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The Role of Anionic, Imidic, and Amidic Forms in Structure–Activity Relationships. Correlation of Electronic Indices and Bacteriostatic Activity in Sulfonamides

Augusto Rastelli,* Pier G. De Benedetti, Giovanna Gavioli Battistuzzi,

Istituto di Chimica Fisica dell'Università di Modena, 41100 Modena, Via Campi 183, Italy

and Albano Albasini

Istituto di Chimica Farmaceutica e Tossicologica dell'Università di Modena, 41100 Modena, P. le S. Eufemia 19, Italy.
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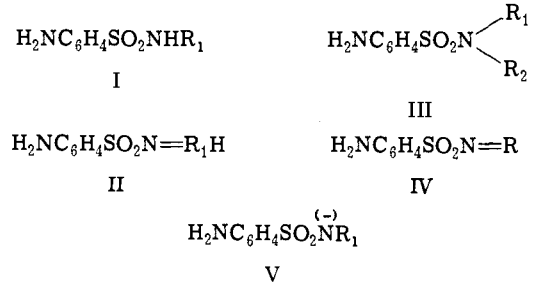
The problem of structure–activity relationships in sulfonamide type compounds is tackled on the ground that both bacteriostatic activities and structural indices must be referred to the specific individual forms assumed by sulfa drugs in the active solutions. The frequency value of the symmetric stretching mode of the sulfonyl group ν_s (SO_2) is chosen as a suitable electronic index and measured for the individual active forms in aqueous and Me_2SO solutions. The *linear* correlation that exists between bacteriostatic parameter and vibration frequency (over the complete range of data at present available) proves a strict relationship between electronic structure and bacteriostatic activity in this class of drugs. Furthermore, it justifies the assumption used for the calculation of the bacteriostatic activity of the anionic form; i.e., in equilibrium with a very active species (the anion) a less active species (the neutral form) gives a negligible contribution or does not contribute at all to the total activity. The results can be summarized as follows: *the lower the frequency of the symmetric stretching mode of the SO_2 group of any active species of sulfonamide type compounds, the higher its bacteriostatic activity.* The existence of a clear structure–activity correlation demonstrates that the whole class of compounds, whatever their form, has a single mechanism of action, while incontrovertible deviations from the general trend indicate differences or complications in the mechanism itself, but does not demonstrate that the group on which the structural index is localized plays a dominant role in the biological process. The usefulness of pK_a and NH_2 proton chemical shift of precursor amine as indirect indices of the electronic structure of the anionic forms is explored on extensive sets of available data.

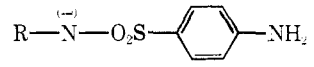
Sulfa drugs antagonized by *p*-aminobenzoic acid (PABA) contain the common moiety *p*-$NH_2C_6H_4SO_2$ which can be considered the minimal structural condition for their bacteriostatic action; the substituents bonded to the SO_2 group therefore serve only to determine the actual values of bacteriostatic activity. In *p*-aminobenzenesulfonamides there is a substituted NH_2 group attached to the SO_2 group; according to the number and type of substituents on this NH_2 group one can distinguish the following structural situations I–V.



The isomeric forms I (amide) and II (imide), which can coexist in aqueous solution, have acid properties and give the common anion V in alkaline solution. These structures (I, II, and V) have markedly different electronic properties, as is demonstrated by their spectroscopic behavior[1–4] which can in turn be used as a tool for analytical purposes. It shows, for example, that in aqueous solutions 2-*p*-aminophenylsulfonamidopyridine (sulfapyridine) and -thiazole (sulfathiazole) assume forms II and V, their ratio depending on the pH of the solution; N^1-phenylsulfanilamide and 2-*p*-aminophenylsulfonamidopyrimidine (1) (sulfapyrimidine), on the other hand, assume forms I and V. *p*-Aminobenzenesulfonylguanidine (sulfaguanidine) has been assigned structure IV by comparison with some ring *N*-methyl derivatives[4] which must necessarily have that structure, whereas structure III is obviously assumed by all the N^1-methyl or N^1-acetyl derivatives of sulfa drugs pertaining to forms I or II.

Although these structures have long been the subject of research,[5–8] their role in structure–activity relationships does not appear to have been fully understood. In fact, even if the anion structure V is commonly claimed to be the most active, the neutral form I has usually been preferred for measuring[9–15] or calculating[16,17] the structural indices. If it is important that the index chosen as representative of electronic structure be related to realistic events (energetically) in which the drug may participate and that, when localized on a particular position or functional group on a molecule, it should reflect a chemical event actually taking

Table I. Anionic Forms. Activity Parameters and Stretching Frequencies of the Symmetric Mode of the Sulfonyl Group



No.	R	pK_a	1/MIC[a]		1/MIC$_V$[b]		ν_s (SO_2)[h] Aqueous solutions Raman, cm^{-1}	Aqueous solutions Ir, cm^{-1}	Me_2SO Ir, cm^{-1}
1	H	10.43[c]	7.81	−03[d]	19.53		1114	1112	1115
2	4-Methoxyphenyl	9.34[e]	2.90	−02[e]	6.37		1117	1115	
3	Phenyl	8.97[e]	6.25	−02[e]	5.90		1118	1116	1121
4	2-Pyridinyl	8.56[f]	1.64	−01[d]	6.12		1120	1119	1125
5	3-Pyridinyl	7.89[c]	5.00	−01[c]	4.38		1119	1118	
6	2-(4-Methylthiazolyl)	7.79[c]	5.00	−01[c]	3.58		1123	1123	1128
7	2-(4,6-Dimethylpyrimidinyl)	7.51[g]	5.62	−01[d]	2.38		1118	1117, 1130	1116 (1118), 1133
8	2-Thiazolyl	7.23[f]	6.67	−01[d]	1.80		1122	1123	1127
9	6-(3-Methoxypyridazinyl)	7.17[f]	1.00[d]		2.47		1120	1121	1128
10	2-(6-Methylpyrimidinyl)	6.85[f]	1.07[d]		1.84		1116, 1125	1123	1128
11	2-Pyrimidinyl	6.37[f]	9.80	−01[d]	1.21		1125	1123	1129
12	2-(3-Methoxypyrazinyl)	6.1[e]	6.29	−01[d]	7.08	−01	1124	1123	1128
13	6-(2,4-Dimethoxypyrimidinyl)	5.98[f]	1.30[d]		1.42		1125	1124	1130
14	$CONH_2$	5.42[c]	3.12	−02[d]	3.21	−02	1120	1119, 1137	1114 (1120), 1137 (1136)
15	$COCH_3$	5.38[c]	3.03	−01[d]	3.10	−01	1129	1130	1130
16	2-(5-Methyl-1,3,4-thiadiazolyl)	5.22[f]	5.00	−01[c]	5.08	−01	1129	1126	
17	1,2,4-Triazolyl	4.66[c]	1.25	−03[c]	1.26	−03	1132	1129	1129

[a]Experimental antibacterial activity against *E. coli*; values are given in exponential form. [b]Calculated activity of the anionic form. [c]Reference 19. [d]Reference 20. [e]Reference 12. [f]M. Yoshioka, K. Hamamoto, and T. Kubota, *Yakugaku Zasshi*, 84, 90 (1964). [g]A. V. Willi and W. Meier, *Helv. Chim. Acta*, 39, 54 (1956). [h]In parentheses, Raman values; underlined, the frequency value of the higher intensity peak (see text); missing values in the last column are due to unavailable pure sodium salts.

place at that position[18] or involving that functional group, then it is all the more essential that it be related to the structural forms actually operating in the biological process in question. It does not seem that the last condition has ever been satisfied in previous attempts. The aim of the present paper is to try a structure–activity relationship with the use of an electronic index fulfilling the above-mentioned requirement and to discuss the success of some previous correlations.

Experimental Section

Raman measurements were recorded on a Cary 81 laser-equipped instrument; ir spectra were done on a Perkin-Elmer 257 spectrophotometer.

Pure sulfonamides were dissolved in dilute NaOH solutions; for each compound a sufficiently high pH was chosen so as to guarantee complete dissociation; this condition satisfied, no changes were registered due to pH variation. The concentrations of the samples were between 3×10^{-1} and 1×10^{-2} mol/l.; in the limit of experimental accuracy (~1 cm^{-1}) the band positions, as well as the intensity ratios of the two peaks in **7**, **10**, and **14**, were not affected by 1:5 dilution.

Sodium salts previously prepared and purified[25] and pure amide and imide compounds were used for recording the spectra in Me_2SO (C. Erba, RS for spectrophotometry).

Bacteriostatic Activity of Individual Forms. The in vitro biological activity that can be expressed as the inverse of the minimum concentration [1/MIC (μmol/l.)$^{-1}$] inhibiting totally or in part the growth of a fixed amount of a certain bacterial strain is defined irrespective of the actual structure(s) taken from the drug in the active solution. When only one form is present it is responsible for the entire activity, and on it the structural indices have to be measured or calculated. When, on the other hand, two or more forms coexist in solution, each of them can be supposed to contribute to the activity of the solution, and one must try to separate the different contributions so as to correlate them with the indices of the corresponding structures. From the data reported by Shepherd et al.,[5] it is possible, in a few cases, to obtain the order of magnitude of the activity ratios in the different structures of a sulfa drug; in fact, according to their findings, N^1-methylsulfapyridine (structure III) is 128 times less active than sulfapyridine (~97% of II and ~3% of V at pH 7), and ring *N*-methylsulfapyridine (IV) is about four times less active than sulfapyridine. If we assume that the bacteriostatic activity in these compounds depends *only* on the electronic structure of the *p*-$NH_2C_6H_4SO_2$ group, since in this part of the molecule the N^1-methyl derivative III can be considered electronically equivalent to the form I and the ring *N*-methyl derivative equivalent to the form II, we are left with only three distinct electronic situations (I ≃ III, II ≃ IV, and V) the activities of which are in the ratios: sulfapyridine, activity I–activity II–activity V = 1:32:3200. For sulfathiazole the corresponding values are activity I–activity II–activity V = 1:64:2500. From these results it follows that even a very small fraction of form V in solution makes the contributions of other forms negligible. Within the limitations of the sundry approximations included in the above analysis, one can calculate the bacteriostatic activities of the anions from the available experimental results.[19–21] Unfortunately, MIC values from a single source were not available; this could introduce additional errors due to the use of different bacterial strains and culture media. However, for those compounds measured by different authors,[19–21] the MIC values were consistent within the experimental errors.

Stretching Frequencies of the SO_2 Group in Amide, Imide, and Anion Forms. Extensive investigations into the assignment of vibrational spectra of *p*-aminobenzenesulfonamides have been carried out by Brandmüller and Wahl[13,22] both in the solid state and in solution. All 11 compounds were examined in their neutral forms; three of them were shown to be in their imide form in the solid state.[2] Other infrared measurements on neutral forms were performed for assay methods of drug analysis[23,24] for the study of amide–imide tautomerism[3,7,8] and for correlation with in vitro activity,[9,10,13] use being made in the latter case of the stretching frequencies of the sulfonyl group and of the aromatic amino group. As far as we know, no extensive study has ever been performed on the anionic forms.

A number of factors prevent assignments from being confidently and accurately given. For example, the relative insolubility of these compounds leads to their being studied in the solid state or in solution of polar solvents, so that the overall picture is obscured by association effects. Furthermore, the SO_2 stretching frequency

bands, mainly the asymmetric band, usually have a multiple structure with subbands on the sides of the main peaks; while this is an aid to identification of the band systems it renders the assignment of the main peak uncertain. However, identification of the bands corresponding to the symmetric stretching modes is generally easy for all three forms; this is why we have chosen this frequency as a suitable index for characterizing the electronic structure of the *p*-$NH_2C_6H_4SO_2$ moiety. Aqueous solutions would be preferable, for then the products would be in the medium where the measured bacteriostatic activity is displayed, but, unfortunately, only the anionic forms reach sufficiently high concentrations in aqueous solutions. Dimethyl sulfoxide (Me_2SO) was chosen because it can dissolve sufficiently high amounts of all the compounds and has a spectral window in the range of interest. Both solvents are highly polar media and also can give specific associations with the solutes which can be so strong to prevent intermolecular associations. This should explain why the frequency values depend on the solvent and do not depend on the concentration. The use of these frequency values as comparative electronic indices of the *free* molecules is justified only on the basis that, in the same solvent, solvation effects in a series of compounds are related to the electronic structure of the free molecule.

Results and Discussion

The bacteriostatic activities for the anionic forms, calculated according to the assumption that the contributions of neutral forms are negligible, are set out in Table I, together with the stretching frequencies (Raman and ir) of the symmetric modes of the SO_2 group [ν_s (SO_2)] of 17 sulfa drugs. The intense band assigned to the symmetric stretching mode of the sulfonyl group often exhibited a shoulder or even a resolved peak near the main peak; in a few cases the two peaks had similar intensities and depolarization ratios, which render the choice of the characteristic frequency uncertain. Attention should, however, be drawn to certain points. (a) Either in the Raman or in the ir spectrum a single peak corresponded, within experimental error, with one of the two peaks observed by the other technique (compounds **7** and **10** in aqueous solutions and compound **7** in Me_2SO solution). In these cases the frequency of the peak occurring both in the Raman and in the ir spectrum, or the average of Raman and ir values, can be taken as the characteristic frequency. (b) The Raman spectrum of *p*-aminobenzenesulfonylurea (sulfanylurea) (**14**) exhibited a very strong peak at about 1120 cm^{-1} and a shoulder at a higher frequency, whereas in the ir spectrum the intensity ratio was inverted; this allows for an alternative choice of the frequency value, and, consequently, as we shall see later, it introduces ambiguity into the classification of this substance as a "biologically anomalous compound".

Frequency and pK_a appear to be correlated to some extent in that decreased pK_a corresponds to increased frequency. It follows that pK_a can be taken as an indirect index of the electronic structure of the anion; a number of inversions in the correlation cannot be explained by invoking only uncertainties of measurement, but also recalling that pK_a refers to a thermodynamic equilibrium between the neutral and the anionic form, and so can be representative of the anion properties only if the neutral form plays the same role in all the series. This cannot be the case for the compounds in question which form quite a heterogeneous series in this respect; moreover, some of the compounds, in aqueous solutions, are known to be in their neutral imide forms, the electronic structures of which can differ markedly from those of the amide forms taken by other compounds. Sulfanylurea does not conform with the correlation, the lower frequency agreeing with a much higher pK_a and the higher frequency suggesting a much lower pK_a than the actual value.

According to reasonings well known in organic chemistry, the stretching frequency of the sulfone group is directly related to π_{S-O} bond order and inversely related to the formal negative charge on sulfonyl oxygens. It follows that a lower stretching frequency, i.e., a higher pK_a, is related to a higher polarity of the SO_2 group, in full agreement with the hypothesis of Bell and Roblin.[19] The bacteriostatic activity calculated for the anionic forms, and reported in the fifth column of Table I, appears to be related to the symmetric stretching frequency of the sulfonyl group, a higher activity corresponding to a lower frequency. Recalling the inverse relation between frequency and S–O bond polarity, the statement by Bell and Roblin[19] concerning the direct relationship between S–O bond polarity and bacteriostatic activity of the anionic form is proven to be tenable.

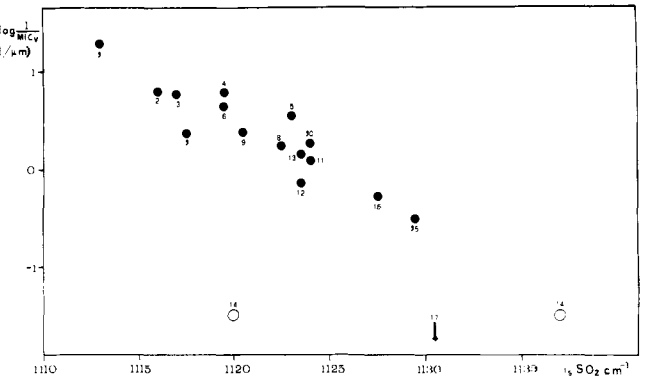
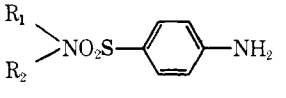


Figure 1. Structure–activity correlation for anionic forms. Activity parameters are plotted as log $1/MIC_V$ where MIC_V is the minimum inhibitory concentration calculated for the anionic form V, expressed in μmol/l. (Table I). Frequency values are the averages of Raman and ir data for aqueous solutions (Table I). Empty circlets refer to sulfanylurea discussed in the text. The point corresponding to **17** lies outside the drawing.

A plot of anion activity (log $1/MIC_V$) vs. ν_s (SO_2) measured in aqueous solution is given in Figure 1, where, in spite of some scatter justified by experimental measurement uncertainties, a good *linear* relationship appears. 4-*p*-Aminophenylsulfonamido-1,2,4-triazole (sulfatriazole) (**17**) clearly deviates from the correlation; sulfonylurea (**14**) activity is well accounted for by the higher frequency (1137 cm^{-1}), while its lower frequency value (1120 cm^{-1}) marks it as an exception in the series of anions.

In an attempt to extend the range of variability of electronic structure and bacteriostatic activity, three imide forms and three amide forms were considered; activity and frequency data in Me_2SO solution are reported in Table II.

Table II. Imide and Amide Forms. Bacteriostatic Parameters and Stretching Frequencies of the Symmetric Mode of the Sulfonyl Group



No.	R_1	R_2	$1/MIC^a$	ν_s (SO_2), Me_2SO, cm^{-1}
18	2-(*N*-Methylthiazolyl)		4.17 –02[b]	1140
19	2-(*N*-Methylpyridinyl)		4.10 –02[b]	1136
20	$C(NH_2)_2$		1.56 –02[c]	1135
21	CH_3	CH_3	3.33 –03[d]	1154
22	CH_3	2-Pyridinyl	1.28 –03[b]	1152
23	CH_3	2-Thiazolyl	6.51 –04[b]	1159

[a] Experimental antibacterial activity against *E. coli;* values are given in exponential form. [b] Calculated from the activity ratios of ref 5 and from MIC values of the parent compounds reported in Table I. [c] Reference 20. [d] Reference 19.

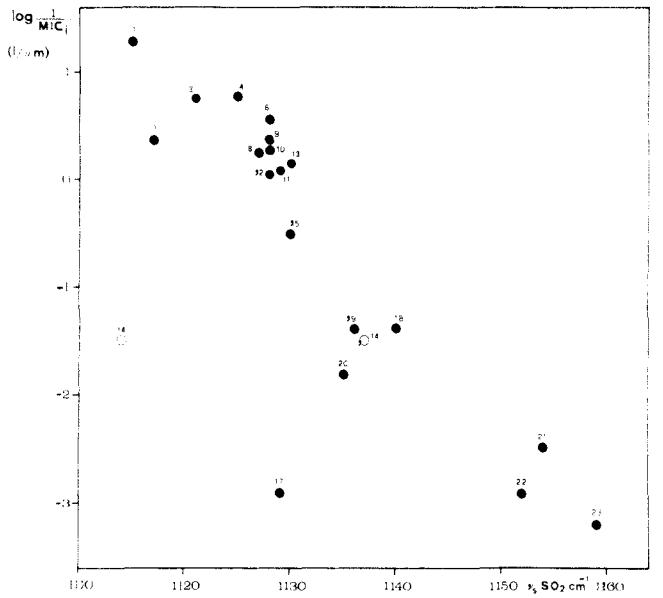


Figure 2. Structure–activity correlation for anionic, imide, and amide forms. Frequency values from ir spectra in Me_2SO solutions. Activity parameters plotted as log $1/MIC_i$ where i = V (anions), i = IV (imides), and i = III (amides). Empty circlets refer to sulfanylurea discussed in the text.

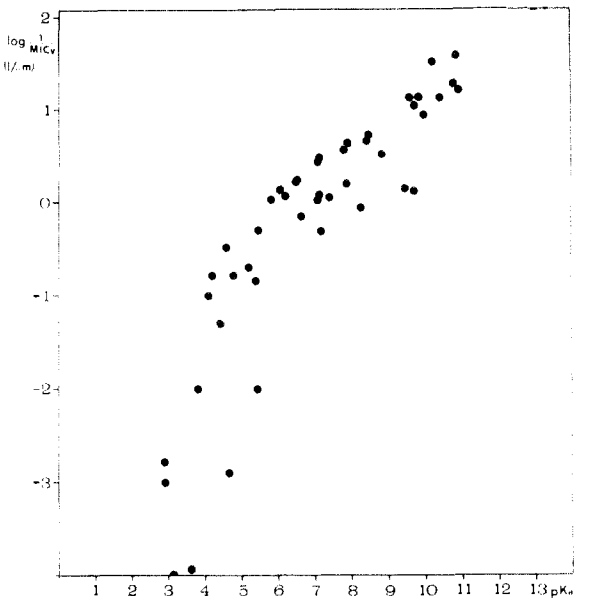


Figure 3. Correlation between the acid pK_a and the activity parameter for the anionic forms. All the data are from Bell and Roblin.[19] Marked deviations from the linear trend correspond to marked deviations from the original bell-shaped trend.

In Figure 2 the bacteriostatic activities (log $1/MIC_i$) of all the compounds of Tables I and II, anionic (i = V), imide (i = IV), and amide (i = III) forms, are plotted against ν_s (SO_2) measured in Me_2SO solutions. The very good correlation obtained is the first unequivocal proof of a strict relationship between electronic structure and bacteriostatic activity in this class of drugs, because both the electronic index and the bacteriostatic activity are defined on the specific electronic structures of the active species; furthermore, it justifies neglecting the contribution of neutral forms to overall activity in calculating the bacteriostatic activities of the anionic forms. Actually, the results are also consistent with the assumption that, in equilibrium with a very active species (the anionic), a less active species (the neutral form) does not contribute at all to total activity. This could be substantiated by the finding that the sulfanylamide anion seems to be well correlated, whereas, overcoming the unfavorable ratio of the activities of the two forms, the very large percentage of the neutral form should give a nonnegligible contribution to the overall activity. Unfortunately, the accuracy of the data (pK_a, MIC, frequency values) is not such as to enable one to adopt a clear-cut attitude toward this problem.

The results of the present study can be summarized as follows. *The lower the frequency of the symmetric stretching mode of the SO_2 group of any active species of sulfonamido type compounds, the higher its bacteriostatic activity.* The relationship of the stretching frequency with SO bond polarity and with pK_a accounts for the basic premises of Bell and Roblin's interpretation of the well-known bell-shaped trend of the activity–pK_a relationship. A reverse relationship obtained by Brandmüller and Wahl[13] between the same structural indices and the bacteriostatic activity does not depend on the accuracy of measurement but on a different approach to the problem.

As for the exceptions, sulfatriazole (**17**) activity clearly does not correlate, while in the case of sulfanylurea doubt remains as to the value of its characteristic frequency; further study seems to be necessary in order to establish whether they can be classified as biologically "anomalous" compounds.[19]

Apart from its possible practical use for drug-design purposes, the existence of a clear structure–activity correlation demonstrates that the whole class of compounds has a common mechanism of action, while marked deviations from the general trend indicate eventual differences or complications in the mechanism itself. In our opinion, it is not possible, simply on the basis of structure–activity correlations, to assign the dominant role in the mode of action to one group (e.g., SO_2) or the other (e.g., NH_2). So the success of the correlation of bacteriostatic activity with the stretching frequency of the symmetric mode of the sulfonyl group does not demonstrate that this group plays the dominant role in the biological mechanism of action. The choice of the experimental index ν_s (SO_2), mainly localized on a part of the common moiety ($H_2NC_2H_4SO_2$), does lead to a relationship over the complete range of available data, but, in principle, other indices mainly localized on the common moiety should offer the same advantages, for they should reflect the behavior of the common part as a whole.

Successful correlations are reported where use was made of indices neither localized on the common part nor explicitly defined on the individual forms. Bell and Roblin[19] and Seydel[21,26] obtained bell-shaped trends by plotting in vitro bacteriostatic activities of a large number of sulfonamides against their pK_a's and the NH_2 proton chemical shifts of their precursor anilines, respectively. Whereas Bell and Roblin's correlation suffered from the limitation that only compounds showing acidic properties could be included, Seydel found that substituted anilines and substituted 3-aminopyridines gave rise to distinct correlations. Although this is only to be expected, it does upset the practical use of the correlations and confuses the issue of the single mechanism of action. According to the present analysis and approximations, the bacteriostatic activities of all the correlated compounds could be assigned to the anionic forms, thus neglecting the contributions of the corresponding neutral forms. The bacteriostatic activities of the anions (log $1/MIC_V$) were calculated from the experimental MIC and pK_a values of ref 19, 21, and 26 and plotted against pK_a (Figure 3) and proton chemical shift (Figure 4). Figure 3 shows a clear correlation between anion activity and pK_a, thus contributing to the view that pK_a is, in general, a good

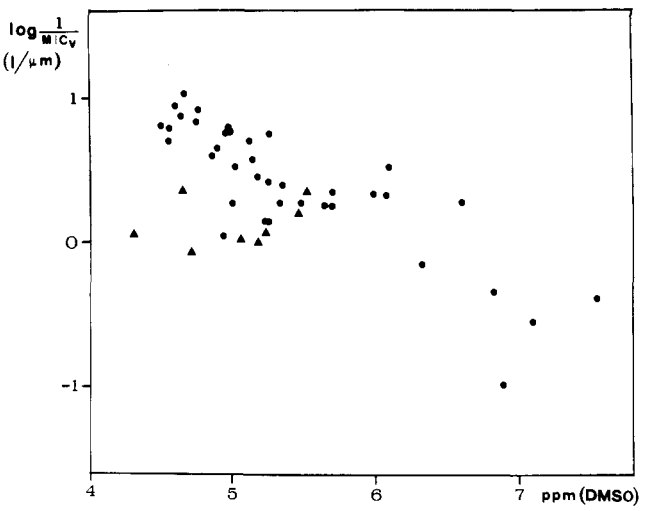


Figure 4. Correlation between the NH_2 proton chemical shift of precursor amine and the activity parameter for the anionic forms. All the data are from Seydel et al.;[21,26] when different values are reported for the same compound, the more recent values[26] have been chosen. ●, substituted N^1-phenylsulfonamides; ▲, substituted N^1-3-pyridinylsulfonamides.

index for the electronic structure of anions: a relatively small number of exceptions could possibly be explained as being due either to inadequacy of pK_a as a structural index in the specific cases or to differences or complications in the mode of action. Even the NH_2 proton chemical shift of precursor amine proves to be a good index of electronic structure of the anions (Figure 4), but, in this case, aniline and 3-aminopyridine derivatives still undergo distinct trends; because of the strict homology between the compounds entering the correlations, no clear deviation occurs over the normal dispersions.

It follows from the above discussion that, although pK_a and NH_2 proton chemical shift of precursor amine do not satisfy the above-mentioned conditions for the optimal choice of structural indices, they can be used as indirect indices of electronic structure for the anionic form; equally, they could be assumed to be representative of the electronic structure for the neutral forms, although, obviously, they cannot be used for correlations extended from the anion to the neutral forms.

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