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Binding of Sulfonylurea-related Compounds with Bovine Serum Albumin[1a,b]

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Thirteen sulfonylurea derivatives were synthesized and their binding with bovine serum albumin was investigated, using an equilibrium dialysis technique. The Scatchard plots for data of 11 sulfonylurea derivatives indicated the presence of more than two classes of a binding site. The binding parameters, k_1, k_2, n_1, and n_2 were computed by the principle of least squares method. Physicochemical properties such as the dissociation constant in water and partition coefficient with octanol-water system were obtained. With the use of Hansh analysis, BSA binding constants could be represented as follows: For the binding constant at the primary site, $\log k_1 = 0.512 \log P + 3.754$, binding constant at the secondary site, $\log k_2 = 0.334 \log P + 0.240\ pK_a + 1.480$. An important role of hydrophobicity must be considered for binding at the primary site, but electrostatic force as well as hydrophobic force must also be included for binding at the secondary site.

Keywords——sulfonylurea-related compounds; bovine serum albumin (BSA); equilibrium dialysis; BSA binding parameter; Hansch analysis; octanol–water partition coefficient; hydrophobic interaction; electrostatic interaction

In our previous paper,[1b] binding of seven commercial sulfonylureas to bovine serum albumin (BSA) using the equilibrium dialysis method was reported and it was observed that they have two classes of binding sites. Thermodynamic calculation, based on the analysis of the temperature dependence of the binding to BSA, was made at pH 7.4. The large contribution of ΔH° to ΔG° may indicate that the main binding force is derived from hydrophobic source. Although there are a few literature[3–5] on the sulfonylureas-BSA binding data, mechanism of this interaction has not been elcidated. The present work was designed to evaluate the binding mechanism by means of the following procedures: synthesis of sulfonylurea-related compounds, further BSA binding studies using the synthesized compounds, and application of the Hansch analysis to the correlation of BSA binding constants, k, with octanol/water partition coefficients, P, and ionic constants, pK_a, of sulfonylurea-related compounds.

Experimental

Materials

Bovine serum albumin, fraction V (Armour pharmaceutical Co., U.S.A.) was used, and its molecular weight was assumed to be 69000. Reagent grade KH_2PO_4 and Na_2HPO_4 were used to prepare the pH 7.4 buffer solution (M/15). The sulfonylurea-related compounds were synthesized. All sulfonylurea-related compounds and BSA solutions were prepared immediately before use for equilibrium dialysis.

Equilibrium Dialysis Method

The general approach, technique, and treatment of data of this study were described previously.[1b] Dialysis cell made of acrylic resin was the same as those employed in our previous experiment.[1b] Binding of sulfonylurea-related compounds by membranes and dialysis cells was found to be insignificant. Equilibrium was established at the end of 6 hr by agitation on a rotational shaker (Toyo incubator Model 100T) at

1) *a*) This paper forms Part II of a series entitled "Interaction Between Drugs and Blood Components"; *b*) Part I: S. Goto, H. Yoshitomi, and M. Kishi, *Yakugaku Zasshi*, **97**, 1219 (1977).
2) Location: *Tsushima-naka 1-1-1, Okayama, 700, Japan.*
3) J. Judis, *J. Pharm. Sci.*, **61**, 89 (1972).
4) K.F. Brown and M.J. Crooks, *J. Pharm. Sci.*, **9**, 75 (1974).
5) M.J. Crooks and K.F. Brown, *J. Pharm. Pharmacol.*, **26**, 304 (1974).

30.0°. Dialysis studies were made with 5×10^{-5} M BSA. All sulfonylurea-related compounds were used at pH 7.4 and their initial concentration ranged from 3 to 100×10^{-5} M.

Analysis for Sulfonylurea-related Compounds after Equilibration

Spectrophotometric analysis of solutions of sulfonylurea-related compounds in the absence of BSA was made on a Hitachi spectrophotometer Model 181. At the end of equilibrium time, 1 or 2 ml aliquot was removed and diluted with pH 7.4 buffer solution. The concentration was determined at the following wavelength; 227 nm for SU-1—SU-3, 245 nm for SU-4 and SU-9, 229 nm for SU-5—SU-8, 226 nm for SU-10—SU-12, and 220 nm for SU-13.

Synthesis of Sulfonylurea-related Compounds

The procedure was the same as described in the literatures.[6-8]

Method 1[6]——This method was used for the preparation of SU-1 to SU-9 and SU-13. To a solution of 0.05 mol of sulfonamide in a mixture of 50 ml of 1 N NaOH and 50 ml of acetone, 0.05 mol of isocyanate (R–NCO) dissolved in 20 ml acetone was added dropwise with stirring and cooling, while the temperature was maintained below 10°. Stirring was continued for 1 hr with cooling and for 3 hr at room temperature. acetone was removed *in vacuo*. The aqueous solution was acidified with 2 N HCl, and filtered. The filtrate cake was washed with water, dried, and recrystallized from dilute EtOH.

Method 2[7,8]——This method was applied for the preparation of SU-10 to SU-12.

a) *p*-Toluenesulfonyl Carbamate: To a mixture of 0.6 mol of *p*-toluenesulfonamide and 1.6 mol of anhydrous K_2CO_3 in 750 ml of acetone, 0.79 mol of ethyl chlorocarbonate was added dropwise with stirring, and the mixture was then stirred and refluxed for 20 hr. The cooled mixture was filtered, the filter cake was dissolved in 2000 ml of H_2O, and the solution was acidified with conc. HCl. The crude product was collected by filtration and used for the reaction with hydrazine.

b) Nitrosamine: To a solution of 0.4 ml of secondary amines in 38 ml of conc. HCl and 16 ml of H_2O, a solution of 0.45 mol of $NaNO_2$ in 50 ml of H_2O was added dropwise with stirring at 70° during 3 hr. The cooled solution was extracted with ether. The solution was dried over anhydrous $MgSO_4$. Ether was evaporated, and its residue was distilled *in vacuo*.

c) Disubstituted Hydrazine: To a mixture of 0.4 mol of $LiAlH_4$ in 400 ml of anhydrous ether, a solution of 0.2 mol of nitrosamine in 60 ml of anhydrous ether was added dropwise with stirring and refluxing. After refluxing for 3 hr, the mixture was decomposed with 100 ml of H_2O and 12 ml of 20% NaOH. The inorganic residue was removed by filtration. Ether solution was dried over anhydrous $MgSO_4$. Ether was evaporated, and the residue was distilled *in vacuo*.

d) Sulfonylurea-related Compounds: A mixture of 0.1 ml of *p*-toluenesulfonyl carbamate and 0.12 mol of disubstituted hydrazine was heated at 130° for 2 hr. Vaccum was applied to the mixture for 2 hr at 130° and under 25 Torr. The cooled residue was recrystallized from EtOH. These syntheses are summarized in Chart 1.

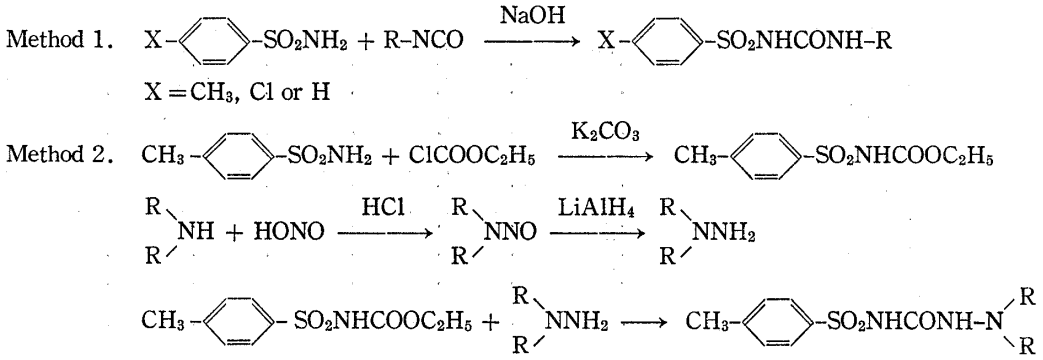


Chart 1

Partition Coefficient

The true partition coefficient, *P*, was determined by equilibration in octanol–M/15 phosphate buffer system, the pH of aqueous phase being 7.4. Each sulfonylurea-related compound was dissolved in 10 ml of phosphate buffer (initial concentration; 1×10^{-4} M) and equilibrated with 10 ml of octanol. After shaking for 30 min at room temperature, samples were shaken in a thermostatic bath (30°) for least 2 hr and centrifug-

6) B. Blank, F.A. Farina, J.F. Kerrin, and H. Saunders, *J. Org. Chem.*, **26**, 1551 (1961).
7) F.J. Marshall and M.V. Sigl, Jr., *J. Org. Chem.*, **23**, 927 (1958).
8) J.B. Wright and R.E. Willette, *J. Med. Pharm. Chem.*, **5**, 815 (1960).

ed at 3000 rpm for 10 min. Concentration in the aqueous phase was measured and P was determined from Eq. (1).[9]

$$P = P_{app}[1+\text{antilog}(\text{pH}-\text{p}K_a)] \quad (1)$$

where P_{app} is apparent partition coefficient, which is calculated from following equation:[1b]

$$P_{app} = \frac{a_0 - a}{a} \quad (2)$$

where a_0 and a are initial and equilibration concentrations of sufonylurea-related compounds in pH 7.4 buffer.

Dissociation Constant

The pK_a values of sulfonylurea-related compounds were measured in the same way as in the literatures.[10,11]

Application of Hansch Analysis

Two equations were developed to correlate with BSA binding using the data on log P as the hydrophobic or lipophilic parameter and on pK_a as the ionic parameter.

$$\log k = a \log P + c \quad (3)$$

$$\log k = a' \log P + b' \text{ p}K_a + c' \quad (4)$$

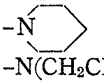
where k is the sulfonylurea-BSA binding constant at pH 7.4 and at 30°, and a, c, a', b', and c' are constants determined by linear and multiple regression analysis.

Results and Discussion

Scatchard Plots for Sulfonylurea-related Compounds

Thirteen sulfonylurea-related compounds were synthesized as listed in Table I, together with their molecular weight, and data of analysis and melting points.

TABLE I. Molecular Structure, Molecular Weight, Data of Analysis, and Melting Point of Sulfonylurea-related Compounds

Compd. No.	R_1	R_2	Molecular weight	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	mp °C
SU-1	CH_3-	$-CH_3$	228.26	47.35	5.30	12.27	47.36	5.26	12.16	172—174
SU-2	CH_3-	$-CH_2CH_3$	242.29	49.57	5.82	11.56	49.68	5.86	11.42	139—140
SU-3	CH_3-		296.38	56.73	6.80	9.45	56.67	6.87	9.33	168—169
SU-4	CH_3-		290.33	57.92	4.86	9.65	57.75	4.93	9.93	161—164
SU-5	Cl-	$-CH_3$	248.67	38.64	3.65	11.26	38.64	3.70	11.29	182—184
SU-6	Cl-	$-CH_2CH_3$	262.70	41.15	4.22	10.66	41.03	4.26	10.65	143—145
SU-7	Cl-	$-CH_2CH_2CH_2CH_3$	276.73	45.44	5.20	9.63	45.45	5.24	9.80	112—114
SU-8	Cl-		316.80	49.29	5.41	8.84	49.15	5.38	9.12	158—160
SU-9	Cl-		310.75	50.25	3.57	9.01	50.05	3.68	9.09	175—177
SU-10	CH_3-		283.35	50.86	6.05	14.83	50.56	5.97	14.81	188—190
SU-11	CH_3-		297.37	52.51	6.44	14.13	52.19	6.36	14.01	203—205
SU-12	CH_3-	$-N(CH_2CH_3)_2$	285.36	50.50	6.71	14.72	50.44	6.79	14.61	150—153
SU-13	H-	$-CH_3$	214.23	44.84	4.71	13.08	44.64	4.63	13.18	145—147

9) J. Cymerman-Craig and A.A. Diamantis, *J. Chem. Soc.*, **1953**, 1619.
10) A. Albert and E.P. Serjeant, "Ionization Constant of Acids and Bases," Methuen and Co., London, 1967, pp. 69—91.
11) S. Asada, T. Nakazato, and S. Takino, *Yakugaku Zasshi*, **93**, 1647 (1973).

TABLE II. Binding Parameter for the Interaction of Sulfonylurea-related Compounds with Bovine Serum Albumin at pH 7.4 and 30°

Compd.	R_1	R_2	k_1 $\times 10^5$/M	k_2 $\times 10^3$/M	n_1	n_2
SU-1	CH_3–	$-CH_3$	0.14	0.97	1.14	3.52
SU-2	CH_3–	$-CH_2CH_3$	0.17	1.36	0.84	4.83
Tolbutamide	CH_3–	$-CH_2CH_2CH_2CH_3$	2.49	5.63	0.75	5.14
SU-3	CH_3–		1.38	3.98	0.99	4.74
SU-4	CH_3–		3.81	3.75	1.63	4.60
SU-5	Cl–	$-CH_3$	0.17	1.14	1.60	2.44
SU-6	Cl–	$-CH_2CH_3$	0.22	1.60	1.23	3.05
Chlorpropamide	Cl–	$-CH_2CH_2CH_3$	1.19	2.38	0.88	4.89
SU-7	Cl–	$-CH_2CH_2CH_2CH_3$	2.51	4.76	1.16	4.34
SU-8	Cl–		1.58	5.59	1.00	5.95
SU-9	Cl–		3.82	4.60	1.76	4.35
SU-10	CH_3–		3.39	2.83	0.86	2.38
SU-11	CH_3–		0.18	2.15	0.92	2.88
Tolazamide	CH_3–		—	4.77[a]	—	4.40[a]
SU-12	CH_3–	$-N(CH_2CH_3)_2$	—	2.07[a]	—	3.52[a]
Chlorpentazide	Cl –		2.75	3.43	0.87	2.56
Azepinamide	Cl –		0.24	3.87	0.92	5.40
SU-13	H–	$-CH_3$	—	—	—	—
Carbutamide	NH_2–	$-CH_2CH_2CH_2CH_3$	0.84	1.52	0.74	4.42
Acetohexamide	CH_3CO–		0.75	2.41	1.13	3.77

a) Tolazamide and SU-12 have only one class binding site and these binding parameters could be looked as the same degree with k_2 and n_2 values of another sulfonylureas.

The general approach, technique, and treatment of data of BSA binding studies for these synthesized compounds were the same as described in our previous paper.[1b] The mathematical analysis of binding data, values for the binding constant, k, and number of binding sites, n, are summarized in Table II. The data for seven commercial sulfonylureas are also included in the same Table (Table II).

The Scatchard plots for binding data indicate the presence of more than two classes of binding sites in the same manner as shown for the seven commercial sulfonylureas in Fig. 1—3. Figure 1 shows data for compounds carrying CH_3 group as R_1 substituent group, and cyclic and chain groups as R_2 substituent group. The profile for compounds in which R_1 group is Cl and R_2 group is cyclic and chain groups is given in Fig. 2. Figure 3 gives data on the compounds containing N-substituted secondary amines as R_2 substituent group.

Effect of R_2 Substituent Group on BSA Binding

According to the results in Fig. 1 and 2, and in Table II, it is possible to consider that the introduction of a higher hydrophobic and lipophilic group as R_2 substituent group will result in an enhanced BSA binding constants, especially k_1. Comparison of the binding constant, k_1 for primary binding site, reveals that the binding increases from R_2=CH_3 to C_2H_5

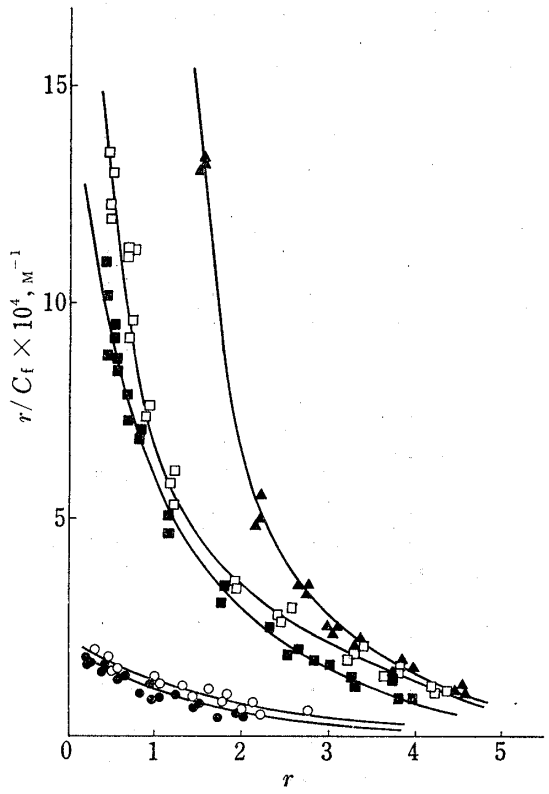
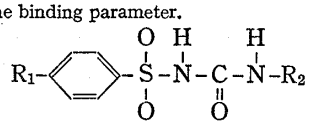


Fig. 1. Scatchard Plots for the Binding of Sulfonylurea Related Compounds (R_1: CH_3) to Bovine Serum Albumin at pH 7.4 and 30°

All points are experimental while the solid lines are computed from the binding parameter.



	R_1	R_2
●, SU-1	CH_3–	–CH_3
○, SU-2	CH_3–	–CH_2CH_3
□, tolbutamide	CH_3–	–$CH_2CH_2CH_2CH_3$
■, SU-3	CH_3–	–C$_6$H$_{11}$
▲, SU-4	CH_3–	–C$_6$H$_5$

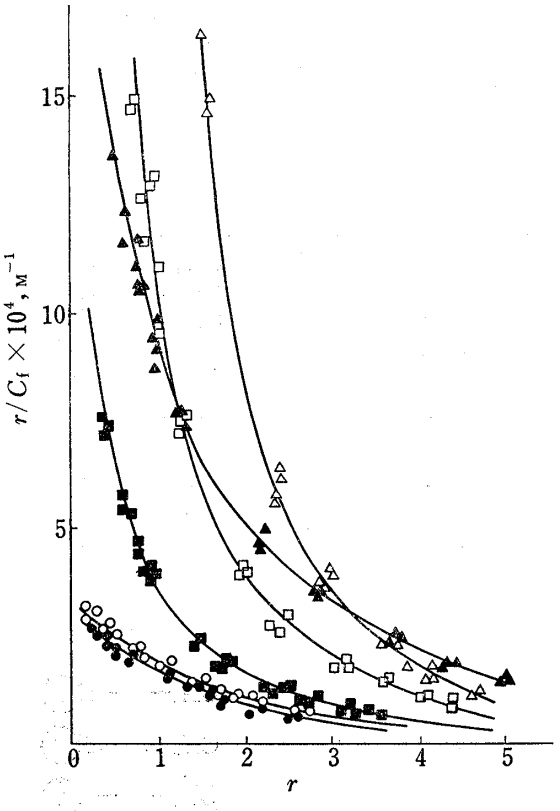
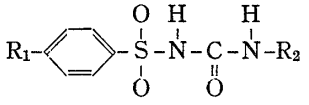


Fig. 2. Schatchard Plots for the Binding of Sulfonylurea Related Compounds (R_1: Cl) to Bovine Serum Albumin at pH 7.4 and 30°

All points are experimental while the solid lines are computed from the binding parameter.



	R_1	R_2
●, SU-5	Cl–	–CH_3
○, SU-6	Cl–	–CH_2CH_3
■, chlorpropamide	Cl–	–$CH_2CH_2CH_3$
□, SU-7	Cl–	–$CH_2CH_2CH_2CH_3$
▲, SU-8	Cl–	–C$_6$H$_{11}$
△, SU-9	Cl–	–C$_6$H$_5$

to C_3H_7 to C_4H_9 and cyclohexyl to phenyl, but the effect or dependency of k_2 for secondary binding site on the above order is relatively small.

Effect of R_1 Substituent Group on BSA Binding

Generally, compounds carrying Cl as R_1 group have a larger binding constants than that carrying CH_3 as R_1 group by comparing binding constants of two compounds carrying the same R_2 group. The order of binding in the compounds where R_2 group is cyclohexyl or butyl groups was as follows: R_1=Cl>CH_3>CH_3CO in the case of cyclohexyl, and R_1=Cl>CH_3>NH_2 (as regards k_1) and R_1=CH_3>Cl>NH_2 (as regards k_2) in the case of butyl. If an electrostatic mechanism is assumed for BSA binding, binding constants is expected to correlate with the Hammett constant, σ,[12] in the same manner as the close correlation between

12) The Hammett constants reported by Jaffe (*Chem. Rev.*, **53**, 191 (1953)) are as follows: NH_2 −0.660, CH_3 −1.70, Cl 0.227, and CH_3CO 0.516.

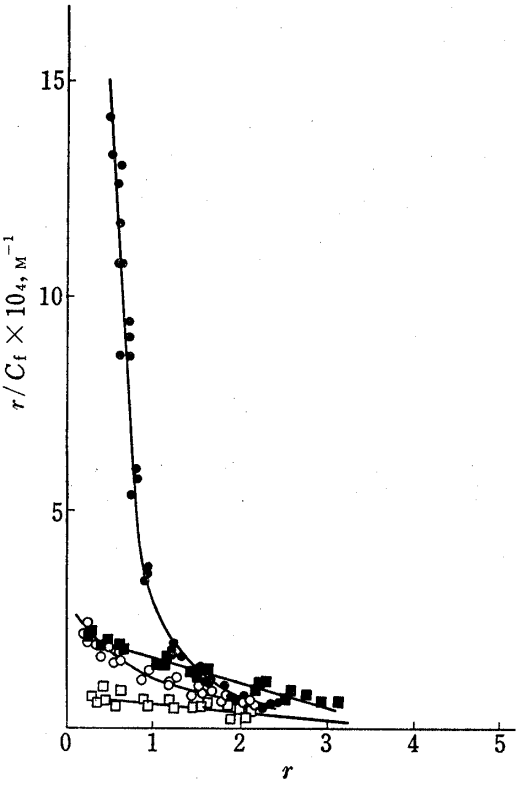
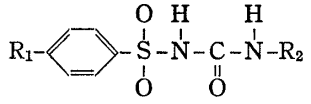


Fig. 3. Scatchard Plots for the Binding of Sulfonylurea Related Compounds (R_1: CH_3, R_2,: N-substituted secondary amine) to Bovine Serum Albumin at pH 7.4 and 30°

All points are experimental while the solid lines are computed from the binding parameter.



	R_1	R_2
●, SU-10	CH_3–	N⑤
○, SU-11	CH_3–	N⑥
■, tolazamide	CH_3–	N⑦
□, SU-12	CH_3–	$-N(CH_2CH_3)_2$

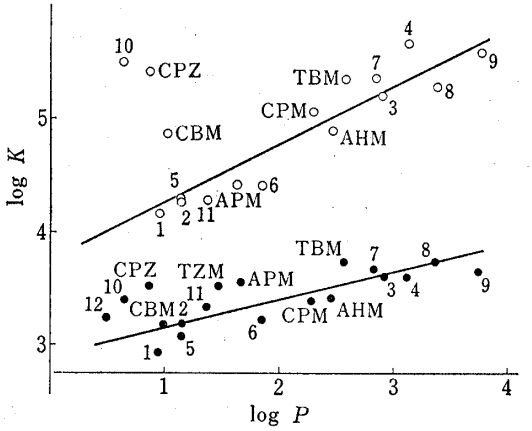


Fig. 4. Relationship between Binding Constants and Octanol/Water Partion Coefficient of Sulfonylurea Related Compounds at pH 7.4 and 30°

Key ○, log k_1, ●, log k_2.
CPM: chlorpropamide, CPZ: chlorpentazide, CBM: carbutamide, TZM: tolazamide, TBM: tolbutamide, APM: azepinamide, AHM: acetohexamide, 1—12: SU-1—12.

pK_a value and Hammett constant in a series of compounds. Unfortunately, the fact that carbutamide (R_1: NH_2, R_2: C_4H_9) has a smaller binding constant than other compounds carrying C_4H_9 as R_2 group, could not be explained from the Hammett constants alone. On the other hand, a new substituent constant, π, derived from the partition coefficient has been proposed by Fujita and his co-workers,[13] as one of the parameters that represents the degree of hydrophobicity of a substituent group. The substituent constants were calculated from the partition coefficients between octanol and water for eight different derivative systems, and the values for π[14,15] obtained with substituted phenols were as follows: NH_2 (−1.63), CH_3CO (−0.11), CH_3 (0.48), and Cl (0.93). If this constant is applied in the present work, a good correlation is observed berween π for R_1 group and BSA binding constants. The increasing hydrophobicity of R_1 group is mutually related to the increase of BSA binding constant if R_2 group is the same.

Effect of R_2 Substituent Group containing N-Substituted Secondary Amines on BSA Binding

When R_2 group contains N-substituted secondary amines, the binding ability changes markedly with increase of ring carbons, from five to six, to seven-membered ring, and the pattern of the Scatchard plots is shown in Fig. 3. SU-12, in which the five-membered ring

13) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).
14) The substituent constant is defined as: $\pi = \log P_X - \log P_H$ where P_X is the partition coefficient of derivatives and P_H is that of the parent compound.
15) H. Terada, S. Muraoka, and T. Fujita, *J. Med. Chem.*, **17**, 330 (1974).

as R_2 substituent group is opened and changed to a linear chain type, has only one class binding site, and its Scatchard plots become a straight line, as shown in Fig. 3.

Sulfonylureas-BSA Binding Mechanism

It is obvious that physico-chemical properties such as the partition coefficient with the octanol–water system, P, is linearly and positively correlated with BSA binding constants as shown in Fig. 4. To obtain a more accurate correlation, Eq. (3) and (4) were used. The results of linear and multiple regression analysis are summarized in Table III. Equation

Table III. Equation Correlating Binding Constants, $\log k = a \log P + c$, $\log k = a' \log P + b' \mathrm{p}K_a + c'$

Compd.[a]	n[b]	Eq	log k	a	b	r[b]	s[b]
—	19	(5)	log k_2	0.159	3.129	0.667	0.179
Tolazamide	17	(6)	log k_1	0.467	3.385	0.732	0.435
SU-12		(7)	log k_2	0.176	3.079	0.698	0.181
Chlorpentazide SU-10	17	(8)	log k_2	0.201	3.015	0.782	0.159
Tolazamide	15	(9)	log k_1	0.512	3.754	0.866	0.279
Chlorpentazide		(10)	log k_2	0.244	2.896	0.853	0.140
SU-10, 12							

Compd.[a]	n[b]	Eq	log k	a'	b'	c'	r[b]	s[b]
—	19	(11)	log k_2	0.282	0.272	1.465	0.837	0.135
Tolazamide	17	(12)	log k_1	0.493	0.064	3.502	0.732	0.449
SU-12		(13)	log k_2	0.288	0.272	1.445	0.839	0.143
Chlorpentazide SU-10	17	(14)	log k_2	0.321	0.369	1.374	0.930	0.980
Tolazamide	15	(15)	log k_1	0.548	0.095	3.195	0.870	0.287
Chlorpentazide		(16)	log k_2	0.334	0.240	1.480	0.947	0.091
SU-10, 12								

a) These compounds were omitted in deriving the numerical coefficient in equation.
b) n is the number of compounds, r is the correlation coefficient, and s is the standard deviation.

(5) for k_2 was derived from 19 data except SU-13 from which k_2 value could not be obtained. Similar analyses were also made except specific sulfonylurea-related compounds containing N-substituted secondary amines, and then Eq. (6) to (10) were derived. Fitting the 19, 17, and 15 data to the two-parameter equation that added electrostatic parameter to the equation for log P, the constants a′, b′, and c′ were obtained as shown in Table III. The difference between single and two-parameter equations was checked statistically by the F-test. Because no significant difference between Eq. (9) and (15) for k_1 was observed, it could be proved that the addition of $\mathrm{p}K_a$ term did not improve the correlation. Therefore, the BSA binding of sulfonylurea-related compounds may occur through the binding nonionic group of the molecule and hydrophobic regions on BSA surface. On the other hand, Eq. (16), addition of the $\mathrm{p}K_a$ term, was justified at better than the 0.95 level of significance (Eq. (15), $F_{1,12}=0.299$, Eq. (16), $F_{1,12}=18.997$, $F_{1,12,0.05}=4.75$). Thus, the BSA binding of this series of compounds seems to be governed by both the hydrophobicity and electrostatic character of the molecule. Implication of the above conclusion could be more clearly indicated by the square of correlation coefficient, r^2, 0.75 for Eq. (9) and 0.90 for Eq. (16). It would be reasonable to say 75% of BSA binding is hydrophobic for the primary binding site and at least 90% of BSA binding can be explained by both hydrophobic and electrostatic interactions for the secondary binding site.

The calculation was made according to Eq. (9) and (16), and the result is shown in Table IV, together with observed values.

$$\log k_1 = 0.512 \log P + 3.754 \quad (9)$$

$$\log k_2 = 0.334 \log P + 0.240\ pK_a + 1.480 \quad (16)$$

TABLE IV. Binding Constants of Sulfonylurea-related Compounds with Bovine Serum Albumin

Compound[a)]	pK_a[b)]	log P	log k_1		log k_2	
			Obs.	Calcd.[c)]	Obs.	Calcd.[d)]
Chlorpropamide	4.92	2.27	5.076	4.918	3.377	3.421
Tolbutamide	5.27	2.52	5.396	5.042	3.751	3.586
Acetohexamide	4.63	2.44	4.874	5.005	3.382	3.408
Carbutamide	5.96[a)]	1.01	4.925	4.273	3.182	3.250
Chlorpentazide*	5.68	0.84	5.439	4.185	3.535	3.125
Azepinamide	5.94	1.64	4.378	4.596	3.588	3.456
Tolazamide*	6.18	1.45	—	4.496	3.526	3.448
SU-1	5.24	0.95	4.145	4.239	2.986	3.055
SU-2	5.37	1.14	4.227	4.337	3.134	3.150
SU-3	5.50	2.90	5.140	5.240	3.600	3.771
SU-4	4.38	3.11	5.581	5.348	3.574	3.572
SU-5	4.77	1.16	4.231	4.349	3.056	3.014
SU-6	4.84	1.79	4.340	4.669	3.203	3.239
SU-7	4.92	2.81	5.399	5.192	3.677	3.600
SU-8	4.94	3.38	5.199	5.485	3.748	3.796
SU-9	3.97	3.61	5.582	5.603	3.663	3.640
SU-10*	5.95[a)]	0.65	5.530	4.086	3.451	3.126
SU-11	5.71[a)]	1.34	4.246	4.440	3.332	3.299
SU-12*	6.08[a)]	0.48	—	3.980	3.316	3.099
SU-13*	5.14[a)]	−0.26	—	3.619	—	2.621

a) The names marked by an asterisk were omitted in deriving the numerical coefficients in Eq. (9), (16).
b) pK_a values were determined by spectrophotometry, except *a*) which was obtained by titration.
c) Calculated from Eq. (9).
d) Calculated from Eq. (16).

Fujita and his co-workers[15,16] reported the hydrophobic binding activity relation of various sulfonamides and fenamic acids, and they stated that the variation of BSA binding depends on the state of the dissociation equilibrium of drugs. Thus, Eq. (17) and (18) are derived for the neutral and ionized forms, respectively.

$$\log k_n = \log k + \log\left(\frac{K_a+[H^+]}{[H^+]}\right)$$

$$\simeq \log k - pK_a + pH \quad (17)$$

$$\log k_i = \log k + \log\left(\frac{K_a+[H^+]}{K_a}\right)$$

$$\simeq \log k \quad (18)$$

where k_n and k_i are BSA binding constants for the neutral and ionized forms, K_a is the ionic constant, and $[H^+]$ is the hydrogen ion concentration.

The same analysis was also applied for sulfonylurea-related compounds. For instance, log k_n and log k_i for SU-9 are as follows: log k_{1n}=9.01, log k_{1i}=5.58 for the primary binding site and log k_{2n}=7.09, log k_{2i}=3.66 for the secondary binding site. Recently, Gillett[17] re-

16) T. Fujita, *J. Med. Chem.*, **15**, 1049 (1972).
17) J.R. Gillette, *Ann. N.Y. Acad. Sci.*, **226**, 6 (1973).

ported that the albumin binding constant could not be considered over the value of the order of 3×10^{10} M^{-1} based on his calculation using other literatures.[18,19] Therefore, the value for k_{1n} in this experiment is quite large and closer to the maximum one stated above. Detailed consideration and continued discussion about such a separated BSA binding constants, k_n and k_i, is considered to be of no significant meaning in this paper.

18) C. Goresky, *Am. J. Physiol.*, **204**, 626 (1963).
19) P. Taylor, "Methods in Pharmacology," C.F. Chignell, Ed., Appleton-Century-Crofts, New York, 1972, Vol. 2, pp. 351—379.