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Carbonic anhydrase inhibitors. Interaction of the antitumor sulfamate EMD 486019 with twelve mammalian carbonic anhydrase isoforms: Kinetic and X-ray crystallographic studies ☆

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ABSTRACT

The new antitumor sulfamate **EMD 486019** was investigated for its interaction with twelve catalytically active mammalian carbonic anhydrase (CA, EC 4.2.1.1) isozymes, hCA I – XIV. Similarly to 667-Coumate, a structurally related compound in phase II clinical trials as steroid sulfatase/CA inhibitor with potent antitumor properties, **EMD 486019** acts as a strong inhibitor of isozymes CA II, VB, VII, IX, XII, and XIV (K_Is in the range of 13–19 nM) being less effective against other isozymes (K_Is in the range of 66–3600 nM against hCA I, IV, VA, VI, and mCA XIII, respectively). The complete inhibition profile of 667-Coumate against these mammalian CAs is also reported here for the first time. Comparing the X-ray crystal structures of the two adducts of CA II with **EMD 486019** and 667-Coumate, distinct orientations of the bound sulfamates within the enzyme cavity were observed, which account for their distinct inhibition profiles. CA II/IX potent inhibitors belonging to the sulfamate class are thus valuable clinical candidates with potential for development as antitumor agents with a multifactorial mechanism of action.

Inhibitors of zinc enzyme carbonic anhydrases (CAs, EC 4.2.1.1) have clinical applications as diuretic, antiglaucoma, antiobesity, or antitumor drugs/diagnostic tools.[1–6] Various CA isoforms are responsible for specific physiological functions, and drugs with such a diversity of actions target different isozymes of the 15 presently known in humans (CA I- CA XIV, but there are two CA V type isoforms, CA VA and CA VB).[2–6] In all of them, the inhibitor is bound usually as anion to the catalytically critical Zn(II) ion, also participating in extensive hydrogen bond networks and van der Waals interactions with amino acid residues both in the hydrophobic and hydrophilic halves of the enzyme active site, as shown by X-ray crystallographic studies of such enzyme-inhibitor complexes.[7–15] Three main classes of potent CA inhibitors (CAIs) were described so far: the sulfonamides, the sulfamates, and the sulfamides, possessing the general formula R-X-SO_2NH_2, where X is nothing, O or NH, respectively.[1–6] X-ray crystal structures are available for many adducts of several isozymes (i.e., CA I, II, IV, V, XII, and XIV) [7–15] mostly with sulfonamides, with several sulfamates (including the simplest one, sulfamic acid)[10] and with few sulfamides, such as the simple derivative $H_2NSO_2NH_2$ [10a] and the topiramate–sulfamide analogue.[10b] A number of such derivatives are clinically used drugs, such as acetazolamide (**AAZ**), methazolamide, ethoxzolamide, dichlorophenamide, dorzolamide, brinzolamide, topiramate (**TPM**), zonisamide, sulpiride, sulthiame, celecoxib, and valdecoxib among others.[1–3] Other compounds are in clinical development, such as the antitumor sulfonamide indisulam and 667-Coumate (**CMT**), a compound acting both as a potent steroid sulfatase and as a CA inhibitor.[1]

CA inhibitors (CAIs) are mainly used in therapy as diuretics and antiglaucoma agents but some of them also show marked anticonvulsant, antiobesity, and antitumor effects.[1,2,5–11] However, most of the presently available compounds in clinical use show undesired side effects due to the indiscriminate inhibition of CA isoforms other than the target ones.[1,2,5–14] Thus, many new CAI classes are being developed in the search of isozyme-selective compounds as potential drugs with less side effects.[1–5,11–15]

In this work, we report a detailed inhibition study of all 12 catalytically active mammalian CA isoforms (i.e., CA I, II, III, IV, VA, VB, VI, VII. IX, XII, XIII, and XIV, of human—h or murine—m origin) with a new sulfamate compound, **EMD 486019**, as well as with its closely related analogue in phase II clinical trials as an antitumor drug, 667-coumate (**CMT**). The high resolution X-ray crystal structure for the adduct of **EMD 486019** with the ubiquitous and physiologically dominant isoform hCA II was also obtained and is presented here. Comparison of the X-ray crystal structures of the adducts of **EMD 486019** with that of the hCA II—**CMT** complex

☆ The coordinates of the hCA II—EMD 486019 adduct have been deposited in PDB, ID code 3DD8.

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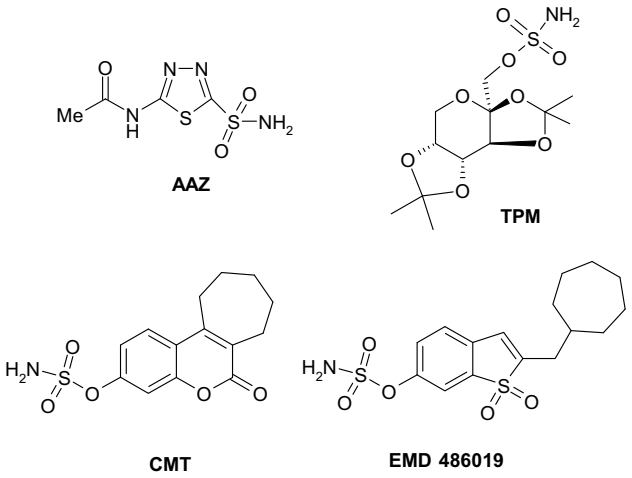
reported earlier by Potter's group[13b] allow us to draw some interesting conclusions regarding the drug design of sulfamate CAIs.

The following should be noted regarding the CA inhibition data of the three sulfamates (**TPM, CMT**, and **EMD 486019**) and standard sulfonamide CA inhibitor **AAZ** presented in Table 1[16]: (i) **EMD 486019** behaves as a potent inhibitor of isozymes hCA II, hCA VB, hCA VII, hCA IX, hCA XII, and hCA XIV, showing inhibition constants in the low nanomolar range (K_Is of 13–19 nM). The compound is also a medium potency mCA XIII inhibitor (K_I of 66 nM), and a weak inhibitor of hCA I, hCA IV, hCA VA, and hCA VI (K_Is in the range of 654–3600 nM). As most sulfonamides and sulfamates,[1–3,15e] the compound is a very weak hCA III inhibitor (Table 1); (ii) 667-Coumate presents a distinct inhibition profile as compared to the new sulfamate **EMD 486019**. Thus, **CMT** is a potent inhibitor of isoforms hCA II, hCA IV, hCA VII, hCA IX, and hCA XII, with inhibition constants in the range of 12–34 nM. On the other hand, all other isozymes (e.g., hCA I, hCA VA, hCA VB, hCA VI, mCA XIII, and hCA XIV) were weakly inhibited by this compound, with K_Is in the range of 653–3450 nM, whereas hCA III is inhibited in the millimolar range (Table 1); (iii) Topiramate (**TPM**), a clinically used sulfamate as antiepileptic drug,[4,6] behaves as a potent inhibitor of isoforms II, VB, VII, and XII (K_Is in the range of 0.9–30 nM), it is a medium potency inhibitor against hCA VA, hCA VI, hCA IX, and mCA XIII (K_Is in the range of 45–63 nM), and weakly inhibits hCA I, hCA III, hCA IV, and hCA XIV (K_Is in the range of 250–7.8 × 10^5nM); (iv) acetazolamide **AAZ**, the classical inhibitor of CAs, in clinical use since 1954,[1–3] behaves as a potent inhibitor against isoforms II and VI-XIII (K_Is in the range of 2.5–25 nM), is a medium potency inhibitor against hCA IV, hCA VA, and VB, as well as hCA XIV, with K_Is in the range of 41–74 nM. It has a rather weak affinity only for hCA I (K_I of 250 nM) and very weak affinity for hCA III (K_I of 2 × 10^5 nM).

The two structurally related sulfamates **CMT** and **EMD 486019** behave thus quite similarly against the following two groups of isozymes: hCA I, hCA III, hCA VA, and hCA VI (both are weak inhibitors); hCA II, VII, IX, and XII (both are strong inhibitors). However, there are net differences between them regarding their interactions with: hCA IV (**CMT** is a potent inhibitor, whereas **EMD 486019** is a weak one); hCA VB and hCA XIV (**CMT** is a weak inhibitor, whereas **EMD 486019** is a potent one); and mCA XIII (**CMT** is a weak inhibitor, **EMD 486019** a medium potency one). Thus, except for isozyme IV, much better inhibited by **CMT**, the sulfamate **EMD 486019** behaves always as a better CAI as compared to the corresponding behavior (against the same isozyme) of **CMT** (against hCA XII the two compounds may be considered equipotent). Considering all compounds investigated here, **EMD 486019** is a quite potent hCA II/hCA XII inhibitor, with potencies in the same range as **AAZ** and **TPM** (similarly also to **CMT**), it is the best hCA VB/hCA IX/hCA XIV inhibitor among the four derivatives considered here, but it is a much weaker inhibitor of hCA VA and hCA VI as compared to **AAZ** and **TPM**. Thus, the inhibition profile of **EMD 486019** is completely different from those of the clinically used derivatives **AAZ** and **TPM**, being more similar to that of **CMT**, but distinct of it. **EMD 486019** generally behaves as a better inhibitor of most isozymes (as compared to **CMT**), except for hCA IV.

Here, we also present the detailed X-ray crystallographic structure of the hCA II—**EMD 486019** adduct, and its comparison with the hCA II—**CMT** adduct reported earlier by Potter's group (at a resolution of 1.95 Å).[13b,17] Crystallographic refinement of the hCA II—**EMD 486019** adduct was performed at a final resolution of 1.90 Å. Crystals of the adduct were isomorphous with those of the native protein,[17] allowing for the determination of the crystallographic structure by difference Fourier techniques. The refined structure presents a good geometry with r.m.s.d. from ideal bond lengths and angles of 0.009 Å and 1.2°, respectively. The overall quality of the model was excellent with all residues in the allowed regions of the Ramachandran plot. Refinement statistics are summarized in Table 2. Inspection of the electron density maps at various stages of

Table 1
Inhibition data with the clinically used compounds **AAZ**, **TPM,** the clinical candidate **CMT,** and **EMD 486019,** against isozymes CAI—XIV



Isozyme*	K_I** (nM)			
	AAZ	**TPM**	**CMT**	**EMD 486019**
hCAI[a]	250	250	3450	3600
hCAII[a]	12	10	21[c]	14
hCAIII[a]	2.0 × 10^5	7.8 × 10^5	7.0 × 10^5	7.4 × 10^5
hCAIV[a]	74	4900	24	842
hCAVA[a]	63	63	765	682
hCAVB[a]	54	30	720	18
hCAVI[a]	11	45	653	654
hCAVII[a]	2.5	0.9	23	19
hCAIX[b]	25	58	34	18
hCAXII[b]	5.7	3.8	12	13
mCAXIII[a]	17	47	1050	66
hCAXIV[a]	41	1460	755	13

* h, human; m, murine isozyme.
** Errors in the range of 5–10% of the reported value (from 3 different assays).
[a] Human (cloned) isozymes, by the CO_2 hydration method.
[b] Catalytic domain of human, cloned isozyme, by the CO_2 hydration method.[16]
[c] IC_{50} value of 25 nM reported by Potter's group.[13b]

Table 2
Crystallographic parameters and refinement statistics for the hCA II—**EMD 486019** adduct

Parameter	Value
X-ray source	Enhance Ultra
Wavelength (Å)	1.5418
Space group	P21
Cell parameters	*a* = 42.1 Å
	b = 41.5 Å
	c = 72.2 Å
	(3 = 104.23°
No of total reflections	46,588
No. of unique reflections	18,403
Completeness (%)[a]	96.0(89.3)
$\langle I/\sigma(I)\rangle$	10.8 (4.0)
Resolution range (Å)	20.00 – 1.90
R-merge (%)[b]	8.0 (21.1)
R-factor (%)[c]	20.0
R-free (%)	25.0
Rmsd of bonds from ideality (Å)	0.009
Rmsd of angles from ideality (°)	1.2

[a] Values in parentheses relate to the highest resolution shell (2.00–1.90).
[b] R-merge = $\sum|I_i - \langle I\rangle|/\sum I_i$.
[c] R-factor = $\sum|F_o - Fc|/\sum F_o$; *R-free* calculated with 5% of data.