Articles

Quantitative Structure-Activity Analysis in Dihydropteroate Synthase Inhibition by Sulfones. Comparison with Sulfanilamides

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Received December 23, 1985

A set of 25 4'-, eight 2',4'-, and five 2',4',6'-substituted 4-aminodiphenyl sulfones were tested for their inhibitory activity on dihydropteroate synthase of *Escherichia coli*. Linear regression analysis shows that enzymic inhibition indices correlate well with both quantum chemical and spectroscopic descriptors of the electronic structure of the common moiety 4-NH_2-C_6H_4-SO_2 of the sulfones (the above descriptors being expressed in relation to the electronic structure of the enzyme substrate, *p*-aminobenzoate). Therefore, the biological activity of the sulfones can be related to the electronic structural resemblance between these inhibitors and the substrate of the target enzyme. Since a similar result was previously obtained for a wide series of sulfanilamides in their different (amidic, imidic, and anionic) forms, it appears possible to consider the antibacterial sulfones and sulfanilamides as a congeneric chemical series. On the basis of the present results, the classical theory of antimetabolites would appear to take on a quantitative and sound rationale.

The diaryl sulfone derivatives (SU), such as 4,4'-diaminodiphenyl sulfone, represent an important class of antibacterial,[1] antimalarial,[2] and antileprotic[3] agents. SU, like sulfanilamides (SA),[4] exert their biological action by inhibiting,[5] competitively with respect to the substrate *p*-aminobenzoate (PAB), the enzyme dihydropteroate synthase (DHPS), which catalyzes the formation of dihydropteroate from PAB and (hydroxymethyl)dihydropteridine pyrophosphate. SA and SU can also react with the dihydropteridine substrate to form dihydropteroate analogues.[6-8]

Previous theoretical research,[9] concerning 14 different 4'-substituted SU, has suggested that SU and SA can be considered as a congeneric chemical series, on the basis of both the electronic properties of the 4-NH_2-C_6H_4-SO_2 common moiety, which are modulated by the substituents via intramolecular interaction,[10] and the activity of these compounds on bacterial growth.[11]

In the present research the inhibitory effect of 25 4'-, eight 2',4'-, and five 2',4',6'-substituted SU on the enzymic activity of DHPS of *Escherichia coli* has been studied. The results have been correlated with the electronic structure of the common moiety of the inhibitors, expressed in terms of spectroscopic data (empirical descriptors) and of quantum chemical indices that give the electronic total net charges on the atoms and groups of the common moiety of the SU molecules (theoretical descriptors). Furthermore, the correlations obtained have been compared among themselves and with similar relationships previously evaluated for the SA.[12,13]

Results and Discussion

Table I reports the measured ap_E values, giving the inhibitory effect of the 38 SU studied on the activity of DHPS, together with the determined values of empirical and theoretical descriptors. Literature data on growth inhibition indices[11] and standard compilation of the Hammett's substituent constants[14] are also given, when available. Proton chemical shift values and net charges of the single atoms or groups of the moiety common to SU and the substrate PAB are expressed as differences with respect to the corresponding values for PAB.

Table I also reports the values of $\delta(NH_2)$ and of the net charges of the single atoms or groups of PAB;[12] therefore, it is possible to calculate the values of $\delta(NH_2)$ and of the net charges for the SU. Some qualitative considerations about the influence of the nature of the substituent groups on the values of the electronic charges of the different atoms or groups of the SU common moiety enable the following conclusions: (a) Electron-donor substituents increase the electron population on all atoms or groups, except for the C(2)-H and C(6)-H groups. The opposite holds for electron-acceptor substituents. (b) The ranges of variation of the electronic charges are small, the greatest

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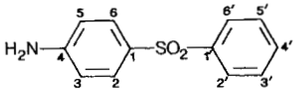
0022-2623/87/1830-0459$01.50/0

Table I. Activity Parameters and Physicochemical and Theoretical Indices of Sulfones[a]



no.	substituents	EII_{50}[b]	ap_E[c] obsd	ap_E[d] calcd	log (1/MIC)[e]	σ_p[f]	$\nu_S(SO_2)$,[g] cm^{-1}	$\Delta\delta(NH_2)$, ppm	$\Delta q(NH_2)$	$\Delta q(C^1)$	$\Delta q(CH^{2,6})$	$\Delta q(CH^{3,5})$	$\Delta q(C^4)$	$\Delta q(SO_2)$	$\Delta q(O)$
1	4′-NO_2	45.28 (±7.54)	−1.66	−1.98	3.36	0.78	1150.0	1.20	66.5	−16.6	28.9	66.5	39.9	652.7	128.5
2	4′-$CON(C_2H_5)_2$	49.06 (±7.54)	−1.69	−1.48			1148.0	1.12	64.3	−12.6	27.6	63.1	40.3	645.6	124.3
3	4′-CN	67.30 (±14.16)	−1.83	−1.49		0.66	1149.0	1.21	63.8	−17.5	28.1	64.5	38.6	645.8	124.5
4	4′-$COOCH_3$	33.96 (±11.32)	−1.53	−1.54		0.45	1150.0	1.17	63.6	−17.9	28.3	64.2	38.4	645.9	124.9
5	4′-COOH	5.09 (±1.51)	−0.71	−1.43		0.45	1150.0	1.15	63.2	−17.8	28.2	64.1	38.3	644.6	124.0
6	4′-$COCH_3$	43.40 (±7.54)	−1.64	−1.39		0.50	1150.0	1.14	63.0	−17.8	28.2	63.9	38.3	644.0	123.7
7	4′-Br	11.07 (±1.60)	−1.04		3.59	0.23	1147.0	1.08							
8	4′-Cl	20.75 (±5.66)	−1.32	−1.30	3.92	0.23	1149.0	1.07	62.9	−17.7	27.7	63.8	38.2	644.3	122.9
9	4′-$CONH_2$	22.60 (±3.78)	−1.35	−1.38		0.36	1151.5	1.12	62.5	−18.5	27.8	63.4	37.9	643.9	123.6
10	4′-F	18.11 (±4.53)	−1.26	−1.22	3.50	0.06	1147.5	1.04	62.1	−17.8	27.6	63.6	38.0	643.9	122.3
11	4′-H	9.56 (±1.52)	−0.98	−1.14		0.00	1147.0	1.02	61.6	−18.2	27.7	63.0	39.9	641.0	121.6
12	4′-OCH_3	6.60 (±1.15)	−0.82	−0.91	4.02	−0.27	1146.5	0.95	61.1	−18.1	27.3	62.5	37.3	639.0	119.7
13	4′-OH	2.45 (±0.32)	−0.39	−0.87		−0.37	1144.0	0.91	61.1	−18.2	27.3	62.4	37.3	638.5	119.4
14	2′-NH_2	2.27 (±0.65)	−0.35	−0.64	4.35		1136.0	0.85	61.0	−21.3	25.7	62.2	37.0	641.8	117.5
15	4′-NHCHO	5.79 (±0.85)	−0.76	−0.91	4.74	0.00	1148.5	0.98	60.8	−18.6	27.2	62.2	37.0	637.7	119.7
16	4′-CH_3	6.04 (±0.94)	−0.78	−0.89		−0.17	1147.5	1.03	60.6	−18.6	27.2	62.1	37.0	637.5	119.5
17	3′-NH_2	6.04 (±1.50)	−0.78	−0.83	4.62		1142.0	0.96	60.0	−19.2	27.4	61.6	36.8	641.6	119.0
18	4′-$NHCOCH_3$	5.75 (±2.27)	−0.76	−0.74		0.00	1148.5	0.98	59.7	−19.0	27.2	61.8	36.5	635.1	118.3
19	4′-$N(CH_3)_2$	3.63(±1.33)	−0.56	−0.52		−0.83	1143.5	0.86	59.1	−19.1	26.3	61.0	36.2	632.7	116.5
20	4′-NHOH	4.53 (±1.36)	−0.65	−0.54	4.46	−0.34	1145.0	0.88	58.9	−19.4	26.1	60.8	36.1	632.7	116.6
21	4′-NH_2	2.64 (±0.76)	−0.42	−0.46	5.09	−0.66	1144.0	0.88	58.8	−19.3	26.2	60.7	36.0	631.8	116.0
22	4′-$N(C_2H_5)_2$	2.64 (±0.50)	−0.42	−0.45			1143.0	0.88	58.8	−19.3	26.1	60.6	36.0	631.1	115.9
23	4′-NHC_2H_5	4.53 (±0.76)	−0.65	−0.46	4.26		1144.0	0.85	58.8	−19.2	26.3	60.9	36.0	631.7	116.0
24	4′-COONa	5.09[h] (±1.51)	−0.71[h]	1.29		0.00	1144.0	0.99	42.3	−22.4	26.4	51.0	29.3	605.9	101.5
25	4′-ONa	1.50[h] (±0.21)	−0.17[h]	3.14	5.96	−0.81	1137.5	0.66	32.9	−24.9	23.1	44.3	24.3	575.1	86.2
26	2′,4′-$(NO_2)_2$	36.31 (±13.72)	−1.56	−3.22			1151.0	1.38	67.8	−6.7	42.4	66.3	40.6	669.0	138.8
27	2′,4′-$(Cl)_2$	2.24 (±0.60)	−0.35	−1.96			1152.0	1.16	63.5	−16.6	29.6	64.1	38.4	656.7	128.4
28	2′,4′-$(CH_3)_2$	1.89 (±0.54)	−0.28	−0.45			1140.0	1.01	60.7	−20.8	26.7	61.9	37.0	628.6	115.9
29	2′,4′-$(OCH_3)_2$	3.21 (±1.13)	−0.51	−0.69			1140.0	0.89	60.6	−16.6	28.3	62.2	37.0	640.8	117.9
30	2′,4′-$(OH)_2$	0.71 (±0.17)	0.15	−0.70			1139.5	0.81	60.0	−17.2	28.7	61.3	36.6	643.5	118.0
31	2′,4′-$(NH_2)_2$	1.59 (±0.20)	−0.20	−0.02			1131.5	0.78	58.3	−22.2	24.3	60.1	35.5	632.7	112.3
32	2′-ONa-4′-OH	0.51[h] (±0.14)	0.29[h]	1.58			1131.0		23.8	−26.2	36.6	36.3	18.9	621.4	99.1
33	2′,4′-$(ONa)_2$	0.005[h] (±0.002)	2.20[h]	5.47			1128.0	0.41	−1.2	−32.6	3.6	16.5	5.0	551.3	67.0
34	2′,4′,6′-$(Cl)_3$	3.31 (±1.18)	−0.52	−2.62			1163.0	1.23	64.0	−15.3	31.4	64.6	38.7	668.8	133.8
35	2′,4′,6′-$(CH_3)_3$	1.69 (±0.55)	−0.23	−0.03			1147.0	0.96	60.5	−22.2	26.0	61.6	37.1	620.2	112.4
36	2′,4′,6′-$(OCH_3)_3$	12.88 (±4.57)	−1.11	−0.52			1128.0	0.72	60.3	−15.0	29.4	61.8	36.7	643.3	116.5
37	2′,4′,6′-$(OH)_3$	1.91 (±0.34)	−0.28	−0.55			1122.5	0.90	58.9	−16.2	30.2	60.2	36.0	649.4	116.7
38	2′-ONa-4′,6′-$(OH)_2$	1.46[h] (±0.27)	−0.16[h]	1.65			1117.0	0.59	22.8	−25.4	29.8	35.1	18.3	629.2	98.5
4-aminobenzoate (PAB)								$\delta(NH_2)$	$q(NH_2)$	$q(C^1)$	$q(CH^{2,6})$	$q(CH^{3,5})$	$q(C^4)$	$q(CO_2^-)$	$q(O)$
								5.13	−62.4	−3.8	5.1	−110.5	146.4	−869.0	−667.5

[a] The values of 4-NH_2 proton chemical shifts [$\delta(NH_2)$] and the theoretical indices ($\times 10^3$) are expressed as differential (Δ) values relative to PAB. Differences of total net charges have been calculated for the 4-NH_2 group, the C(1) atom, the equivalent C(2)-H or C(6)-H groups, the equivalent C(3)-H or C(5)-H groups, the C(4) atom, the SO_2 group with respect to the COO^- group of PAB, the single O atom of the SO_2 group with respect to the single O atom of the COO^- group of PAB. At the bottom of the table, the values of $\delta(NH_2)$ and total net charges of PAB (ref 12) are reported. [b] Enzyme inhibition index values, measured on DHPS of *E. coli* (see the Experimental Section). Standard deviations are given in parentheses. [c] $ap_E = \log (1/EII_{50})$. [d] Calculated by eq 4. [e] MIC = minimal inhibitory molar concentration values, measured on *Mycobacterium* sp. 607 (ref 11). [f] Hammett substituent constants. [g] Symmetric stretching frequency values of the SO_2 group. [h] These values are calculated from the activities measured for the corresponding acidic forms (see the Experimental Section).

Table II. General Correlation Matrix between the Indices Given in Table I

	ap_E	log (1/MIC)	σ_P	ν_S-(SO_2)	$\Delta\delta$ (NH_2)	Δq-(NH_2)	Δq-(C^1)	Δq-($CH^{2,6}$)	Δq-($CH^{3,5}$)	Δq-(C^4)	Δq-(SO_2)	Δq-(O)
ap_E	1	0.847 (12)[a]	−0.849 (18)	−0.500 (33)	−0.671 (32)	−0.706 (28)	−0.574 (28)	−0.415 (28)	−0.703 (28)	−0.715 (28)	−0.477 (28)	−0.577 (28)
log (1/MIC)		1	−0.858 (9)	−0.648 (12)	−0.861 (12)	−0.792 (10)	−0.652 (10)	−0.677 (10)	−0.829 (10)	−0.821 (10)	−0.751 (10)	−0.793 (10)
σ_P			1	0.849 (20)	0.948 (20)	0.903 (17)	0.800 (17)	0.922 (17)	0.908 (17)	0.773 (17)	0.906 (17)	0.935 (17)
$\nu_S(SO_2)$				1	0.804 (37)	0.602 (32)	0.217 (32)	0.228 (32)	0.663 (32)	0.586 (32)	0.355 (32)	0.662 (32)
$\Delta\delta(NH_2)$					1	0.897 (32)	0.548 (32)	0.600 (32)	0.887 (32)	0.834 (32)	0.672 (32)	0.887 (32)
$\Delta q(NH_2)$						1	0.676 (32)	0.664 (32)	0.971 (32)	0.929 (32)	0.755 (32)	0.910 (32)
$\Delta q(C^1)$							1	0.888 (32)	0.572 (32)	0.648 (32)	0.779 (32)	0.762 (32)
$\Delta q(CH^{2,6})$								1	0.589 (32)	0.568 (32)	0.778 (32)	0.777 (32)
$\Delta q(CH^{3,5})$									1	0.888 (32)	0.728 (32)	0.895 (32)
$\Delta q(C^4)$										1	0.662 (32)	0.831 (32)
$\Delta q(SO_2)$											1	0.902 (32)
Δq(O)												1

[a] Numbers in parentheses represent the data points considered in each case.

variations being found on the oxygen atoms of the SO_2 group and on the 4-NH_2 group. (c) The variation of the spectroscopic indices of the different SU shows a trend similar to that of the corresponding electronic charges.

On the other hand, the use of difference values for the theoretical data furnishes a simple, direct indication of the resemblance between the inhibitors SU and the substrate PAB: the lower the absolute value of the differences, the higher will be the electronic structural resemblance and, correspondingly, the higher will be the inhibitory effects observed on the DHPS activity. The only exception, represented by the Δq(C1) values, can be explained as due to the peculiar position of the C(1) atom in bridged 4′-substituted diaryl systems, where a reverse substituent effect is observed;[10,15] this situation is present in the SU, but of course not in PAB.

Linear regression analysis allows a quantitative expression of the qualitative conclusions reported above: the general correlation matrix of Table II furnishes the values of the correlation coefficients between all the theoretical and experimental parameters reported in Table I for the SU studied and the number of data points considered in each case. The bromine derivative **7** was not considered in the correlations involving theoretical indices, since the parameterization is not available. In the correlations among the theoretical indices and between these and the spectroscopic indices, the anionic compounds **24**, **25**, **32**, **33**, and **38** have been omitted; deviation from linearity in these cases was previously interpreted[10] as due to an overestimation of the computed electronic charge transfer from the formally negative substituent to the rest of the molecule. In the correlations between the theoretical indices and the ap_E values, the acidic compounds **5**, **13**, **30**, and **37** have been omitted also; these compounds ionize at pH 8.2, which is the pH of the enzymic inhibition measurements, giving rise to anionic forms, with different electronic properties (see the Experimental Section). Likewise, in the correlations between the spectroscopic indices and the ap_E values, the acidic compounds **5**, **13**, **30**, and **37** must be omitted, but the corresponding monoanionic compounds **24**, **25**, **32**, and **38** have been considered. In these cases, the $\nu_S(SO_2)$ and $\Delta\delta(NH_2)$ values are those measured for the salts and the ap_E values are those calculated for the monoanionic forms; to the latter the entire inhibitory activity is ascribed[13] because of the well-known higher activity of the anionic forms of acidic SA[16-18] and of the SU bearing ionizable substituents[9,19] with respect to that of the corresponding acidic ones (see the Experimental Section). The activity of the bianionic compound **33** has also been calculated, but this compound cannot be considered because it is present at a very low concentration at pH 8.2.

Considering the correlations among the theoretical indices, it can be noted that, although the correlations involving the carbon atoms of the ring are scarcely significant in some cases, good correlations are observed among the indices of the groups that appear to be the most important for the biological effects of the SU as the 4-NH_2, the SO_2, and the O atoms of the SO_2.

It is worth stressing that a good linear correlation holds between $\Delta q(NH_2)$ and Δq(O), which refer to groups at the extremes of the common moiety of the SU, as is shown by the following equation:

$$\Delta q(NH_2) = 0.364\ (\pm 0.050)\Delta q(O) + 17.54\ (\pm 6.13) \qquad (1)$$

$$n = 32,\ r = 0.910,\ s = 0.960,\ F = 144.09$$

where n represents the number of SU considered, r is the correlation coefficient, s is the standard deviation from the regression, F is the significance Fisher test, and the values in parentheses give the 95% confidence intervals.

This result seems to disagree with Koch and Moffitt's analysis[20] of the conjugation in aromatics sulfones: according to this analysis, symmetry considerations of case

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II type SU (no coplanarity of phenyl rings) suggest that mesomeric interaction between the conjugated π-system and the sulfur group should not affect the S=O bonds. Very recently, however, the substituent effects in SU have been interpreted[10] in terms of electronic charge perturbation, which is linearly transmitted from the substituent to the whole molecule, the bridging SO_2 group included, by means of a strong and linearly related $\sigma-\pi$ electron interaction (hyperconjugation) in the C(1)–SO_2–C(1′) part of the molecule.

The linear correlation between $\Delta q(NH_2)$ and $\Delta q(O)$ is paralleled by a similar relationship between the corresponding empirical parameters, $\Delta\delta(NH_2)$ and $\nu_S(SO_2)$, as shown by the following equation:

$$\Delta\delta(NH_2) = 0.017\ (\pm 0.003)\nu_S(SO_2) - 18.68\ (\pm 4.11) \quad (2)$$

$$n = 37,\ r = 0.804,\ s = 0.113,\ F = 63.79$$

In this correlation all the SU reported in Table I have been considered, with the sole exception of compound **32** because of the unavailability of the corresponding $\delta(NH_2)$ value (see the Experimental Section).

Also the Hammett's σ_p values of the 4′-substituted SU show linear relationships with theoretical and spectroscopic parameters (see Table II).

As far as the correlations between the theoretical and spectroscopic indices and the ap_E values are concerned, a more accurate analysis than that reported in Table II is in order. The following equations show the relationships between the most significant indices and the ap_E values:

$$ap_E = -0.209\ (\pm 0.042)\Delta q(NH_2) + 11.88\ (\pm 2.55) \quad (3)$$

$$n = 23,\ r = 0.879,\ s = 0.241,\ F = 71.41$$

$$ap_E = -0.121\ (\pm 0.016)\Delta q(O) + 13.57\ (\pm 2.19) \quad (4)$$

$$n = 23,\ r = 0.942,\ s = 0.169,\ F = 166.13$$

$$ap_E = -0.084\ (\pm 0.017)\nu_S(SO_2) + 95.58\ (\pm 19.51) \quad (5)$$

$$n = 28,\ r = 0.852,\ s = 0.286,\ F = 68.96$$

$$ap_E = -2.921\ (\pm 0.517)\Delta\delta(NH_2) + 2.00\ (\pm 0.52) \quad (6)$$

$$n = 27,\ r = 0.884,\ s = 0.236,\ F = 89.38$$

In addition to compounds that have not already been considered in the general matrix of Table II for the reasons reported in the discussion of this table, compounds **26**, **27**, and **34–36** have been also omitted in eq 3 and 4, and compounds **27**, **34–36**, and **38** have been omitted in eq 5 and 6, because of the large deviations from linearity. Compounds that exhibit deviations from linearity in eq 3–6 are the dichloro derivative **27** and all the trisubstituted derivatives. The deviations shown by the dinitro derivative **26**, which appear only in equations involving theoretical indices, could be explained by calculation uncertainties, which are frequently observed with similar derivatives.[21]

It is interesting to note that compounds **27**, **34**, and **35** are more inhibitory on DHPS than is predicted by the corresponding $\nu_S(SO_2)$ and $\Delta\delta(NH_2)$ values (see Table I), whereas an opposite behavior is shown by compounds **36** and **38**. With these compounds, the concurrence of factors different from the electronic structural ones (such as hydrophobic interaction with the enzymic site, in the first case, or steric interference by groups present in the ortho position with respect to the SO_2 group, in the second case) could be invoked. This point may warrant further investigation.

Values of ap_E measured in the present research correlate well with the published values of growth inhibition indices (log 1/MIC), as shown in Table II.

Lastly, eq 7 considers 28 SU (see eq 5 in the present research) and 20 SA (see eq 5 in ref 13) together:

$$ap_E = -0.049\ (\pm 0.009)\nu_S(SO_2) + 55.49\ (\pm 9.85) \quad (7)$$

$$n = 48,\ r = 0.810,\ s = 0.368,\ F = 87.92$$

where the symmetric stretching frequency is used as electronic index. For comparative purposes, eq 5 in ref 13 is reported here:

$$ap_E = -0.048\ (\pm 0.011)\nu_S(SO_2) + 53.44\ (\pm 12.12) \quad (8)$$

$$n = 20,\ r = 0.871,\ s = 0.350,\ F = 56.47$$

Equation 7 is the best one among the different linear regressions involving the molecular descriptors considered. The correlation appears acceptable for a unifying description of the more relevant aspects of the relationship between electronic properties and biological activities of SU and SA.

The present results are in full agreement with the original proposal of Bell and Roblin,[16] which, as well as considering the geometrical similarity between the SA inhibitors and the PAB molecule, postulated a relationship between the growth inhibition effects of different SA and the corresponding values of the negative charges on the oxygen atoms of the SO_2 group of these compounds (indirectly evaluated by their acidic dissociation constant values), the SO_2 group itself being considered as the analogue of the COO^- group of PAB. In the previous[13] and in the present research, Bell and Roblin's hypothesis has been verified not only for dissociable SA but also for nonacidic SA in their imidic and amidic forms and for a large series of different SU derivatives.

Thus, the classical theory of antimetabolites, which is based on their competitive inhibitory effect, appears to take on, at least in the case of SU and SA, a sound rationale by the evaluation of indices describing the extent of the resemblance of electronic structure of the inhibitor common moiety to the molecule of PAB. The present results could also provide useful information about the nature of the intermolecular forces operating between the inhibitors SU and SA and the active site of the enzyme DHPS.

The conclusion, previously[9] reached for the case of 14 4′-substituted SU and 30 amidic, imidic, and anionic SA, that SA and SU belong to one and the same congeneric chemical series on the basis of their theoretical properties can now be extended also to the 2′,4′-substituted SU. In particular, the electronic features of the common moiety 4-NH_2-C_6H_4-SO_2 of SU bearing neutral substituents are intermediate between those of the neutral imidic and amidic SA, whereas SU with substituents bearing a negative charge fall between the anionic and imidic SA. It seems useful to recall that PAB shows theoretical values of $q(NH_2)$ and $q(O)$ that place this compound at the extremity of the sequence: PAB, anionic SA, anionic SU, imidic SA, neutral SU, and amidic SA.[9]

The present results are in essential agreement with the recent ones by Bawden and Tute[22] and Coats et al.[19] on 4′-monosubstituted SU. According to the results reported by these authors, factors different from the electronic ones appear to give only minor contributions to the SU and SA activity or could be interpreted again as electronic factors in agreement with the present work.

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Experimental Section

Enzymic Inhibition Measurements. The enzyme preparation, containing *E. coli* dihydropteroate synthase (E.C. 2.5.1.15), was obtained essentially by the method of Richey and Brown,[23] as described previously.[13]

The enzyme substrate 2-amino-4-hydroxy-6-(hydroxymethyl)-7,8-dihydropteridine pyrophosphate (dihydropteridine-PP) was prepared essentially by the method of Ho et al.[24] After elution from charcoal with 3.5 N ammonium hydroxide and evaporation under reduced pressure of excess ammonia, the pteridine pyrophosphate derivative was precipitated with excess $BaCl_2$. The yellow-green precipitate was washed with water, suspended in a minimal volume of H_2O and treated with sulfate ions at pH ~7; after centrifugation, the $BaSO_4$ precipitate was washed with water. The combined supernatants were reduced with $Na_2S_2O_4$, and the final compound was recovered according to Viscontini and Furuta.[25]

4-Amino[7-^{14}C]benzoic acid (sp act. 53 μCi/mg) was purchased from the Radiochemical Centre, Amersham, U.K.

The enzymic formation of dihydropteroate was measured according to Richey and Brown.[23] Complete reaction mixtures contained in a final volume of 1 mL: 0.1 M Tris-HCl buffer, pH 8.6; 0.01 M $MgCl_2$; 0.05 M mercaptoethanol; 0.12 mM dihydropteridine-PP; 5 μM 4-amino[7-^{14}C]benzoic acid (16000 cpm); and different concentrations of SU, when present. The reaction was started by addition of 0.05 mL of the enzyme preparation and was stopped after a 20-min incubation at 37 °C by adding 1 mL of absolute ethanol and heating at 100 °C for 3 min. After cooling and centrifugation, chromatographic separation of unreacted [7-^{14}C]PAB was accomplished on the supernatant according to Richey and Brown,[23] and the radioactivity incorporated into synthesized dihydropteroate was measured with a Packard Tri-Carb 460-C scintillator. The values of the SU concentrations giving 50% inhibition of enzyme activity were calculated by interpolation from linear regressions of 1/dpm vs. SU concentrations. These values divided by PAB concentration give the enzyme inhibition indices, EII_{50}. These values are expressed as $ap_E = \log (1/EII_{50})$. Each reported ap_E value represents the mean of at least three independent measures.

The pH values of the different reaction mixtures (with or without the inhibitors) were measured in separate experiments and resulted to be 8.2 ± 0.1, throughout the duration of the experiment.

pK_a Measurements. The pK_a values of the acidic derivatives (compounds **5**, **13**, **30**, and **37**) were measured spectrophotometrically, with a Beckman DU8 spectrophotometer, at 25 ± 2 °C according to the method outlined by Albert and Serjant.[26] The measured values of pK_a are as follows: compound **5**, pK_a = 3.60; compound **13**, pK_a = 8.00; compound **30**, pK_{a1} = 7.67, pK_{a2} = 10.14; compound **37**, pK_{a1} = 7.69, pK_{a2} = 9.62.

From the pK_a values reported above and from the pH value (8.2) at which the determinations of the enzymic activity have been performed, it was possible to calculate for the acidic compounds (**5**, **13**, **30**, and **37**) the percentages of the corresponding monoanionic forms (**24**, **25**, **32**, and **38**) present in the solution at pH 8.2. These values are respectively: 99.9%, 61.3%, 77.2%, and 76.5%. The bianionic forms corresponding to the second dissociation of compounds **30** and **37** are present at the percentages of 1% and 2.9%, respectively. By ascribing the whole inhibitory effect of the acidic compounds to their monoanionic forms only,[9,12,13] the ap_E values measured for compounds **5**, **13**, **30**, and **37** were corrected to obtain the values assigned in Table I to the salts: **24**, **25**, **32**, and **38**. The activity of the bianionic compound **33** has also been calculated, but it must be considered that this compound is present at a very low concentration at pH 8.2.

Spectroscopic Measurements. Spectral data relative to SU compounds of Table I were measured in the present research or taken from the previous one.[10] NMR spectra were recorded at 25 °C for solutions in [$^2H_6$]Me_2SO with a Varian Associates XL-200 spectrometer. Sample concentrations were in the range 0.1–0.01 M. The 4-NH_2 group proton signals were reproducible to less than 0.02 ppm and showed no concentration dependence in the range 0.2–0.0125 M. It was not possible to assign the $\delta(NH_2)$ value of compound **32**. IR spectra were recorded for solutions in Me_2SO with a Perkin-Elmer 180 spectrometer. The concentration of the samples was between 0.3 and 0.01 M; within the limits of experimental accuracy (±0.5 cm^{-1}) the band positions were not affected by 1:5 dilution.

Calculations. LCAO–MO results were obtained by using the CNDO/2 approximation and parameterization already applied to the monosubstituted SU in the previous research.[10] Standard geometries were used for the substituents, whereas for the common moiety 4-NH_2-C_6H_4-SO_2 a constant geometry was assumed for all the derivatives of Table I. A modified version of program QCPE 141 was used. The calculations were performed at the Centro di Calcolo Elettronico of the University of Modena.

For compound **7** (bromine derivative), the parameterization is not available.

When the substituents bear negative charges, the comparison between the computed charges and the corresponding spectroscopic values indicate that the electron population of the atoms of the common moiety results exceedingly high (except for the C(2)-H and C(6)-H groups) with respect to the case of neutral substituents. This fact is probably due to an overestimation of the computed electronic charge transfer from the formally negative substituent to the rest of the molecule.[10]

Chemistry. Melting points were determined on Büchi apparatus and are uncorrected. Microanalyses were within ±0.4% of the theoretical values.

Commercial bis(4-aminophenyl) sulfone (**21**) from Ega Chemie was purified by crystallization from water. Compounds **1–13**,[10] **14**,[27] **15**,[28] **16**,[10] **17**,[29] **18–20**,[10] **22** and **23**,[10] **26**,[30] **29**,[31] **31**,[32] **37**[33] were prepared according to literature methods.

The 4-aminophenyl 2,4-dihydroxyphenyl sulfone (**30**) (mp 134–136 °C (lit.[33] mp 134–136 °C)) was prepared from the amino derivative **29** as previously reported[33] for compound **13**.

Compounds **25**, **32**, **33**, and **38** were obtained from the corresponding hydroxyphenyl sulfones by a calculated amount of NaOH.

2,4,6-Trichlorothiophenol. Granulated tin (36.6 g) and 2,4,6-trichlorobenzenesulfonyl chloride[34] (27.8 g, 100 mmol) were added in small portions and simultaneously to a stirred solution of 37% HCl (55 mL, 662 mmol), while the temperature was maintained below 55 °C.

Additional HCl (18 mL, 216 mmol) was added, and the reaction mixture, after stirring for 4 h, was cooled and extracted three times with ether. The extracts were washed with water and evaporated. 2,4,6-Trichlorothiophenol was isolated by steam distillation and extracted from the distillate with ether. After evaporation of the solvent, 18 g (85%) of 2,4,6-trichlorothiophenol was obtained; mp 62–63 °C (lit.[35] mp 57–60 °C).

2,4,6-Trimethylthiophenol. In a similar manner 2,4,6-trimethylbenzenesulfonyl chloride[36] afforded 83% of 2,4,6-trimethylthiophenol, bp 69–70 °C (1.3 mm), n^{20}_D 1.5692 (lit.[37] bp 79–82 °C (2 mm)).

2,4-Dichlorothiophenol. 2,4-Dichlorobenzenesulfonyl chloride[38] (45 g, 183 mmol) was added, in small portions, with vigorous

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Table III. Aryl 4-Nitrophenyl Sulfones (4-NO_2-C_6H_4-SO_2-C_6H_2-2′X,4′Y,6′Z)

X	Y	Z	method	time, h	yield,[a] %	mp, °C	recrystn solvent[b]	formula[c]
H	Cl	Cl	B	360	80	174–176	*d*	$C_{12}H_7Cl_2NO_4S$
H	CH_3	CH_3	A	4	84	109–111	E	$C_{14}H_{13}NO_4S$
Cl	Cl	Cl	B	360	82	190–191	*e*	$C_{12}H_6Cl_3NO_4S$
CH_3	CH_3	CH_3	A	8	80	155–157[f]	E–D	$C_{15}H_{15}NO_4S$
OCH_3	OCH_3	OCH_3	B	24	76	217–219[f]	*g*	$C_{15}H_{15}NO_7S$

[a] No attempt was made to maximize yield. [b] E = ethanol, D = dioxane. [c] Analyzed for C, H, N, and S; analytical results were within ±0.4% of theoretical values. [d,e] Crude products were recrystallized from ethyl acetate and acetonitrile, respectively, and were purified by chromatography on silica gel ($CHCl_3$ as eluant). [f] The corresponding sulfides were prepared according to the literature method.[41] [g] The crude product was recrystallized from ethyl acetate and was chromatographed on silica gel (CH_2Cl_2 as eluant).

Table IV. Aryl 4-Aminophenyl Sulfones (4-NH_2-C_6H_4-SO_2-C_6H_2-2′X,4′Y,6′Z)

no.	X	Y	Z	yield,[a] %	mp, °C	recrystn solvent[b]	formula[c]
27	H	Cl	Cl	60	146–147	B	$C_{12}H_9Cl_2NO_2S$
28	H	CH_3	CH_3	67	143–144	*d*	$C_{14}H_{15}NO_2S$
34	Cl	Cl	Cl	71	185–186	*e*	$C_{12}H_8Cl_3NO_2S$
35	CH_3	CH_3	CH_3	89	190–191	B	$C_{15}H_{17}NO_2S$
36	OCH_3	OCH_3	OCH_3	76	217–219	M	$C_{15}H_{17}NO_5S$

[a,c] See corresponding footnotes in Table III. [b] B = benzene, M = methanol. [d,e] Crude products were recrystallized from benzene and were purified by chromatography on silica gel with CH_2Cl_2 and 30% $CHCl_3$ in CH_2Cl_2 as eluant, respectively.

stirring, to a −5 °C cooled mixture of 416 g of crushed ice and 40 mL (751 mmol) of concentrated sulfuric acid. Then 69 g of zinc dust was added in small portions as rapidly as possible without the temperature rising above 0 °C. The reaction mixture was stirred for 2 h and was slowly allowed to reach room temperature. After stirring for 2 h, the mixture was slowly heated and refluxed for 5 h. 2,4-Dichlorothiophenol was isolated by steam distillation and extracted with ether. The ether layer was washed with water, dried ($MgSO_4$), and evaporated. The crude product was purified by distillation to give 29 g (88%) of the desired thiol, bp 88 °C (3.2 mm), n^{20}_D 1.6202 (lit.[39] bp 108–110 °C (10 mm)).

4-Nitrophenyl 2,4-Dimethylphenyl Sulfide. To a solution of sodium (3.3 g) in absolute ethanol (90 mL) was added 2,4-dimethylthiophenol[40] (18 g, 130 mmol) followed by 20.5 g (130 mmol) of 4-nitrochlorobenzene dissolved in 90 mL of absolute ethanol. The mixture was refluxed for 3 h, and after the mixture was allowed to stand overnight at room temperature, the precipitate obtained was collected and washed with ethanol and water and crystallized from hexane to yield 23 g (68%) of sulfide, mp 73–74 °C. Anal. ($C_{14}H_{13}NO_2S$) C, H, N, S.

4-Nitrophenyl 2,4-Dichlorophenyl Sulfide. In a similar manner, 17.9 g (100 mmol) of 2,4-dichlorothiophenol afforded 17 g (58%) of the title compound, mp 89–91 °C (from hexane). Anal. ($C_{12}H_7Cl_2NO_2S$) C, H, N, S.

4-Nitrophenyl 2,4,6-Trichlorophenyl Sulfide. In a similar manner, 21.3 g (100 mmol) of 2,4,6-trichlorothiophenol afforded 16.7 g (50%) of the title compound, mp 126–128 °C (from ethanol/dioxane, 3:1). Anal. ($C_{12}H_6Cl_3NO_2S$) C, H, N, S.

Synthesis of Aryl 4-Nitrophenyl Sulfones (Table III). The sulfones were prepared according to the following methods.

Method A. A solution of the sulfide (20 mmol) in acetic acid was heated at 100 °C and hydrogen peroxide (30% v/v, 50 mmol) was added dropwise. After the mixture was allowed to stand overnight at room temperature, the precipitate obtained was collected, washed with ethanol, and crystallized.

Method B. To a stirred solution of the sulfide (20 mmol) in chloroform (80 mL) was added slowly *m*-chloroperbenzoic acid (85%, 50 mmol) in 150 mL of chloroform at room temperature. The *m*-chlorobenzoic acid and unchanged peroxy acid were removed by washing with dilute alkali and dilute aqueous sodium sulfite. Removal of the solvent gave the crude product, which was purified by crystallization and chromatography.

Synthesis of Aryl 4-Aminophenyl Sulfones (27, 28, 34–36) (Table IV). The amino derivatives were prepared by catalytic hydrogenation (RaNi catalyst, 1 atm, room temperature) of the corresponding nitro compounds. When the calculated amount of H_2 had been absorbed, the catalyst was removed by filtration. Crude products obtained after removal of methanol were purified by recrystallization or chromatography on silica gel.

Registry No. **1**, 1948-92-1; **2**, 101533-58-8; **3**, 101533-57-7; **4**, 34037-45-1; **5**, 46948-43-0; **6**, 100866-99-7; **7**, 6626-22-8; **8**, 7146-68-1; **9**, 24454-46-4; **10**, 312-35-6; **11**, 7019-01-4; **12**, 17078-72-7; **13**, 25963-47-7; **14**, 27147-69-9; **15**, 21501-24-6; **16**, 4094-38-6; **17**, 34262-32-3; **18**, 565-20-8; **19**, 86552-09-2; **20**, 32695-27-5; **21**, 80-08-0; **22**, 51688-32-5; **23**, 3572-34-7; **24**, 101533-59-9; **25**, 101533-60-2; **26**, 75333-79-8; **27**, 105456-57-3; **28**, 105456-58-4; **29**, 105456-59-5; **30**, 105456-60-8; **31**, 35880-91-2; **32**, 105456-61-9; **33**, 105456-62-0; **34**, 105456-63-1; **35**, 33597-78-3; **36**, 105456-64-2; **37**, 105456-65-3; **38**, 105456-66-4; DHPS, 9055-61-2; 2,4,6-trichlorobenzenesulfonyl chloride, 51527-73-2; 2,4,6-trichlorothiophenol, 24207-66-7; 2,4,6-trimethylbenzenesulfonyl chloride, 773-64-8; 2,4,6-trimethylthiophenol, 1541-10-2; 2,4-dichlorobenzenesulfonyl chloride, 16271-33-3; 2,4-dichlorothiophenol, 1122-41-4; 2,4-dimethylthiophenol, 13616-82-5; 4-nitrochlorobenzene, 100-00-5; 4-nitrophenyl 2′,4′-dimethylphenyl sulfide, 105456-67-5; 4-nitrophenyl 2′,4′-dichlorophenyl sulfide, 42583-60-8; 4-nitrophenyl 2′,4′,6′-trichlorophenyl sulfide, 105456-68-6; 4-nitrophenyl 2′,4′-dichlorophenyl sulfone, 105456-69-7; 4-nitrophenyl 2′,4′-dimethylphenyl sulfone, 105456-70-0; 4-nitrophenyl 2′,4′,6′-trichlorophenyl sulfone, 105456-71-1; 4-nitrophenyl 2′,4′,6′-trimethylphenyl sulfone, 105456-72-2; 4-nitrophenyl 2′,4′,6′-trimethoxyphenyl sulfone, 105456-73-3; 4-nitrophenyl 2′,4′,6′-trimethylphenyl sulfide, 77189-94-7; 4-nitrophenyl 2′,4′,6′-trimethoxyphenyl sulfide, 77189-95-8.

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