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R_m VALUES, RETENTION TIMES AND π VALUES OF A SERIES OF POTENTIALLY MUTAGENIC NITROIMIDAZO[2,1-*b*]THIAZOLES

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SUMMARY

The R_m values and retention times of a series of nitroimidazo[2,1-*b*]thiazoles were obtained by means of thin-layer and high-performance liquid chromatography, respectively. Extrapolated R_m and log k' values were correlated in a statistically significant way with π values calculated according to the literature.

INTRODUCTION

Nitroheterocyclic compounds play an important role in chemotherapy, mainly for their antibacterial, antitrichomonal and antiamebic activity. More recently they have been investigated for a possible sensitizing effect of hypoxic tumour cells toward radiotherapy[1–3]. However, nitroheterocyclic drugs are suspected to be genotoxic. In fact, although data on their carcinogenicity are still conflicting, several reports have demonstrated the mutagenic activity of these drugs[4]. The risk is related to the dosage, which is particularly high in radiosensitization and chemotherapeutic applications.

The purpose of this work was to study the relationship between chromatographic R_m values, retention times and π values of a series of nitroimidazo-[2,1-*b*]thiazoles with a view to investigating their structure–activity relationship as mutagenic agents. The use of the substituent or hydrophobic bonding constant, π, in the correlation of biological activity with chemical constitution is well established[5–8]. Substituent constants have also been used in the prediction of partition coefficients[9,10] and chromatographic retention times[11,12]. The usefulness of chromatographic R_m values as an expression of the lipophilic character of molecules has been shown previously for several series of compounds[13–17].

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EXPERIMENTAL

Determination of R_m values

The reversed-phase thin-layer chromatographic (TLC) technique for the determination of R_m values was similar to that described for a series of 5-nitroimidazoles[13]. The polar mobile phase was glycocoll buffer (0.1 *M*) of pH 13[18], alone or in various mixtures with acetone or methanol. The non-polar stationary phase was a silica gel GF 254 layer impregnated with a 5% (v/v) solution of silicone oil [silicone DC 200 (350 cSt) from Applied Science Labs. (State College, PA, U.S.A.)] in diethyl ether. The concentration of acetone in the mobile phase ranged from 10 to 70% and that of methanol from 10 to 90%. The nitroimidazo[2,1-*b*]thiazoles were dissolved in methanol (1 mg/ml) and 1–3-μl volumes of solution were spotted on the plates in random locations. The developed plates were dried and sprayed with an alkaline solution of potassium permanganate. Most of the compounds were also visible owing to their fluorescence when the plates were viewed under an ultraviolet lamp. The R_m values were expressed as $\log (1/R_F - 1)$.

Determination of retention times

High-performance liquid chromatography (HPLC) was performed on a Waters Assoc. liquid chromatograph equipped with an M 6000 pump, using a μBondapak C_{18} column (300 × 3.9 mm I.D.), packed with silica gel (particle size 10 μm) with a C_{18} chemically bonded non-polar stationary phase. A Waters Assoc. Model 440 UV detector at 313 μm and Hamilton 802 chromatographic syringes (25 μl) were used. The compounds were dissolved in methanol (1 mg/ml) and applied to the column in 5-μl volumes. All solutions and reagents were first filtered through Millipore filters (Type FH, pore size 0.5 μm). The separation was carried out using methanol–water mixtures as the mobile phase at a flow-rate of 1 ml/min. The methanol concentration ranged from 60 to 80%.

The retention times were expressed as

$$\log k' = \log \left(\frac{t_x - t_0}{t_0} \right)$$

where t_x = retention time of the compound and t_0 = retention time of the solvent front.

RESULTS AND DISCUSSION

R_m and $\Sigma\pi$ values

Reversed-phase TLC showed that none of the test compounds migrated when the mobile phase was glycocoll buffer alone. When methanol was added to the mobile phase in order to obtain suitable R_F values, 24 compounds did not move from the starting line even at a 90% methanol concentration. In contrast, a linear relationship between R_m values and acetone concentration in the mobile phase was found for all the compounds (Fig. 1). The equations describing the linear relationship were used to calculate a theoretical R_m value at 0% methanol in the mobile phase. The extrapo-

lated R_m values are reported in Table I, which also gives the $\Sigma\pi$ values calculated according to the literature[19–21]. Referring to generic structures in Table I in order to calculate the $\Sigma\pi$ values, the substituents R_2, R_3, R_5 and R_6 were considered. In particular for CH_3, Cl, NO_2 and NO groups, π values of 0.56, 0.71, −0.28 and −1.20 respectively were used[19].

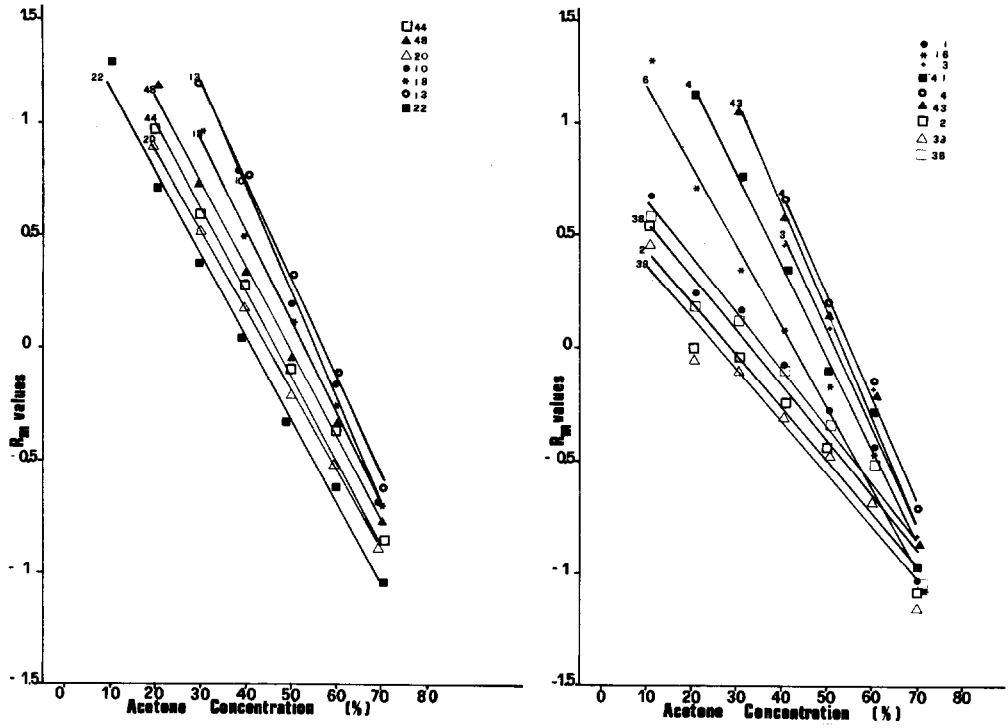


Fig. 1. Relationship between R_m values and acetone concentration in the mobile phase. Compound numbers as in Table I.

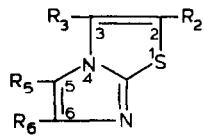
Leo *et al.*[20] and Rekker[21] calculated for one double bond a π value of −0.30 as obtained from non-heterocyclic systems. However, from the data of Hansch and Leo[19], the following π values for each double bond from heterocyclic rings could be calculated:

log *P*(pyridine) − log *P*(piperidine) =	0.67	− 0.76	= −0.09/3	=	−0.03
log *P*(pyrrole) − log *P*(pyrrolidine) =	0.75	− 0.46	= 0.29/2	=	0.14
log *P*(pyrazine) − log *P*(piperazine) =	−0.22	− (−1.17)	= 0.95/3	=	0.32
			Mean π double bond:		0.14

Therefore, in calculating the $\Sigma\pi$ values of compounds 38–49 the lack of one double bond was taken into account by adding −0.14.

TABLE I

R_m, LOG k' AND $\Sigma\pi$ VALUES OF NITROIMIDAZO[2,1-*b*]THIAZOLES



No.	*Structure*				*Empirical formula*	R_m	*Log k'*	$\Sigma\pi$
	R_2	R_3	R_5	R_6				
1	H	H	NO_2	Cl	$C_5H_2ClN_3O_2S$	0.88	1.72	0.43
2	H	H	NO_2	CH_3	$C_6H_5N_3O_2S$	0.66	1.51	0.28
3	H	H	NO_2	O_2N-Ph	$C_{11}H_6N_4O_4S$	1.80	2.91	1.57
4	H	H	NO_2	Cl-Ph(NO_2)	$C_{11}H_5ClN_4O_4S$	2.27	–	2.28
5	H	H	NO_2	H_3C-Ph(NO_2)	$C_{12}H_8N_4O_4S$	2.18	2.74	2.13
6	CH_3	H	NO_2	CH_3	$C_7H_7N_3O_2S$	0.99	1.61	0.84
7	CH_3	H	NO_2	O_2N-Ph	$C_{12}H_8N_4O_4S$	2.20	3.03	2.13
8	CH_3	H	NO_2	Cl-Ph(NO_2)	$C_{12}H_7ClN_4O_4S$	2.59 (calcd)	3.14	2.84
9	H	H	NO_2	Cl-Ph	$C_{11}H_{16}ClN_3O_2S$	2.25	2.89	2.56
10	H	CH_3	NO_2	Cl-Ph(NO_2)	$C_{12}H_7ClN_4O_4S$	2.64	3.57	2.84
11	H	CH_3	NO_2	H_3C-Ph(NO_2)	$C_{13}H_7N_4O_4S$	2.50 (calcd)	3.40	2.69
12	CH_3	H	NO_2	H_3C-Ph(NO_2)	$C_{13}H_7N_4O_4S$	2.50 (calcd)	3.76	2.69
13	CH_3	H	NO_2	Cl-Ph	$C_{12}H_8ClN_3O_2S$	2.51	3.44	3.12
14	H	CH_3	NO_2	Cl-Ph	$C_{12}H_8ClN_3O_2S$	2.42	3.45	3.12
15	H	CH_3	NO_2	O_2N-Ph	$C_{12}H_8N_4O_4S$	2.06	2.66	2.13
16	H	H	NO	Ph	$C_{11}H_7N_3OS$	1.45	2.48	0.93
17	CH_3	H	NO	Ph	$C_{12}H_9N_3OS$	1.60	2.79	1.49
18	H	H	NO	Cl-Ph	$C_{12}H_8ClN_3OS$	1.54	2.07	1.64
19	H	H	NO	H_3C-Ph	$C_{12}H_9N_3OS$	1.79	2.66	1.49
20	H	CH_3	NO	Ph	$C_{12}H_9N_3OS$	1.59	2.17	1.49
21	H	CH_3	NO	O_2N-Ph	$C_{12}H_8N_4O_3S$	1.51	2.73	1.21
22	H	CH_3	NO	Cl-Ph	$C_{11}H_6ClN_3OS$	2.19	2.63	2.20
23	H	CH_3	NO	H_3C-Ph	$C_{13}H_8N_3OS$	1.94	–	2.05

TABLE I (*continued*)

No.	*Structure*				*Empirical formula*	R_m	*Log k'*	$\Sigma\pi$
	R_2	R_3	R_5	R_6				
24	CH_3	CH_3	NO		$C_{13}H_{11}N_3OS$	1.88	3.06	2.05
25	CH_3	H	NO		$C_{12}H_8N_4O_3S$	1.31	1.59	1.21
26	CH_3	H	NO		$C_{12}H_8ClN_3OS$	2.26	3.57	2.20
27	CH_3	H	NO		$C_{13}H_{10}N_3OS$	2.07	3.22	2.05
28	CH_3	H	NO		$C_{12}H_7ClN_4O_3S$	1.82	–	1.92
29	CH_3	H	NO		$C_{13}H_{10}N_4O_3S$	1.72	2.74	1.77
30	H	CH_3	NO		$C_{12}H_7ClN_4O_3S$	2.03	–	1.92
31	CH_3	H	H		$C_{12}H_9N_3O_2S$	1.72	2.99	2.41
32	H	CH_3	H		$C_{12}H_9N_3O_2S$	1.84	2.65	2.41
33	H	CH_3	H		$C_{12}H_8ClN_3O_2S$	2.27	3.43	3.12
34	CH_3	H	H		$C_{13}H_{11}N_3O_2S$	2.15	3.11	2.97
35	CH_3	H	H		$C_{12}H_8ClN_3O_2S$	2.34	3.39	3.12
36	H	H	H		$C_{12}H_9N_3O_2S$	1.75	2.55	2.41
37	H	CH_3	H		$C_{13}H_{11}N_3O_2S$	2.02	3.00	2.97
38	H	H	NO_2	Cl	$C_5H_4ClN_3O_2S$	0.79	1.23	0.29
39	H	H	NO_2	CH_3	$C_6H_7N_3O_2S$	0.59	1.28	0.14
40	H	H	NO_2		$C_{11}H_9N_3O_2S$	1.90	2.48	1.71
41	H	H	NO_2		$C_{11}H_8N_4O_4S$	1.82	2.73	1.43
42	H	H	NO_2		$C_{11}H_8ClN_3O_2S$	2.12	3.17	2.42
43	H	H	NO_2		$C_{11}H_7ClN_4O_4S$	2.27	3.22	2.14
44	H	H	NO_2		$C_{12}H_{11}N_3O_2S$	2.22	2.82	2.27
45	H	H	NO_2		$C_{12}H_{10}N_4O_4S$	2.13	2.99	1.99
46	H	H	NO_2	H	$C_5H_5N_3O_2S$	0.32	0.04	−0.42
47	H	H	H		$C_{11}H_9N_3O_2S$	1.52	2.52	1.71
48	H	H	H		$C_{11}H_8ClN_3O_2S$	1.87	2.78	2.42
49	H	H	H		$C_{12}H_{11}N_3O_2S$	1.56	2.74	2.27

The equation describing the relationship between the R_m values and the $\Sigma\pi$ values shows a good correlation coefficient:

	n	r	s	
$R_m = 0.702 + 0.588\ \Sigma\pi$ ($F = 240.40$; $P < 0.005$)	49	0.915	0.220	(1)

However, as the compounds with no NO_2 or NO groups at position 5 are among those showing the greater deviation from linearity, we turned our attention to the role of each substituent group. The π values used in calculating the $\Sigma\pi$ values in Table I are reported in Table II, where they can be compared with the ΔR_m values obtained from the data in Table I. The ΔR_m values for the CH_3 and Cl groups and for the double bond in the thiazole ring seem to be in good agreement with the π values[19–21]. In particular, the π value for the double bond as calculated in this work is very close to the ΔR_m value. The NO_2 and NO groups deserve more attention. In fact, in the R_m system the NO_2 and NO groups at position 5 seem to be much more lipophilic than in the π system. This is less evident for the NO_2 group on the phenyl ring at position 6. A similar chromatographic behaviour for the NO_2 group was observed in a series of xanthone derivatives[22]. In a series of benzodiazepines the N → O group was found to be more lipophilic in the R_m than in the π system[23]. The reason could be an interaction between the molecule bearing one such substituent and the silica gel layer.

As a consequence of the above considerations, eqn. 2 was calculated with only ten compounds, *viz.*, those without any substituent at position 5 and eqn. 3 was calculated with the remaining 39 compounds.

	n	r	s	
$R_m = 0.387 + 0.588\ \Sigma\pi$ ($F = 58.96$; $P < 0.005$)	10	0.938	0.104	(2)
$R_m = 0.613 + 0.685\ \Sigma\pi$ ($F = 677.02$; $P < 0.005$)	39	0.974	0.136	(3)

A z-test[24] showed that the difference between the correlation coefficients of eqns. 3 and 1 is statistically highly significant ($P < 0.01$).

The slopes of eqns. 1–3 are less than unity. This is clearly due to the narrower range of the R_m values, which range from 0.32 to 2.64, while the $\Sigma\pi$ values range from −0.42 to 3.12. However, a general multiple linear regression equation calculated with all the compounds was obtained by introducing an indicator variable, I, into eqn. 1. This variable assumed values of 1 or 0 in the presence or absence, respectively, of an NO_2 or NO group at position 5 and accounted for the already mentioned interaction between the molecules bearing one such substituent at position 5 and the silica gel layer. The following equation was obtained:

	n	r	s	
$R_m = 0.154 + 0.678\ \Sigma\pi + 0.471\ I$ ($F = 386.06$; $P < 0.005$)	49	0.971	0.130	(4)

An analysis of variance showed the significant improvement yielded by the introduction of the I term into eqn. 1.

It is of interest to note that in a previous series of 5-nitroimidazoles[13], binding to the silica gel layer was suggested in order to explain a very low correlation coefficient between R_m and log P values. The introduction of a ΣMR term as an expression of the molar refractivity of substituent groups was necessary in order to obtain a significant improvement in the equation. The molar refractivity could account for the binding of 5-nitroimidazoles to the silica gel layer. In the present series of nitroimidazo[2,1-*b*]thiazoles, owing to the high correlation ($r^2 = 0.890$) between ΣMR and $\Sigma\pi$ values, the molar refractivity of substituents could not be taken into consideration.

Log k' values

HPLC showed that most of the compounds were not eluted when the methanol concentration in the mobile phase was 50% or lower. Concentrations ranging between 60 and 80% yielded reliable log k' values.

The plots in Fig. 2 show the linear relationships between log k' and the methanol concentration in the mobile phase. The extrapolated log k' values at 0% methanol are reported in Table I.

A good correlation between the R_m and log k' values is shown by eqn. 5 calculated with 45 compounds, as the log k' values were not available for compounds 4, 23, 28 and 30:

	n	r	s	
$R_m = -0.050 + 0.701 \log k'$	45	0.924	0.216	(5)
$(F = 250.73; P < 0.005)$				

The slope of eqn. 5 shows that the range of the log k' values (0.04 to 3.76) is wider than that of the R_m values.

The Δlog k' values reported in Table II show that in the HPLC system the NO_2 and NO groups at position 5 have a lower lipophilic character than that shown in the R_m system and closer to that calculated in the π system. In fact, eqn. 6, calculated without the ten compounds that do not have any substituent at position 5, is not significantly different from eqn. 5:

	n	r	s	
$R_m = -0.095 + 0.724 \log k'$	35	0.932	0.226	(6)
$(F = 219.66; P < 0.005)$				

On the other hand, eqns. 7 and 8, calculated with and without the above ten compounds, respectively, are very similar:

	n	r	s	
$\log k' = 1.247 + 0.743 \Sigma\pi$	45	0.881	0.352	(7)
$(F = 149.73; P < 0.005)$				
$\log k' = 1.165 + 0.831 \Sigma\pi$	35	0.903	0.351	(8)
$(F = 146.80; P < 0.005)$				

TABLE II

INFLUENCE OF SUBSTITUENT GROUPS AND DOUBLE BOND ON THE LIPOPHILIC CHARACTER OF NITROIMIDAZO[2,1-*b*]THIAZOLES

Group	*Position*	*Compounds**	ΔR_m	*Δlog k'*	π	*Group*	*Position*	*Compounds**	ΔR_m	*Δlog k'*	π
CH_3	2	2 *vs.* 6	0.33	0.10		NO_2	Phenyl ring	9 *vs.* 4	0.02	–	
	2	9 *vs.* 13	0.26	0.55			Phenyl ring	14 *vs.* 10	0.22	0.12	
	2	36 *vs.* 34	0.40	0.56			Phenyl ring	42 *vs.* 43	0.15	0.05	
	2	16 *vs.* 17	0.15	0.31			Phenyl ring	44 *vs.* 45	−0.09	0.17	
	2	20 *vs.* 24	0.29	0.89			Phenyl ring	40 *vs.* 41	−0.08	0.25	
	2	18 *vs.* 26	0.72	1.40			Phenyl ring	13 *vs.* 8	0.08	−0.30	
	2	19 *vs.* 27	0.28	0.56			Phenyl ring	17 *vs.* 25	−0.29	−1.20	
	2	3 *vs.* 7	0.40	0.12			Phenyl ring	20 *vs.* 21	−0.08	0.56	
	2	4 *vs.* 8	0.32	–			Phenyl ring	26 *vs.* 28	−0.44	–	
	2	5 *vs.* 12	0.32	1.02			Phenyl ring	22 *vs.* 30	−0.16	–	
	3	36 *vs.* 37	0.27	0.45			Phenyl ring	27 *vs.* 29	−0.35	−0.48	
	3	18 *vs.* 22	0.65	0.56							
	3	19 *vs.* 23	0.15	–							
	3	16 *vs.* 20	0.14	−0.31					$\bar{x}$ = 0.09 ± 0.06	$\bar{x}$ = −0.10 ± 0.19	−0.28
	3	17 *vs.* 24	0.28	0.27							
	3	3 *vs.* 15	0.26	−0.25		5		31 *vs.* 7	0.48	0.04	
	3	4 *vs.* 10	0.27	–		5		32 *vs.* 15	0.22	0.01	
	3	5 *vs.* 11	0.32	0.66		5		33 *vs.* 10	0.37	0.14	
	3	9 *vs.* 14	0.17	0.56		5		34 *vs.* 12	0.35	0.65	

	Phenyl ring	16 *vs.* 19	0.34	0.18		5	35 *vs.* 8	0.25	−0.25		
	Phenyl ring	20 *vs.* 23	0.35	–		5	36 *vs.* 5	0.43	0.19		
	Phenyl ring	17 *vs.* 27	0.47	0.43		5	37 *vs.* 11	0.43	0.40		
	Phenyl ring	40 *vs.* 44	0.32	0.34		5	47 *vs.* 41	0.30	0.21		
	6	46 *vs.* 39	0.27	1.24		5	48 *vs.* 43	0.40	−0.44		
						5	49 *vs.* 45	0.57	0.25		
			$\bar{x}$ = 0.32 ± 0.03	$\bar{x}$ = 0.48 ± 0.10	0.56			$\bar{x}$ = 0.38 ± 0.03	$\bar{x}$ = 0.12 ± 0.10	−0.28	
Cl	Phenyl ring	40 *vs.* 42	0.22	0.69	NO	5	31 *vs.* 25	−0.41	−1.40		
	Phenyl ring	16 *vs.* 18	0.09	−0.41		5	32 *vs.* 21	−0.33	0.08		
	Phenyl ring	17 *vs.* 26	0.66	0.78		5	33 *vs.* 30	−0.24	–		
	Phenyl ring	20 *vs.* 22	0.60	0.46		5	34 *vs.* 29	−0.43	−0.37		
	6	46 *vs.* 38	0.47	1.19		5	35 *vs.* 28	−0.52	–		
			$\bar{x}$ = 0.41 ± 0.11	$\bar{x}$ = 0.54 ± 0.26	0.71			$\bar{x}$ = −0.39 ± 0.05	$\bar{x}$ = −0.56 ± 0.43	−1.20	
						Double bond	38 *vs.* 1	0.09	−0.51		
						at position	49 *vs.* 36	0.19	−0.19		
						2 3	39 *vs.* 2	0.07	0.91		
							42 *vs.* 23	0.13	–		
							43 *vs.* 4	0.00	–		
							45 *vs.* 5	0.05	−0.25		
							41 *vs.* 3	−0.02	0.18		
								$\bar{x}$ = 0.07 ± 0.03	$\bar{x}$ = 0.03 ± 0.24	0.14	

* See Table I.

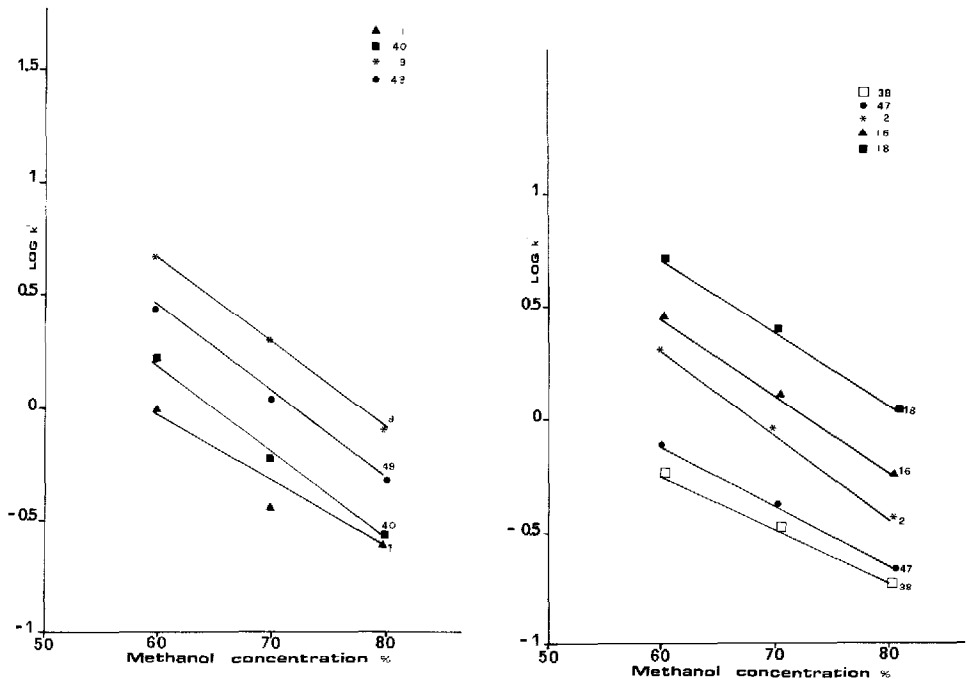


Fig. 2. Relationship between log k' values and methanol concentration in the mobile phase. Compound numbers as in Table I.

The data in Table I show that the range of the log k' values is very similar to that of the $\Sigma\pi$ values. Because of poorer statistical parameters, this is not reflected in the slopes of eqns. 7 and 8, which should be closer to unity.

The introduction of the I term into eqn. 7 improves the correlation coefficient in a significant way, as shown by the analysis of variance:

	n	r	s	
$\log k' = 0.807 + 0.817\,\Sigma\pi + 0.381\,I$	45	0.904	0.323	(9)
($F = 93.53$; $P < 0.005$)				

However, eqns. 7–9 indicate that in the HPLC system the interaction of the compounds with the stationary phase, presumably through the NO_2 or NO group at position 5, should play a less important role. This is again in agreement with the previous findings obtained on correlating the R_m and log P values of 5-nitroimidazoles[13].

CONCLUSIONS

The lipophilic character of nitroimidazo[2,1-*b*]thiazoles can be determined by means of both TLC and HPLC. The best correlations are obtained between the R_m and $\Sigma\pi$ values. On the other hand, the HPLC data have the advantage of a range of log k' values very close to that of the $\Sigma\pi$ values. The role of the NO_2 or NO group

at position 5 indicates the possibility of an interaction through these groups with the stationary phase in both TLC and HPLC. However, the log k' values seem to be much less influenced by such an interaction. The NO_2 or NO groups are strongly electron withdrawing and this might provoke the interaction with active sites of the stationary phase.

In previous work on 5-nitroimidazoles it was similarly observed that the interaction with the HPLC stationary phase is less important than that with the silica gel layer. As both series of compounds share an imidazole ring, it would be interesting to suggest a common mechanism for the interaction with the stationary phase. However, whereas for the nitroimidazo[2,1-*b*]thiazoles the binding could be attributed only to the NO_2 or NO group, for the nitroimidazoles it could be related to the molar refractivity of two substituent groups. The presence of an NO_2 group only at position 5 in all the nitroimidazoles previously investigated and the lack of a variety of side-chains in the series of nitroimidazo[2,1-*b*]thiazoles does not allow a more detailed analysis.

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REFERENCES

1 G. E. Adams, J. Denekamp and J. F. Fowler, *Chemotherapy*, 7 (1976) 187.
2 J. F. Fowler, G. E. Adams and J. Denekamp, *Cancer Treat. Rev.*, 3 (1976) 227.
3 P. Wardman, *Curr. Top. Radiat. Res. Q.*, 11 (1977) 347.
4 C. E. Voogd, *Mutat. Res.*, 86 (1981) 243.
5 M. E. Pickup and A. H. Beckett, *J. Pharm. Pharmacol.*, 29 (1977) 715.
6 C. Hansch and A. R. Steward, *J. Med. Chem.*, 7 (1964) 691.
7 T. Fujita, J. Iwasa and C. Hansch, *J. Amer. Chem. Soc.*, 86 (1964) 5175.
8 J. C. Dearden and E. Tomlinson, *J. Pharm. Pharmacol.*, 23, Suppl. (1971) 735.
9 D. J. Currie, C. E. Lough, R. F. Silver and H. L. Holmes, *Can. J. Chem.*, 44 (1966) 1035.
10 G. L. Flynn, *J. Pharm. Sci.*, 60 (1971) 345.
11 B. A. Knights and G. H. Thomas, *Nature (London)*, 194 (1962) 833.
12 J. C. Dearden, A. M. Patel and H. J. Tubby, *J. Pharm. Pharmacol.*, 26, Suppl. (1974) 749.
13 M. C. Guerra, A. M. Barbaro, G. Cantelli Forti, M. T. Foffani, G. L. Biagi, P. A. Borea and A. Fini, *J. Chromatogr.*, 216 (1981) 93.
14 J. K. Seydel and K. J. Schaper, *Chemische Struktur und Biologische Aktivität von Wirkstoffen*, Verlag Chemie, Weinheim, 1979.
15 N. B. Chapman and J. Shorter, *Correlation Analysis in Chemistry*, Plenum Press, New York, 1978.
16 E. Tomlinson, *J. Chromatogr.*, 113 (1975) 1.
17 A. M. Barbaro, M. C. Pietrogrande, M. C. Guerra, G. Cantelli Forti, P. A. Borea and G. L. Biagi, *J. Chromatogr.*, 287 (1984) 259.
18 *Documenta Geigy, Tables Scientifiques*, J. R. Geigy, Basle, 6th ed., 1963.
19 C. Hansch and A. J. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979.
20 A. Leo, C. Hansch and E. Elkins, *Chem. Rev.*, 71 (1971) 525.
21 R. F. Rekker, *The Hydrophobic Fragmental Constants*, Elsevier, Amsterdam, Oxford, New York, 1977.
22 A. M. Barbaro, M. C. Guerra, G. Cantelli Forti, G. Aicardi, G. L. Biagi, P. Da Re, P. Valenti and P. A. Borea, *J. Chromatogr.*, 242 (1982) 1.
23 G. L. Biagi, A. M. Barbaro, M. C. Guerra, M. Babbini, M. Gaiardi, M. Bartoletti and P. A. Borea, *J. Med. Chem.*, 23 (1980) 193.
24 G. W. Snedecor and W. G. Cochran, *Statistical Methods*, Iowa State University Press, Ames, IA, 1967, p. 186.