Lucky Switcheroo: Dramatic Potency and Selectivity Improvement of Imidazoline Inhibitors of Human Carbonic Anhydrase VII

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Supporting Information

ABSTRACT: A substantial improvement of potency and selectivity of imidazoline-based inhibitors of hCA VII (a promising target for the treatment of seizures and neuropathic pain) was achieved by simply switching the position of the benzenesulfonamide moiety from N¹ (as in the earlier reported series) to C². Selectivity indices vs the off-target isoforms (hCA I, I, I and IV) greater than 100 were reached, which is exceedingly rare for hCA VII inhibitors. The drastic profile improvement of the new series has been rationalized by an additional hydrogen bonding with the nonconserved Q69 residue in the active site of hCA VII (absent in the other three isoforms studied), which also results in a favorable accommodation of the inhibitor's lipophilic periphery in the nearby hydrophobic pocket. The robustness of the docking simulations was tested and confirmed by molecular dynamics simulations.

KEYWORDS: Privileged scaffold, 2-imidazolines, N-arylimidazolines, carbonic anhydrase inhibitors, isoform selectivity, zinc binding group, primary sulfonamide, docking simulation, hydrogen bonding, nonconserved residue, molecular dynamics

Human carbonic anhydrases (hCAs, EC 4.2.1.1) regulate the pH inside and outside the living cell by catalyzing reversible hydration of carbon dioxide to produce bicarbonate anion and proton. The fundamental significance of their catalytic function links hCAs to many important physiological processes and therefore allows considering these 15 enzymes (hCA I–XIV, including hCA VA and VB isoforms) as drug targets for a range of diseases such as glaucoma, cancer, epilepsy, obesity, and others.^{1–5} Development of isoform-selective inhibitors of hCA is desirable from the standpoint of delineating the therapeutic effect caused by inhibition of the isoform in question from that resulting from the inhibition of the “off target” ones.⁶ Moreover, the ongoing validation of certain hCA isoforms as targets for therapeutic intervention requires reliable, selective tool compounds.⁷ These can be employed to study, at the cellular or whole-organism levels, the consequences of inhibiting a particular hCA isoform, to gain structural insights into their binding mode via X-ray

crystallography and thus serve as well-characterized leads for the development of future therapeutic agents.⁸

One hCA isoform, whose potential as a drug target is currently being unveiled, is hCA VII. First characterized in 1991,⁹ it is mostly expressed in various regions of the brain where it is involved in GABAergic neuronal excitation.¹⁰ hCA VII has been stipulated as a target for the treatment of epileptic seizures¹¹ and neuropathic pain.¹² Additionally, it has been suggested that cysteine-rich regions of the enzyme may be involved in cellular protection against oxidative damage.¹³ The crystal structure of mutant hCA VII obtained with nonselective inhibitor acetazolamide¹⁴ has been successfully employed to understand the binding mode and the origin of isoform selectivity of newly designed inhibitors¹⁵ as well as for virtual

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screening for new compounds in the commercial domain, for which selective hCA VII inhibition was later confirmed experimentally.¹⁶

Potent inhibition of hCA VII isoform with high selectivity over other isoforms (particularly, the abundant cytosolic hCA I and II, which have a high catalytic activity and are the usual off-targets in this case¹⁷) is extremely rare; hCA VII selectivity indices (SIs) greater than 100 are virtually absent in the current literature. Low-nanomolar¹⁸ and even subnanomolar¹⁹ levels of hCA VII inhibition are certainly achievable but typically accompanied by hCA I and II inhibition in the single- to double-digit nanomolar range. Another isoform primarily localized in the central nervous system, for which a clean profile of hCA VII inhibitors would be desirable, is the membrane-bound hCA IV.²⁰ The latter has been shown to be involved in supporting the functioning and nutrition of the glial cells.²¹ Being an approach to the treatment neurologic disorders on its own, hCA IV inhibition should be separated from that of hCA VII for the sake of targeted therapy development.

Recently, we described a series of imidazoline-based primary sulfonamides **1**, which were synthesized via a Pd-catalyzed *N*-arylation²² followed by sulfonamide deprotection. They displayed preferential inhibition of hCA VII over hCA I and IV (however, with modest selectivity) while also inhibiting hCA II in the same concentration range as they did hCA VII.²³ The observed separation of hCA VII potency from its usual off-targets was viewed as a valuable, chemotype-related finding. However, we sought to improve the desired potency of inhibitors **1** and also eliminate or lower the inhibition of hCA II, which has a wide tissue distribution and, preferably, should not be affected in the course of neurologic drug intervention. 2-Imidazolines²⁴ and, more specifically, *N*-aryl-imidazolines²⁵ are privileged scaffolds for drug design, and therefore, we were initially not keen to depart from this chemotype and decided to take a more palliative approach and investigate a different presentation of the vicinal aromatic groups on the imidazoline scaffold. Compounds **2**, where the pharmacophoric benzenesulfonamide moiety (acting as prosthetic zinc binding group, or ZBG, in hCA inhibitors of this chemical class) is at position 2 of the imidazoline core, would be difficult to access using the Pd-catalyzed (Buchwald–Hartwig) protocol as it only allows introducing electron-deficient (hetero)aromatic moieties.²² To circumvent this obstacle, we recently developed an alternative imidazoline *N*-arylation approach using the Chan–Evans–Lam procedure²⁶ and expected to efficiently employ it to append various aromatic groups to protected benzenesulfonamide imidazoline **3** on reaction with boronic acids **4** (Figure 1).

The starting imidazoline **3** was synthesized from nitrile **5** using the CS₂-catalyzed reaction with ethylenediamine. The Chan–Evans–Lam arylation with boronic acids **4** in DMSO using the open-flask reaction format²⁶ furnished DMB-protected compounds **6a–i**. The latter were transformed into target primary sulfonamides **2a–i** on brief treatment with TFA in ice-cold dichloromethane (Scheme 1).

Having synthesized compounds **2a–i**, we proceeded to interrogate hCA VII and other isoforms with this set of tools. Biochemical testing of compounds **2a–i** against the same panel of hCA as was described for compounds **1** (hCA I, II, IV, and VII)²³ using hCA pan-inhibitor acetazolamide (AAZ) as a comparator revealed a promising (and desired in the context of present study) SAR trend (Table 1).

Even a brief comparison of chemotypes **1** and **2** in terms of their inhibitory profiles against the panel of hCAs shown in

Figure 1. Approaches to imidazoline-based sulfonamides **1** and **2**.

Scheme 1. Synthesis of Compounds **2a–i**^a

^aReagents and conditions: (a) (DMB)₂NH, TEA, CH₂Cl₂, r.t., overnight (93%); (b) ethylenediamine, cat. CS₂, 120 °C, 2 h (80%); (c) (Het)ArB(OH)₂, K₂CO₃, Cu(OAc)₂·H₂O, DMSO, open flask, r.t., overnight (33–43%); (d) TFA, CH₂Cl₂, 0 °C, 30 min (52–68%).

Table 1. Inhibitory Profile of Compounds **2a–i** against hCA I, II, IV, and VII^a

compd	(Het)Ar	K _i (nM)			
		hCA I	hCA II	hCA IV	hCA VII
2a	Ph	466.1	376.5	3912.1	6.8
2b	4-ClC ₆ H ₄	453.0	182.1	608.2	0.96
2c	4-FC ₆ H ₄	428.7	742.2	1036.5	9.4
2d	4-MeOC ₆ H ₄	317.9	53.3	3643.4	0.97
2e	2-MeC ₆ H ₄	70.7	61.7	961.2	0.93
2f	2-EtOC ₆ H ₄	216.3	866.5	2299.7	0.84
2g	3,4-(MeO) ₂ C ₆ H ₄	847.6	723.6	8302.6	0.87
2h	3-pyridyl	316.0	848.3	1826.6	0.84
2i	5-pyrimidinyl	274.5	703.2	2296.0	0.83
1a	4-FC ₆ H ₄	2343.0	76.1	9726.0	303.5
1b	4-ClC ₆ H ₄	960.5	46.7	8142.0	269.0
AAZ		250.0	12.1	74.0	6.0

^aMean K_i values from three different stopped-flow assays (errors were in the range of ±5–10% of the reported values). Inhibitory profile of compounds **1a–b**²³ is provided for comparison.

Table 1 and reported earlier²³ allows one to note a remarkable, up to two orders of magnitude on average, improvement of hCA VII potency, with some of the compounds reaching into the subnanomolar range. At the same time, the inhibition

profile of **2** against hCA I and hCA IV remains relatively low, similarly to **1**. However, we were particularly pleased to find that the potency against hCA II (the principal “off-target” of **1** as hCA VII inhibitors) was significantly lowered, thus even more improving the selectivity of **2** for hCA VII. This is clearly illustrated by comparing the pairs of direct analogues in series **1** (**1a–b**) and **2** (**2b–c**) where swapping the positions of sulfonamide- and halogen-substituted aryl groups (**1a** → **2c** and **1b** → **2b**) leads to dramatic improvement of hCA VII selectivity, particularly for within the latter pair (Figure 2).

Figure 2. Selectivity indices ($K_i(\text{hCA N})/K_i(\text{hCA VII})$) of hCA I, II, and IV vs hCA VII inhibition by compounds **1a–b** and **2b–c**.

In order to rationalize the hCA VII selectivity improvement trend observed on going from series **1** to series **2**, we performed a back-to-back docking simulation of the binding of compounds **1b** and **2b** within the active site of hCA VII and compared it to the docking poses of the same compounds in the active site of hCA I, II, and IV. Figure 3 shows the binding site interactions of compound **1b** into the four hCA isoforms. In all the binding sites the compound shows almost the same binding disposition: the sulfonamide group acts as a ZBG and forms hydrogen bonds with the protein backbone and the hydroxy group of the threonine residue; the phenyl ring attached to the ZBG does not show important interactions. The 4-chlorophenyl substituent is partially exposed to the solvent, and it is in contact with the external region of the binding site cavity. With regard to the imidazoline ring, in the hCAI, IV, and VII binding sites, it shows the same disposition with the presence of lipophilic interactions with L142 (isoleucine for hCAIV) and L199 (hCAI sequence number). In the hCAII binding site, due to the presence of I91 and F130, the imidazole ring of compound **1b** is shifted by about 3 Å and shows lipophilic interactions with these two residues.

The analysis of compound **2b** suggests that for the hCAI, II, and IV isoforms, it shows the same binding disposition of compound **1b**, with the 4-chlorophenyl substituent exposed to the solvent without important interactions with the protein and the interaction of the imidazoline ring with L142 (isoleucine for hCAIV) and L199 (hCAI sequence number) for hCAI and IV and with I91 and F130 for hCAII (Figure 4). Interestingly, in

Figure 3. Docking of compound **1b** into hCAI (A), hCAII (B), hCAIV (C), and hCAVII (D).

Figure 4. Docking of compound **2b** into hCAI (A), hCAII (B), hCAIV (C), and hCAVII (D).

the hCAVII binding site, due to the presence of the nonconserved Q69, the compound shows a completely different binding orientation (Figure 4D). As for the other ligand–enzyme complexes, the sulfonamide group acts as a ZBG and forms hydrogen bonds with the protein backbone and the hydroxy group of the T201, the nitrogen of the imidazole ring forms an H-bond with the nonconserved Q69, and this interaction allows the disposition of the 4-chlorophenyl group in the lipophilic region of the binding site mainly delimited by L143, L200, and F133, thus suggesting this as a likely reason for the high hCAVII inhibition activity observed for this compound.

In order to evaluate the reliability of the docking results²⁷ and to carry out an analysis of the ligand–protein interaction, the hCAVII–**2b** complex was used as a starting structure for 11 ns of molecular dynamics (MD) simulation. As shown in Figure 5, the complex is stable during the simulations, and the analysis of the root-mean-square deviation (rmsd) of all the heavy

Figure 5. Analysis of the MD simulation of **2b** complexed with hCAVII. (A) Minimized average structure of the complex. (B) Rmsd analysis of the heavy atoms of the receptor and the ligand from the starting model structure during the simulation.

atoms from the X-ray structures highlights a stabilization of the rmsd value around 1.0 Å. Regarding the geometry of the compound, analyzing the rmsd of the position of the ligand during the simulations with respect to the starting structure, it maintains its starting disposition with an rmsd value between 0.2 and 0.5 Å. Finally, the H-bond between Q69 and the imidazoline nitrogen appears to be very stable as this interaction is maintained for about 90% of the MD simulation.

In summary, we reported a substantial improvement of potency and selectivity of imidazoline-based inhibitors of hCA VII by switching the position of the benzenesulfonamide moiety from the N¹ (as in the earlier reported series) to the C² position of the imidazoline ring. This minor alteration in the chemotype resulted in subnanomolar inhibition of hCA VII, a promising target for the treatment of seizures and neuropathic pain. This eloquently illustrates the privileged character of imidazolines for drug design and a possibility to fine-tune affinity profiles against a panel of closely related targets by altering the positions of the periphery appendage groups around this polar, nonflat scaffold.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmchemlett.7b00300](https://doi.org/10.1021/acsmchemlett.7b00300).

Experimental procedures for the synthesis of **5**, **3**, **6a–i**, **2a–i**; docking and molecular dynamics simulations protocol for compounds **1b** and **2b** (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

hCA, human carbonic anhydrase; ZBG, zinc-binding group; DMSO, dimethyl sulfoxide; DMB, 2,4-dimethoxybenzyl; TEA, triethylamine; TFA, trifluoroacetic acid; AAZ, acetazolamide; SAR, structure–activity relationship; MD, molecular dynamics; RMSD, root-mean-square deviation

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