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Inhibition of Bovine Carbonic Anhydrase by New Sulfonamide Compounds

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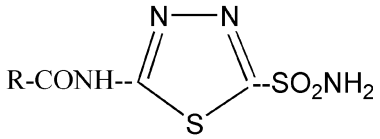
Abstract—Inhibitory effects of three new derivatives of 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide on bovine carbonic anhydrase have been investigated. The new compounds are 2-(3-chloropropionylamino)-1,3,4-thiadiazole-5-sulfonamide, 2-(2,2-dichloroacetylamino)-1,3,4-thiadiazole-5-sulfonamide, and 2-(3-phenylpropionylamino)-1,3,4-thiadiazole-5-sulfonamide. The new compounds inhibit the esterase activity of carbonic anhydrase noncompetitively and have inhibition constants and I_{50} values very similar to those for 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, the latter being clinically used in the treatment of glaucoma.

Key words: inhibition, bovine carbonic anhydrase, sulfonamides

Carbonic anhydrase (EC 4.2.1.1) catalyses the reversible hydration of CO_2 and some other reactions. The enzyme is composed of a single polypeptide with no disulfide bonds and contains one tightly bound Zn^{2+} ion, which is required for activity. The only known physiological function of carbonic anhydrase is to facilitate the interconversion of CO_2 and HCO_3^-, so it plays key roles in diverse processes such as physiological pH control, gas balance, and calcification [1].

Carbonic anhydrase inhibitors have been used in the treatment of glaucoma for almost three decades. The background and history of the use of sulfonamide carbonic anhydrase inhibitors in the treatment of glaucoma has been reviewed [1]. Finding the sulfonamide acetazolamide to be a potent secretory inhibitor useful in the management of glaucoma [2-5] stimulated the introduction of other carbonic anhydrase inhibitors possessing a similar effect. Currently used carbonic anhydrase inhibitors are administered systemically and include acetazolamide, dichlorophenamide, ethoxzolamide and methazolamide. Each of these drugs possesses a free sulfamoyl group attached to an aromatic heterocyclic ring and each inhibits the enzyme *in vitro* with potency in the nanomolar range [4]. Intraocular pressure is decreased by reduction in humor formation stemming from the inhibition of carbonic anhydrase present in the ciliary epithelium. A number of carbonic anhydrase inhibitors have been reported to lower intraocular pressure when instilled topically in animals [6].

In the present paper, *in vitro* inhibition of bovine carbonic anhydrase by new sulfonamide compounds of the general structure shown below is reported.



MATERIALS AND METHODS

2-Acetylamino-1,3,4-thiadiazole-5-sulfonamide (acetazolamide) was obtained from Sigma Chemical Co. (USA). 2-(3-Chloropropionylamino)-1,3,4-thiadiazole-5-sulfonamide (CTS), 2-(2,2-dichloroacetylamino)-1,3,4-thiadiazole-5-sulfonamide (DTS), and 2-(3-phenylpropionylamino)-1,3,4-thiadiazole-5-sulfonamide (PTS) were synthesized in this laboratory [7].

Bovine carbonic anhydrase with specific activity of 1.4 U/mg was isolated from bovine erythrocytes using a Sepharose 4B-L-tyrosine-sulfonamide affinity column [8]. Carbonic anhydrase activity in eluates obtained during purification was determined by measuring CO_2 hydration according to the method of Wilbur and Anderson [9]. The purified carbonic anhydrase migrated as a single band during SDS polyacrylamide gel electrophoresis (data not shown).

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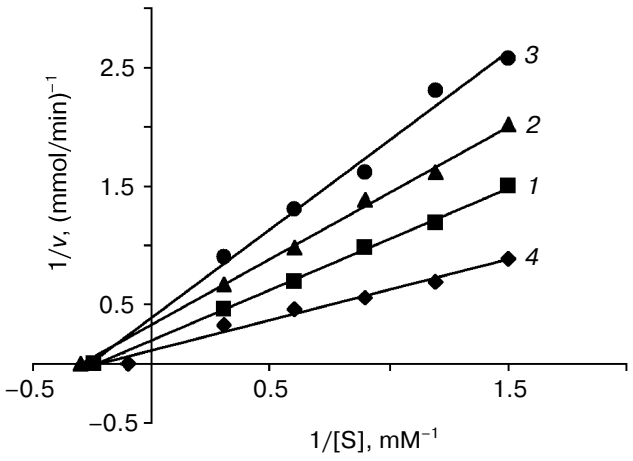

Lineweaver–Burk plot for inhibition of carbonic anhydrase with 2-(3-chloropropionylamino)-1,3,4-thiadiazole-5-sulfonamide. Inhibitor concentrations: 150 (*1*), 300 (*2*), 450 nM (*3*); *4*) in the absence of the inhibitor

Values of K_i and I_{50} for the inhibition of carbonic anhydrase by different sulfonamides

Compound	R-	K_i, μM	I_{50}, μM
Acetazolamide	CH_3-	0.12 ± 0.03	0.121 ± 0.005
CTS	$ClCH_2CH_2$-	0.59 ± 0.03	0.6 ± 0.1
DTS	Cl_2CH-	0.22 ± 0.03	0.22 ± 0.01
PTS	$C_6H_5CH_2CH_2$	0.29 ± 0.05	0.28 ± 0.03

Values of K_i were calculated from Lineweaver–Burk plots obtained using three different concentrations of the inhibitor and five different concentrations of the substrate—*p*-nitrophenylacetate [10]. I_{50} values were obtained using five different inhibitor concentrations at 0.66 mM substrate concentration.

RESULTS AND DISCUSSION

Although carbonic anhydrase is mainly involved in reversible hydration of CO_2, it can also catalyze hydrolysis of esters [1]; this reaction is often used in kinetic studies of the enzyme [7]. All three new derivatives of acetazolamide were found to be noncompetitive inhibitors of the esterase activity of bovine carbonic anhydrase, as illustrated in the figure for one of them. This result shows that sulfonamides do not compete with *p*-nitrophenylacetate for the active site of carbonic anhydrase. A similar result was obtained with human carbonic anhydrases I and II [7].

The values of the inhibition constant K_i, derived from the figure and similar plots for the other inhibitors are summarized in the table along with the values of I_{50} measured at 0.66 mM substrate concentration. These values are very similar to those for acetazolamide, which is used in the treatment of glaucoma. Therefore, the new compounds could be considered as potential agents for treatment of glaucoma in animals.

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