[Chem. Pharm. Bull. 26(4)1049—1053(1978)]

UDC 577.157.04 : 547.794.3.09

1,3,4-Thiadiazole-5-sulfonamides as Carbonic Anhydrase Inhibitors: Relationship between Their Electronic and Hydrophobic Structures and Their Inhibitory Activity[1]

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(Received July 20, 1977)

Hückel molecular orbital method and Hansch and Fujita's extrathermodynamic method were employed to characterize the chemical structure of 1,3,4-thiadiazole-5-sulfonamide derivatives which are potent carbonic anhydrase inhibitors.

Regression analysis showed that a good correlation exists between the positive charge on nitrogen-10 and the inhibitory activity. It is also shown that the hydrophobicity of the side chain is another factor of development of enzyme inhibition.

These results are consistent with the findings of X-ray crystallographic works.

Keywords——carbonic anhydrase; enzyme inhibition; extrathermodynamic method; molecular orbital method; thiadiazolesulfonamide

Introduction

Carbonic anhydrase (E.C. 4.2.1.1) catalyzes the reversible hydration of carbon dioxide,[3] the hydration of certain aldehydes[4] and the hydrolysis of certain esters.[5] The enzyme is widely distributed both in animals and in plants.[6] The enzyme plays an important role in respiration, secretory processes[7] and photosynthesis.[8]

The enzyme has a molecular weight of about 30000, and has one atom of Zn^{2+} per molecule[6] which is essential for enzyme activity.

X-ray works showed that the active site of the enzyme forms a cavity; at the bottom of this cavity the essential zinc ion is located.

Certain aromatic and heterocyclic sulfonamides are very powerful inhibitors of the enzyme, and the mechanism of inhibition is considered to be caused by binding to the enzyme.

The existence of sulfonamide-Zn^{2+} linkage as well as other interactions between the inhibitor and the enzyme was confirmed by X-ray studies.[9]

It is the purpose of this study to clarify the inhibitory mechanism of the thiadiazole-5-sulfonamides against the enzyme. Hückel molecular orbital method[10] and Hansch and Fujita's extrathermodynamic method were used.[11]

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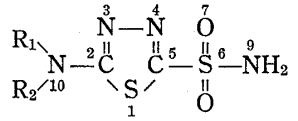
Methods

Compounds Studied——Nine derivatives of 1,3,4-thiadiazole-5-sulfonamide selected in this study were those studied by Young *et al.*[12] and their chemical structures are shown in Table I with the values of inhibitory activity.

Table I. Chemical Structures of the Thiadiazole-5-sulfonamides and Its Inhibitory Activity Against Carbonic Anhydrase



	Compounds	R_1	R_2	Inhibitory activity ($-pI_{50}$)[b]
	1,3,4-Thiadiazole-5-sulfonamide			
I	2-acetylamino-[a]	CH_3CO-	H–	0.0000
II	2-amino	H–	H–	−0.7109
III	2-(N-methylacetamido)-	CH_3CO-	CH_3-	0.1727
IV	2-methylamino-	CH_3-	H–	−0.5144
V	2-(N-phenylacetamido)-	CH_3CO-		0.1279
VI	2-anilino-	H–		0.3841
VII	2-(N-ethylacetamido)-	CH_3CO-	CH_3CH_2-	0.0059
VIII	2-(N-butylacetamido)-	CH_3CO-	$CH_3CH_2CH_2CH_2-$	0.3765
IX	2-(N-methylpropionamido)-	CH_3CH_2CO-	CH_3	0.1308

a) Diamox.®
b) Logarithmic value of 50% inhibition was expressed as the base of molar ratio. Original value of 50% inhibition was cited from the report of Young *et al.*[12]

Calculations of LCAO-MO Method——Hückel molecular orbital approximation (LCAO-MO method) was employed for the calculation of the π-electron system of the molecules. Coulomb and resonance integrals (α_x and β_{xy}) were given as usual forms by the following equations, respectively.

$$\alpha_x = \alpha + h_x\beta \qquad \beta_{xy} = k_{xy}\beta$$

The methyl group and methylene group were treated as two-atom model. The inductive effect onto the adjacent carbon atom was taken into account only in the case of the methyl group. The values of h_x and k_{xy} were mostly those recommended by B. Pullman and are listed in Table II.

Table II. Coulomb and Resonance Integrals (α_x and β_{xy}) of Heteroatom used in Hückel Molecular Orbital Calculation

$\alpha_x = \alpha + h_x\beta$, $\beta_{xy} = k_{xy}\beta$

Atom	h_x	Bond	k_{xy}
$-\dot{N}=$	0.4	$\dot{C}—\dot{N}$	1.0
$-\ddot{N}-$ (H)	1.0	$\dot{C}—N$	0.9
$\dot{O}=$	1.6	$\dot{N}—\dot{N}$	1.0
$=\ddot{S}=$	0.2	$\ddot{S}=\dot{O}$	1.0
$-\ddot{S}-$	0.0	$\ddot{S}—\dot{C}$	0.6
		$\ddot{S}—\ddot{N}$	0.9
Hyperconjugation			
. 2.0 . 0.7 $H_3{=}C—R$ −0.2 0.0 (−0.1)		. 2.0 . 0.7 $H_2{=}C—R$ −0.2 0.0	

12) R.W. Young, K.H. Wood, J.A. Eichler, J.R. Vaughan, and G.W. Anderson, *J. Am. Chem. Soc.*, **78**, 4649 (1956).

The substituted groups, R_1 as well as R_2, at nitrogen-10 were assumed to conjugate with thiadiazole ring except the case of the phenyl group of compound V. This phenyl group cannot be coplaner to the ring according to the construction of CPK models.

Net charge on r-th atom (Q_r), electrophilic and nucleophilic superdelocalizabilities of r-th atom (S_r^E and S_r^N),[13] and mutual polarizability by atom r and atom s ($M_{r,s}$)[14] were calculated with the following equations as reaction indices of π-electron structure.

$$Q_r = q_r - 2\sum_i^{o}(C_{ir})^2$$

$$S_r^E = 2\sum_i^{o}(C_{ir})^2/\lambda_i$$

$$S_r^N = 2\sum_i^{u}(C_{ir})^2/(-\lambda_i)$$

$$M_{r,s} = 4\sum_i^{o}\sum_k^{u}C_{ri}C_{si}C_{rk}C_{sk}/(\varepsilon_i-\varepsilon_k)$$

where q_r is the number of π-electrons contributing to the conjugation system from the r-th atom, C_{ir} is the LCAO coefficient of r-th atomic orbital in the i-th molecular orbital, λ_i is the coefficient of its energy (given as $\varepsilon_i=\alpha+\lambda_i\beta$) and the summations $\sum_i^{o}$ and $\sum_i^{u}$ cover occupied and unoccupied orbitals, repectively.

Calculations were carried out by a high speed digital computer, NEAC 2200/500, in Osaka University Computation Center.

Estimation of Hydrophobic Parameter——As a parameter of hydrophobicity, the π value of substituted groups was calculated. The π value here was defined as a simple summation of substituent constants.[15]

Results and Discussion

The importance of the reactivity of the sulfamoyl group of various sulfonamides in the mechanism of their inhibitory action against the carbonic anhydrase has been reported by previous investigators on the basis of both experimental[16] and theoretical considerations.[17]

The author calculated the electrophilic and nucleophilic superdelocalizabilities of each atom of the sulfamoyl group as well as the net charge on each atom. The results are shown in Tables III and IV, respectively. Although the value of the inhibitory activity of the compounds varies with their chemical structure, the reaction indices mentioned above were almost constant. This fact means that the reactivity of the sulfamoyl group is not the

Table III. Electrophilic and nucleophilic superdelocalizabilities (S_r^E and S_r^N) of atoms in sulfamoyl group. The suffix "r" denotes atomic numbering as coded in Table I.

Compounds	S_6^E	S_7^E	S_9^E	S_6^N	S_7^N	S_9^N
I	0.3018	1.0596	1.4703	1.3046	0.2013	0.2826
II	0.3040	1.0607	1.4728	1.3026	0.2008	0.2817
III	0.3033	1.0603	1.4717	1.3041	0.2012	0.2824
IV	0.3067	1.0619	1.4753	1.3020	0.2007	0.2815
V	0.3018	1.0596	1.4703	1.3046	0.2013	0.2826
VI	0.3052	1.0611	1.4734	1.3039	0.2012	0.2824
VII	0.3022	1.0598	1.4707	1.3046	0.2014	0.2826
VIII	0.3022	1.0598	1.4707	1.3046	0.2014	0.2826
IX	0.3032	1.0603	1.4717	1.3042	0.2013	0.2825

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Table IV. Energy Coefficients of HOMO and LUMO, Net Charge on Each Atom, and Their Correlation Coefficient (R) to the Value of Inhibitory Activity, $-pI_{50}$

Compounds	HOMO	LUMO	Q_1	Q_2	Q_3	Q_4	Q_5	Q_6	Q_7	Q_9	Q_{10}
I	0.3053	−0.7896	0.6350	−0.0001	−0.3232	−0.2516	−0.1104	1.2940	−0.8125	0.2590	0.2520
II	0.2716	−0.7942	0.6133	0.0114	−0.3373	−0.2528	−0.1139	1.2935	−0.8127	0.2587	0.1525
III	0.2740	−0.7897	0.6330	−0.0049	−0.3240	−0.2510	−0.1109	1.2938	−0.8126	0.2589	0.2993
IV	0.2327	−0.7944	0.6101	0.0056	−0.3388	−0.2520	−0.1146	1.2932	−0.8128	0.2586	0.2051
V	0.3052	−0.7896	0.6350	−0.0001	−0.3232	−0.2516	−0.1104	1.2940	−0.8125	0.2590	0.2520
VI	0.2340	−0.7871	0.6320	−0.0042	−0.3249	−0.2503	−0.1112	1.2937	−0.8126	0.2589	0.2732
VII	0.2935	−0.7891	0.6372	−0.0040	−0.3124	−0.2512	−0.1101	1.2940	−0.8125	0.2590	0.2815
VIII	0.2936	−0.7891	0.6372	−0.0040	−0.3214	−0.2512	−0.1101	1.2940	−0.8125	0.2590	0.2818
IX	0.2747	−0.7894	0.6338	−0.0053	−0.3235	−0.2509	−0.1108	1.2938	−0.8126	0.2589	0.3031
R	0.2344	0.9449	0.8805	−0.9195	0.7397	0.8912	0.8535	0.7031	0.7179	0.7973	0.8848

major factor. This does not necessarily deny the importance of the sulfamoyl group. Other factors must be considered.

The net charge on each atom, and energy coefficients of LUMO (lowest unoccupied molecular orbital) and HOMO (highest occupied molecular orbital) are summarized in Table IV. Good correlations are found between Q_2 and $-pI_{50}$ ($R=-0.9195$), and between Q_{10} and $-pI_{50}$ ($R=0.8848$). The facts suggest that carbon-2 and/or nitrogen-10 play important roles in the inhibitory action.

It is obvious that the two atoms are closely correlated to each other electronically, because the correlation coefficient between Q_2 and Q_{10} is calculated to be -0.9921.

The absolute value of Q_2 is on the order ranging from 0.001 to 0.01, while that of Q_{10} is on the order of 0.1. The fact means that nitrogen-10 plays a decisive role in the reaction of the enzyme inhibition.

Furthermore, the energy level of HOMO is not correlated to the inhibitory activity ($R=0.2344$), but that of LUMO is highly correlated ($R=0.9449$). This means that thiadiazole-5-sulfonamides act as electron acceptors, *i.e.* a positively charged atom (s) plays a rather important role.

It is therefore concluded that Q_{10} is an adequate reaction index for the inhibitory action of thiadiazole-5-sulfonamide against the enzyme. As far as these compounds are concerned, inhibitory activity increases with an increase of the positive charge on nitrogen-10 as shown in Fig. 1. This relationship is expressed in the following regression equation:

$$-pI_{50} = -1.7397 + 6.7940Q_{10} \qquad \text{(Eq. 1)}$$

$$R = 0.8848, \quad t = 10.781 \ (t_{7,\,0.001}=3.499)$$

The correlation is significant and Eq. 1 explains 78% of the variance in the inhibitory activity.

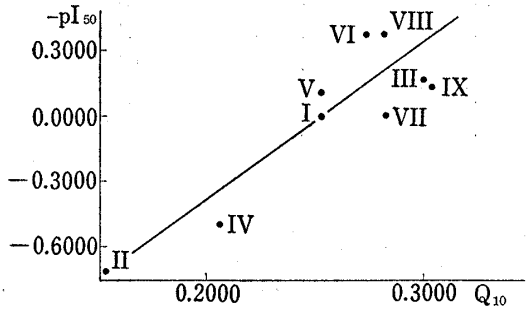

Fig. 1. Correlation between the Net Charge on Nitrogen-10, Q_{10}, and the Inhibitory Activity, $-pI_{50}$

Table V. Mutual Polarizability by Nitrogen-1 and Nitrogen-10 ($M_{9,10}$)

Compounds	$M_{9,10}$
I	−0.0009
II	−0.0013
III	−0.0009
IV	−0.0014
V	−0.0009
VI	−0.0010
VII	−0.0008
VIII	−0.0008
IX	−0.0009

In recent years, X-ray crystallographic works have yielded many fruitful results in the field of study of this enzyme.[9] Waara *et al.*[9b] presented a scheme of the state of binding of acetazolamide to human carbonic anhydrase C, *i.e.* EI-complex, in which the sulfamoyl group binds to the essential Zn^{2+} as well as Thr-177, and the nitrogen-10 to His-128. (His-128 was corrected to Tyr-126 by a later study on the primary structure of the enzyme.[18])

It is expected from the above discussion that the nitrogen-10 of thiadiazole-5-sulfonamide interacts electronically with the enzyme to form a stable EI-complex. There still remains a possibility that an interaction between the enzyme and nitrogen-10 may cause a change in electronic state of the sulfamoyl group, resulting in the change of reactivity of the group. However, this possibility was excluded by calculation of mutual polarizability by nitrogen-9 and nitrogen-10, $M_{9,10}$, of each compound (Table V). Since the value of $M_{9,10}$ of each compound is about -0.0010, a change in electronic state of nitrogen-10 cannot cause a significant change in the reactivity of nitrogen-9.

In such cases as chymotrypsin,[19] hydrophobicity of the molecule is also an important factor in enzyme reaction. As carbonic anhydrase also has a hydrophobic region,[20] multiple regression analysis was tried using π (Table VI), as another parameter in addition to Q_{10} in expectation of improvement of the correlation coefficient. The following equation was obtained:

$$-pI_{50} = -1.7313 + 6.3877Q_{10} + 0.1393\pi \qquad \text{(Eq. 2)}$$

$$R = 0.9438, \quad F_{2,6} = 24.46 \ (F_{2,6,0.01} = 10.93)$$

TABLE VI. The Value of Hydrophobic Parameter, π, of Each Compound

Compounds	$\pi^{a)}$	Compounds	$\pi^{a)}$
I	−0.71	VI	2.13
II	0.00	VII	0.29
III	−0.21	VIII	1.29
IV	0.51	IX	0.29
V	1.42		

a) The value was obtained by simple summation of substituent constant of R_1 and R_2.

The correlation coefficient is improved to 0.9438, and the equation explains 89% of the variance in the inhibitory activity. This means that the enzyme inhibition is dependent on both Q_{10} and π. This fact suggests that the substituted groups interact with the hydrophobic amino acid residues of the enzyme.

Acknowledgement The author is grateful to professor A. Imamura, Shiga Medical University, for his helpful discussion during this work.

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