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Determination of Lipophilicity and Hydrogen-bond Donor Acidity of Bioactive Sulphonyl-containing Compounds by Reversed-phase HPLC and Centrifugal Partition Chromatography and their Application to Structure-activity Relations

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Abstract—The lipophilic character of two large series of substituted benzenesulphonamides (BzSA) and 4-aminodiphenylsulphones (4-ADS) has been assessed by two chromatographic methods, i.e. reversed-phase HPLC using a relatively novel octadecylpolyvinyl packing and centrifugal counter-current chromatography (CPC). The octadecylpolyvinyl stationary phase proved an interesting alternative to the more common octadecylsilane type stationary phase for obtaining retention parameters correlated to partition coefficients (i.e. log P). The CPC method, being far less time-consuming and markedly more precise than the classical shake-flask method, offers a promising alternative for measuring partition coefficients. The parameter $\Delta\log P_{oct\text{-}hep}$, i.e. $\log P_{octanol}$ minus $\log P_{heptane}$, was also determined for both congeneric series and was indicative of a similar H-bonding capacity for the SO_2NH_2 and $4\text{-}NH_2\text{-}C_6H_4\text{-}SO_2$ groups. QSAR analyses of carbonic anhydrase inhibition by BzSA and antimycobacterial activity of 4-ADS show the capacity of the new lipophilicity parameters to express the hydrophobic component of the drug-enzyme interactions and to reveal a possible role of H-bond donor capacity in governing the antimycobacterial activity of 4-ADS.

Lipophilicity is a molecular property influencing the pharmacokinetic and pharmacodynamic behaviour of many classes of drugs (Topliss 1983; Hansch et al 1987). Among the different lipophilicity descriptors, partition coefficients are particularly significant. They are traditionally obtained by the so-called shake-flask technique, generally using 1-octanol and water as a biphasic liquid system ($\log P_{oct}$) (Leo et al 1971; Martin 1978).

From an experimental viewpoint the shake-flask technique has several drawbacks (Dearden & Bresnen 1988), and chromatography in reversed-phase high-performance liquid chromatography (RP-HPLC) has been proposed as an alternative means of obtaining descriptors of lipophilicity (Braumann 1986; Terada 1986; van de Waterbeemd & Testa 1987). Alkylsilane-bonded phases, in particular octadecylsilane (ODS), are the non-polar stationary phases most frequently used to determine isocratic capacity factors which, when extrapolated to 100% water eluent, yield $\log k_w$ as a lipophilicity index. However, compounds with strong hydrogen-bond accepting groups, and most sulphonyl-containing compounds, present $\log k_w$ values determined on ODS phases that are much larger than expected from their $\log P_{oct}$ values (El Tayar et al 1988). This effect is due to silanophilic interactions and is not completely eliminated by masking agents (Altomare et al 1989).

In order to avoid silanophilic interactions, a new octadecylpolyvinyl copolymeric (ODP) stationary phase has been recently proposed and made commercially available as a promising alternative to the ODS phase for lipophilicity measurements by RP-HPLC (Bechalany et al 1989). Even more recently, centrifugal partition chromatography (CPC) also called centrifugal counter-current chromatography has revealed several marked advantages over other lipophilicity measurement techniques (Terada et al 1987a, b; El Tayar et al 1989; Vallat et al 1990). CPC is a liquid–liquid chromatographic method in which two non-miscible solvents are used as the stationary and mobile phases (Berthod & Armstrong 1988a, b). The method directly yields partition coefficients (and not capacity factors) in a variety of solvent systems with significantly greater precision and considerable time saving compared with the shake-flask method.

Partition coefficients are important parameters in drug design not only because they allow empirical correlations with biological data, but because they encode a wealth of structural information (Pearlman 1986; Yang et al 1986; Dunn et al 1987). Studies aimed at unfolding this information have shown that lipophilicity can be factorized into two terms, one representing bulk or steric properties, while the other is related to electrostatic and polar properties (van de Waterbeemd & Testa 1987; van de Waterbeemd et al 1989).

$$\text{lipophilicity} = \text{bulk} + \text{polarity} \qquad (1)$$

The cavity term (bulk) mainly accounts for hydrophobic and Van der Waals interactions and can be described by steric parameters such as molar volume or molar refractivity. In contrast, the expression of polarity terms is more complex and related to dipolarity-polarizability, H-bond donor acidity and H-bond acceptor basicity (Kamlet et al 1983, 1988; Taft et al 1985a, b). Also relevant is the $\Delta\log P$ parameter

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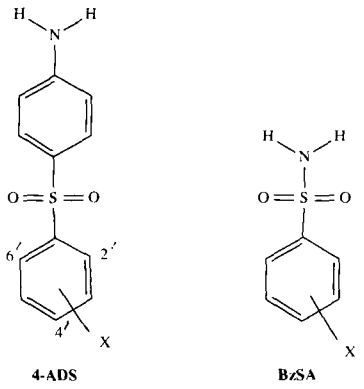

FIG. 1. Structures of the investigated compounds.

earlier developed by Seiler (1974) as a measure of hydrogen-bonding ability. Some of us have recently analysed the physicochemical information content of $\Delta\log P_{oct\text{-}hep}$ (i.e. log $P_{octanol}$ minus log $P_{heptane}$) and found it to express the ability of solutes to donate hydrogen-bonds (El Tayar et al 1991a). This property has been shown to influence brain penetration (Young et al 1988) and skin permeation (El Tayar et al 1991b). In addition, the H-bond donor acidity has been recently postulated to play an important role in the interactions with membranes and enzymes and in the recognition process responsible for the natural selection of molecules for their biological roles (Meot-Ner 1988).

The objectives of this study were to examine the advantages in RP-HPLC of the ODP stationary phase, as well as to demonstrate the superiority of the CPC over the shake-flask method in the experimental measurement of lipophilicity. For this purpose two congeneric series of sulphonyl-containing drugs were selected, namely *meta*- and *para*-substituted benzenesulphonamides (BzSA) as inhibitors of carbonic anhydrase (Altomare et al 1989; Carotti et al 1989), and 4-aminodiphenylsulphones (4-ADS) as inhibitors of dihydropteroate synthase (De Benedetti 1987; De Benedetti et al 1987, 1989) (Fig. 1). In addition, a possible role of H-bond donor acidity (as revealed by $\Delta\log P_{oct\text{-}hep}$) in determining enzyme inhibition potency was explored.

Materials and Methods

Substituted benzenesulphonamides (BzSA) and 4-aminodiphenylsulphones (4-ADS) were synthesized as already reported (De Benedetti et al 1987; Carotti et al 1989). Their identity and purity were checked by ^{1}H-NMR, IR, elemental analysis and HPLC. Methanol, 1-octanol and sodium 2-(*N*-morpholino)ethanesulphonate (MES) were obtained from Merck (Darmstadt, Germany), and were of analytical grade.

RP-HPLC measurements

All the experiments were carried out on a Siemens S101 chromatograph equipped with an Orlita DMP-AE 10-4 pump. The detector was a Uvikon 740 LC (Kontron) operating at 254 nm. A Spectraphysics 4100 computing integrator was used for peak registration and calculation of retention times (precision 0·01 min).

A μ-Bondapak C_{18} (10 μm) column (150 × 3·9 mm i.d.) and an Asahipak ODP-50 (5 μm) column (150 × 6·0 mm i.d.) were used as the ODS and ODP stationary phases, respectively. The mobile phases consisted of different volume fractions of methanol in water buffered to pH 5·50 with MES. The pK_a of BzSA and 4-ADS are such that the compounds exist practically exclusively in their neutral form at this pH value. Solutions of 0·1–1·0 mg mL^{-1} of the compounds were prepared using the eluent as solvent. The flow rate (1·00 ± 0·01 mL min^{-1}) was strictly controlled using a digital flowmeter (Phase Separations Ltd, Queensferry, UK) and the retention data were collected at a temperature of 30·0 ± 0·2°C (thermostated column and eluent reservoir). The methanol signal provided the value of the column dead time. The logarithm of the isocratic capacity factors (log k_i) was determined for at least five different methanol—water mixtures. The extrapolation to 100% water eluent yielded log k_w values reported in Table 1.

Shake-flask determination of partition coefficients

As a set of reference measurements of lipophilicity, partition coefficients in the 1-octanol–water solvent system (log P_{oct}) were determined by the classical shake-flask method. Log P_{oct} values for BzSA were taken from Carotti et al (1989), whereas the partition coefficients of 4-ADS were determined for the present study. At equilibrium, concentrations were measured in the aqueous phase by UV after centrifugation. The pH values of phosphate buffer 0·01 M were selected to ensure that the percent of ionized form was always < 1% (pH = 5·50 for the hydroxy derivatives 59, 60, 61; pH = 7·40 for the other congeners).

Centrifugal partition chromatography

Measurements were performed at room temperature (25 ± 1°C) using an Ito Multi-Layer Coil Separator-Extractor (P. C. Inc., Potomac, MD, USA) equipped with a preparative coil #10 (i.d. 2·6 mm, total capacity 370 mL) (Ito 1988). The rotation speed of the rotor was about 1000 rev min^{-1}. To measure partition coefficients within the range of log P values between −3 and 3, the volume ratio between stationary and mobile phases and the flow rate (0·5–8·0 mL min^{-1}) were adjusted, depending on the solute lipophilicity, to obtain reasonable retention times (< 1 h). The retention time of potassium dichromate or anthracene was taken as column dead time (t_0) when water or organic solvent (1-octanol or n-heptane), respectively, was used as the mobile phase.

Other instrumental details have been reported (Vallat et al 1990), whereas the experimental conditions (buffers, pH of aqueous phase) are as those reported for the shake-flask measurements (Carotti et al 1989). The log P value of each compound was determined at least three times, the relative standard deviations always being < 2·0%.

Results and Discussion

Relationships between RP-HPLC lipophilicity descriptors (ODS phase) and logP_{oct} values

In agreement with previous results for BzSA (Altomare et al 1989), the capacity factors (log k_i) of 4-ADS increased linearly with decreasing methanol concentration in the eluent when using the ODS stationary phase ($r^2 > 0·98$), allowing linear extrapolations to log k_w[ODS] values (Table 1).

Table 1. Lipophilic indices determined by various chromatographic methods.

No.	Substituent(s)	log k_w[ODS][a]	log k_w[ODP][b]	log $P_{oct/SF}$[c]	log $P_{oct/CPC}$[d]	log $P_{hep/CPC}$	Δlog $P_{oct-hep}$
BzSA							
1	H	0·88	1·63	0·35	0·33	−2·54	2·89
2	4-CH_3	1·45	2·01	0·80	0·83	−2·42	3·22
3	3-CH_3	1·49	2·07	0·90	0·85	−2·35	3·25
4	4-Cl	1·55	2·39	1·10	—[e]	−2·27	3·37
5	3-Cl	1·55	2·40	1·20	—[e]	−2·21	3·41
6	4-Br	1·71	2·63	1·38	—[e]	−2·17	3·55
7	3-Br	1·70	2·65	1·39	—[e]	−2·04	3·43
8	4-I	1·97	2·84	1·59	1·77	−1·91	3·50
9	3-I	1·93	2·84	1·62	1·58	1·91	3·53
10	4-$CH(CH_3)_2$	2·33	2·85	1·75	1·96	−1·37	3·12
11	3-$CH(CH_3)_2$	2·39	2·84	1·70	1·96	−1·41	3·11
12	4-C_6H_5	2·89	3·67	2·28	2·60	−1·24	3·52
13	4-CN	1·09	1·90	0·22	0·40	−2·54	2·76
14	3-CN	1·09	1·83	0·28	0·27	−2·33	2·61
15	4-$COCH_3$	1·38	1·92	0·24	0·31	−2·74	2·98
16	3-$COCH_3$	1·41	1·88	0·25	0·23	−2·74	2·99
17	4-OCH_3	1·34	1·98	0·45	0·48	−2·71	3·16
18	3-OCH_3	1·45	2·09	0·57	0·65	−2·33	2·99
19	4-NO_2	1·19	2·34	0·75	0·72	−2·96	3·71
20	3-NO_2	1·19	2·31	0·56	0·60	−2·98	3·54
21	4-OC_4H_9	2·82	3·31	2·09	2·19	−1·12	3·21
22	3-OC_4H_9	2·81	3·37	2·10	2·09	−0·94	3·04
23	4-OC_6H_{13}	3·87	4·06	2·93	2·83	−0·41	3·34
24	4-SO_2NH_2	0·38	1·51	−0·96	−0·70	—[f]	>2·0
25	3-SO_2NH_2	0·57	1·54	−0·46	−0·56	—[f]	>2·5
26	4-$CONH_2$	0·59	1·06	−0·79	−0·66	—[f]	>2·2
27	3-$CONH_2$	0·74	1·11	−0·80	−0·55	—[f]	>2·2
28	4-NH_2	0·30	1·33	−0·62	−0·64	—[f]	>2·4
29	3-NH_2	0·46	1·40	−0·28	−0·38	—[f]	>2·7
30	4-$NHSO_2CH_3$	0·83	1·83	−0·36	−0·73	—[f]	>2·6
31	4-$NHCOCH_3$	1·17	1·91	0·00	−0·10	—[f]	>3·0
32	4-OH	0·55	1·52	−0·06	−0·06	—[f]	>3·0
4-ADS							
33	H	2·68	3·13	1·76	1·84	−0·97	2·73
34	4′-CH_3	2·89	3·37	2·40	—[e]	−0·49	2·89
35	2′,4′-$(CH_3)_2$	3·35	3·56	2·73	2·69	−0·63	3·36
36	2′,4′,6′-$(CH_3)_3$	3·65	3·89	3·06	—[e]	—[g]	—
37	4′-F	2·68	3·34	2·01	2·17	−0·76	2·77
38	4′-Cl	3·11	3·49	2·57	—[e]	−0·91	3·48
39	2′4′-$(Cl)_2$	3·43	3·95	3·12	—[e]	—[g]	—
40	4′-Br	3·29	3·69	2·85	3·15	−0·44	3·29
41	4′-CN	2·56	3·37	1·63	—[e]	−1·43	3·06
42	4′-$COCH_3$	2·83	3·21	1·67	—[e]	−1·34	3·01
43	4′-$COOCH_3$	3·21	3·66	2·25	2·13	−1·18	3·43
44	4′-OCH_3	2·87	3·43	1·96	1·99	−0·98	2·94
45	2′,4′-$(OCH_3)_2$	2·91	3·18	1·63	1·65	−1·50	3·13
46	2′,4′,6′-$(OCH_3)_3$	2·60	2·84	1·03	—[e]	−2·27	3·30
47	4′-NO_2	2·64	3·33	2·13	2·03	−1·00	3·13
48	2′,4′-$(NO_2)_2$	2·56	4·28	2·04	1·84	−1·22	3·26
49	4′-$CONH_2$	2·18	2·74	0·90	—[e]	—[f]	>4·0
50	4′-$CON(C_2H_5)_2$	3·16	3·26	1·44	—[e]	−2·03	3·47
51	2′-NH_2	2·37	3·06	1·56	1·75	−1·70	3·26
52	3′-NH_2	2·22	2·79	1·03	—[e]	—[f]	>4·0
53	4′-NH_2	2·05	2·76	0·94	—[e]	—[f]	>4·0
54	2′,4′-$(NH_2)_2$	1·77	2·48	0·38	0·38	—[f]	>3·4
55	4′-$N(CH_3)_2$	3·30	3·42	2·04	1·93	−1·19	3·23
56	4′-NHC_2H_5	2·97	3·41	1·98	—[e]	−1·38	3·36
57	4′-$N(C_2H_5)_2$	3·73	4·13	3·00	—[e]	—[g]	—
58	4′-NHOH	1·91	2·66	0·88	—[e]	−2·30	3·18
59	4′-OH	2·20	3·03	1·57	—[e]	—[f]	>4·6
60	2′.4′-$(OH)_2$	1·91	2·68	1·29	—[e]	—[f]	>4·3
61	2′,4′,6′-$(OH)_3$	1·73	3·18	1·53	1·75	—[f]	>4·5

[a] Log k_w[ODS] is the lipophilic index extrapolated linearly to 100% water using the ODS column. Data for BzSA derivatives (1–32) were taken from Altomare et al (1989). [b] Log k_w[ODP] is the lipophilic index extrapolated linearly to 100% water using the ODP column. [c] Log of partition coefficient in n-octanol–water system measured by the shake-flask method. Data for BzSA derivatives (1–32) were taken from Altomare et al (1989). [d] Log of partition coefficient in n-octanol–water system measured by CPC. [e] Not determined. [f] Not measurable; the lowest methodological limit of log P_{hep} is about −3·0. [g] Not measurable because of negligible solubility both in water and n-heptane.

By comparing log P_{oct} (shake-flask procedure) and log k_w[ODS] values for 4-ADS congeners, the following correlation equation was formulated:

$$\log k_w[\text{ODS}] = 0{\cdot}67(\pm 0{\cdot}16) \log P_{oct} + 1{\cdot}49(\pm 0{\cdot}32) \quad (2)$$
$$n = 29 \quad r = 0{\cdot}851 \quad s = 0{\cdot}301$$

where n is the number of data points, r is the correlation coefficient, s is the standard deviation of the regression and 95% confidence limits are given in parentheses.

The statistics of equation 2 are rather poor compared with the previously published correlation equation obtained for BzSA (Altomare et al 1989):

$$\log k_w[ODS] = 0{\cdot}79(\pm 0{\cdot}10) \log P_{oct} + 0{\cdot}92(\pm 0{\cdot}11) \quad (3)$$
$$n = 32 \quad r = 0{\cdot}950 \quad s = 0{\cdot}263$$

The discriminative power of the ODS phase, as revealed by the slopes in equations 2 and 3, is similar for the two sets of sulphonyl-containing compounds. Log k_w[ODS] values for 4-ADS are much higher than expected from their log P_{oct} values, and by comparing the intercepts in equations 2 and 3 it can be noted that a stronger retention on the ODS phase exaggerated the chromatographically measured lipophilicity of 4-ADS compared with BzSA. Since the hydrogen-bonding capacity of solutes is mainly responsible for their silanophilic interactions with the ODS phase, we believe that the different behaviour of the two sets of compounds can be attributed to the presence among 4-ADS congeners of a greater number of compounds bearing one or more hydrogen-bond donor and acceptor substituents. Interestingly, the combination of equations 2 and 3, linked through an indicator variable (I) which takes the value of 0 for BzSA and 1 for 4-ADS, gives rise to the following regression equation for the whole set of compounds:

$$\log k_w[ODS] =$$
$$0{\cdot}75(\pm 0{\cdot}08) \log P_{oct} + 0{\cdot}38(\pm 0{\cdot}17)I + 0{\cdot}95(\pm 0{\cdot}12) \quad (4)$$
$$n = 61 \quad r = 0{\cdot}956 \quad s = 0{\cdot}284$$

Equation 4 indicates a reasonably good correlation, and we note the high intercept value due to the strongly interacting sulphonyl moiety.

Relationships between RP-HPLC lipophilicity descriptors (ODP phase) and log P_{oct} values

The ODP stationary phase (Arai et al 1987; Yasukawa et al 1987) is claimed to offer some advantages over the ODS phase, e.g. expanded applicability with efficient separation of basic substances, stability over a wide pH range, and absence of silanophilic interactions. From data in Table 1 it can be seen that log k_w[ODP] values are always greater than log k_w[ODS] values. This difference could be related to problems of mass transfer, since the more dense structure and the smaller particle size (5 μm) of the ODP phase render it more compact than the ODS phase. However, despite the longer retention times, 40 compounds could be investigated with eluents containing less than 40% methanol and for more than 10 compounds reasonable chromatographic runs with mobile phase containing up to 90% water were possible. A linear increase of log k_i by decreasing the percent of methanol was observed, with r^2 always greater than 0·99. The relationship between log P_{oct} and log k_w[ODP] was examined for BzSA and 4-ADS separately, revealing good correlations with identical slopes but different intercepts (equations not shown). This allowed the use of the indicator variable I (with the same meaning as in equation 4) to yield equation 5:

$$\log k_w[ODP] =$$
$$0{\cdot}66(\pm 0{\cdot}06) \log P_{oct} + 0{\cdot}31(\pm 0{\cdot}13)I + 1{\cdot}76(\pm 0{\cdot}09) \quad (5)$$
$$n = 61 \quad r = 0{\cdot}967 \quad s = 0{\cdot}211$$

The coefficient of I, namely +0·31, indicates that, once lipophilicity has been accounted for, the 4-ADS compounds have a greater affinity than the BzSA compounds for the ODP phase. This may be due to hydrophobic and Van der Waals interactions contributing more to their retention compared with the BzSA compounds. The statistical quality of equation 5 is slightly better than that of equation 4. The slopes in equations 4 and 5 are not significantly different, indicating a comparable discriminative power toward the lipophilic character of solutes, whereas the higher intercept in equation 5 can be interpreted in terms of intermolecular forces and/or physical features of the ODP phase. Specifically, the two phases should possess a different hydrogen-bonding capacity toward solute molecules.

Within the limits of the above considerations, our results indicate that the ODP phase gives log k_w values that are slightly better correlated with log P_{oct} than the log k_w values given by ODS, especially when sulphonyl groups result in deviant partitioning behaviour on the ODS stationary phase.

Measurements of partition coefficients by centrifugal partition chromatography

With the aim to further explore the applicability of CPC as a technique for measuring lipophilicity, we selected a set of 41 compounds, belonging to BzSA and 4-ADS, having log P_{oct} values ranging from −0·70 to 3·15 (Table 1). Log P_{oct} values measured by CPC (log $P_{oct/CPC}$) were highly correlated with those determined by the shake-flask method (log $P_{oct/SF}$):

$$\log P_{oct/CPC} = 1{\cdot}01(\pm 0{\cdot}04) \log P_{oct\,SF} + 0{\cdot}03(\pm 0{\cdot}06) \quad (6)$$
$$n = 41 \quad r = 0{\cdot}992 \quad s = 0{\cdot}148$$

In equation 6 the slope is almost equal to unity and the intercept to zero, implying that within the lipophilicity range investigated log P values determined by the CPC method can be equated to log P values obtained by the shake-flask method without any calibration or proportionality factor. This constitutes another piece of experimental evidence that both methods are governed by the same partitioning mechanism and that the CPC method, being far less time-consuming and much more reproducible, offers a promising alternative for measuring partition coefficients.

The method was therefore applied to the measurement of the lipophilicity of BzSA and 4-ADS in the n-heptane–water system. Only 42 compounds were lipophilic enough (log $P_{hep} > -3$) to yield measurable log P_{hep} values (Table 1). Log P_{oct} and log P_{hep} values are reasonably well correlated, as shown by the following "Collander type" equation:

$$\log P_{oct} = 0{\cdot}97(\pm 0{\cdot}11) \log P_{hep} + 3{\cdot}14(\pm 0{\cdot}20) \quad (7)$$
$$n = 42 \quad r = 0{\cdot}942 \quad s = 0{\cdot}260$$

Equation 7 has a slope almost equal to unity, but a large intercept, which expresses the difference in polar and H-bonding interactions in the two organic solvents (El Tayar et al 1991a). Indeed, it is well known that the difference between partition coefficients in 1-octanol–water and in n-heptane–water systems is not due to the cavity term, but rather to solute–solvent interactions. Polar and hydrogen-bonding interactions occur only in the water phase of the heptane–water partitioning system, being negligible in n-heptane (an aprotic apolar solvent containing only 0·009% water at saturation). When the slope in a "Collander type" equation approaches unity, the intercept can be considered as an index of H-bonding ability and mainly H-bond donor acidity of the solutes (Seiler 1974; El Tayar et al 1991a).

Separate examination of the two congeneric series yielded equation 8 for the BzSA:

$$\log P_{oct} = 1{\cdot}03(\pm 0{\cdot}19) \log P_{hep} + 3{\cdot}29(\pm 0{\cdot}41) \quad (8)$$
$$n = 23 \quad r = 0{\cdot}928 \quad s = 0{\cdot}292$$

and equation 9 for the 4-ADS:

$$\log P_{oct} = 0{\cdot}88(\pm 0{\cdot}20) \log P_{hep} + 3{\cdot}02(\pm 0{\cdot}27) \quad (9)$$
$$n = 19 \quad r = 0{\cdot}912 \quad s = 0{\cdot}301$$

The slopes and the intercepts are not significantly different in equations 8 and 9, indicating that both SO_2NH_2 and 4-NH_2-C_6H_4-SO_2 are almost equipotent as hydrogen-bond donors. This finding is unexpected in view of the known higher acidity of the SO_2NH_2 group and suggests a marked intramolecular interaction between the SO_2 and NH_2 groups in the 4-NH_2-C_6H_4-SO_2 moiety. From the available log P_{oct} (shake-flask methods) and log P_{hep} data, $\Delta\log P_{oct\text{-}hep}$ were calculated (Table 1). By analysing this data set and after excluding non-measurable compounds with log $P_{hep} < -3{\cdot}00$, we note that the $\Delta\log P$ values for the two series of sulphonyl-containing compounds span a very limited range of 1·10 log units. Unfortunately, the methodological limit did not allow us to measure log P_{hep} for compounds containing one or more substituents capable of participating as donors in intermolecular hydrogen-bonds. However, it is clear that among the 4-ADS derivatives, H-bond donor substituents raise the $\Delta\log P$ parameter to values higher than 4 units, particularly the hydroxyl groups in derivatives 59, 60, 61. In contrast, such high $\Delta\log P_{oct\text{-}hep}$ values were not observed for congeners bearing H-bond acceptor substituents, providing additional evidence that $\Delta\log P_{oct\text{-}hep}$ is a measure of H-bond donor acidity (El Tayar et al 1991a).

Application to structure-activity relationships

Compounds investigated in this study were BzSA as inhibitors of carbonic anhydrase (Carotti et al 1989) and 4-ADS as inhibitors of dihydropteroate synthase (De Benedetti et al 1987), the latter activity being only negligibly influenced by non-electronic parameters (Bawden & Tute 1981; Coats et al 1985).

For the BzSA listed in Table 1, the following structure-activity correlation was published earlier (Carotti et al 1989):

$$\log 1/K_i = 0{\cdot}95(\pm 0{\cdot}33)\sigma + 0{\cdot}54(\pm 0{\cdot}12)\pi - 0{\cdot}35(\pm 0{\cdot}11)B_{5,3} + 6{\cdot}29(\pm 0{\cdot}22) \quad (10)$$
$$n = 31 \quad r = 0{\cdot}914 \quad s = 0{\cdot}294 \quad F_{4,27} = 45{\cdot}6$$

where K_i is the inhibition constant, σ the Hammett electronic constant of the *meta*- or *para*-substituent, π the hydrophobic parameter of the *meta*- or *para*-substituent calculated from log P_{oct}, and $B_{5,3}$ the STERIMOL steric parameter for the *meta*-substituents.

As indicated by equation 10 and further confirmed by molecular graphic analysis, *meta*- and *para*-substituents in BzSA interact with a hydrophobic region in the active site. Furthermore, *meta*-substituents suffer from a detrimental steric effect as indicated by the negative sign associated with the STERIMOL parameter $B_{5,3}$.

To investigate the capability of the chromatographic parameters to express the hydrophobic nature of substituents in the set of carbonic anhydrase inhibitors, the RP-HPLC-derived hydrophobic substituent constants π^* were calculated as π^*_{ODS} and π^*_{ODP} in analogy with the Hansch hydrophobic substituent constant π from log P_{oct} (Braumann 1986). Replacing π in equation 10 with the new chromatographically derived hydrophobic substituent constant π^*_{ODP} leads to an equation with the same physicochemical meaning:

$$\log 1/K_i = 0{\cdot}91(\pm 0{\cdot}32)\sigma + 0{\cdot}72(\pm 0{\cdot}15)\pi^*_{ODP} - 0{\cdot}35(\pm 0{\cdot}11)B_{5,3} + 6{\cdot}08(\pm 0{\cdot}23) \quad (11)$$
$$n = 31 \quad r = 0{\cdot}918 \quad s = 0{\cdot}287 \quad F_{4,27} = 47{\cdot}8$$

Equation 11 is superimposable on equation 10 and possesses the same good predictive value. Indeed, 3-NO_2,4-OC_6H_{13}-BzSA, a compound designed on the basis of equation 10 to maximize the inhibitory potency of BzSA and recently synthesized (Carotti et al 1989), has been found to have an inhibitory activity that is well predicted by equation 11 (log $1/K_i$ pred. = 7·25 vs log $1/K_i$ obs. = 7·56).

A minor, but significant, contribution of lipophilicity to antimycobacterial activity (inhibition of *M. smegmatis*) by sulphones was evidenced by using the Rekker hydrophobic fragmental constant f in combination with the resonance component (R) (Bawden & Tute 1981). We carried out a regression analysis on 12 4-ADS common with the set examined by Bawden & Tute (equations 12–14):

$$\log 1/C = -1{\cdot}52(\pm 1{\cdot}02)R - 0{\cdot}44(\pm 0{\cdot}38)\pi - 2{\cdot}43(\pm 0{\cdot}44) \quad (12)$$
$$n = 12 \quad r = 0{\cdot}848 \quad s = 0{\cdot}388 \quad F_{1,9} = 6{\cdot}8$$

$$\log 1/C = -1{\cdot}64(\pm 0{\cdot}95)R - 0{\cdot}61(\pm 0{\cdot}47)\pi^*_{ODS} - 2{\cdot}52(\pm 0{\cdot}40) \quad (13)$$
$$n = 12 \quad r = 0{\cdot}865 \quad s = 0{\cdot}367 \quad F_{1,9} = 8{\cdot}7$$

$$\log 1/C = -1{\cdot}72(\pm 0{\cdot}91)R - 0{\cdot}83(\pm 0{\cdot}60)\pi^*_{ODP} - 2{\cdot}43(\pm 0{\cdot}40) \quad (14)$$
$$n = 12 \quad r = 0{\cdot}875 \quad s = 0{\cdot}355 \quad F_{1,9} = 10{\cdot}0$$

The activity is indeed dependent on resonance (R) and lipophilicity, again indicating that the three indices of lipophilicity π, π^*_{ODS} and π^*_{ODP} have essentially the same information content. The negative influence of lipophilicity on the transport into the mycobacterial cells was already noted and interpreted as a reflection of hydrophobic binding to cell wall lipids (Bawden & Tute 1981). However, qualitative examination of the antimycobacterial activity data suggests a possible positive influence of hydrogen-bond donor capacity on transport and consequently on whole-cell activity. Indeed, we can note that the most active compounds are not only the more hydrophilic ones, but they always bear H-bond donor substituents. In fact, the following equation was obtained by introducing an indicator variable (I_1) with the value of 1 when H-bond donor substituents are present and 0 when they are absent:

$$\log 1/C = -0{\cdot}93(\pm 0{\cdot}91)R + 0{\cdot}85(\pm 0{\cdot}47)I_1 - 2{\cdot}64(\pm 0{\cdot}33) \quad (15)$$
$$n = 12 \quad r = 0{\cdot}910 \quad s = 0{\cdot}303 \quad F_{1,9} = 18{\cdot}8$$

The higher statistical reliability of equation 15 over equations 12–14 seems to indicate that the H-bond donor acidity, rather than lipophilicity, could play a major role in influencing antimycobacterial activity. In addition, with only two exceptions (cpds 56 and 58), all 4-ADS analogues having $I_1 = 0$ show $\Delta\log P_{oct\text{-}hep} < 3{\cdot}5$ whereas those with $I_1 = 1$ show $\Delta\log P_{oct\text{-}hep} > 4$. This last observation suggests that $\Delta\log P$

has potential as a QSAR parameter accounting in part for the biological activity of 4-ADS, and could even be used to assess the role of hydrogen-bonding capacity in biological interactions such as cell membrane penetration and enzymic inhibition (De Benedetti 1987).

Conclusions

This study confirms the interchangeability of lipophilic descriptors obtained by various chromatographic methods. In particular, the ODP phase proved an interesting alternative to the popular ODS phase in measuring lipophilicity of compounds capable of adsorption interactions on silanol sites (e.g. sulphonyl-containing compounds in our case). Moreover, our findings confirmed the superiority of the CPC over the shake-flask technique in the experimental determination of lipophilicity.

Using the new lipophilic descriptors in QSAR studies offered a good opportunity to further verify the capacity of chromatographic parameters obtained by RP-HPLC to assess hydrophobic interactions occurring in the formation of enzyme-inhibitor complexes. Finally, the relatively modest influence of lipophilicity on the 4-ADS-mediated inhibition of dihydropteroate synthase offers an incentive to explore other lipophilicity-related parameters such as $\Delta\log P_{oct\text{-}hep}$, a molecular indicator accounting for hydrogen-bond donor capacity. The latter property is of interest to medicinal chemists since it plays a role in governing penetration into cells and/or binding to enzymes (De Benedetti 1987).

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