacted with Lys83 and Arg120, which act as a channel gate that opens the hCOX active site.[49]

The hCA isoforms docking simulations were performed with a previously applied protocol.[43,44] We improved cross docking

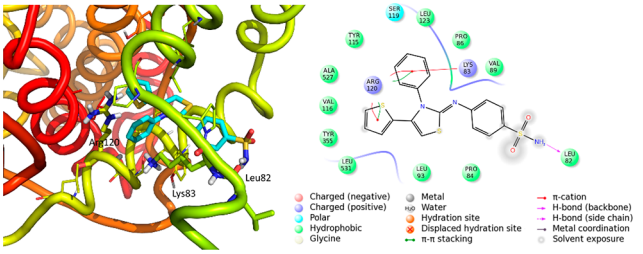


Figure 2. 3D representation of the putative binding mode obtained by docking experiment of (*Z*)-**EMAC10111g** into hCOX-2 and 2D representation of the complex stabilizing interactions with the binding site residues.

validation and compared the previously utilized crystal structures with the newest and with better resolution pdb entries. While in the case of hCA XII and hCA IX we did not change the receptor, when hCA II was investigated, we considered both the previously applied 3F8E[50] and the 3K34 crystal (resolution 0.9 Å),[51] where the His64 shows an alternate conformation compared to the previously considered 3F8E. The new receptor model improved cross-docking RMSD results. Concerning the validation, it was observed that the docking program was able to reproduce the binding mode of the Zn^{2+} interacting portion, with both the ion and the other catalytic site residues. On the contrary, different binding conformations, due to the absence of anchoring residues, were observed when the solvent accessible ligand portion was docked. Furthermore, in our theoretical protocol, we considered the receptor without waters and other agents, such as glycerol and ethylene glycol, used for crystallization. Considering that these latter compounds often occupy the entrance cavity in the crystals, more space was available to accommodate the cap of docked hCAIs. The binding mode of the most promising compound (*Z*)-**EMAC10111g** in all

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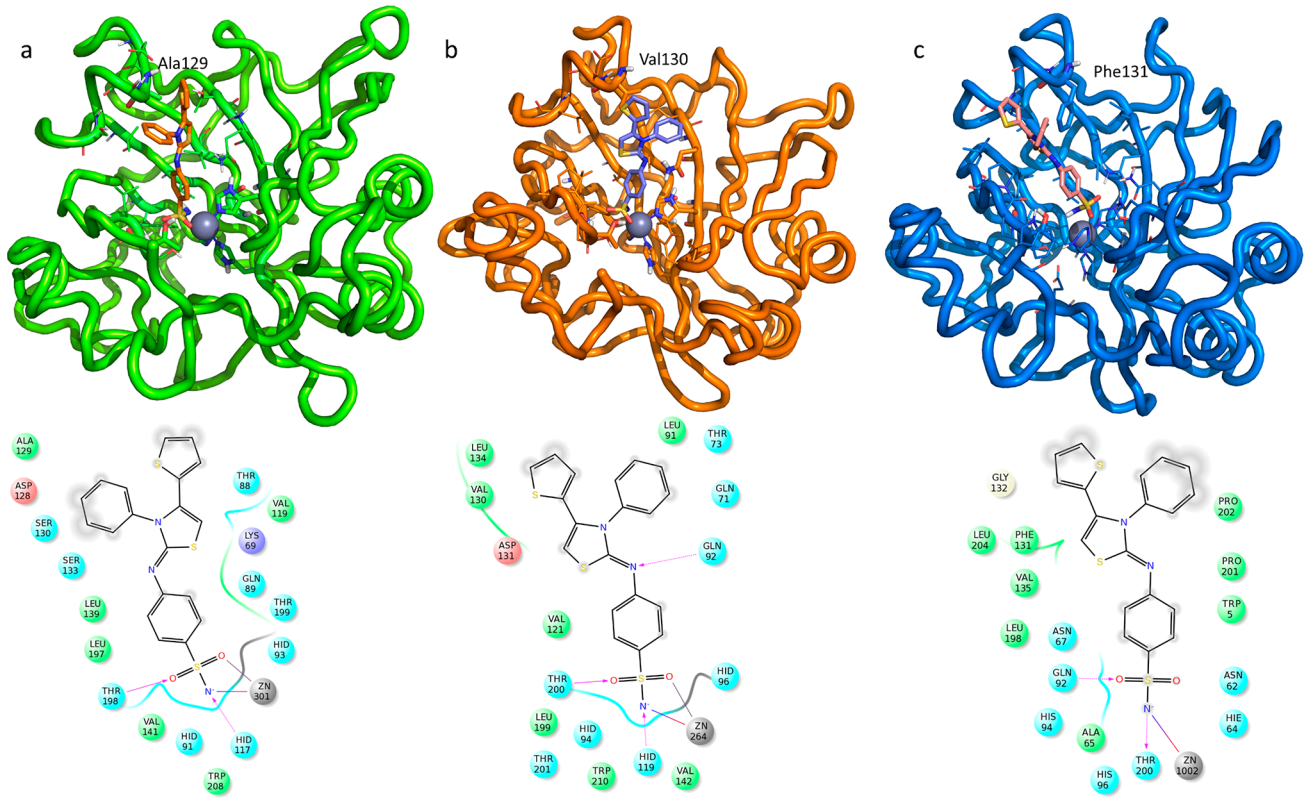

Figure 3. 3D representation of the putative binding mode obtained by docking experiment of **(Z)-EMAC10111g** into (a) hCA XII, (b) hCA IX, and (c) hCA II. Below each is depicted the relative 2D representation of the complexes stabilizing interactions with the residues of the binding site.

isoforms showed how the compound was able to reach the catalytic site with the benzene sulfonamide group. Furthermore, despite the bulky cap exposed to solvent, the "*Z*" configuration allowed for a Y shaped geometry of the three aromatic groups that can, therefore, be accommodated in hCA II, IX, and XII. However, when docked in hCA II, the cap aromatic rings are both pushed toward one side (Figure 3c). The reason was mainly addressed to the presence of Phe131, which is replaced by an Ala by a Val in hCAXII and hCAIX respectively.

Our data indicated that 4-[(3-phenyl-4-aryl-2,3-dihydro-1,3-thiazol-2-ylidene)amino]benzene-1-sulfonamide derivatives could be considered as a promising scaffold for the dual inhibition of hCA and hCOX-2 enzymes. The information on the putative binding modes in both targets and relative isoforms of the newly synthesized inhibitors encourage us to further investigate and optimize these derivatives in order to improve their activity and selectivity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00352.

Experimental procedures and compounds' characterization (PDF)

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○R.M. and S.D. contributed equally. 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

hCOX, human cyclooxygenase; hCA, human carbonic anhydrase; hCOXIs, human cyclooxygenase inhibitors; hCAIs, human carbonic anhydrase inhibitors

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