Application of Regression Analysis to the Hypoglycemic Activities of a Series of Substituted Benzenesulfonamidopyrimidines

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Quantitative structure–activity relationship studies have been performed on two types of sulfonamides with hypoglycemic activity. In the case of the 2-benzenesulfonamidopyrimidines, substituted in the 5 position of the pyrimidine ring, a correlation between hydrophobic forces, expressed as R_m values, and the binding to serum albumin as well as to the heights of the equipotent dose has been found. In the series of 2-benzenesulfonamidopyrimidines additionally substituted in the 4 position of the benzene ring, however, a correlation between electronic parameters, expressed as the chemical shift of the anilide NH- (type IIa) and benzylamide NH- (type IIb) protons and the biological response was observed. This correlation indicates a charge-controlled second fixation of these molecules to the receptor. This is supported by the observation of stereospecificity of the blood-glucose lowering effect and also by the importance of a constant distance between the nitrogen in the side chain and the nitrogen atom in the sulfonamido group. The correlation between the logarithm of the biological response and the electronic effects of the substituents is linear as long as one homologous series is considered. If the anilide and benzylamide derivatives are combined, a linear correlation can only be obtained if a dummy parameter is included which may account for differences in conformation within these two series of compounds.

The possibility to optimize the effect of molecules for specific therapeutic purposes by the application of regression analysis to structure–activity data is of considerable interest. Extrathermodynamic models such as the Hansch approach, empirical mathematical models such as the Free–Wilson method, or quantum chemical approaches have shown to be of value in this respect.

Several preconditions are necessary for a satisfactory application of regression analysis to structure–activity data: the series to be studied should consist of closely related analogs so that a change in mechanism of action becomes unlikely, the biological activity data must be obtained under uniform conditions, and the biological response should be of low complexity. For instance, different bioavailability resulting from different solubility or/and absorption rates and different unspecific binding to serum proteins should be excluded or at least be considered. A large number of compounds and a wide range in biological response are desirable.

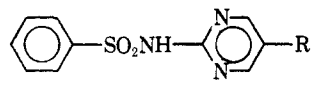
It is generally accepted that the blood glucose lowering effect of sulfonylureas and related compounds of the sulfonamide type is due to a stimulation of insulin secretion from the β cells of the pancreatic islets. The detailed mechanism of this action as well as the specific receptors involved is not known.[1-5]

Since the early work of Loubatieres and Franke and Fuchs[6,7] and the detection of the blood glucose lowering effect of certain sulfonamides, thousands of derivatives have been synthesized to gain more potent compounds with suitable pharmacokinetic properties. Structure–activity relations derived were, however, only of a qualitative nature so far.[8]

The first quantitative approach to structure–activity studies with this type of drug was reported by Smithfield and Purcell[9] using the activity data from McManus, *et al.*[10] In this paper we have tried to correlate various physicochemical properties of benzenesulfonamidopyrimidines to their blood glucose lowering properties.

Results and Discussion

I. 2-Benzenesulfonamidopyrimidines. At first we want to report on quantitative structure–activity studies in a series of antidiabetic drugs of the benzenesulfonamidopyrimidine type (I)[11] with the general formula



I

where R are alkyl, alkoxy, or aryloxy groups (Table I).

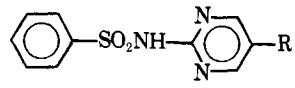
In this series of 2-benzenesulfonamidopyrimidines (I) the general importance of the 2-benzenesulfonamidopyrimidine structure has been evaluated and the influence of the substituent R on strength and differences of the biological effect was demonstrated.[11] To quantify this dependency the physicochemical influence of the various substituents R on the molecule has been characterized by the determination of the pK_a, for the electronic influence, and by the determination of the partition coefficient, P, or the R_m value by reversed phase thin-layer chromatography,[12] for the change in hydrophilic and lipophilic properties.

In addition, the degree of albumin binding, B (in per cent free drug), has been evaluated at a single similar concentration for each compound using a three-chamber dialysis. It was not possible to determine the dissociation constants of the drug–albumin complex because of the limited range of solubility of the compounds.

To get further information on the interaction between the benzenesulfonamidopyrimindines (I) and the albumin macromolecule, nmr measurements were performed with some compounds of this series. The broadening of the proton signals, especially of the pyrimidine ring protons as a function of albumin concentration, revealed a strong interaction of the pyrimidine ring protons with the macromolecules. The interaction [expressed as $(T_2)_{free}/(T_2)_{bound}$] becomes more pronounced with increasing lipophilic properties of the substituents R (Table I). Figure 1 demonstrates a linear correlation between the relaxation rate $(1/T_2)$ of the pyrimidine protons (given as the line width at one-half maximum peak height) and the albumin concentration. From these data the ratio $(T_2)_{free}/(T_2)_{bound}$ has been calculated according to Jardetzky.[13] The ranking of this ratio, which is a parameter for the degree of binding, is in reasonable agreement with the degree in binding determined by dialysis experiments (Table I and Figure 1).

The influence of lipophilic properties on binding to serum albumin has been reported several times.[14,15] There-

Table I. Physical Constants, Protein Binding, and Biological Activity of Benzenesulfonamidopyrimidines[b]



R	Mol wt	Deg of protein binding (4% albumin), % free drug B	$(T_2)_{free}$ / $(T_2)_{bound}$[a]	pK_a	Partitn coeff, P	R_m	Dose (uncor/cor) for albumin binding: C, mg/kg	C, μmol/kg	Dose, log 1/C, kg/μmol	Log dose calcd from eq 3, log C
$-CH_3$	249.3	15.2	87.3	6.15	1.58	0.28	62	37.9	-1.578	-1.699
$-C_2H_5$	263.3	4.7	173.9	6.14	7.58	0.81	125	21.6	-1.335	-1.398
$-n-C_3H_7$	277.4	3.4	217.8	6.18	18.26	1.41	125	15.1	-1.180	-1.046
$-CH_2CH(CH_3)_2$	291.4	2.0		6.07	30.00	1.90	125	8.6	-0.932	-0.770
$-OC_2H_5$	279.3	6.4		5.78	3.40	0.74	125	28.5	-1.455	-1.398
$-O-n-C_3H_7$	293.4	4.6	198.5	5.83	13.80	1.40	31	4.9	-0.688	-1.046
$-O-i-C_3H_7$	293.4	9.7	207.7	5.77	7.34	1.24	62	20.4	-1.311	-1.097
$-O-n-C_4H_9$	307.4	2.4		5.80	26.00	1.92	62	4.7	-0.677	-0.745
$-OC_6H_5$	327.4	1.3		5.33	24.00	1.80	125	4.9	-0.694	-0.824

[a] See ref 13. [b] For determination, see the Experimental Section.

fore we tried to correlate the obtained parameters for lipophilicity (P) to the degree of protein binding (B). The result is given in eq 1 together with the parameters for statistic significance. The statistics for eq 1–14 are the standard errors of estimate, s, the multiple correlation coefficient, r, and the F test. The value in parentheses below the coefficients is the t test. The interrelationship between the two parameters for lipophilic properties, P and R_m, is given in eq 2.

The albumin binding diminishes the biologically active fraction of the different 2-benzenesulfonamidopyrimidines to a different degree. In order to obtain a physically significant correlation between structural parameters and the dose to exert a biological effect, the different degrees of albumin binding were considered in the equipotent doses (Table I, column 7). The biological effect is expressed as the dose necessary to obtain a decrease of 25% of blood glucose concentration in fasted rabbits after oral application of the drugs. The data are arithmetic means of 3–6 animals (dose gradients, 8, 16, 31, 62, 125, 250, 500 mg/kg) (Table I).[16] The doses were corrected for protein binding and molecular weight (Table I, corrected dose C). The reciprocal of the corrected dose [Table I, 1/C (kg/μmol)] showed a significant correlation to the lipophilic properties of the compounds as expressed by the partition coefficient, P, and R_m values, respectively (eq 3a).

As no important structural change with respect to the "essential" components in molecular structure I takes place in this series of homologous compounds, the differences in biological response seem explainable by changes in the hydrophobic properties only.

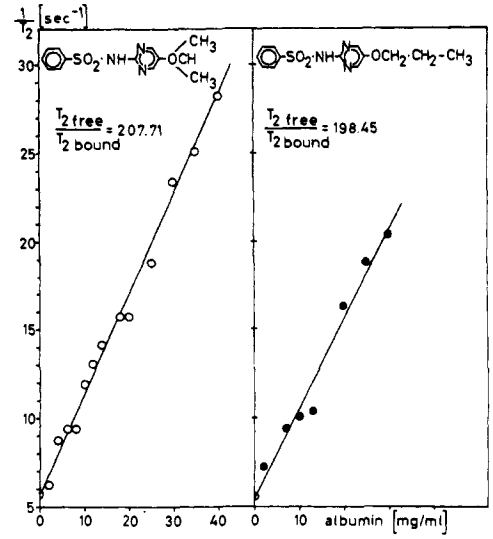
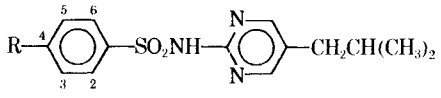


Figure 1. Relaxation rates of the pyrimidine protons of 36 mM 2-benzenesulfonamido-4-isopropoxypyrimidine (○) and 2-benzenesulfonamido-4-propoxypyrimidine (●) as a function of BSA concentration.

Because of the small variance in pK_a values in this series

	n	r	s	F	S	
$\log B = -0.51R_m + 1.27$ (4.40)	9	0.86	0.19	19.3	>99.5	(1)
$\log P = 0.74R_m + 0.08$ (9.85)	9	0.97	0.12	97.1	>99.9	(2)
$\log 1/\text{dose} = 0.54R_m - 1.79$ (4.77)	9	0.87	0.18	22.71	>99.5	(3a)
$\log 1/\text{dose} = 0.49R_m - 0.24\ pK_a - 0.30$ (3.90) (0.92)	9	0.89	0.19	11.52	>99.0	(3b)

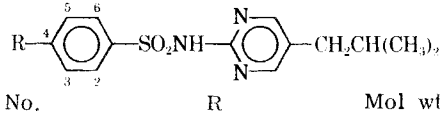
Table II. Physical Constants and Biological Activity of 4-Substituted Benzenesulfonamidopyrimidines of Anilide Type IIa[a]



No.	R	Mol wt	$\bar{R}_m$	ppm	$E_{s(m)}$ Kutter[22]	$E_{s(m)}$ Taft[21]	$E_{s(o)}$ Kutter[22]	$E_{s(o)}$ Taft[21]	Dose, mg/kg, iv (with 95% confidence limits), blood glucose reduction to: y_1, 50% of the initial value	Dose, mg/kg, iv (with 95% confidence limits), blood glucose reduction to: y_2, 50 mg/100 ml	Log 1/dose, log $1/y_2$, kg/μmol	Log 1/dose calcd from eq 4	Log 1/dose calcd from eq 13	Log 1/dose calcd from eq 14
1	$C_6H_5NHCOCH_2$–	424.5	1.32	10.20	1.24	1.24	1.24	1.24		4.47 (0.7–6.38)	−1.022	−0.921	−0.921	−1.097
2[b]	2-OCH_3-5-Cl-$C_6H_3NHCOCH_2$–	491.0	2.98	9.56	0.27	0.18	0.69	0.99	0.40 (0.29–0.92)	0.28 (0.16–0.36)	0.244	0.149	0.161	0.130
3	5-CF_3-C_6H_4NH-$COCH_2$–	493.5	3.21	9.81	−0.99	−1.16	1.24	1.24	2.21 (1.66–3.43)	1.62 (1.29–2.22)	−0.516	−0.260	−0.260	−0.469
4	6-C_6H_5-C_6H_4-$NHCOCH_2$–	501.6	2.45	9.46	−2.58	−0.90	−2.58	−0.99	0.33 (0.25–0.72)	0.21 (0.17–0.36)	0.378	0.320	0.330	0.320
5	6-OCH_3-C_6H_4-$NHCOCH_2$–	455.6	1.73	9.37	0.69	0.99	0.69	0.99	0.93 (0.56–3.72)	0.35 (0.27–0.56)	0.114	0.470	0.480	0.490
6	2-Cl-5-CF_3-$C_6H_3NHCOCH_2$–	529.0	2.49	10.00	−0.99	−1.16	0.27	0.18	1.78 (1.47–2.64)	1.19 (0.15–1.71)	−0.352	−0.569	−0.586	−0.699
7	4-Cl-C_6H_4-$NHCOCH_2$–	460.0	2.28	10.33	1.24	1.24	1.24	1.24	18.18 (12.77–102.9)	6.10 (0.31–8.33)	−1.123	−1.097	−1.155	−1.301
8	2-OCH_3-5-F-$C_6H_3NHCOCH_2$–	474.6	2.05	9.56	0.78	0.49	0.69	0.99	0.19 (0.15–0.39)	0.13 (0.09–0.17)	0.562	0.149	0.161	0.130
9	2-CF_3-C_6H_4-$NHCOCH_2$–	493.6	2.00	9.79	1.24	1.24	−0.99	−1.16	1.72	0.93	−0.275	−0.229	−0.229	−0.301
10	2-OCH_3-5-Cl-$C_6H_3N(CH_3)COCH_2$–	505.1	2.29		0.27	0.18	0.69	0.99	1.71 (1.33–2.43)	1.03 (0.81–1.31)	−0.310			

[a] For determination, see the Experimental Section. [b] Proposed WHO (generic) name, glidanil.

Table III. Physical Constants and Biological Activity of 4-Substituted Benzenesulfonamidopyrimidines of Benzylamide Type IIb[c]



No.	R	Mol wt	$\bar{R}_m$	ppm	$E_{s(m)}$ Kutter[22]	$E_{s(m)}$ Taft[21]	$E_{s(o)}$ Kutter[22]	$E_{s(o)}$ Taft[21]	Dose, mg/kg, iv (with 95% confidence limits), blood glucose reduction to: y_1, 50% of the initial value	Dose, mg/kg, iv (with 95% confidence limits), blood glucose reduction to: y_2, 50 mg/100 ml	Log 1/dose, log $1/y_2$, kg/μmol	Log 1/dose calcd from eq 13	Log 1/dose calcd from eq 14
1	$C_6H_5NHCOCH_2$–	424.5	1.32	10.20	1.24	1.24	1.24	1.24	20.95 (16.40–29.93)	14.92 (12.70–18.06)	−1.545		

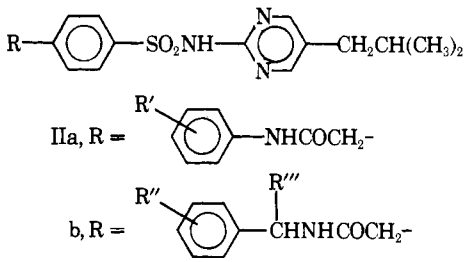
2[a]	2-OCH_3-5-Cl-C_6H_3-$NHCOCH_2$-	491.0	2.98	9.56	0.27	0.18	0.69	0.99	0.64 (0.45–1.21)	0.39 (0.25–0.53)	0.100		
3	$C_6H_5CH_2NHCOCH_2$-	438.6	1.42	8.62	1.24	1.24	1.24	1.24	0.85 (0.66–1.23)	0.551 (0.46–0.69)	−0.099	0.170	0.149
4	$C_6H_5CH(CH_3)$-$NHCOCH_2$-	452.6	1.58	8.57	1.24	1.24	1.24	1.24	0.59 (0.46–0.85)	0.317 (0.25–0.40)	0.155	0.250	0.250
5	2-OCH_3-5-F-C_6H_3-$CH(CH_3)NHCOCH_2$- racemate	502.6	2.21	8.51	0.78	0.49	0.69	0.99	0.19 (0.14–0.33)	0.119 (0.09–0.19)	0.626	0.350	0.360
6[b]	2-OCH_3-5-F-C_6H_3-$CH(CH_3)NHCOCH_2$- S-(−) enantiomer	502.6					0.07 (0.05–0.10)		0.07 (0.05–0.10)	0.048 (0.04–0.07)	1.020		
7	2-OCH_3-5-Cl-C_6H_3-$CH_2NHCOCH_2$-	505.1	1.93	8.48	0.27	0.18	0.69	0.99		0.16 (0.09–0.47)	0.499	0.400	0.420
8	$C_6H_5CH(CH_3)N(CH_3)$-$COCH_2$-	466.6	2.02		1.24	1.24	1.24	1.24	11.66 (9.70–14.16)	7.324 (4.56–10.1)	−1.196		

[a] Proposed WHO (generic) name, glidanil. [b] Proposed WHO (generic) name, glifumide. [c] For determination, see the Experimental Section.

of compounds (Table I) this statement is, however, limited. Comparative F statistics for eq 3a over 3b demonstrate no superiority of eq 3a over 3b ($S < 90.0$).

As expressed in eq 2 and 3a an increase in lipophilic properties (R_m) of the molecule responded in an increase in binding to albumin and simultaneously in a decrease in the equipotent dose. This might be of some interest with respect to the mode of action theory put forward by Madsen and others,[17] where a competition for the binding site at the serum protein between insulin and this type of antidiabetic drugs is discussed.

II. 4-Substituted Benzenesulfonamidopyrimidines. (a) Anilides of 4-[*N*-(2-Pyrimidinyl)sulfonamido]-phenylacetic Acid and (b) Benzylamides of 4-[*N*-(2-Pyrimidinyl)sulfonamido]phenylacetic Acid. IIa. If benzenesulfonamidopyrimidines (I) are substituted in the 4 position of the benzene ring by certain groups represented by formulas IIa and IIb, in most cases a remarkable increase in activity is observed.



In these experiments the hypoglycemic activity was determined in fed female rats after iv injection. The potency of the compounds was expressed as the dose causing a decrease in blood glucose concentration to 50% of the initial value and to 50 mg % within 1 hr (Tables II and III, y_1 or y_2, respectively).

Since we do not know the receptor and mechanism involved in the biological reaction we can only empirically correlate various physicochemical parameters of the molecule group with the biological activity parameters of the corresponding molecules.

The first series included only compounds of type IIa, *i.e.*, the anilide type. It is remarkable that the distance between the nitrogen in the side chain and the nitrogen atom in the sulfonamido group was constant for highly active derivatives. This observation has led to the assumption of a second binding site of these molecules at the receptor, where probably the second nitrogen atom is involved.[18–20]

Therefore we have tried to obtain characteristic parameters for the anilide (IIa) and later for the benzylamide (IIb) nitrogen atoms, respectively. The chemical shift of the adjacent protons in nmr measurements seems especially suitable for this purpose. As given in Tables II and III a considerable difference in chemical shift (ppm) can be observed for the NH proton as a function of changes of different substituents in the phenyl ring. The selection of substituents was such that type and position of the substituents in the phenyl ring covered a wide range of electronic, hydrophobic, and steric properties. The compound with the largest shift downfield at the proton of the anilide nitrogen atom has the lowest biological activity (Table II, 1 and 7) comparable with the activity of the unsubstituted compounds of type I. In addition, R_m values have been determined to characterize the hydrophobic influences of the various substituents. The steric parameters have been taken from the compilations of Taft[21] and Kutter.[22] Multiparameter regression analysis reveals eq 4–8 and results.

There is a significant correlation between the electronic effects expressed as the chemical shift (ppm) of the proton

	n	r	s	F	S	
log 1/dose = −1.64 ppm + 15.90 (6.25)	9	0.92	0.25	39.12	>99.9	(4)
log 1/dose = $0.20R_m$ − 0.67 (0.52)	9	0.19	0.63	0.27	<90.0	(5)
log 1/dose = −1.64 ppm + $0.04R_m$ + 15.69 (5.70) (0.25)	9	0.92	0.27	16.97	>99.5	(6)
log 1/dose = −1.58 ppm − $0.04E_s$(Kutter) + 15.25 (5.08) (0.49)	9	0.92	0.26	17.57	>99.5	(7)
log 1/dose = 11.33 ppm − 0.66 ppm^2 − 47.93 (0.55) (0.63)	9	0.93	0.26	18.08	>99.5	(8)

adjacent to the anilide nitrogen atom and the logarithm of the equipotent dose (eq 4). In contrast, no significant contribution of hydrophobic forces, expressed as R_m values, can be detected (eq 5). It was not possible to discriminate between hydrophobic and steric influences. A test shows an overlap of R_m and E_s values of approximately 70% (r = 0.84). The combination of electronic and hydrophobic or steric parameters results not in an improvement. The t test on each of the coefficients in eq 4–8 (indicated in parentheses below each variable) suggests that only the ppm term is significant at the 99% confidence level.

The observed linearity between the "electronic distribution" at the anilide nitrogen atom and the biological response may justify the discussion of a charge controlled second fixation of the nitrogen atom of these compounds to the receptor which in turn causes a considerable increase in biological effect compared to the effect of the "simple" benzenesulfonamidopyrimidines (I) (see also ref 23).

IIb. In the second series some compounds of the benzylamide type (IIb) were tested. For better comparison compounds 1 and 2 of the anilide type, IIa, were included in the test. The determination of the hypoglycemic activity was performed under the same conditions except that male rats were used instead of female rats (Table III). Unfortunately, a lower activity was observed for compounds 1 and 2 in this experiment possibly because of a higher response of female rats. There is, however, still an overlap within the limits of confidence in the case of compound 2. Equations 9 and 10 were obtained from a regression analysis for compounds of the benzylamide type (IIb).

In contrast to the results of the first series a significant correlation was obtained not only for the electronic parameter (ppm), but also for the hydrophobic or steric forces expressed by R_m. However, the significance of these correlations has to be considered in view of the limited number of compounds and the smaller variance in the biological activity data.

Combination of the benzylamide series (IIb) with the anilide series (IIa) results in a decrease in the significance of the correlation.

Since the two sets of biological data are available for compounds 1 and 2, two sets of equations are obtained depending on the use of data from either Table II (eq 11 and 13) or Table III (eq 12 and 14; doses marked with asterisk). A small improvement of the correlation is obtained if R_m or E_s values are included, as expressed by the t test for the coefficients and the standard deviations (eq 11 and 12).

The decrease in significance in the obtained correlation compared to the results with the separate series (eq 4 and 9) could be due to differences in conformation in the two series of compounds. The phenyl ring is attached directly to the nitrogen in one type of compound (IIa) and is separated by a methylene group in the second series (IIb). From quantum chemical studies of Hoyer and Herrmann[23] such a difference in conformation is very likely. For the anilide type compounds a gain in energy is obtained if the phenyl ring is perpendicular to the molecule's plane. This is not observed for the benzylamide derivatives. If this is consid-

	n	r	s	F	S	
log 1/dose = −4.95 ppm + 42.60 (3.81)	4	0.94	0.14	14.55	>90.0	(9)
log 1/dose = $0.91R_m$ − 1.33 (6.59)	4	0.98	0.08	43.48	>97.5	(10)
log 1/dose = −0.64 ppm + 5.95 (3.58)	13	0.73	0.41	12.81	>99.5	(11a)
log 1/dose = $0.31R_m$ − 0.72 ppm + 6.06 (1.48) (4.04)	13	0.79	0.39	8.18	>99.0	(11b)
log 1/dose* = −0.72 ppm + 6.69 (3.43)	13	0.73	0.47	12.46	>99.5	(12a)
log 1/dose* = $0.43R_m$ − 0.84 ppm + 6.85 (1.89) (4.31)	13	0.81	0.42	9.47	>99.5	(12b)
log 1/dose = −1.69 ppm + $1.58x$ + 14.73 (6.57) (4.50)	13	0.92	0.24	27.73	>99.9	(13)
log 1/dose* = −1.89 ppm + $1.76x$ + 16.46 (6.03) (4.10)	13	0.91	0.30	23.62	>99.9	(14)

ered in a multiparameter regression analysis by a dummy parameter (for the anilides and the benzylamides the values of 1 and 0, respectively, have been chosen) both series can be combined and correlated in an equation with high statistical significance where x could indicate the difference in conformation in the two series.

The N-methyl compounds **10** (Table II) and **8** (Table III) could not be included in the above correlations because of lack of the proton attached to the nitrogen; therefore, a determination of the electron density by nmr was not possible. Both of the compounds show a decrease in biological activity compared to the nonmethylated parent compounds (**2**, Table II, and **4**, Table III, respectively). The increase of the negative charge around the nitrogen atom by an additional methyl group is very likely. This is supported by the observed chemical shift of the surrounding protons. The observed decrease in activity could be explained by a bypass of an optimal negative charge (see, however, Hoyer and Herrmann[23]). Another explanation could be a steric hindrance in the approach of the nitrogen atoms to the receptor site. Similar steric effects were reported by Rufer, *et al.*,[19] and Biere, *et al.*,[20] on blood glucose lowering effects with compounds of type IIb bearing an asymmetric carbon atom. The stereospecificity of the effect is clearly demonstrated. An example in this contribution is compound **5** (Table III) whose S enantiomer (**6**) shows a significant increase in biological activity. Hindrance in approaching the receptor is also possible as a consequence of additional intramolecular forces which limit the freedom of rotation. Such an intramolecular steric effect in case of N-methylation is observed for compound **8** (Table III). A split of the methylene proton signal occurs (Figure 2) which collapses on heating the probe to higher temperatures.

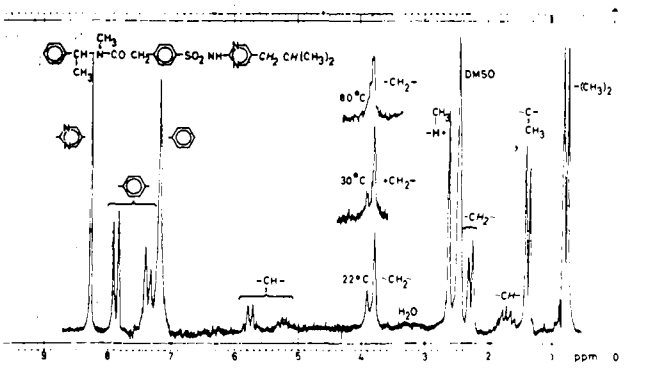


Figure 2. Nmr spectrum of N-methyl-N-(1-phenylethyl)-4-[N-(isobutyl-2-pyrimidinyl)sulfonamido]phenylacetamide in DMSO-d_6 at different temperatures.

Experimental Section

Sulfonamidopyrimidines. The compounds used in this study (Tables I–III) were synthesized in the laboratories of Schering AG, Berlin.[11,18–20]

Determination of Biological Activity. The hypoglycemic activity of benzenesulfonamidopyrimidines not substituted in position 4 was determined in male rabbits (body weight 2–4 kg)[16] 24 hr before the experiment's food (Altromin K pellet diet) was withdrawn. Blood glucose concentration was measured hypoglycemically with glucose oxidase and peroxidase[25,26] using commercial test kits (Boehringer, Mannheim GmbH, Germany) and a semiautomatic measuring system (Braun Systematik, Braun, Melsungen, Germany, with spectrophotometer type PL 4, Zeiss, Oberkochen, Germany). Blood was withdrawn by puncture of an ear vein before as well as 1, 2, 4, and 6 hr, respectively, after oral application of the drugs. The compounds were given as freshly prepared sodium salts dissolved in distilled water (mg/kg of body weight). The following doses were chosen: 8, 15, 31, 62, 125, 250, and 500 mg/kg of body weight. The blood glucose values obtained after treatment were expressed as percentage of the initial concentration. The minimal active dose leading to an at least 25% reduction of blood glucose at any time was taken as an indicator of biological activity. The hypoglycemic activity of 4-substituted benzenesulfonamidopyrimidines was determined in male or female Wistar rats, respectively. Their body weight varied between 140 and 190 g. The test compounds were dissolved in 0.9% saline solution after addition of equimolar concentrations of NaOH. They were injected into a tail vein in a volume of 10 ml/kg of body weight. Controls were treated with the vehicle. From each compound four doses differing by the factor 2 were tested. Each dose group consisted of four (controls, ten) animals. Food (Altromin R pellet diet) was withdrawn after application of the substances; however, the animals were allowed to drink tap water throughout the experiment.

Before and 1 hr after injection of the compounds blood was withdrawn from the retrobulbar vein plexus.[24] Blood glucose concentration was determined as described above.[25,26] The blood glucose values measured after 1 hr were given as absolute concentrations (mg/100 ml) and percentage of the initial concentration. Based on both variables dose–response curves were calculated by means of regression analysis (log dose/linear response)[27] using computer programs elaborated by the Section of Biometrics and Statistics, Schering AG, Berlin/Bergkamen.

The doses which decrease blood glucose to 50% of the initial value (y_1) or to 50 mg/100 ml (y_2) were calculated from these dose–response curves (with 95% confidential limits).

Nmr Measurements. All spectra were determined on a Varian HA-100 high-resolution spectrometer, equipped with a 5512A Hewlett-Packard electronic counter; the solvent used was DMSO-d_6 (Uvasol grade, Merck, Darmstadt, Germany). Anilide group proton signals were reproducible to 0.2 Hz (temperature 23°) and showed no dependence on compound concentration in the concentration range used (0.05–0.1 M). The chemical shift values of the anilide group protons are expressed in parts per million downfield (TMS) and are listed in Tables II and III. Binding measurements were performed with the same equipment, however, in D_2O phosphate buffer, pH 8. Line width determinations were made at a sweep width of 5 Hz/cm and a sweep rate of 0.5 Hz. Relaxation rates $1/T_2$ were calculated from at least three measurements using the equation, $1/T_{2\,(obsd)} = \pi\Delta\nu_{1/2}$, where $\nu_{1/2}$ is the line width of the proton signals of the pyrimidine ring protons at one-half maximum peak height (Figure 1). $1/T_2$ is an average of the relaxation rates of the bound and free nuclei, each term being weighed by the appropriate mole fraction.[13] In this study it was assumed that the longitudinal relaxation time, T_1, is equal to T_2; therefore, the measurements are reported as $1/T_2$. Human serum albumin (Behring, Marburg/Lahn, Germany) was used as the receptor in concentrations between 0 and 40 mg/l.

Acid Dissociation Constants (pK_a). The pK_a values of the acid NH atom of the sulfonamidopyrimidines (Table I) were determined spectrophotometrically according to the method outlined by Albert and Sergeant[28] and Yoshioka, *et al.*,[29] and are listed in Table I.

R_m Values. R_m values were determined according to Biagi, *et al.*,[30] by reversed thin-layer chromatography on paraffin oil coated (5% v/v) silica gel GF_{254} plates. The compounds were dissolved in acetone and 5 μl of solution was spotted on the plates. An aqueous mobile phase was used (phosphate buffer, pH 7.4) in various proportions with acetone. The experimental R_m values were calculated by regression analysis as the R_m with buffer alone as the mobile phase (Tables I–III).

Binding to Serum Albumin. Binding to serum albumin (Behring, Marburg/Lahn, Germany) was studied by equilibrium dialysis using a three-chamber dialysis cell. Concentration of free drug was determined by uv spectrometry after equilibrium was obtained. Concentration of albumin was 4 g/100 ml of 0.2 M phosphate buffer, pH 7.4, + 0.15 M NaCl (temperature 20°). Listed is the per cent free drug (Table I). The drug concentration used was ≈0.2 mM.

Determination of Partition Coefficients. Partition coefficients were determined in the system octanol–phosphate buffer (0.01 M, pH 7.2). Usually 20-ml portions of the solvents were used. The octanol phase was saturated with carbon dioxide free water and the water phase was saturated with octanol before partitioning was performed. The mixtures were shaken on a mechanical shaker for 2 hr after which time the water layer was drawn off and centrifuged at 3000 rpm for 30 min. Drug concentration in the water

phase was determined before (usually ≈1 mg/10 ml) and after partitioning in the water phase by uv spectrophotometry against the appropriate blank. The given partition coefficient, P (Table I), is corrected for the ionized fraction at pH 7.2.

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Homologous *N*-Alkylnorketobemidones. Correlation of Receptor Binding with Analgesic Potency

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For a homologous series of *N*-alkylnorketobemidones a statistically significant correlation was found between the relative abilities to bind mouse brain homogenate *in vitro* and their *in vivo* mouse hot-plate analgesic potencies. The correlation between *in vitro* binding in the presence of 100 m*M* sodium and analgesic potency was not as good as that found in the absence of sodium. A statistically significant correlation was also found between their analgesic potencies and their abilities to antagonize electrically induced contractions of the guinea pig ileum.

It has been demonstrated previously that the analgesic potencies of a wide range of narcotic analgesics generally parallel their binding affinities to a stereospecific opiate receptor in brain homogenates.[1] Some notable exceptions are etorphine,[1] meperidine,[2] and codeine.[1] The first two compounds probably deviate because their high lipid solubilities[3] allow very rapid penetration into the brain. Codeine may first require metabolic O-demethylation before it is active.[1] A statistical correlation between *in vitro* binding and *in vivo* analgesic or antagonist potency has not been reported heretofore. One difficulty which can be foreseen is that of possible metabolic and/or distribution differences between the various classes of analgesics (*vide supra*). However, if such a correlation were achieved within some series of analgesics in which differences due to metabolism and/or distribution were minimized, it would provide additional evidence that events occurring at the *in vitro* receptor were in fact related to *in vivo* analgesic or antagonist activity. Herein we report the correlation between *in vitro* binding and *in vivo* analgesic potencies of a homologous series of analgesics all of which appear to have similar profiles of metabolism and distribution.

Results and Discussion

The preparation and analgesic potencies of the *N*-alkylnorketobemidones (1–7) have been reported.[4] We have extended this series through the decyl homolog (see Experimental Section) and the analgesic data in mice are presented in Table I. Binding assays were performed using mouse brain homogenate in the presence and absence of 100 m*M* sodium as described previously[2] and the concentration of drug necessary to displace one-half of the stereospecific