CARBONIC ANHYDRASE INHIBITORS. Part 87¹ INHIBITION OF CARBONIC ANHYDRASE ISOZYMES I, II AND IV WITH ARSINOLIPIDS

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Abstract: A series of mono- and bis-arsinolipids possessing C₁₂ – C₂₂ aliphatic chains showed interesting inhibitory activity against three isozymes of carbonic anhydrase (CA), i.e., CA I, II and IV. For the mono arsinolipids, CA inhibitory power increased with the length of the aliphatic chain, whereas for the bis-derivatives, best activity was correlated with the presence of smaller groups substituting the hydroxy moieties.

Introduction

In previous works [2-4] of our group it was shown that arsonolipids of type **1a,b** showed good carbonic anhydrase (CA, EC 4.2.1.1) inhibitory properties and this prompted us to investigate other derivatives possessing the arsonic acid and related moieties in their molecule, for their interaction with this ubiquitous zinc enzyme [5]. A simple aromatic derivative of this kind, p-aminobenzenearsonic acid **2a** and its sulfonamide **2b**, were among the first to be investigated, showing indeed interesting CA inhibitory activities [4]. Since the AsO₃H₂ moiety shows some similarities with the SO₂NH₂ group, present in the most powerful class of CA inhibitors [5-10], it appeared of interest to explore some more derivatives possessing such moieties. In this context, one should note that in a recent study we showed sulfenamides RSNH₂ and sulfinamides RSONH₂ to act as strong CA inhibitors, with potencies sometimes similar to those of the structurally related sulfonamides RSO₂NH₂ [11]. In this paper we report an inhibition study of isozymes CA I, II and IV with mono- and bis-arsinolipids **3-11** [12].

Materials and Methods

Racemic arsino lipids **3-11** were prepared as described in ref. [12]. Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/HCA I and pACA/HCA II (the two plasmids were a gift from Prof. Sven Lindskog, Umea University, Sweden). Cell growth conditions were those described by Lindskog's group [13] and enzymes were purified by affinity chromatography according to the method of Khalifah et al. [14]. Enzyme concentrations were determined spectrophotometrically at 280 nm, using a molar absorptivity of 49 mM⁻¹.cm⁻¹ for CA I and 54 mM⁻¹.cm⁻¹ for CA II, respectively, based on M_r = 28.85 kDa for CA I, and 29.3 kDa for CA II, respectively [15]. CA IV was isolated from bovine lung microsomes [16].

Initial rates of 4-nitrophenyl acetate hydrolysis were monitored spectrophotometrically, at 400 nm and 25°C, with a Cary 3 apparatus interfaced with an IBM compatible PC by the method of Pocker and Stone [17]. Solutions of the substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 10^{-2} and 10^{-6} M. A molar absorption coefficient $\epsilon = 18,400 \text{ M}^{-1}\text{cm}^{-1}$ was used for the 4-nitrophenolate formed by hydrolysis, in the experimental conditions (pH 7.80), as reported by Pocker and Stone [17]. Non-enzymatic hydrolysis rates were always subtracted from the observed rates. Inhibitors were dissolved in DMSO (0.01 mM stock solutions) and dilutions up to 0.001 μM were then obtained with distilled deionized water. Duplicate experiments were done for each inhibitor, and the values reported throughout the paper are the averages of such results. IC_{50} represents the molarity of inhibitor producing a 50% decrease of enzyme-catalyzed hydrolysis of 4-nitrophenyl acetate.

Results and Discussion

The compounds **3-11** assayed for their interaction with isozymes CA I, II and IV, are shown in Table I.

Table I. CA inhibition data with the arsinolipids **3-11** against isozymes CA I, II and IV compared to those of compounds **2a** and **2b**.

No	R	IC_{50} (μM)		
		hCA I ^a	hCA II ^a	bCA IV ^b
2a		600	110	210
2b		22	3	13
3	$\text{C}_{12}\text{H}_{25}$	40	31	26
4	$\text{C}_{14}\text{H}_{29}$	25	22	20
5	$\text{C}_{16}\text{H}_{33}$	15	14	19
6	$\text{C}_{18}\text{H}_{37}$	12	8	16
7	$\text{C}_{22}\text{H}_{45}$	8	4	12
8	$\text{C}_{12}\text{H}_{25}$	65	54	62
9	$\text{C}_{14}\text{H}_{29}$	110	68	76
10	$\text{C}_{16}\text{H}_{33}$	175	75	93
11	$\text{C}_{18}\text{H}_{37}$	180	110	124

^a Human (cloned) isozymes; ^b From bovine muscle; ^c From bovine lung microsomes.

The following facts should be noted regarding data of Table I: (i) the mono-substituted derivatives **3-7** behave as moderate-weak inhibitors against all three investigated isozymes, with hCA II the most susceptible to inhibition, followed by bCA IV and hCA I the less susceptible. Inhibitory efficiency increases with the increase of the length of aliphatic carbon chain substituting the hydroxy moieties of these arsinolipids, the C_{22} derivative being a really potent inhibitor (compare with **2b**); (ii) for the bis-substituted

derivatives **8-11**, best inhibitors were those with smaller groups substituting the hydroxy moieties, such as **8** and **9**. Increasing the aliphatic chain led to less active compounds, probably due to steric hindrance and difficult access within the enzyme active site.

These new inhibitors probably directly bind to the Zn(II) ion within the CA active site, as deprotonated moieties, similarly to the classical inhibitors of the sulfonamide type [5-10] as well as the arsonolipids previously investigated [2-4].

In conclusion, we report here a novel class of inhibitors of the zinc enzyme carbonic anhydrase. The interest in this type of metal-containing enzyme inhibitors [18] has recently been revived by the report that some As(III) and As(V) derivatives induce a high proportion of complete remissions in relapsed patients with acute promyelocytic leukemia [19,20]. Our study might reveal new insights in the search of this type of enzyme inhibitors, with possible applications as drugs, fungicides or plant growth inhibitors.

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