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NOVEL CARBONIC ANHYDRASE ISOZYMES I, II AND IV ACTIVATORS INCORPORATING SULFONYL-HISTAMINO MOIETIES

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Abstract: Sulfonylamido(ureido) derivatives of histamine were synthesized by an original procedure in order to obtain tight-binding activators of the zinc enzyme carbonic anhydrase (CA), exploiting the binding energy of the alkyl/arylsulfonyl moieties with amino acid residues at the entrance of the active site. In contrast to the lead molecule, histamine, the new derivatives possessed higher affinity for three different CA isozymes, as evidenced by compairing the affinity constants of these compounds for isozyme CA II.

Introduction: Carbonic anhydrase (CA, EC 4.2.1.1) inhibitors of the unsubstituted sulfonamide type, RSO_2NH_2, are widely used drugs for the treatment or prevention of a variety of diseases, such as: glaucoma,[1,2] epilepsy,[3] gastric and dueodenal ulcers,[4] or acid-base disequilibria[5] among others. In contrast to inhibitors, activators of this enzyme (for which at least 8 different isozymes were isolated up to now in higher vertebrates)[6] were much less investigated. CA-s play an important role in the animal, vegetal and bacterial kingdoms, in processes such as photosynthesis, respiration, homeostasis and pH regulation.[4-7] Only recently the X-ray crystallographic structures of the first adducts of the physiologically relevant isozyme II (hCA II) with the activators histamine[7] and phenylalanine (in this case a tertiary complex, in which azide is also bound to the Zn(II) ion)[8] have been reported by this group. Furthermore, few other QSAR[9] or synthetic chemistry[10] studies were reported in the field of CA activators, although some of these compounds might be used in the treatment of the CA deficiency syndrome, a genetic disease of bone, brain and kidney affecting a large enough number of patients.[11] In this condition, a certain CA isozyme gene (generally CA II, I or IV) is either not expressed, or its protein product is unstable due to deleterious mutations, and the corresponding CA isozyme is absent in the blood, kidney or lung of such patients. No pharmacologically specific treatment for this condition is available up to now. CA activators are also important for understanding the CA catalytic and inhibition mechanisms.[7-10]

The lead molecule considered by us for obtaining tighter binding CA activators was histamine **1** itself. As seen from Fig.1, the activator molecule is bound at the entrance of the hCA II active site cavity, where it is anchored by hydrogen bonds to amino-acid side-chains and to water molecules. Such hydrogen bonds involve

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only the nitrogen atoms of the imidazole moiety, whereas the terminal aliphatic amino group is not experiencing any contact with the enzyme, but is extending away from the cavity into the solvent. On the other hand, the Nδ1 and Nε2 atoms of the histidine imidazole ring are engaged in hydrogen bonds with the side-chains of Asn 62, His 64, Gln 92 and with Wat152.[7] Thus, it appeared of interest to derivatize the lead at its aliphatic NH_2 moiety, just in order to exploit the energy of binding of such modified groups with amino acid residues at the edge of the active site. This approach has been successfully used both by Whiteside's[12] and our groups[7,13] for the design of tight-binding, isozyme-specific sulfonamide CA inhibitors.

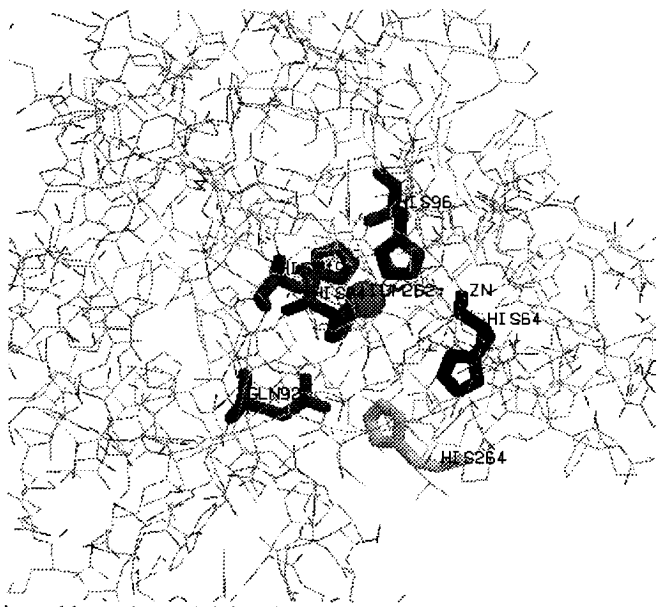
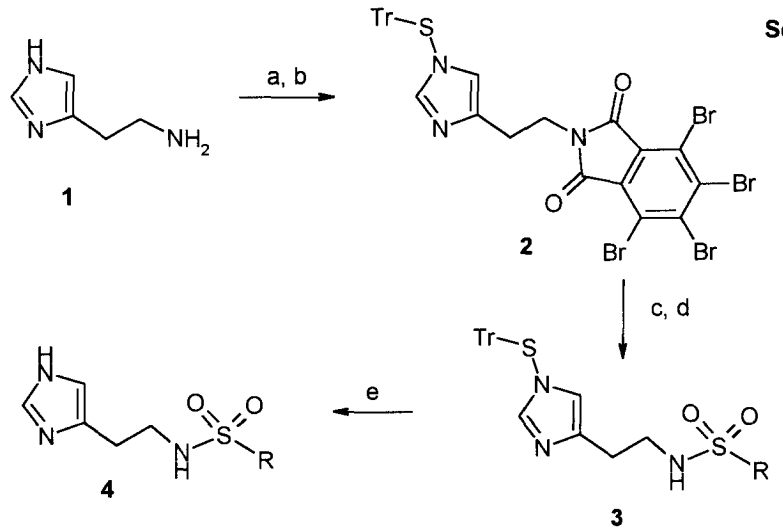


Fig. 1: hCA II - histamine adduct: the Zn(II) ion (green sphere) and its three histidine ligands (His 94, His 96 and His 119, in red) are shown at the center of the active site, whereas histamine (numbered as His 264, in yellow) is situated at the entrance in it, between residues His 64 (blue) and Gln 92 (red). The figure was generated from the X-ray coordinates of the hCA II-histamine adduct,[7] with the program RasMol for Windows 2.6. The coordinates of this structure are depositated in the Brookhaven Protein Database (PDB entry 4TST).

In this paper we report the synthesis of a series of sulfonylated histamine derivatives, obtained as outlined in Scheme 1. Treatment of histamine **1** with tetrabromophthalic anhydride afforded the corresponding phthalimide, which was further protected at the imidazole NH moiety with triphenylmethanesulfenyl chloride (tritylsulfenyl chloride), leading to **2**. The key intermediate (N-1-tritylsulfenyl histamine) was easily obtained by room temperature hydrazinolysis of **2**, whereas its treatment with alkyl/arylsulfonyl halides in the presence of triethylamine afforded sulfonamides **3** in high yields. These last compounds were then deprotected with 4M HCl-dioxane, giving the target compounds **4**. Alternatively, a smaller series of compounds was obtained by treating N-1-tritylsulfenyl histamine with arylsulfonylisocyanates, followed by deprotection of the imidazolic moiety in a similar manner as that described above, leading to derivatives of type **5** (Scheme 2). The first

procedure mentioned above is relatively similar to that reported recently by Wolin et al.[14] for the syntheses of some sulfonylated homologs of histamine possessing H_3 antagonistic properties, except that different protecting

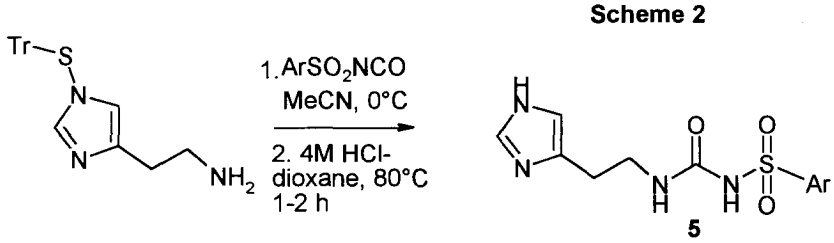
Scheme 1



groups were used by us, being in this way possible to work in much milder reaction conditions.

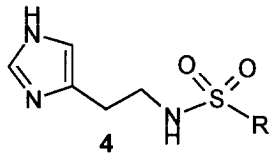
Reagents and Conditions: (a) tetrabromophthalic anhydride, toluene, reflux, 5h, 92 %; (b) trityl sulfenyl chloride, Et_3N, MeCN, 2h, room temp., 97 %; (c) H_2N-NH_2, EtOH, 4h, room temp., 89 %; (d) RSO_2Cl, Et_3N, MeCN, 2-10 h, room temp., 49 - 95 %; (e) 4M HCl – dioxane, 80 °C, 2h, 85-94 %.

Scheme 2



In vitro activation data of table 1 (determination of the activation constants, K_a) show significant differences between the investigated isozymes in their behavior towards both "classical" activators, such as histamine **1**, as well as the new class of activators synthesized in the present work. Thus, histamine **1** is a potent hCA I activator, and a relatively weak hCA II activator, whereas isozyme bCA IV possesses an intermediate behavior. The most interesting finding of the present study is represented by the high susceptibility of the cytosolic isozyme, hCA II to be activated by some of the sulfonylated histamines of types **4** and **5**, as compared to the lead molecule (compounds with activation constants in the 0.10-0.15 μM were frequently obtained). Moreover, the highly abundant and most prone to activation (by histamine) isozyme hCA I was also susceptible

Table 1: CA isozymes I, II and IV activation with histamine **1** and its sulfonylated derivatives of type **4** and **5**.



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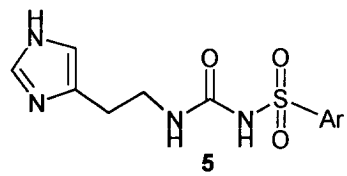

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Compound	R, Ar	K_a* (μM)		
		hCA I[a]	hCA II[a]	bCA IV[b]
1 (histamine)	-	2	125	41
4a	CF_3	0.013	1.2	0.15
4b	CCl_3	0.018	13	3.6
4c	C_4F_9	0.010	0.9	0.05
4d	C_8F_{17}	0.007	0.5	0.03
4e	$PhCH_2$	0.18	34	5.4
4f	Ph	0.21	30	5.0
4g	4-F-C_6H_4	0.11	12	2.4
4h	4-Cl-C_6H_4	0.10	13	2.5
4i	4-Br-C_6H_4	0.09	10	3.0
4j	4-I-C_6H_4	0.08	10	3.2
4k	4-Me-C_6H_4	0.24	28	7.0
4l	4-AcNH-C_6H_4	0.21	20	6.0
4m	4-H_2N-C_6H_4	0.13	14	4.1
4n	4-HOOC-C_6H_4	0.06	0.8	0.10
4o	3-HOOC-C_6H_4	0.05	0.5	0.07
4p	2-HOOC-C_6H_4	0.06	1.2	0.12
4q	4-O_2N-C_6H_4	0.14	10	1.8
4r	3-O_2N-C_6H_4	0.12	9	1.5
4s	2-O_2N-C_6H_4	0.13	8	1.6
4t	3-Cl-4-O_2N-C_6H_3	0.12	6	1.4
4u	4-MeO-C_6H_4	0.20	22	6.5
4v	C_6F_5	0.006	0.1	0.04
4w	2,4,6-$Me_3C_6H_2$	0.25	35	5.9
4x	2-HO-3,5-$Cl_2C_6H_2$	0.10	7	0.09
4y	2,5-$Cl_2C_6H_3$	0.08	5	0.10
4z	2-HOOC-C_6Br_4	0.05	0.3	0.10
4aa	1-naphthyl	0.26	10	3.6
4ab	2-naphthyl	0.24	9	3.5
4ac	5-Me_2N-1-naphthyl	0.28	9	3.2
4ad	2-thienyl	0.09	5	1.1
4ae	Me_2N	0.15	13	5.3
4af	10-camphorsulfonyl	0.17	15	5.4
5a	4-F-C_6H_4	0.004	0.09	0.02
5b	4-Cl-C_6H_4	0.003	0.08	0.01
5c	4-Me-C_6H_4	0.005	0.10	0.02
5d	2-Me-C_6H_4	0.006	0.15	0.03

* Mean from at least three determinations by the esterase method.[15] Standard error was in the range of 5-10 %.
[a] Human cloned isozyme; [b] Purified from bovine lung microsomes.[16]

to activation by the sulfonylated derivatives reported here (with constants in the nanomolar range for the most active derivatives), but differences of activity are not so pronounced as compared to the situation for the rapid isozyme hCA II. bCA IV on the other hand had an intermediate behavior towards the new class of activators, with activation constants in the 0.01-0.10 μM range for the most active such compounds.

Substitution patterns leading to efficient CA activators were: (1) perfluoroalkyl- and perfluoroaryl, such as in derivatives **4a**, **4c**, **4d** or **4v**, which were among the most active compounds in the whole series of derivatives **4**, against all three isozymes. Longer aliphatic chains or a perfluorophenyl moiety greatly enhanced activity; (2) For the arylsulfonyl-substituted derivatives, good CA activation was detected for compounds possessing 4-halogenophenyl; 4-, 3- or 2-carboxyphenyl, 4-, 3- or 2-nitrophenyl as well as 2,5-dichloro- or 2-hydroxy-3,5-dichlorophenyl moieties in their molecule. The only hetaryl-substituted derivative, **4ad**, also possessed an activity of this type; (3) Slightly less active compounds were those containing benzyl, phenyl, 4-tolyl, 4-acetamidophenyl, or 10-camphorsulfonyl moieties; (4) The arylsulfonylureido derivatives of type **5** were the best CA activators reported up to now, with affinities in the 3-6 nM range for hCA I, 80 – 150 nM for hCA II and 10-30 nM for bCA IV, being thus 1500 times stronger hCA II activators as compared to histamine (Table 1). The halogeno-containing derivatives **5a** and **5b** were slightly more active than the tosyl derivatives **5c** and **5d**, but for the moment this subseries of compounds is too small for having a detailed SAR. Work is in progress in our laboratory for developing other derivatives of type **5** in order to obtain even more efficient CA activators.

Similarly to all CA activators reported up to now, the compounds obtained in the present work presumably intervene in the catalytic cycle, leading to the formation of an enzyme-activator complex (similarly to the enzyme-inhibitor adducts, but without substitution of the metal bound solvent molecule), in which the activator bound within the active site facilitates proton transfer processes (which represent the rate-limiting step in catalysis).[7-10] The driving force of this effect might be the fact that intramolecular reactions are more rapid than intermolecular ones. Thus, in the presence of activators (symbolized as "A"), the rate-limiting step is described by equation *1* below:[7-10]

$$EZn^{2+}\text{—}OH_2 + A \Leftrightarrow [EZn^{2+}\text{—}OH_2 - A] \Leftrightarrow [EZn^{2+}\text{—}HO^- - AH^+] \Leftrightarrow EZn^{2+}\text{—}HO^- + AH^+ \qquad (1)$$

enzyme - activator complexes

Obviously, compounds of type **4** and **5** reported here possess the imidazolic moiety which can participate in the proton transfer processes between the active site and the environment (similarly to histamine **1**) but due to the presence of alkyl/arylsulfonyl(ureido) tails in their molecule, they can bind more effectively to the enzyme, allowing thus for more efficient activation processes as compared to **1**. Indeed, the active site edge of all three CA isozymes investigated by us contain a high proportion of polar amino acid residues which might interfere with polar groups such as RSO_2NH or $RSO_2NHCONH$. In fact such amino acid residues might explain the different catalytic properties of the diverse isozymes, as well as their diverse susceptibility to be

inhibited/activated by modulators of activity.[7,8] For instance, the entrance of the active site of isozyme hCA II contains a cluster of 6 histidine residues (His 3, His 4, His 10, His 15, His 17 and His 64), some of which possess different conformations (as shown by X-ray crystallography)[7,8] which could easily participate in hydrogen bond formation (as well as other types of interactions) with the sulfonylated histamine derivatives reported here. This might explain in fact the greater efficiency of the compounds reported in the present work in activating this isozyme, as compared to histamine, which is a relatively weak hCA II activator.

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15. Pocker, Y.; Stone, J.T. *Biochemistry* **1967**, *6*, 668-678. Initial rates of 4-nitrophenyl acetate hydrolysis catalysed by different CA isozymes were monitored spectrophotometrically, at 400 nm, 25 °C, with a Cary 3 instrument interfaced with an IBM compatible PC. A molar absorption coefficient ε of 18,400 $M^{-1}.cm^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.40). Non-enzymatic hydrolysis rates were subtracted from the observed rates. Stock solutions of activator (1 mM) were prepared in distilled-deionized water and dilutions up to 0.1 nM were done thereafter with the same solvent. Activator and enzyme solutions were preincubated together for 10 min at room temperature prior to assay, in order to allow for the formation of the E-A complex. The activation constant K_a was determined as described in ref.[7] Enzyme concentrations were 3.5 nM for hCA II, 12 nM for hCA I and 31 nM for bCA IV.
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